Synthesis and Anticancer Activity Studies of Some Novel Indole Related Heterocycles

THESIS

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

P. O. VENKATARAMANA REDDY

Under the supervision of

Prof. Dalip Kumar

Department of Chemistry

Birla Institute of Technology and Science, Pilani

Pilani Campus, Rajasthan

November-2018

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

CERTIFICATE

This thesis is submitted under Regulation 8.20 (a) of the Academic Regulations for Doctoral Programmes which allows a faculty member of the Institute/Professional to do Ph.D. research without the benefit of a supervisor.

This is to certify that the thesis entitled **"Synthesis and Anticancer Activity Studies of Some Novel Indole Related Heterocycles"** and submitted by **Mr. P. O. Venkataramana Reddy** ID No **2012PHXF0409P** for award of Ph.D. Degree of the Institute embodies my original work.

Signature

Name in Block Letters: **P. O. VENKATARAMANA REDDY**

ID No. **2012PHXF0409P**

Designation: **Research scholar**

Date:

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

CERTIFICATE

This is to certify that the thesis entitled **"Synthesis and Anticancer Activity Studies of Some Novel Indole Related Heterocycles"** and submitted by **Mr. P. O. Venkataramana Reddy** ID No **2012PHXF0409P** for award of Ph.D. of the Institute embodies original work done by him under my supervision.

Signature of the Supervisor

Name in capital letters: **PROF. DALIP KUMAR**
Designation: **Professor in Chemistry**

Date:

Dedicated to

My Family, Friends and Teachers

ACKNOWLEDGEMENTS

First and foremost, I would like to express that it is a great pleasure and privilege for me for being associate with Prof. Dalip Kumar, my research supervisor, who has introduced me to an intriguing realm of synthetic organic and medicinal chemistry. With his enthusiasm, inspiration, and his great efforts to explain things clearly and simply, he helped to make chemistry fun for me. During the period of my PhD tenure, he provided encouragement, sound advice, good teaching, good company, and lots of good ideas. I would have been lost without him who helped me a lot to learn and think more about chemistry. I do sincerely acknowledge the freedom rendered by him for independent thinking, planning and executing research.

I am immensely gratified to the Prof. Souvik Bhattacharyya, Vice-Chancellor, Prof. A. K. Sarkar, Director, Deputy Directors and Deans of Birla Institute of Technology & Science, Pilani (BITS Pilani) for giving me the opportunity to pursue my doctoral degree by providing necessary facilities and support.

My whole-hearted gratitude to Prof. Srinivas Krishnaswamy, Dean, Academic Graduate Studies & Research Division (AGSRD), Prof. S. K. Verma (Former Dean, ARD), BITS Pilani and Prof. Inamur Rahaman Laskar, Convener, Departmental Research Committee (DRC), Department of Chemistry, BITS Pilani, Pilani Campus for their official support and encouragement. I owe my sincere thanks to Prof. Jitendra Panwar, Associate Dean, AGSRD and Prof. Hemanth Jadav (Former, Associate Dean, ARD). I overwhelmingly acknowledge the office staff of AGSRD, whose secretarial assistance helped me in submitting the various evaluation documents in time.

I am indebted to the members of my Doctoral Advisory Committee, Dr. Paritosh Shukla and Prof. Rajeev Sakhuja for their great cooperation in refining my thesis. I also would like to extend my sincere gratitude to Prof. Saumi Ray, Prof. Ajay Kumar Sah, Prof. Madhushree Sarkar, Prof. Indresh Kumar and Dr. Prashant U Manohar members of DRC, Department of Chemistry, BITS Pilani, Pilani Campus for their constant guidance.

I am thankful to Prof. Kavita Shah, Purdue University, Prof. Rachna Sadana, University of Houston–Downtown and Prof. Kazuhito Tanabe, Aoyama Gakuin University, Japan for extending their support for biological screening and valuable discussions.

I am grateful to all the respected faculty members of the Department of Chemistry, BITS Pilani for their generous help and support along with fruitful discussions during the different stages of my doctoral study. Thanks, are also due to the office staff members and lab technicians of the Department for their help during my work. My sincere thanks to Mr. Giridhar Kunkur, Librarian, BITS Pilani and other staff of library for their support and help rendered while utilizing the library services.

I would like to extend my gratitude to Dr. Chakravarthy, Dr. Sudhakar Babu, Dr. P.V. Ramana, Dr. M.N.M. Reddy and Dr. Padma Reddy for their valuable suggestions and support. My sincere thanks to Dr. Prabhakar Reddy (Professor, Osmania University) and Mr. R. Reddeppa Reddy (Former IPS (IGP), Ex. M.L.C) for their support to my family.

I can't restrict myself to thank my close friends Mr. Prakash, Mr. Kondappa, Mr. Manohar Kumar Reddy, Mr. Uday, Mr. Baskar, Mr. Nagaraja Reddy, Mr. Govinda Rao, Mr. Fayaz, Mrs. Pallavi, Mr. Santosh B. K., Mr. Vimal, Dr. Ravi and Dr. Abdul for their never-ending support. I am fortunate and blessed to meet a wonderful couples Mr. Pradeep Choudary and Mrs. Santosh Kumari, and Mr. Hitesh and Mrs. Priyanka.

The great inspiring and congenial atmosphere along with the achievements made in Lab 3110 are one of the most memorable things in my life. My sincere thanks to Dr. Bhupendra Mishra, Dr. K. P. Chandra Shekar, Dr. Mukund P. Tantak, Dr. V. Arun and Dr. Meenakshi for their friendly guidance. I am very delighted to work with my group members Mr. Santosh B. Khandagale, Mr. Manish K. Mehra, Mr. MVSK Chaitanya, Mr. Bintu Kumar and Ms. Manu Bala thanks to you all for your untiring and continued support during my thesis work. My research life in BITS Pilani would have been imperfect without your support. I am thankful to graduate students Ms. Dhanya Lakshmi, Ms. Shriprada and Mr. Hridhay with whom I have worked. I want to thank other lab mates of 3110, Dr. Kiran, Dr. Noorualha, Dr. Abdul, Mrs. Savita, Mrs. Santosh Kumari, Mr. Vimal, Mr. Santosh Mishra, and Mr. Mahesha. I extend my acknowledge to department colleagues Dr. Kameswara Rao**,** Dr. Manoj, Dr. Amit, Dr. Sonu, Dr. Amrit, Dr. Parvej, Dr. Kasi, Dr. Ganesh, Dr. Sunita, Dr. Pinku, Mr. Hitesh, Ms. Moyna, Mrs. Susheela, Mrs. Khima, Ms. Saroj, Mr. Shiv, Mr. Devesh, Mr. Nitesh, Mr. Vikki, Dr. Pankaj, Mrs. Vaishali, Ms. Prachi, Mrs. Pragati, Mrs. Poonam, Dr. Rama Raju, Dr. Nisar, Mr. Sachin, Mr. Saleem, Dr. Ashok, Mr. Anoop, Ms. Sunita, Mr. Dinesh Kumar, Dr. Archana and Dr. Suman, Mr. Vishal, Mrs. Chavi, Ms. Mamata, Mr. Amol, Ms. Jyothi, Dr. Satish, Dr. O. P. Singh, Dr. Dinesh, Mr. Pramod, Mr. Sayantan,

Ms. Karisma, Ms. Rishika, Ms. Jagrithi, Mr. Roshan, Mr. Abid, Ms. Aishwarya, Ms. Sonam, Ms. Bijoya, Ms. Sumona, Mr. D. Pal and all other research scholars of the department. I also extend my heartfelt thanks to other research scholars of BITS Pilani Dr. Subash, Dr. Rajnish, Dr. Pankaj, Dr. Almesh, Mr. Sandeep, Mr. Vikram, Mr. Sourab, Mr. Sridhar, Dr. Santosh, Dr. Gagan and Mr. Sahid for their help and charming company.

Words are inadequate to express my respect for my parents (Smt. Seethamma and Late Sh. P. Krishna Reddy) for their vision, ethical principles, moral support, endless patience and eternal inspiration to face any situation in life have guided me to the successful completion of this work. I express my heartfelt thanks to my sisters Mrs. Alivelamma, and Mrs. Parvathamma and their children Mrs. Devi, Mrs. Renuka, Ms. Srikanya and Mr. Siva Kumar Reddy for their love and motivation. They always sacrificed their personal life for my convenience and I truly do appreciate it. I also extent my thanks to close relatives Mr. Purusottham Reddy, Mr. Krishna Reddy, Mr. Jagannadha Reddy, Mr. Sheshadri Reddy, and Mr. Munivenkata Reddy for their moral support.

I deeply thank my brother Mr. P. Govinda Reddy along with his family Mrs. Sowjanya and their children Ms. Deeksha and Master Siddarth for their continuous motivation and never-ending support. Most importantly, the deepest thanks from the bottom my heart to an important and most valuable person in my life, my brother, Mr. P. Govinda Reddy, who always considered my success as his own which gave me immense pleasure to achieve the best in my life. He always sacrificed his personal life for my convenience and I truly do appreciate it. It is not possible to write all what I have in my heart, so I can only *"*dedicate this achievement to my brother".

Last but not least, a special acknowledgement goes to my wife Mrs. Sukanya and my child Ms. Hedvika for being present in my life to achieve and accomplish my dreams. Finally, I am thankful to God, through whom all things be.

I gratefully acknowledge valuable support in the form of Research Fellowship from DBT, New Delhi and BITS Pilani and DST-FIST for instrumentation facilities.

P. O. Venkataramana Reddy

ABSTRACT

Cancer is one of the prominent causes of death worldwide after heart disease. Identification of potent and selective anticancer drugs with reduced side effects is a serious concern to medicinal chemists. The thesis deals with the design, synthesis and anticancer activity studies of some indole-related heterocycles. The thesis is divided into six chapters.

The **first chapter** briefly highlights the synthesis and chemical properties of indole related heterocycles such as carbazole and *β*-carboline derivatives. In addition, this chapter includes description about the importance of natural and synthetic carbazole and *β*-carboline based anticancer agents.

The **second chapter** is subdivided into two parts dealing with design, synthesis and anticancer activity studies of two different series of novel *β*-carbolinium bromides. **Part A** of the chapter reports microwave-assisted rapid and high yielding synthesis of phenacyl-*β*-carbolinium bromides by N^2-quaternization of *β*-carbolines with 1-aryl-2-bromoethanones in ethanol. The most potent *β*-carbolinium bromides were found to display broad spectrum of anticancer activity against all the tested cancer cell lines (IC_{50} = 3.16-7.93 μM) *via* apoptosis inducing pathway. **Part B** of the chapter describes the synthesis and anticancer activity studies of *β*-carboline-1-chalcones and their bromide salts which led to identify a potent compound with an IC_{50} value lower than 22.5 μM against all the tested cancer cell lines. Further, preliminary mechanism of action studies indicated that chalcone tethered *β*-carbolinium bromides triggered apoptosis in MDA-MB-231cells.

The **third chapter** illustrates a significant exploration of molecular iodine and triphenylphosphine combination enabled the facile construction of natural *β*-carboline skeletons. The desired products were prepared from easily available indoles and tryptamines by *in-situ* cyclization of bis(indolyl)ketoamides involving the use of molecular iodine and triphenylphosphine in DMSO. The present strategy provides an attractive approach to access Pityriacitrin, Hyrtiosulawesine, Alangiobussinine and their derivatives in good to excellent yields from readily accessible and easy to handle substrates under mild reaction conditions.

The **fourth chapter** comprises the rational design, synthesis and photocytotoxicity studies of water soluble porphyrin-*β*-carboline conjugates connected through amide linker. Cationic porphyrin-*β*-carboline conjugates were rapidly prepared in high yields by using MW-assisted coupling of *β*-carboline acids with 5-(4-aminophenyl)tripyridylporphyrin in the presence HATU as coupling reagent, DIPEA as base in DMF, followed by *N*-methylation of neutral porphyrin-*β*-carboline conjugates with excess of methyliodide. All the cationic porphyrin-*β*-carboline conjugates displayed remarkable photocytotoxicity with IC_{50} value ranging 39-173 nM against lung as well as colon cancer cell lines.

The **chapter five** describes synthesis and *in vitro* anticancer and antibacterial activity studies of carbazolyl glyoxamides. A library of twenty-four glyoxamides was achieved from easily accessible carbazolyl glyoxalic acids and various aryl/heteroarylamines by employing HATU as a coupling reagent under MW-irradiation in good yields. The most potent analogues exhibited potent cytotoxicity with IC_{50} values ranging between 9.3-9.8 μM against MCF-7 cancer cell line through apoptotic cell death. In addition, antibacterial activity studies revealed four carbazolyl glyoxamides with prominent potency against Gram-positive and Gram-negative bacteria (MIC = 8-16 μg/mL) by inhibiting the growth of bacteria in few hours of initial interactions.

Finally, summary and conclusions of the thesis are reported in **chapter six**. Future directions in continuation of the results achieved in the thesis are also described in this chapter.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS / SYMBOLS

Abbreviation/ Symbol	Description	Abbreviation/ Symbol	Description
α	Alpha	CNS	Central nervous system
β	Beta	Conc	Concentration
γ	Gamma	d	Doublet
δ	Lowercase delta	DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
Δ	Uppercase delta	dd	Doublet of doublet
°C	Degree centigrade	DCE	Dichloroethane
Å	Angstrom	DCM	Dichloromethane
Ac	Acetyl	DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
ACN	Acetonitrile	DHBCs	3,4-dihydro-β-carbolines
Ar	Aryl	DIB	Diacetoxy iodobenzene
ATP	Adenosine triphosphate	DMF	*N*,*N*-Dimethylformamide
BCs	β-Carbolines	DMSO-d_6	Deuterated dimethylsulfoxide
bFGF	Basic fibroblast growth factor	DNA	Deoxyribonucleic acid
Bn	Benzyl	EC_{50}	Maximal effective concentration
Bu	Butyl	ED_{50}	Effective dose 50%
Bz	Benzoyl	EDDA	Ethylenediamine diacetate
t-BuOK	Potassium *tert*-butoxide	EDTA	Ethylenediaminetetraacetic acid
Calcd.	Calculated	EGFR	Epidermal growth factor receptor
^{13}C	Carbon-13	ESI	Electrospray ionization
Cat.	Catalyst	EtOAc	Ethyl acetate
$CDCl_3$	Deuterated chloroform	EtOH	Ethanol
Chalcones	1,3-Diarylprop-2-en-1-ones	G4	G-quadruplex

Abbreviation/ Symbol	Description	Abbreviation/ Symbol	Description
GI_{50}	The concentration for 50% of maximal inhibition of cell proliferation	MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
h	Hours	mmol	Millimole
HFIP	Hexafluoro-2-propanol	mL	Milliliter
HRMS	High resolution mass spectra	MW	Microwave
5-HT	5-Hydroxytryptamine	NCI	National Cancer Institute
HUVECs	Human umbilical vein endothelial cells	NH_3	Ammonia
IBX	2-Iodoxybenzoic acid	N_2	Nitrogen gas
IC_{50}	Half maximal inhibitory concentration	NBS	*N*-Bromosuccinimide
IR	Infrared	NCS	*N*-Chlorosuccinimide
Hz	Hertz	NIS	*N*-Iodosuccinimide
J	Coupling constant	nM	Nano molar
KSP	Kinesin spindle protein	NMR	Nuclear magnetic resonance
$K_2S_2O_8$	Potassium persulfate	O_2	Oxygen gas
Lit.	Literature	p53	Protein 53
LDA	Lithiumdiisopropylamide	PPA	Polyphosphoric acid
m-CPBA	*m*-Choroperbenzoic acid	PEG	Polyethylene glycol
Me	Methyl	Ph	Phenyl
MIC	Minimum inhibitory concentration	ppm	Parts per million
MRSA	Methicillin-resistance *S. aureus*	rt	Room temperature
MS	Mass spectrometry	m	Multiplet
Equiv	Equivalent	MeOH	Methanol
g	Gram	MHz	Mega hertz

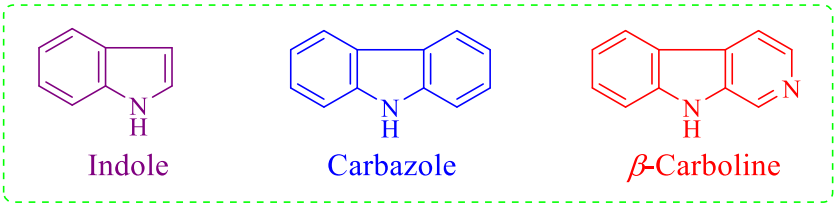
Abbreviation/ Symbol	Description	Abbreviation/ Symbol	Description
min	Minutes	*t*-Bu	Tertiary butyl
Mp	Melting point	TFA	Trifluoroacetic acid
T3P®	Propylphosphonic anyhydride	t	Triplet
TBAB	Tetrabutylammonium bromide	TLC	Thin layer chromatography
TBHP	*tert*-Butyl hydroperoxide	TMS	Tetramethylsilane
TCCA	Trichloroisocyanuric acid	Top I	Topoisomerase I
TfOH	Trifluoromethanesulfonic acid	Top II	Topoisomerase II
Tf_2O	Trifluoromethanesulfonic anhydride	TGF-β	Transforming growth factor beta
THBCs	1,2,3,4-tetrahydro-β-carbolines	TNF-α	Tumor necrosis factor α
THF	Tetrahydrofuran	UV	Ultraviolet
s	Singlet	VEGF	Vascular endothelial growth factor
SAR	Structure-activity relationship	w	Watt
SRB	Sulphorhodamine B	μM	Micromolar

TABLE OF CONTENTS

Appendices

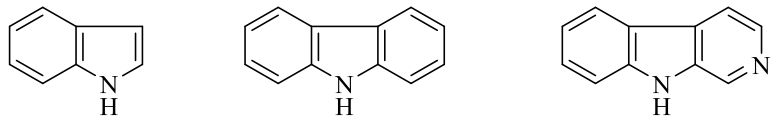
Chapter 1

Introduction



1.1 Introduction

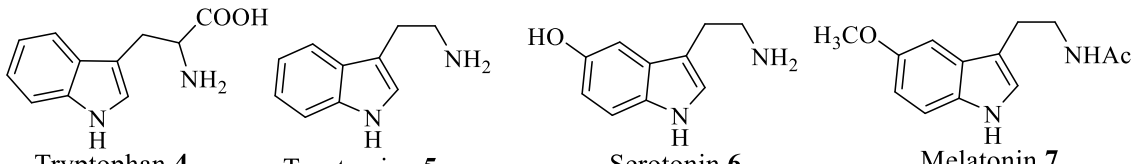
Natural products isolated from plants and microorganisms are unsurpassed as a source of various biologically active compounds.[1] Among the isolated compounds especially, nitrogen containing heterocycles are in major amount due to their unique ability to mimic the structures of peptides and to bind reversibly to proteins.[2] Indole nucleus is one such *N*-containing privileged heterocycle present in most of the naturally occurring bioactive molecules with diverse therapeutic utilities. Over past several years, many indole-derived synthetic analogues have been discovered with excellent anticancer activity that is comparable to their parent natural product counterparts.[3] Indole and indole-fused systems are probably the most widely distributed heterocyclic compounds in nature having medicinal importance. At present, there are approximately 1600 indole-based alkaloids described, which include simple and more complex functionalized indole derivatives.[4] The simple indole **1** is comprised of a pyrrole ring fused with a benzene ring and more complex indole derivatives usually contain an additional fusion ring, and in most cases a six-membered ring, for example carbazole **2** and *β*-carboline **3** as shown in Figure 1.1.



Indole **1** Carbazole **2** *β*-Carboline **3**

Figure 1.1 Structures of indole and related heterocycles

Several simple indole derivatives are known to possess important biological as well as pharmaceutical properties (Figure 1.2). For example, tryptophan **4** is an essential amino acid and as such a constituent of most proteins; it also serves as a biosynthetic precursor for a wide variety of indole-containing secondary metabolites.[5,6]



Tryptophan **4** Tryptamine **5** Serotonin **6** Melatonin **7**

Figure 1.2 Structures of some naturally occurring simple indoles

Tryptamines **5** and **6** are known to possess a significant pharmacological profile in addition to synthetic precursors in the preparation of carbolines and other polynitrogenated compounds.[7] Serotonin **6** is a very important neurotransmitter in CNS, endogenous agonist of 5-HT receptors, and in the cardiovascular and gastrointestinal systems.[8] Serotonin activates at

least seven distinct receptors (5-HT1 to 5-HT7) in the central and peripheral nervous systems to produce important modulatory effects. The structurally similar hormone melatonin **7** is known to control the diurnal rhythm of physiological functions.[9]

The wide distribution of the indole nucleus in biological systems and biologically active natural products has prompted medicinal chemists to explore indole chemistry for drug synthesis, and these efforts have culminated in the discovery of several successful drugs for different diseases. Some of the representative investigational drugs are outlined in Figure 1.3. The drug indomethacin **8** is one of the first non-steroidal anti-inflammatory agent that cures pain, fever and inflammation by reducing the production of prostaglandins.[10-12] Sertindole **9,** an antipsychotic drug used for the treatment of schizophrenia.[13] Pindolol (Visken) **10**, a beta blocker used for the treatment of hypertension and a partial agonist for depression.[14] An aza-indole-based drug, Vemurafenib (Zelboraf™) **11** act as a selective inhibitor of the activated BRAFV600E gene that is found in 70% of malignant melanoma cancers.[15-17] The 5-HT_3 receptor antagonists, Frovatriptan **12** was developed by Vernalis for the treatment of menstruation associated headaches[18] and Ondansetron **13** used for the treatment of chemotherapy or radiation-induced nausea and vomiting and there are indications that they may be effective in the treatment of migraine or the pain associated with it.[19] The two *β*-carboline-based drugs Tadalafil **14** and Abecarnil **15** are clinically used for erectile dysfunction and CNS disorders, respectively.[20-23]

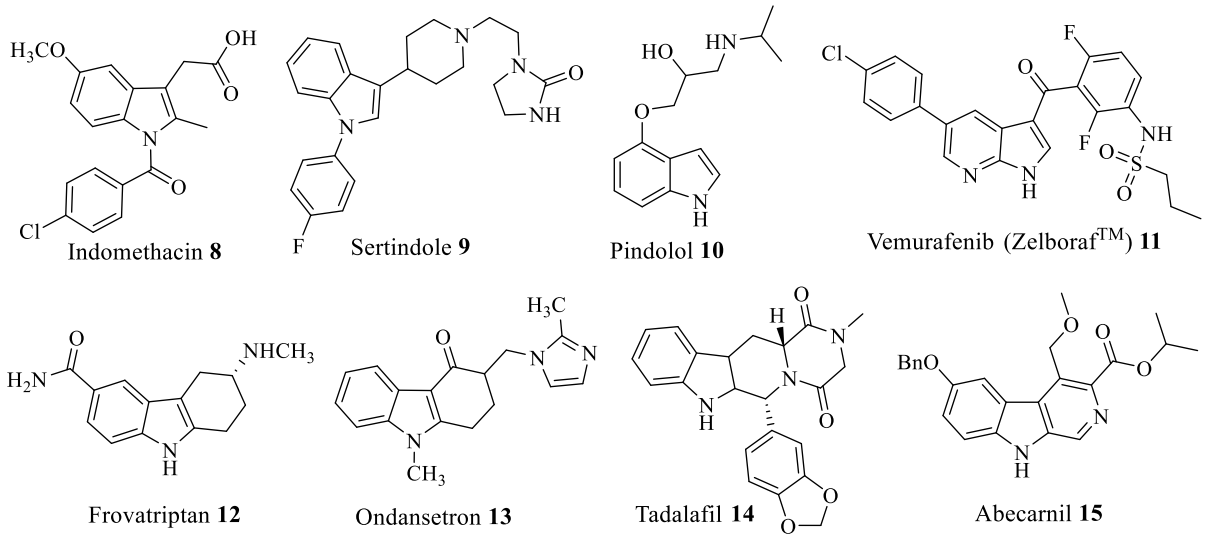


Figure 1.3 Representative marketed drugs with indole scaffold

Especially, since last two decades, most of the synthetic and natural compounds possessing indole nuclei exhibited their anticancer effects through multiple mechanisms such as tubulin polymerization/de-polymerization, DNA intercalation and topoisomerase inhibition.[24] In

view of successful identification of many indole-based bioactive compounds, indole and related heterocyclic systems (Phidianidine B **16**, Labradorin 1 **17**, Granulatimide **18**, Calothrixin **19,** Harmine **20**, Azatoxin **21**) offer an excellent opportunity for further structural modifications to identify novel anticancer agents (Figure 1.4).[25-31]

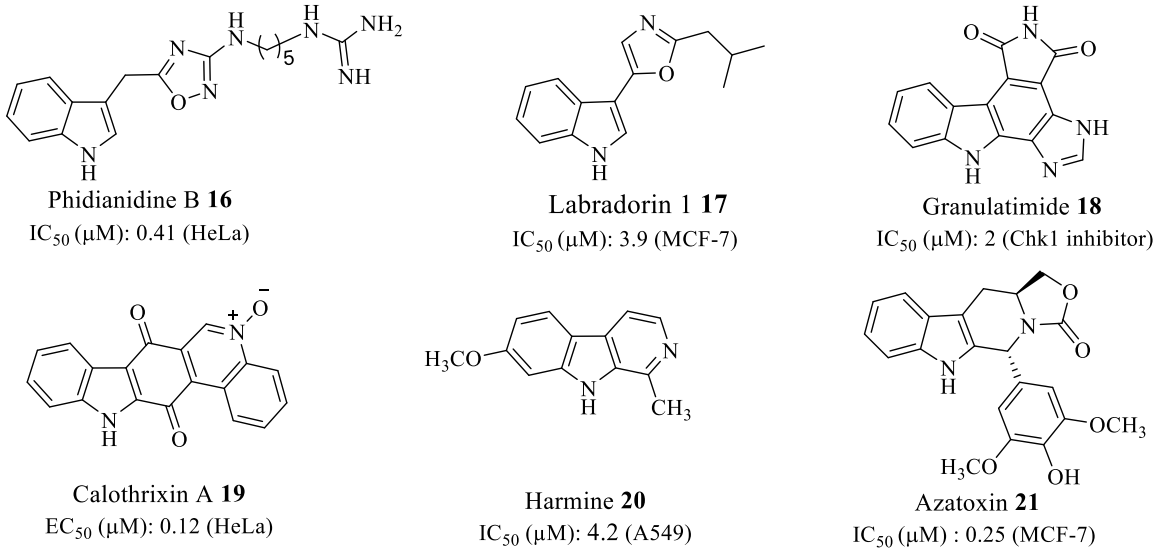


Figure 1.4 Some natural antitumor agents possessing indole-derived systems

Fusion of bioactive indole nucleus with benzene led to another interesting class of compound, carbazole **2** which is commonly found in a wide variety of natural products and pharmaceutical agents endowed with antitumor, psychotropic, anti-inflammatory, antihistaminic, antibiotic and anti-oxidative properties.[32] In particular, carbazole derivatives have been reported as potential antitumor agents.[33]

1.2 Carbazole

Carbazole **2** was first isolated in 1872 by Graebe and Glaser from crude anthracene fraction of coal tar distillate (Figure 1.5).[34] The term carbazole refers to a 9*H*-carbazole based on nomenclature. Conventionally tricyclic ring systems are denoted by A, B and C and the numbering starts from ring A. The classification of the carbazoles is based on the substitution pattern of ring A, although ring C may also carry various substituents. The first carbazole alkaloid, murrayanine **22,** isolated in 1964 as antimicrobial agent from the plant *Murrraya koenigii* Spreng,[35-37] generated a substantial interest among chemists and biologists to initiate research in the field of carbazole. Since then, the fascinating structural features and potent pharmacological activities of these naturally occurring carbazole alkaloids have led to extensive progress in carbazole chemistry.[38-43] In the recent years, most of the carbazole alkaloids have been isolated from the higher plants of the genus *Murraya* (murrastinine A **23**

and C **24**), *Glycosmis* (glycoborine **25** and glybomine A **26**), *Clausena* (sansoakamine **27**, claulansine R **28** and F **29**, clauemarazole **30**, clausenawalline D **31** and clausenaline E **32**) and *Micromelum* (micromeline **33** and microfalcatine **34**) from the family *Rutaceae* (Figure 1.5) and these alkaloids are known to exhibit pharmacological properties such as antioxidant, antimicrobial, antidiarrhoeal, antidiabetic antiprotozoal, and anti-inflammatory, antiplatelet, anti-HIV and anticancer activities.[44-47]

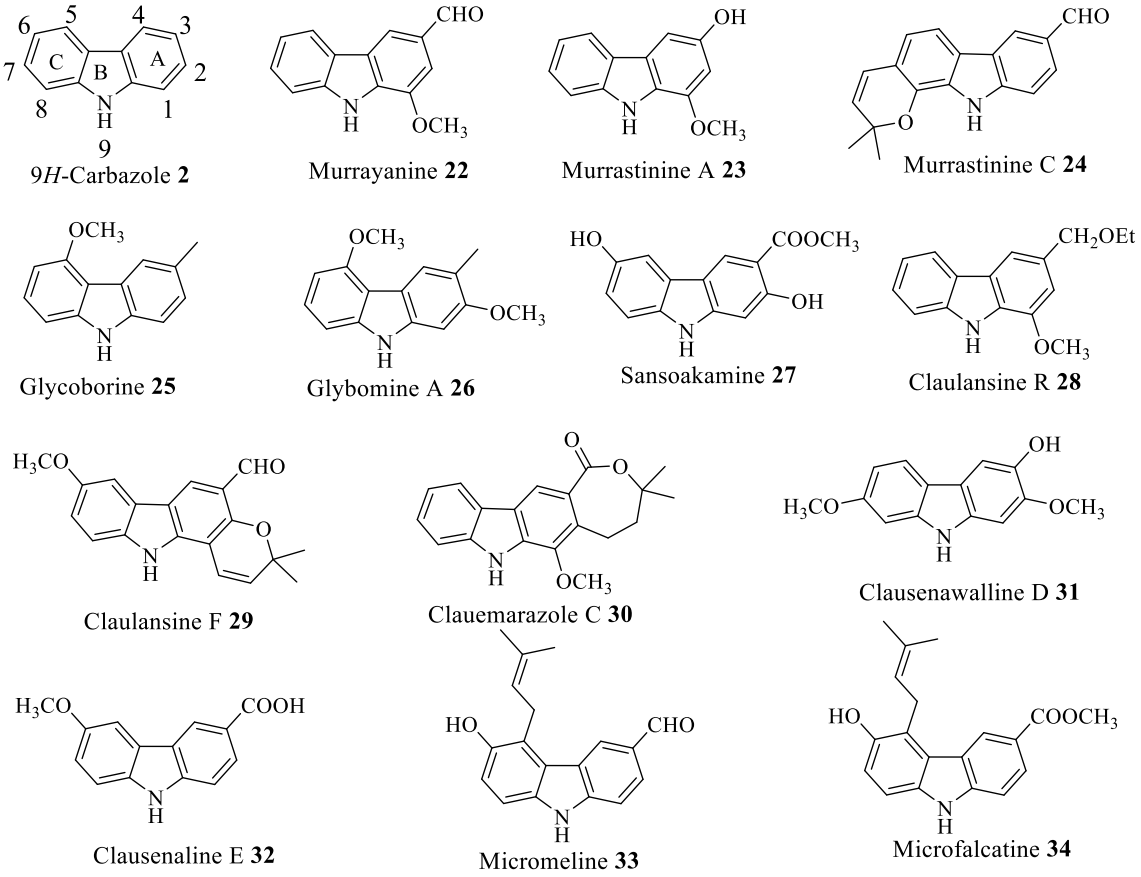


Figure 1.5 Selected examples of carbazole alkaloids

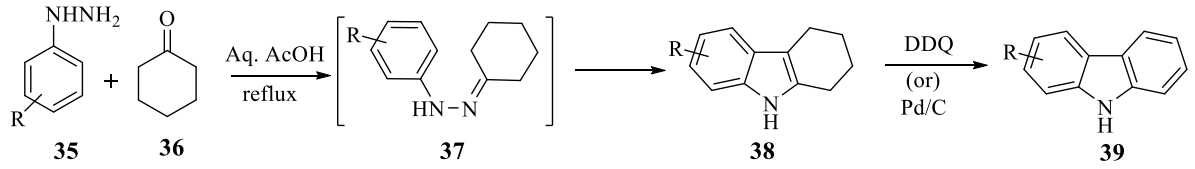
1.2.1 Synthesis of carbazole

Herein, we summarize a brief overview of some important literature reported methods that have been frequently used for the construction of carbazoles and its derivatives.[38,48,49]

1.2.1.1 Fischer-Borsche Synthesis

Fischer-Borsche reaction or Borsche-Drechsel cyclization is a general classical approach for the synthesis of carbazoles **39**, wherein the first step, the arylhydrazine **35** condensed with cyclohexanone **36** to form the cyclohexanone arylhydrazone **37,** which was further cyclized under acidic conditions to produce tetrahydrocarbazoles **38.** Oxidation of tetrahydrocarbazole

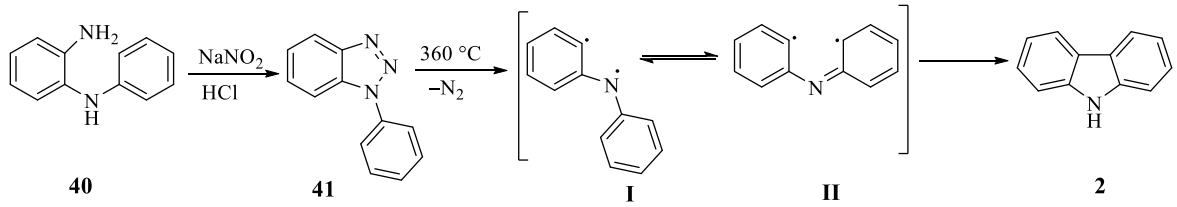
38 with appropriate oxidants like DDQ, chloranil and Pd/C produced desired carbazole **39** (Scheme 1.1).[50,51]



Scheme 1.1 Synthesis of carbazoles by Fischer-Borsche reaction

1.2.1.2 Graebe-Ullmann Synthesis

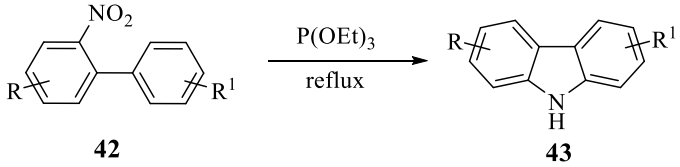
In 1896, Carl Graebe and Fritz Ullmann reported another classical approach for the transformation of 1-phenylbenzotriazole to carbazole **2** under thermal conditions known as the Graebe-Ullmann synthesis.[52,53] The diazotization of *o*-aminodiphenylamine **40** yielded 1-phenyl-1,2,3-benzotriazole **41**, which upon heating at high temperature produced carbazole **2** *via* diradical **I** & **II** (Scheme 1.2).



Scheme 1.2 Graebe-Ullmann carbazole synthesis

1.2.1.3 Cadogan synthesis

In this classical method reductive intramolecular cyclization of 2-nitrobiphenyls **42** in excess of triethylphosphite led to carbazoles **43** (Scheme 1.3).[54] This method has a number of advantages including variety of substrates and functional group tolerance, and more precise regio-control of functional group placement within the product.[55]



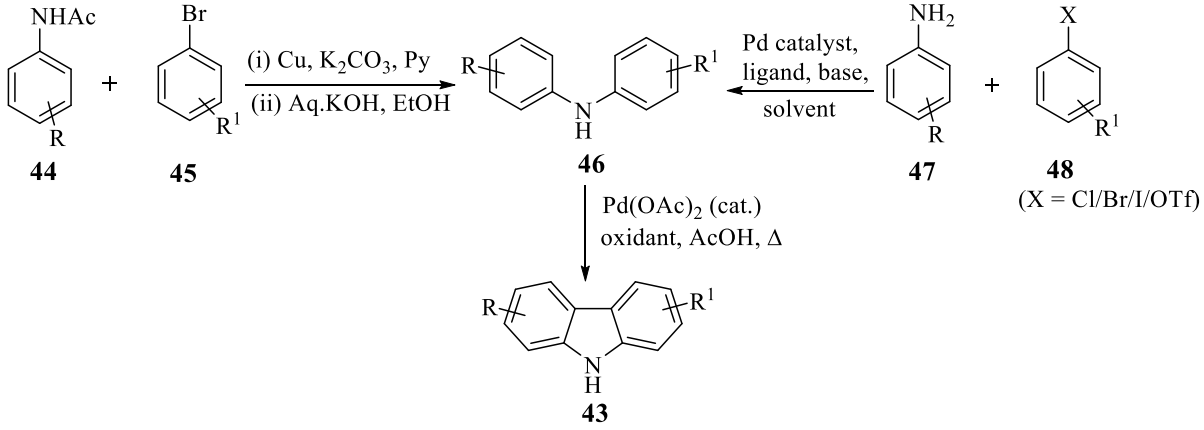
Scheme 1.3 Cadogan synthesis of carbazole derivatives

1.2.1.4 Palladium(II)-mediated carbazole synthesis

The easiest way to access fully aromatized carbazoles is the use of palladium(II) catalysts in the construction of the central pyrrole ring through cyclodehydrogenation of diarylamines **46**. Recently, a variety of palladium metal catalyzed synthetic procedures for the construction of

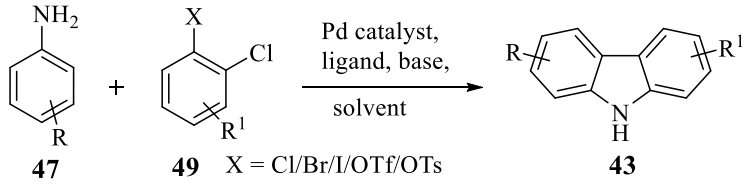
carbazoles employing mild reaction conditions have been developed by utilizing either commercially available or easily prepared starting materials in good to excellent yields.[38]

The preparation of required diarylamines **46** involves either Ullmann-Goldberg coupling of acetanilides **44** with arylbromides **45** or palladium(0)-catalyzed Buchwald-Hartwig amination reaction of arylamines **47** with aryl halides/triflates **48** (Scheme 1.4).[56-63] Further, the diarylamines **46** undergo palladium(II)-mediated oxidative cyclization to afford respective carbazoles **43** in good to excellent yields (Scheme 1.4).[64-67] These protocols offer advantages such as use of only catalytic amount of the transition metal under mild reaction conditions.



Scheme 1.4 Transition metal catalyzed synthesis of carbazoles

In recent past, Ackermann and Althammer reported an extension of above methodology for a one-pot synthesis of carbazoles **43** *via* a domino *N*-H/C-H bond activation promoted by palladium catalyst. Variety of substituted carbazoles **43** were prepared in good yields by the coupling of anilines **47** with 1,2-dihaloarenes **49** (Scheme 1.5).[68,69]

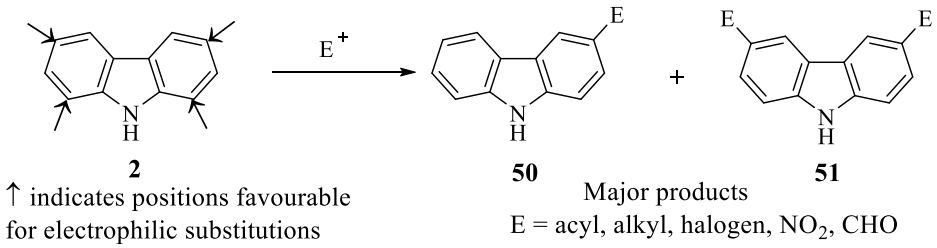


Scheme 1.5 Palladium-mediated direct synthesis of carbazoles

1.2.2 Reactivity of carbazoles

Carbazole and its derivatives are colorless crystalline solids found to be stable in air and soluble in most of the organic solvents. Carbazole is planar conjugated system with 14 π-electrons are reported to show diverse physical and chemical properties in addition to their interesting chemical reactions. Owing to its high electron-rich nature, the carbazole skeleton is a modest nucleophile that can readily react with various electrophiles (tertiary alkyl, acyl,

nitro, halogen etc.).[70] Introduction of electrophiles on carbazole is limited to the positions on the ring system as presented in Scheme 1.6.[71-74] The most reactive positions for electrophilic substitution are the 3 (**50**) and 6 (**51**) positions, and to a lesser extent, the 1 and 8 positions, which often require more forcing reaction conditions.[75-77] Although, the functionalization of 2, 4, 5, or 7 ring positions or mono and/or unsymmetrical substitution on carbazole, and/or when functional groups are not readily introduced by electrophilic substitution, alternate synthetic strategies are required to functionalize carbazole.



Scheme 1.6 Electrophilic substitution reactions on carbazole

1.3 Carbolines

Another interesting bioactive indole related heterocyclic compound, pyrido-fused indole (carboline) is the parent core present in many bioactive naturally occurring compounds. Carbolines and its derivatives have received significant attention due to their wide range of biological activities.[78,79] Carbolines are classified as *α, β, γ* or *δ* according to the mode of pyridine ring fusion with indole (Figure 1.6).

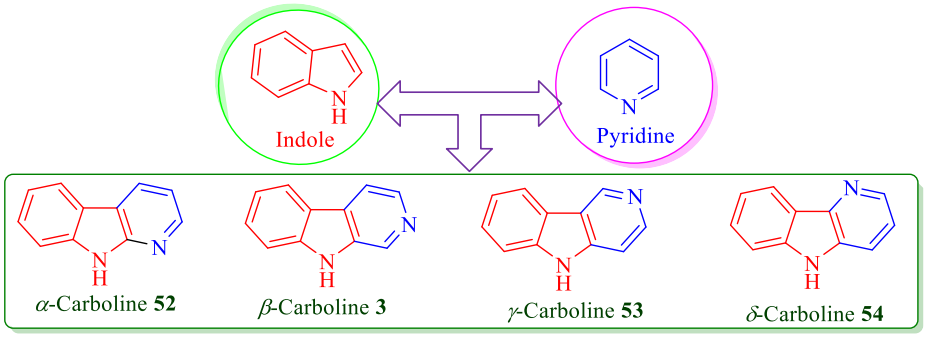


Figure 1.6 Classification of carboline alkaloids-based on position of nitrogen atom

In the family of carbolines [*α* (**52**), *β* (**3**), *γ* (**53**) *or δ* (**54**)], *β*-carbolines **3** belonging to a large family of natural and synthetic indole alkaloids have attracted considerable attention of medicinal chemists because *β*-carbolines possess wide range of biological activities such as antiparasitic, antitumor, and antiviral properties. Mostly, simple and complex *β*-carboline derivatives displayed their antitumor activity mainly through DNA intercalation, topoisomerase and kinase inhibition.[80-82]

1.3.1 *β*-Carboline

β-Carboline **3** (9*H*-pyrido[3,4-*b*]indole), also named as norharmane, is a nitrogen heterocycle being found in several families of natural products.[83] The pyridine nitrogen atom is characterized by a more basic character than the acidic indolic nitrogen. The fully aromatic members of this family are named *β*-carbolines (BCs) **3** whereas the members with partially saturated C-rings are known as 3,4-dihydro-*β*-carbolines (DHBCs) **55** and 1,2,3,4-tetrahydro-*β*-carbolines (THBCs) **56** as illustrated in Figure 1.7.

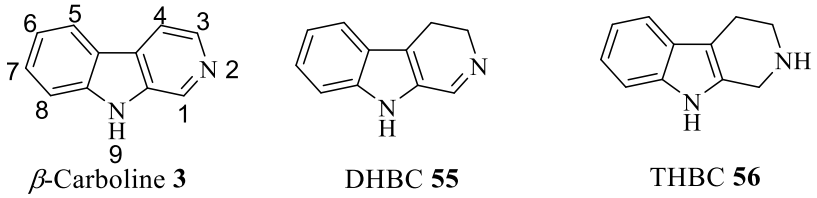


Figure 1.7 Structures of BC, DHBC and THBC

Harmaline **57** was recognized as the first alkaloid from the *β*-carboline family, originally isolated from *Peganum harmala* in 1841, also known as *Syrian rue*.[84] *Peganum harmala L.* (Zygophylaceae) is a medicinal plant known to possess hypothermic and hallucinogenic properties from ancient times. The seeds of *Peganum harmala* contain about 2-6% of pharmacologically active *β*-carboline alkaloids such as harmaline **57**, harmane **58**, harmalol **59** and harmine **18** (Figure 1.8).[85] The structures of harmine **18**, and harmaline **57** alkaloids were determined about 50 years after their initial isolation, and their chemical syntheses are well established. Later, harmaline **57** and harmine **18** alkaloids were also isolated from *Banisteriopsis caapi* (Malpighiaceae), and harmane **58** was isolated from the stem bark of *B. inebrians*. These plants are used as monoamine oxidase inhibitors in ayahuasca, in the western Amazon and Orinoco basins, in conjunction with Psychotria virida (Rubiaceae). The occurrence of *β*-carbolines in nature is widespread, presumably due to their simple biogenesis from tryptamine **5** and today *β*-carbolines have been isolated from various plant families, fungi, animal tissues and marine sources such as tunicates, sponges, soft corals, or bryozoans.[4,86]

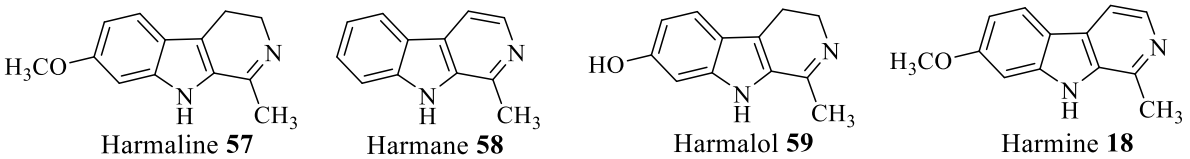


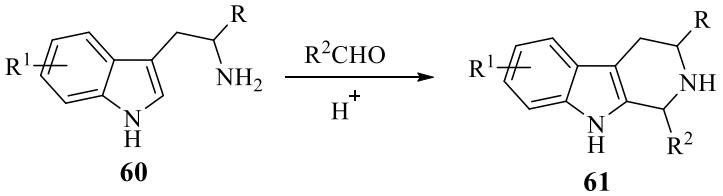
Figure 1.8 Structures of simple *β*-carbolines and dihydro-*β*-carbolines

1.3.1.1 Synthesis of *β*-carbolines

β-Carboline motif is an important structural component that is embedded with many biologically active natural and synthetic compounds. By considering the medicinal as well as pharmaceutical importance of *β*-carbolines, now its synthesis has been the object of researchers since 1928 and reports of new methods for the construction of *β*-carbolines appear often.[87] The formation of fully aromatized *β*-carbolines involves two steps or sequential one-pot methods as reported in the literature. Most importantly, the first step employs by two important classical methods for C-C bond formation reactions such as Pictet-Spengler and Bischler-Napieralski reactions leading to tetrahydro- and dihydro-*β*-carbolines ring systems and the second step involves the oxidative aromatization of dihydro-/tetrahydro-*β*-carboline precursors with appropriate oxidants to obtain *β*-carbolines. Additionally, there are reports for the direct synthesis of *β*-carboline derivatives as discussed below.

1.3.1.1.1 Pictet-Spengler reaction

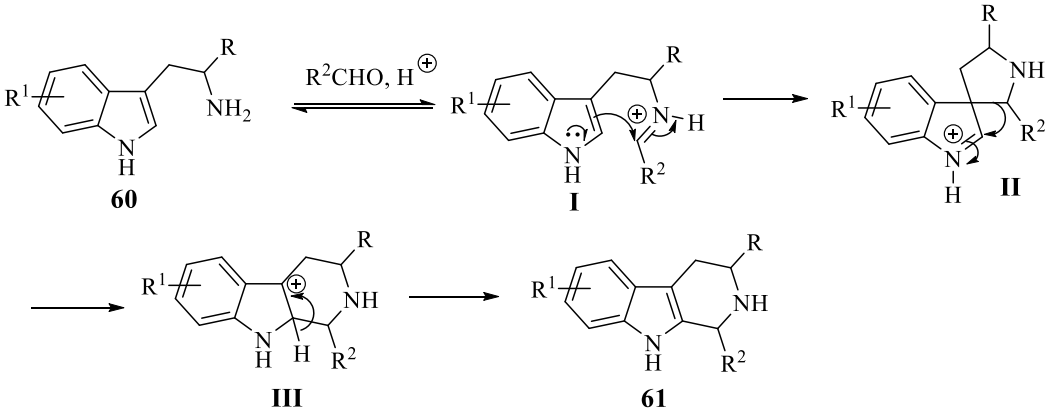
Pictet-Spengler reaction was first reported by Pictet and Spengler in 1911.[88] Initially this reaction was developed for the preparation of tetrahydro-isoquinolines.[89] A few years later, Tatsui developed this process for the preparation of 1-methyl-1,2,3,4-tetrahydro-*β*-carboline involving the acid catalyzed reaction of tryptamine with acetaldehyde.[90] The Pictet-Spengler reaction is one of the key synthetic methods for the formation of tetrahydro-*β*-carbolines **61** by the condensation of indole analogues **60** like tryptophan **4**, tryptamine **5**, serotonin **6** and melatonin **7** with a carbonyl compound, primarily an aldehyde (Scheme 1.7).[91] In addition, this reaction also referred to as Pictet-Spengler cyclization, or Pictet-Spengler condensation. A comparable Pictet-Spengler reaction of ketones with arylethylamines **60** under acidic conditions led to respective tetrahydro-*β*-carbolines, whereas certain aldehydes and ketones including acetone and some benzaldehydes with an *ortho*-electron-donating group are not suitable candidates for this reaction.[92,93]



60 **61**

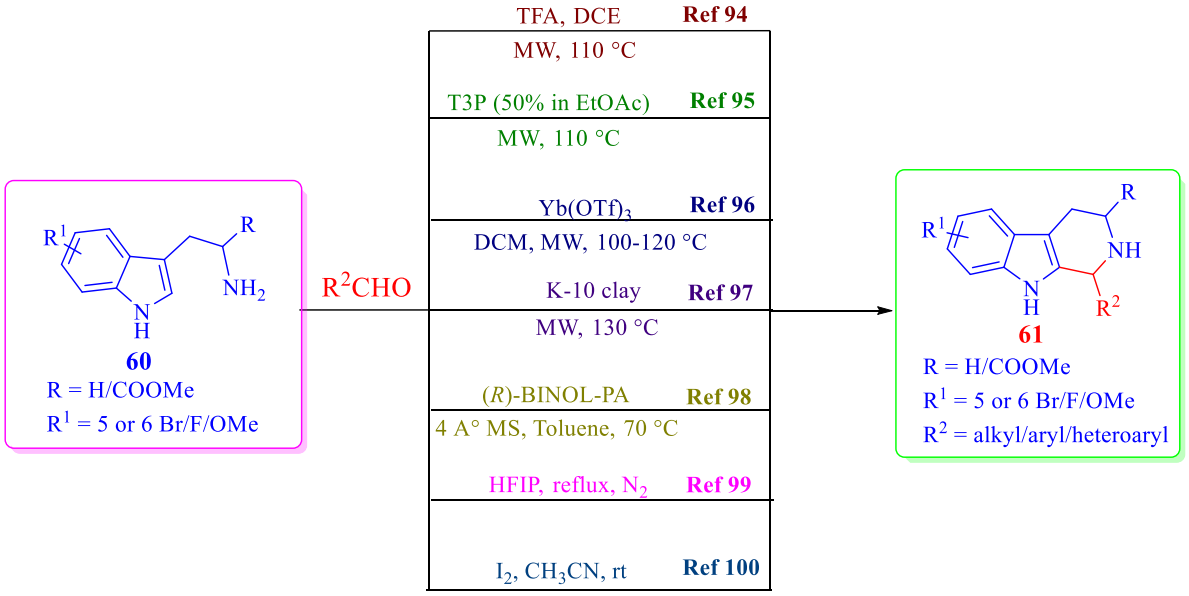
Scheme 1.7 Synthesis of tetrahydro-*β*-carbolines by Pictet-Spengler cyclization

Mechanistically, the acid catalyzed reaction of aldehyde and indolylethylamine **60** involves the formation of iminium ion **I.** Further, the iminium species **I** undergoes intramolecular electrophilic addition at the 3-position of indole, forming a spirocycle **II** that rearranges to a positively charged intermediate **III** which then finally undergoes aromatization *via* deprotonation to yield the tetrahydro-*β*-carbolines **61** as illustrated in Scheme 1.8.



Scheme 1.8 Mechanism of Pictet-Spengler reaction

Since its discovery, the Pictet-Spengler reaction has been studied extensively and continues to be a focus of research in total synthesis of natural and unnatural products of *β*-carbolines and in combinatorial applications. In the recent years, many efforts have been made to improve upon the methodology for the reaction of indolylethylamines **60** with various aldehydes to provide tetrahydro-*β*-carbolines **61** by applying new reaction conditions as shown in Scheme 1.9.



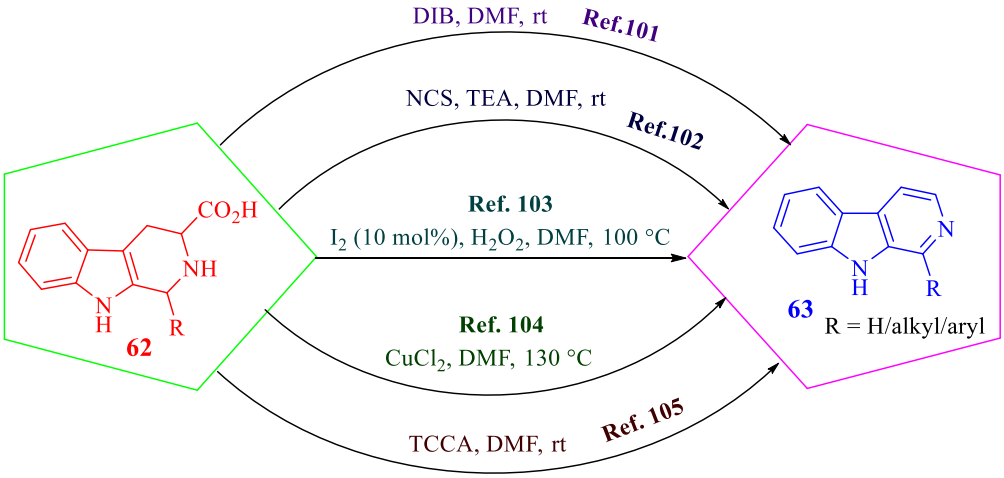
Scheme 1.9 Construction of tetrahydro-*β*-carbolines **61**

In 2014, Eagon and coworkers developed a versatile microwave (MW)-mediated Pictet-Spengler procedure utilizing the reaction of indolylethylamines **60** with various aldehydes in the presence of DCE and TFA to provide tetrahydro-*β*-carbolines **61** within 20 min. and the product precipitates from solution in high yields.[94] Recently, Desroses and group members identified facile reaction conditions to traditional Pictet-Spengler reaction by accessing tetrahydro-*β*-carbolines **61** from the reaction of tryptamines **60** with aromatic aldehydes in the presence of a mild acidic catalyst and efficient dehydrating reagent propane phosphonic acid anhydride (T3P®) under MW irradiation in 10 minutes of time with excellent yield.[95] In 2003, Srinivasn et al. demonstrated a rapid and MW-assisted high yielding Lewis acid-mediated one-pot Pictet-Spengler cyclization of indolylethylamines **60** with variety of aliphatic and aromatic aldehydes to tetrahydro-*β*-carbolines **61**.[96]In 2009, Kulkarni et al. developed an effective and one-pot MW-assisted direct cyclization/dehydrogenation involving the reaction of tryptamines/tryptophan **60** and aromatic aldehydes or glyoxals in the presence of bifunctional catalyst (Pd/C/K-10) and obtained respective *β*-carbolines with excellent selectivity.[97] Sewgobind research group established (*R*)-BINOL-phosphoric acid catalyzed asymmetric Pictet-Spengler reaction of *N*-benzyltryptamine **60** with a library of aromatic or aliphatic aldehydes to obtain enantiomeric tetrahydro-*β*-carbolines **61** in moderate to good yields.[98] Jin Qu research group optimized HFIP-promoted a simple and the environmentally benign condition for the Pictet-Spengler reaction between tryptamine derivatives **60** with various aldehydes and activated ketones to afford tetrahydro-*β*-carbolines **61** in high yields.[99] Using the application of molecular iodine Prajapati et al developed a mild and efficient reaction conditions for the Pictet-Spengler condensation of tryptamines **60** with aldehydes to generate tetrahydro-*β*-carbolines **61** in high yields.[100] These all methods tolerate a wide range of functionality in good to excellent yields and reactions can be performed on milligram to gram scales.

1.3.1.1.1.1 Oxidative aromatization of tetrahydro-*β*-carbolines to *β*-carbolines

General methods for the one-pot decarboxylation and oxidative aromatization of tetrahydro-*β*-carboline-3-carboxylic acids **62** to *β*-carbolines **63** involve the use of reagents including molecular iodine, diacetoxyiodobenzene (DIB), copper catalysts, *N*-chlorosuccinimide (NCS) and trichloroisocyanuric acid (TCCA) as presented in Scheme 1.10. In 2015, Kamal et al. utilized DIB as a mild reagent for practical and highly efficient one-pot oxidative decarboxylative aromatization of tetrahydro-*β*-carboline acids **62** to access the respective aromatic *β*-carbolines **63** at ambient temperature in excellent yields.[101] By employing NCS as

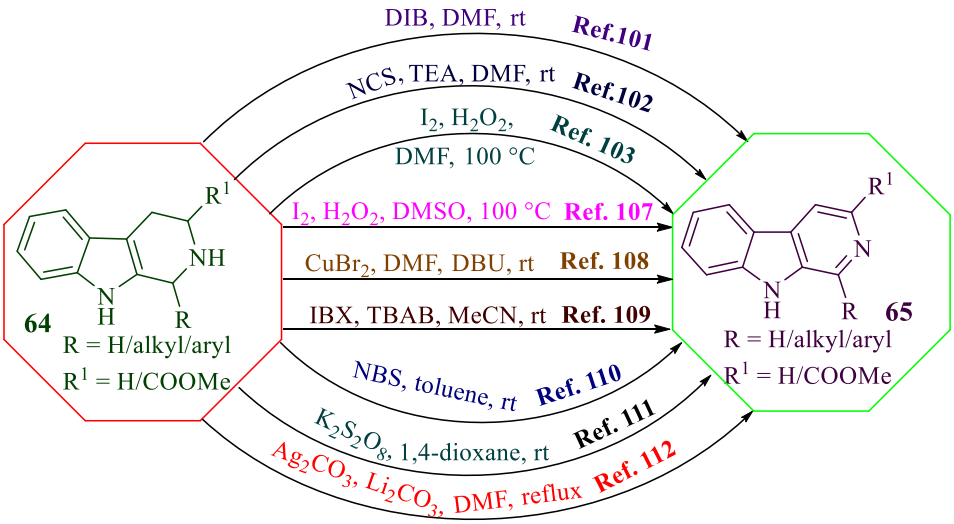
a mild and efficient reagent, the same research group described a facile and good yielded method for the decarboxylative aromatization of tetrahydro-*β*-carboline acids **62** into their corresponding *β*-carbolines **63**.[102] Later, Meesala and coworkers developed a simple and convenient reagents such as molecular iodine/hydrogen peroxide and copper(II)chloride in catalytic amounts for decarboxylation and aromatization of tetrahydro-*β*-carbolin-3-carboxylic acids **62** into respective *β*-carbolines **63** with 60-90% yields.[103,104] Very recently, Manasa and teammates established an operationally simple and mild oxidant TCCA for the tandem oxidative decarboxylation followed by dehydrogenative aromatization of various tetrahydro-*β*-carboline acids **62** at ambient temperature with greater than 75% yields.[105]



Scheme 1.10 Decarboxylative aromatization of tetrahydro-*β*-carboline-3-carboxylic acids **62**

The aromatization is a significant part of simple and complex tetrahydro-*β*-carbolines **64** because fully aromatized *β*-carboline **65** nucleus exists in various natural as well as medicinally important molecules.[106] In view of immense significance of *β*-carbolines, many research groups developed various methods for the aromatization of tetrahydro-*β*-carbolines **64** into *β*-carbolines **65** as outlined in Scheme 1.11. For example, Kamal and coworkers developed a mild and efficient DIB mediated synthesis *β*-carbolines **65** at ambient temperature in excellent yields from dehydrogenation of tetrahydro-*β*-carbolines.[101] Later, other research groups revealed a simple and convenient methods for the oxidative aromatization of tetrahydro-*β*-carboline esters **64** to respective *β*-carboline esters **65** in good to excellent yields by employing a catalytic amounts of molecular iodine/hydrogen peroxide and copper(II)bromide.[103,104,107,108] By using the application of versatile reagent 2-iodoxy-benzoic acid (IBX), Waters research group disclosed dehydrogenation of tetrahydro-*β*-carbolines **64** to *β*-carbolines **65** in 75-91% yields.[109] Recently, a mild and efficient reagent *N*-chlorosuccinimide (NCS) and *N*-bromosuccinimide (NBS) utilized for the aromatization of

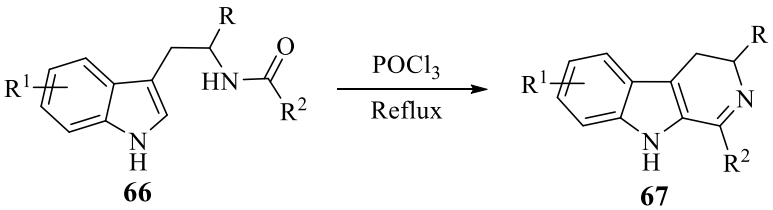
tetrahydro-*β*-carbolines **64** into their corresponding *β*-carbolines **65** in 60-90% yields.[102,110] Very recently, an operationally simple and mild oxidant like potassium persulfate ($K_2S_2O_8$), TCCA and silver carbonate/lithium carbonate were applied for the tandem oxidative aromatization of various tetrahydro-*β*-carbolines in modest to higher yields.[105,111,112]



Scheme 1.11 Conversion of tetrahydro-*β*-carbolines into *β*-carbolines

1.3.1.1.2 Bischler-Napieralski reaction

Another general approach to access *β*-carbolines involving Bischler-Napieralski reaction conditions for the intramolecular cyclization of *β*-arylethylamides **66** to afford dihydro-*β*-carbolines **67** followed by its dehydrogenative aromatization with an appropriate oxidant**.** Bischler-Napieralski reaction was first discovered by August Bischler and Bernard Napieralski, in affiliation with Basle chemical works and the University of Zurich in 1893.[113] The Bischler-Napieralski reaction is an electrophilic aromatic substitution reaction that allows the cyclization of *β*-arylethylamides **66** to dihydro-*β*-carbolines **67** as shown in Scheme 1.12, which can be subsequently oxidized to *β*-carbolines.

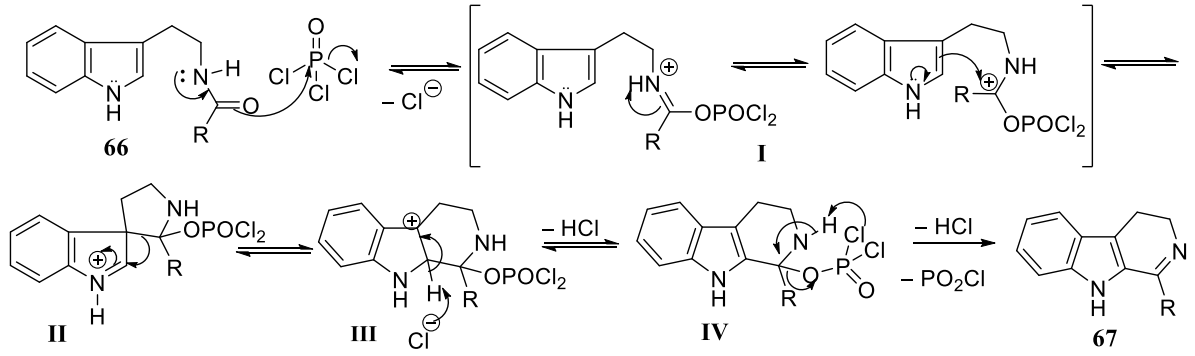


Scheme 1.12 Synthesis of dihydro-*β*-carbolines by Bischler–Napieralski reaction

The Bischler–Napieralski reaction is carried out mainly in the presence of dehydrating agents like $POCl_3$, PCl_5, $SOCl_2$, $ZnCl_2$, $SnCl_4$, BF_3-etherate, triflic anhydride (Tf_2O) and polyphosphoric acid (PPA); but $POCl_3$ is widely cited and used dehydrating agent for

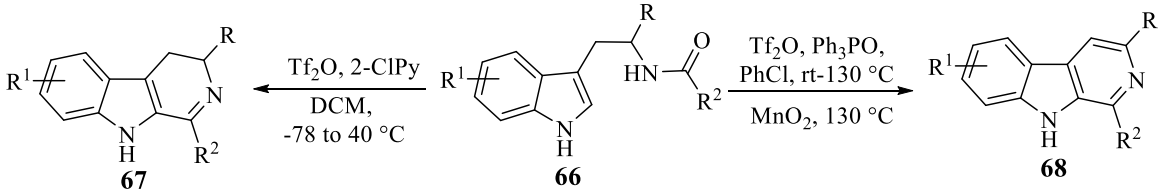
Bischler-Napieralski cyclization under refluxing conditions. Combination of P_2O_5 and $POCl_3$ is most effective reagent to produce dihydro-*β*-carbolines **67,** when the reactant **66** lacking electron-donating groups on the indole ring.[114,115]

Fodor demonstrated detailed mechanistic studies for the Bischler–Napieralski cyclization of indolylethyl amide **66** in the presence of $POCl_3$. Initially, an amide **66** reacts with $POCl_3$ to form imidoyl phosphate intermediate **I,** phosphate being a good leaving group. Upon heating, intermediate **I** undergoes intramolecular electrophilic aromatic substitution leading to spiro intermediate **II** that migrates to form intermediate **III** which undergoes deprotonation to yield **IV** and then loss of phosphate produced the dihydro-*β*-carbolines **67** (Scheme 1.13).[116,117]



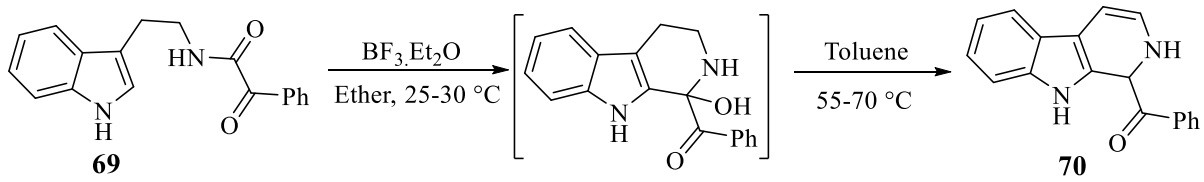
Scheme 1.13 Mechanism of Bischler-Napieralski Reaction

Bischler-Napieralski reaction temperature varies from room temperature to 100 °C depending on the dehydrating reagent used for the cyclization of indolylethyl amide **66**. For example, Movassaghi group described a protocol for the direct conversion of various amides **66** to dihydro-*β*-carboline analogues **67** *via* mild electrophilic amide activation with Tf_2O in the presence of 2-chloropyridine at low-temperature followed by cyclodehydration providing the dihydro-*β*-carbolines **67** in short time as shown in Scheme 1.14.[118] Later, Wang et al. developed a one-pot protocol for direct access to *β*-carbolines **68** by utilizing a combination of triflic anhydride and triphenylphosphine oxide for intramolecular cyclization of *β*-indolyl-ethylamides **66** followed by oxidative aromatization with MnO_2 (Scheme 1.14).[119]



Scheme 1.14 Synthesis of dihydro-*β*-carbolines and *β*-carbolines by using Tf_2O

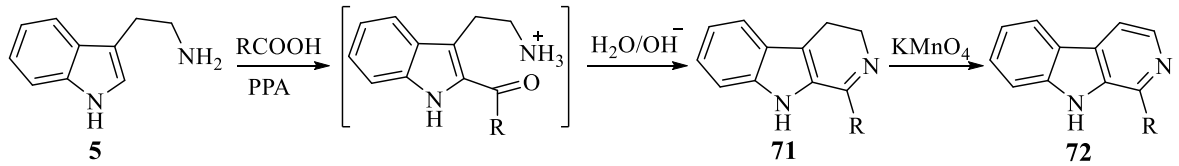
Recently Meruva et al. reported an efficient and mild conditions for the high yielding synthesis of dihydro-*β*-carbolines **70** by the cyclization of ketoamide **69** with BF_3-etherate under Bischler–Napieralski reaction conditions in 70-90% yields (Scheme 1.15).[120]



Scheme 1.15 Synthesis of 1-acyl-dihydro-*β*-carboline analogues.

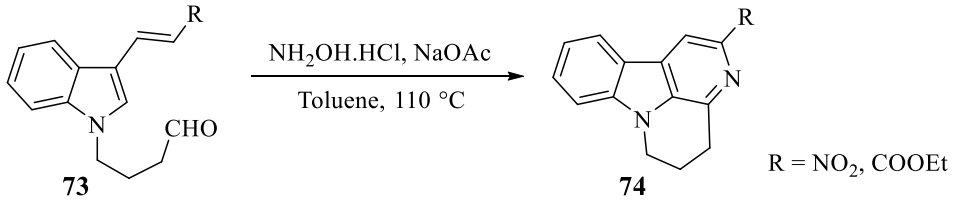
1.3.1.1.3 Other synthetic methods

The Pictet-Spengler and BischIer-Napieralski reactions are perhaps the most commonly used methods for the preparation of simple, complex and natural tetrahydro-*β*-carbolines and *β*-carboline analogues. Other methods have also been used to construct *β*-carbolines directly in one-pot step or multiple steps. For example, Ivanov research group optimized a simple and convenient route to obtain 1-substituted *β*-carbolines **72** by treating of tryptamine **5** with various carboxylic acids in polyphosphoric acid (PPA) produced dihydro-*β*-carbolines **71**. Oxidation of **71** with $KMnO_4$ led to target compound **72** in good yields (Scheme 1.16).[121]



Scheme 1.16 Synthesis of 1-substituted *β*-carboline derivatives

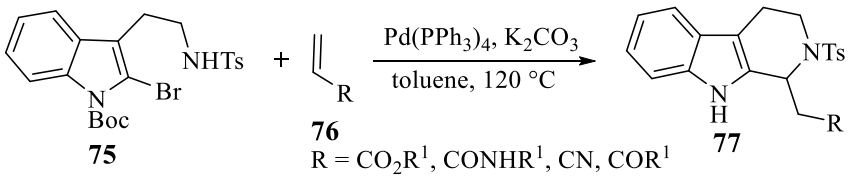
A natural *β*-carboline, canthine derivatives **74** were prepared in good yields by utilizing intramolecular aza-Diels-Alder reaction of aldehyde **73** with hydroxylamine hydrochloride and sodium acetate under refluxing conditions as shown in Scheme 1.17.[122]



Scheme 1.17 Preparation of canthine analogues by intramolecular aza-Diels-Alder reaction

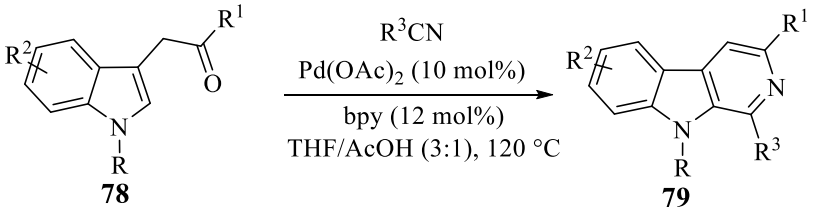
A palladium-catalyzed domino reaction described for the synthesis of C-1-substituted tetrahydro-*β*-carbolines **77** by employing the initial intermolecular Heck reaction of

2-bromoindole **75** with an electron deficient alkene **76**, followed by an *in-situ* intramolecular aza-Michael reaction in high yield (Scheme 1.18).[123,124]



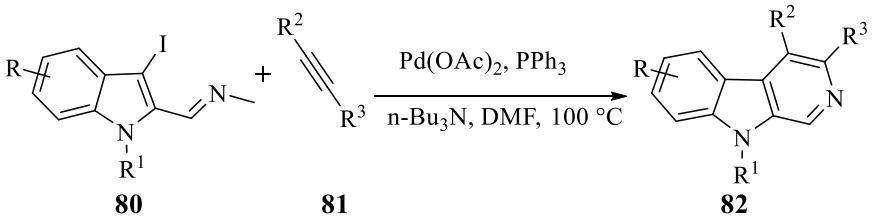
Scheme 1.18 Synthesis of tetrahydro-*β*-carbolines using Heck-aza-Michael process.

In 2018, Liao's group investigated a versatile method for the synthesis of direct *β*-carbolines **79** (Scheme 1.19) by Pd-catalyzed reaction of readily accessible indole analogues **78** with various nitriles in good to excellent yields under reflux conditions.[125]



Scheme 1.19 Synthesis of *β*-carbolines by Pd-catalyzed C–H coupling of indoles

An alternative method for the palladium-catalyzed synthesis of substituted *β*-carbolines **82** was developed by Zang et al. *via* iminoannulation of various internal and terminal alkynes **81** employing the imines of *N*-substituted 3-iodoindole-2-carboxaldehydes **80** with moderate to excellent yields as delineated in Scheme 1.20.[126]



Scheme 1.20 Palladium-catalyzed formation of substituted *β*-carbolines

1.3.1.2 Synthetic applications of *β*-carboline derivatives

The various possibilities for functionalization associated with this *β*-carboline heterocycle paid more attention of synthetic and medicinal chemists towards the synthesis and study of their biological activities. Even though, the possible strategies for the generation of a *β*-carboline core that bears a functional group (CHO, $COCH_3$, COOR, NH_2 and halogens) at a suitable position (either at C-1 **(83)** or C-3 (**86**)) are reported in literature, that could be

further synthetically engineered for producing aryl/heteroaryl substituted (**84** & **87**) and fused *β*-carbolines (**85** & **88**) as outlined in Figure 1.9.[127-129]

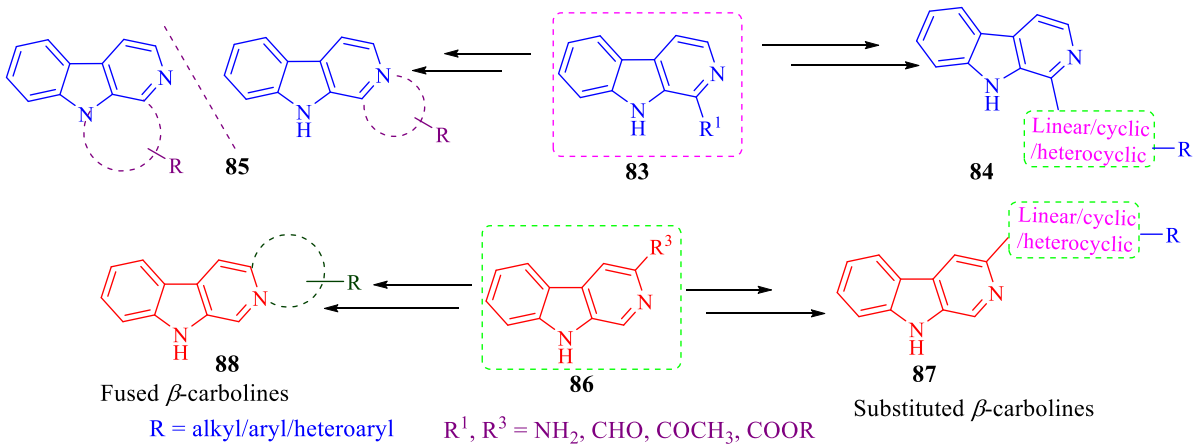


Figure 1.9 Various possibilities for substitution or fusion at C-1/C-3 of *β*-carboline

1.4 Biological importance of indole related heterocycles

Indole related heterocycles such as carbazole and *β*-carbolines are represented in literature as most privileged heterocyclic scaffolds due to their outstanding significance in chemical, biological and pharmaceutical fields. Diverse varieties of carbazole and *β*-carboline containing new chemical entities (natural as well as synthetic molecules) have been emerging as clinical candidates for several diseases. Several academic and industrial laboratories are working on indole related scaffolds in diverse therapeutic areas. Many of designed molecules in medicinal chemistry have their roots from natural products. Naturally occurring, several alkaloids contain either carbazole or *β*-carboline nucleus, which motivated researchers to pay attention towards developing synthetic routes and biological activity studies of different carbazole and *β*-carboline derivatives in various therapeutic areas.[43,130-136] The significance of these heterocycles is well recognized in medicinal chemistry due to their wide spectrum of biological activities including antioxidant, anti-Alzheimer, antileishmanial, anti-HIV, antitumor, antiviral, anticonvulsant and antimicrobial effects as presented in Figure 1.10.

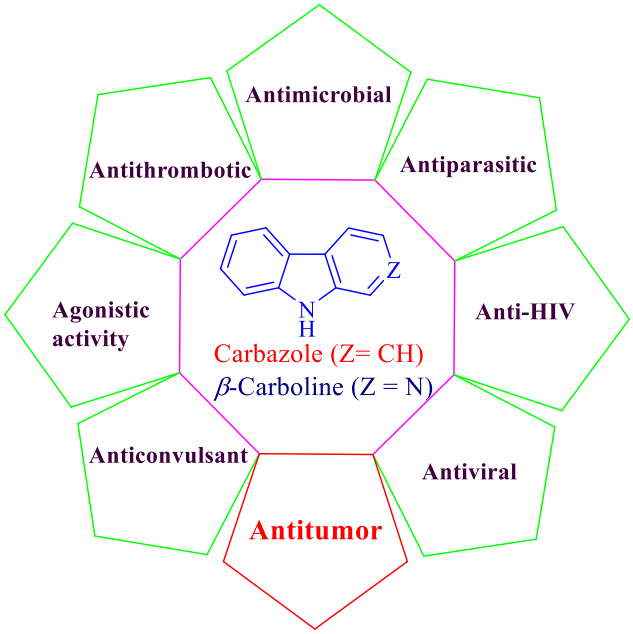


Figure 1.10 Pictorial presentation of biological importance of carbazole and *β*-carboline

Presently, anticancer activity study of carbazole and *β*-carboline derivatives is one of the emerging area of research despite their investigation for various biological activities[137] because, cancer is one of the major life threatening disease in the past several decades and also second largest death causing disease worldwide.[138] Although, considerable advances have been made in curbing the progression of this devastating disease, till date a complete cure for cancer is still a dream. The majority of the cancer treatment involves surgery, radiation, photodynamic therapy (PDT), and chemotherapy.[139] Surgery and radiation, two therapies are conventional methods to treat metastatic cancer that are not amenable to surgical removal, with a major drawback of severe side effects. Chemotherapy and PDT involve the usage of chemical agents to stop/prevent the growth of cancer cells. However, cancer chemotherapy is a very difficult and a tedious task.[140] Despite the impressive performance of many chemotherapeutic agents present in the market such as taxanes, vinca alkaloids etc., and their potentials are somewhat restricted due to several drawbacks like low bioavailability, drug toxicity, solubility and complex structures.[141] This leaves a vast scope for the development of new chemical entities with improved efficacies and reduced side effects.

1.4.1 Carbazoles with anticancer activity

The carbazole core is found in a large range of compounds, both from natural and synthetic sources. Presence of additional rings and/or various substituents on carbazole provide a large structural diversity and biological properties. A large number of carbazoles have shown significant antitumor properties and some of them have entered into clinical trials. Many cytotoxic carbazoles are known to exert their activity *via* interactions with DNA, and inhibition of protein kinases, platelet-derived growth factor signal transduction and topoisomerase etc.[33,130,137] Antitumor activity properties of selected natural and synthetic carbazoles are discussed in the proceeding sections.

1.4.1.1 Natural carbazoles

Carbazoles prominently embody a wide range of plant natural products. The structural attributes of such carbazole-based natural products are multifarious. One of their major structural features is the presence of nuclear hydroxyl groups. In addition, prenyl groups are often found in some natural carbazoles. Many of them are endowed with profound antitumor activities (Figure 1.11).[142] For example, Shen and colleagues isolated clauszoline-I, **89**, a simple cytotoxic carbazole alkaloid in 2012 from the plant *Clausena vestita* D. D. Tao. Carbazole **89** showed potent anticancer activity against tested cancer cell lines with IC_{50} values ranging 13.3-71.6 μM. Furthermore, compound **89** was found to inhibit the phosphorylation of PKCδ at residue Ser643 in a concentration-dependent manner in HepG2 cells.[143] Koenimbine **90,** a carbazole alkaloid was isolated from Sri Lankan curry leaves *Murraya koenigii* by Nakamura et al. The inhibitory effects on melanogenesis at 10 μM concentration of alkaloid **90** was investigated in theophylline-stimulated murine B16 melanoma 4A5 cells. The inhibitory activity results shown that alkaloid **90** is found to suppress melanogenesis by displaying cytotoxicity with IC_{50} = 1.2 μM against melanoma 4A5 cells.[144] Nagappan et al. isolated structurally similar carbazole alkaloid, mahanimbine **91** from the ethanolic extract of the leaves of *Murraya koenigii* (Rutaceae) and screened for antiproliferative effect on three tumor cell lines using MTT assay in a dose-dependent manner. Mahanimbine **91** was found to elicit potent antitumor activity with IC_{50} values of 1.98 and 2.12 μg/mL for HeLa and MCF-7 tumor cell lines respectively.[145] In another medicinal plant *Murraya koenigii*, Yoshikawa et al. found a cytotoxic carbazole alkaloid, euchrestine B **92** with an IC_{50} of 4.1 μM against melanogenesis in B16 melanoma 4A5 cancer cells.[144]

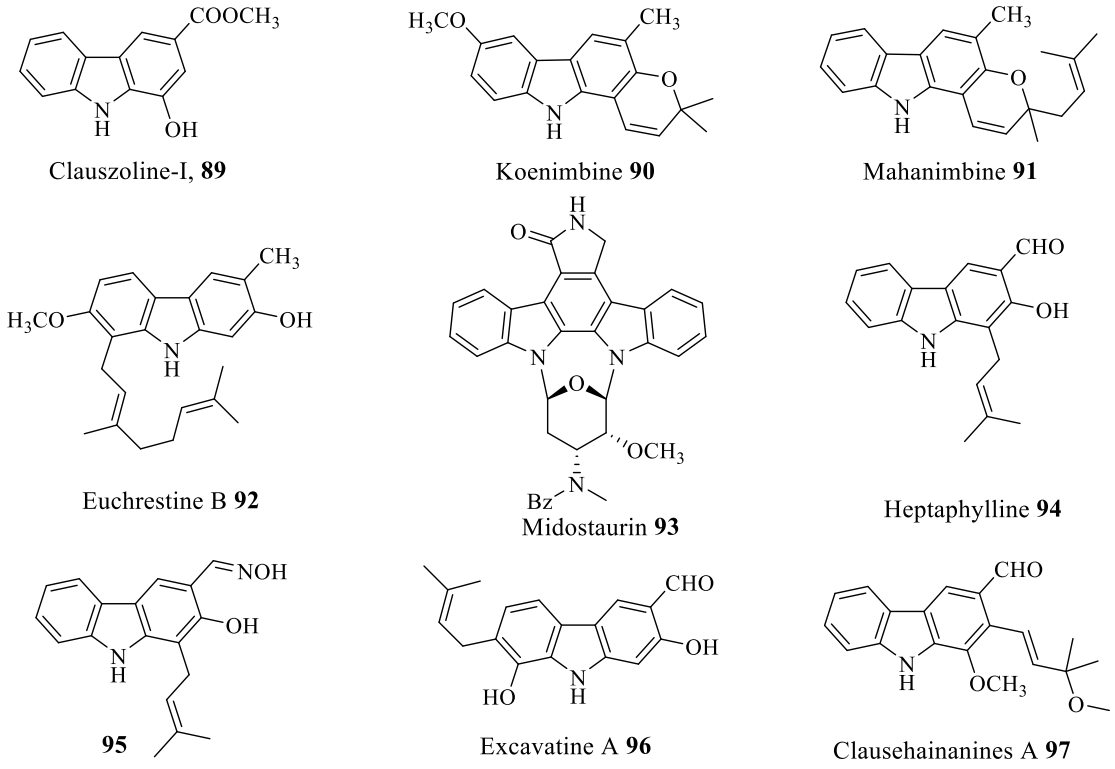


Figure1.11 Anticancer properties of naturally occurring carbazoles

A staurosporine derivative, midostaurin **93** is a multi-targeted protein kinase suppressor, it has reached Phase III clinical trials as a therapy for acute myeloid leukaemia, but also shown to suppress protein kinase C (PKC) in the glioblastoma cell line U373 MG. Midostaurin **93** exhibited *in vitro* antiproliferative activity against 29 cell lines, including five glioblastoma cell lines with IC_{50} values in the sub-micromolar range (0.04-1.92 μM).[146,147] In 2011, Yenjai and colleagues reported the isolation of cytotoxic heptaphylline **94** from the roots of the medicinal plant *Clausena harmandiana* with significant activity (IC_{50} = 1.32 μM, NCI-H187). Additionally, carbazole **94** showed relatively low cytotoxicity with an IC_{50} of 92.47 μM against Vero cells.[148] Oxime derivative **95** displayed 66 fold enhancement in the cytotoxicity (IC_{50} = 0.02 μM) against NCI-H187 cancer cell lines.[149] Tan and teammates identified a carbazole alkaloid named excavatine A **96** from the leaves and stems of the *Clausena excavata* in 2013, which revealed antitumor activity against A549 and HeLa cancer cells with IC_{50} values of 5.2 and 1.9 μM, respectively.[150] Very recently, Yan-Lei et al. isolated carbazole alkaloids, clausehainanines A-E from the stems and leaves of *Clausena hainanensis* and evaluated for their antiproliferative activities against a panel of human cancer cell lines. From this class of alkaloids, clausehainanine A **97** found to elicit significant antiproliferative effects against various human cancer cell lines with IC_{50} values ranging from 0.12 to 1.9 μM.[151]

1.4.1.2 Synthetic carbazoles

In addition to their isolation from the natural sources, advanced synthetic routes have also developed to provide access to diversified natural and synthetic carbazoles to facilitate their antitumor activity studies.[152] In particular, there has been intense research efforts in recent years in the design and development of carbazole derivatives as a new class of potent anticancer agents (Figure 1.12).[33,153] For example, Very recently, Jiang and group utilized a series of prepared carbazole-rhodanine **98** conjugates to study their anticancer properties. The cytotoxicity results suggest that compound **98** was found to display significant activity (IC_{50} = 0.12 μM) against HL-60 carcinoma cells. Mechanistic studies revealed that conjugate **98** selectively impede Topo II function at 20 μM without affecting Topo I catalytic activity.[154] Li and coworkers synthesized a library of carbazolyl chalcones **99** from the reaction of easily accessible 3-formylcarbazoles with various acetophenones and studied their anticancer properties. The anticancer activity results suggest that compound **99** exhibited most potent cytotoxicity against the four cancer cell lines with an IC_{50} values ranging from 0.22 to 2.16 μM. Additionally, carbazole analogue **99** demonstrated as potent Topo II-targeting anticancer agent with apoptosis-inducing activity.[155] In 2010, a study of investigating the binding mode of carbazole **100** derivatives with the G-quadruplex DNA formed by human telomeric sequence, Tang and colleagues identified carbazole **100** containing two charged pyridyl groups to induce the formation of mixed G-quadruplex of Hum22. Additionally, carbazole analogue **100** disclosed significant anticancer activity against Bel-7402 and HCT-8 cell lines with IC_{50} values of 0.22 and 0.18 μM, respectively.[156] From the series of benzopsoralen analogues, Esteves and colleagues identified a simple carbazole **101,** which possess higher antiproliferative activity against TCC-SUP and MDA-MB-231 tumor cell lines with GI_{50} values of 0.025 and 0.198 μM, respectively.[157] A carbazole derivative **102** bearing naphthalene-1-sulfonate was found to inhibit IL-6-induced activation of endogenous signal transducers and activators of transcription 3 (STAT3)-mediated transcription and phosphorylation of STAT3 (Y705) in a dose-related manner by displaying anticancer activity in triple-negative breast cancer (TNBC) cell lines HS578T and SUM149PT. Compound **102** also exhibited *in vitro* antiproliferative activities (GI_{50} = 0.16 μM) towards A431 carcinoma cell lines.[158]

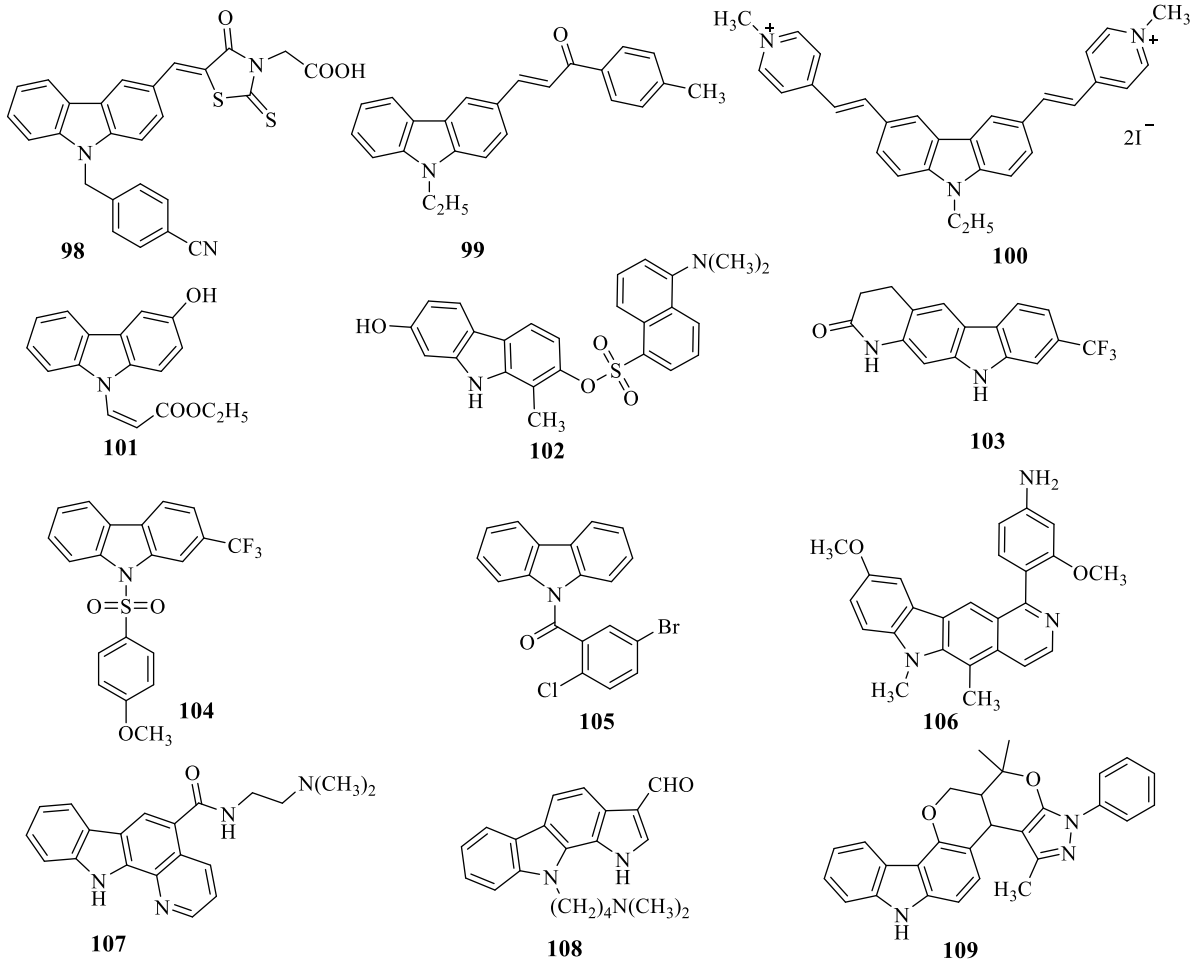


Figure1.12 Synthetic carbazoles as anticancer agents

Fujii and co-workers described the synthesis and SAR studies of lactam-fused carbazole derivative **103** to illustrate the anticancer activity properties by targeting kinesin spindle protein (KSP). The carbazole analogue **103** exhibited remarkable activity against KSP ATPase (IC_{50} = 31 nM) and the consequent cytotoxicity (IC_{50} = 91 nM) against HeLa cells by effective arresting of cell-cycle at the M-phase.[159] In 2018, Xin et al. synthesized a chemical library of *N*-substituted carbazole **104** and screened for potential anticancer activity against five cancer cells. Results demonstrated that the *N*-arylsulfonyl substituted carbazole **104** showed most potent antiproliferative ability towards HCT-116 tumor cell lines with IC_{50} value of 1.9 μM. Moreover, compound **104** exhibited promising *in vivo* anticancer therapeutic efficacy in PANC-1 and Capan-2 xenograft mice models without an apparent side effect.[160] The *in vitro* anticancer activities of a series of *N*-acylcarbazole **105** derivatives were evaluated *via* sulphorhodamine B (SRB) assay against MDAMB231 and CAL 27 cancer cell lines at 10 μM. After three days of treatment, compound **105** was found to inhibit the growth of CAL 27 cells significantly with an IC_{50} value of 28 nM.[161] Beata *et al.* examined the

synthesis and *in vitro* cytostatic activities of pyridocarbazole derivative **106** against human kidney cancer (A498) and human lung cancer (A549) cell lines with IC_{50} values of 0.43 and 0.86 μM, respectively.[162] Various pyrido[3,2-*α*]carbazole **107** derivatives were synthesized and subsequently tested for their anticancer activity against A549 and colon cancer HT29 cells *via* SRB assay. Among all the synthesized compounds, pyridocarbazole **107** offered the highest cytotoxicity against A549 (IC_{50} = 70 nM) and HT29 (IC_{50} = 110 nM) cancer cell lines.[163] In 2012, Akue-Gedu et al. synthesized a series of pyrrole fused carbazoles **108** to study their antiproliferative activity. The prepared *N*-substituted pyrrolocarbazole **108** was found to be most potent inhibitor for pim-kinase activity with IC_{50} in the 46-80 nM range and demonstrated antiproliferative activities against three human cancer cell lines, PA1 (ovarian carcinoma), PC3 and DU145 (prostatic carcinoma) with IC_{50} values in the range of 0.48-0.96 μM.[164] Padmaja and group reported the synthesis of pyrano[3,2-*c*]carbazole **109** analogues by domino Knoevenagel–hetero-Diels–Alder cyclization of the 3-formylcarbazoles with cyclic 1,3-diketones or 5-pyrazolones in the presence of ethylenediamine diacetate (EDDA). All the prepared pyrano[3,2-*c*]carbazole **109** analogues exhibited their antiproliferative activity by inhibiting the growth of tested cancer cell lines (GI_{50} = 0.01-4.45 μM). Predominantly, the compound **109** displayed pronounced antiproliferative activity against HeLa cells (GI_{50} = 0.01 μM).[165]

1.4.2 *β*-Carbolines in cancer drug research

β-Carboline nucleus plays a vital role in anticancer research. Many of the natural and synthetic *β*-carboline analogues have shown remarkable anticancer activities.[166] *β*-Carboline derivatives have displayed anticancer activity through diverse mechanism of actions to kill the cancer cells.[167] Majority of the synthetic and natural *β*-carbolines exhibited anticancer activity through DNA intercalators.[132] Although in the last two decades, the copious literature upon *β*-carboline based classic DNA intercalators indicate that the interest in this topic is still alive, and further studies may lead to a better understanding of the mechanisms involved in the process of intercalation pointing the way for the design of more selective and potent intercalators.[168] In the following sections we deliberated an up to date report regarding anticancer properties of various *β*-carboline derivatives.

1.4.2.1 Natural *β*-carbolines

Historically, numerous natural *β*-carbolines have been reported to exert anticancer activity *via* diverse mechanisms such as intercalating into DNA, inhibiting topoisomerases I and II, blocking the process of cell mitosis, or targeting a specific cancer signaling pathway etc (Figure 1.13).[167] For example, Evodiamine **110,** a quinazolinocarboline alkaloid isolated from the fruits of traditional Chinese herb *Evodiae fructus.* Evodiamine analogue **110** showed remarkable *in vitro* (GI_{50} = 7 nM, HCT116) as well as *in vivo* antitumor efficacy and low toxicity at the dose of 1 mg/kg or 2 mg/kg.[169] Another two cytotoxic *β*-carboline alkaloids ingenine E **111** (IC_{50} = 0.67 μg/mL) and annomontine **112** (IC_{50} = 0.42 μg/mL) were isolated from the Indonesian marine sponge *Acanthostrongylophora ingens* with significant cytotoxic activity against HCT116 cell lines.[170]

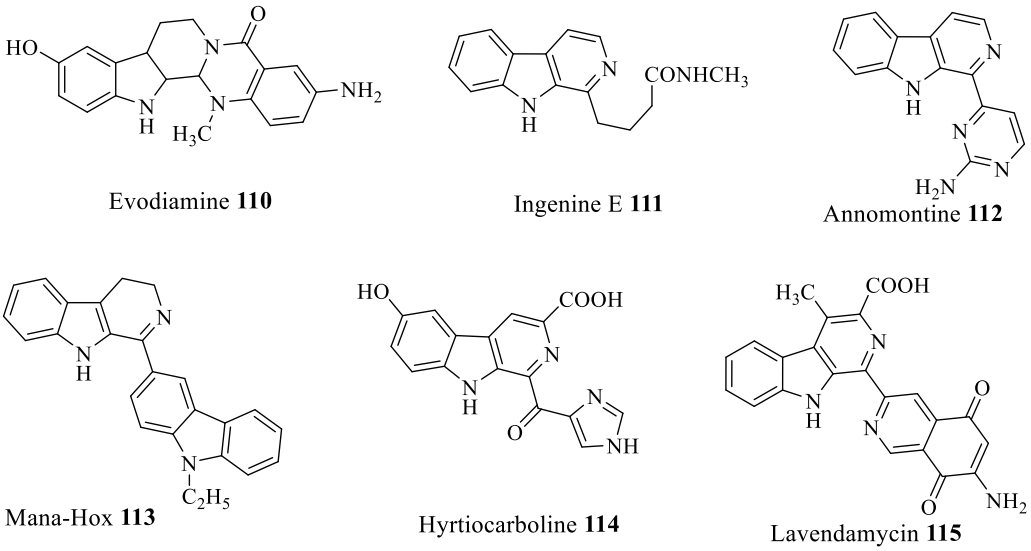


Figure 1.13 Natural *β*-carbolines as anticancer agents

Mana-Hox **113**, a natural analogue of *β*-carboline binding to DNA through intercalation and/or through minor groove binding plays a critical role in the induction of aberrant mitosis and contributes to its anticancer activity against a panel of human cancer cells (IC_{50} = 1.3-6.5 μM). Furthermore, Mana-Hox **113** rapidly penetrated into cells (within 1 min) and concentrated on disorganized metaphase chromosomes after 13 h of exposure.[171,172] Hyrtiocarboline **114** was isolated from a Papua New Guinea marine sponge, *Hyrtios reticulatus* and displayed selective antiproliferative activity against non-small cell lung (H522-T1) cancer cells with IC_{50} value 1.2 μg/mL.[173] Lavendamycin **115** is a bacterially derived antibiotic, isolated from *Streptomyces lavendulae* and reported to exhibit remarkable *in vitro* antiproliferative effects against lung cancer cell lines (IC_{50} = 10 nM; A549).[174,175]

1.4.2.2 Functionalized *β*-carbolines as anticancer agents

Presence of *β*-carboline nucleus in many of the natural products which are used for cancer chemotherapy had provoked the researcher to work on *β*-carboline-based new chemical entities. Varieties of functional groups are tailored at C-1, C-3 and N^2 and N^9 positions of the *β*-carboline ring and evaluated for their anticancer activity; these structural modifications are mostly rational to the anticancer natural products. Moreover, investigation of small molecules for cancer therapy is advantageous in terms of synthetic efforts, cost and environmental hazards. Synthesis of these novel *β*-carboline molecules involves either construction of *β*-carboline ring itself or derivatization at different positions. This section further divided into three parts based on substitution present on the position of the *β*-carboline ring as follows.

1.4.2.2.1 C-1 Functionalized *β*-carbolines

C-1 substituted *β*-carbolines are one of the important class of *β*-carbolines containing natural products, in which *β*-carboline is linked either with heterocyclic ring or any functional group showing significant role in anticancer research (Figure 1.14). For example, quinazolino-*β*-carbolinone derivatives **116** showed good *in vitro* anticancer activity in the range 1-8 μM concentration towards tested cancer cells.[176] 1-Amino-substituted *β*-carbolines **117** demonstrated significant DNA intercalation and anticancer activity (IC_{50} = 0.38-5.2 μM) against a panel of cancer cell lines.[177]

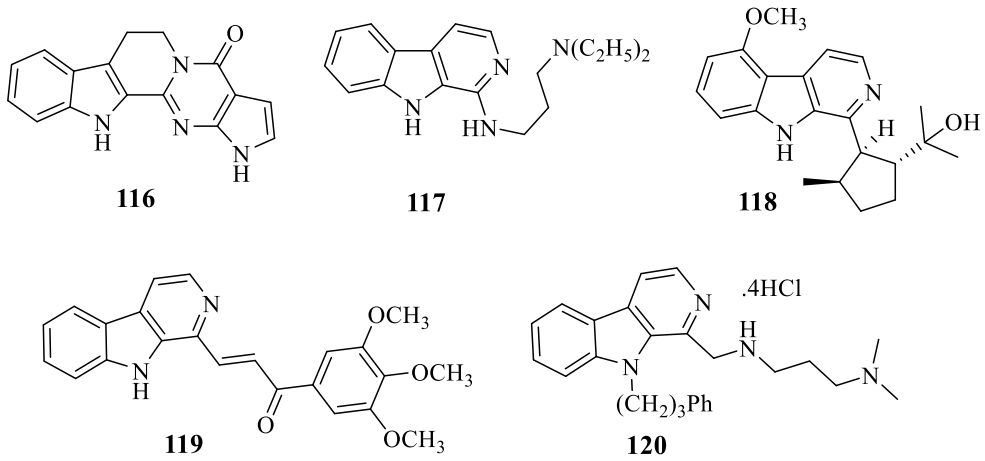


Figure 1.14 C-1 functionalized *β*-carbolines as antitumor agents

A cytotoxic *β*-carboline alkaloid **118**, was isolated from roots of Galianthe thalictroides (Rubiaceae). In addition, alkaloids **118** exhibited potent cytotoxicity (GI_{50} = 3 μM) against MCF-7 cancer cells and DNA targeting antitopoisomerase I and II*α* activities.[178] From a

series of novel *β*-carboline-based chalcones Chauhan et al. identified compound **119** with high cytotoxicity (IC_{50} =2.25 μM; MCF-7) against breast cancer cell lines.[179] In 2011, Chen et al. designed and synthesized a series of 1,9-disubstituted *β*-carbolines **120** as DNA intercalating and cytotoxic agents with IC_{50} values ranging 1.7-18.1 μM against ten human tumor cell lines.[180]

1.4.2.2.2 C-3 Functionalized *β*-carbolines

Various functional groups, linear linkers or heterocycles connected to C-3 position of *β*-carboline ring are classified under this category. There are several C-3 functionalized *β*-carbolines with natural as well as synthetic origin exhibiting interesting antitumor activity (Figure 1.15). For example, alkylamino side chain substituted *β*-carboline derivative **121** showed significant DNA binding by intercalation and exhibited anticancer activity (IC_{50} = 1.9 μM) against human leukemia (HL-60) cancer cell lines.[181] In 2011, Ikeda and teammates reported a series of benzylamino substituted *β*-carboline derivatives based on the C-1 and C-3 positions. From this work, 3-(3-phenoxybenzyl)amino-*β*-carboline **122** showed extremely high cytotoxicity with IC_{50} values 74 nM and 30 nM against HeLa S-3 and Sarcoma180 cancer cells. Mechanistic studies revealed that compound **122** induced apoptosis by various ways like chromatin condensation, DNA fragmentation, caspase-3 activation etc. by arresting cell cycle in the G2/M-phase.[182-184] *β*-Carbolines possessing the phenanthrenyl group at C-1 position **123** exhibited *in vitro* anticancer activity with IC_{50} values < 3 μM against five human cancer cell lines.[185] *β*-Carboline-dithiocarbamate derivatives **124** exhibited significant cytotoxic activity (IC_{50} = 0.79 μM, DU-145) and displayed DNA topoisomerase II (topo II) inhibition by binding to DNA.[186] Very recently, Kamal group investigated a podophyllotoxin linked *β*-carboline congeners **125** for its cytotoxic activity (IC_{50} = 1.07 μM) against DU-145 cell line. Additionally, compound **125** displayed interaction with DNA topoisomerase-II though binding to DNA.[187] *β*-Carboline-hydroxamic acid hybrid **126** demonstrated potent anticancer activity (IC_{50} = 0.83 μM) against HCT116 cancer cells related to histone deacetylases (HDAC) inhibition (IC_{50} = 0.026 μM), DNA damage, and activation of the p53 signaling pathway.[188] *β*-Carboline-oxindole hybrids **127** showed potent cytotoxicity against HCT-15 cancer cells (IC_{50} = 1.43 μM and GI_{50} = 0.89 μM). Notably, compound **127** accomplished induction of apoptosis and DNA intercalation on HCT-15 cell lines.[189] In 2011, Barbosa et al. reported *β*-carboline-carbohydrazide **128** as the most active compound against renal (786-0) cancer cell line (IC_{50} = 0.04 μM) from a series of *β*-carboline derivatives bearing a substituted carbohydrazide moiety at C-3 position.[190]

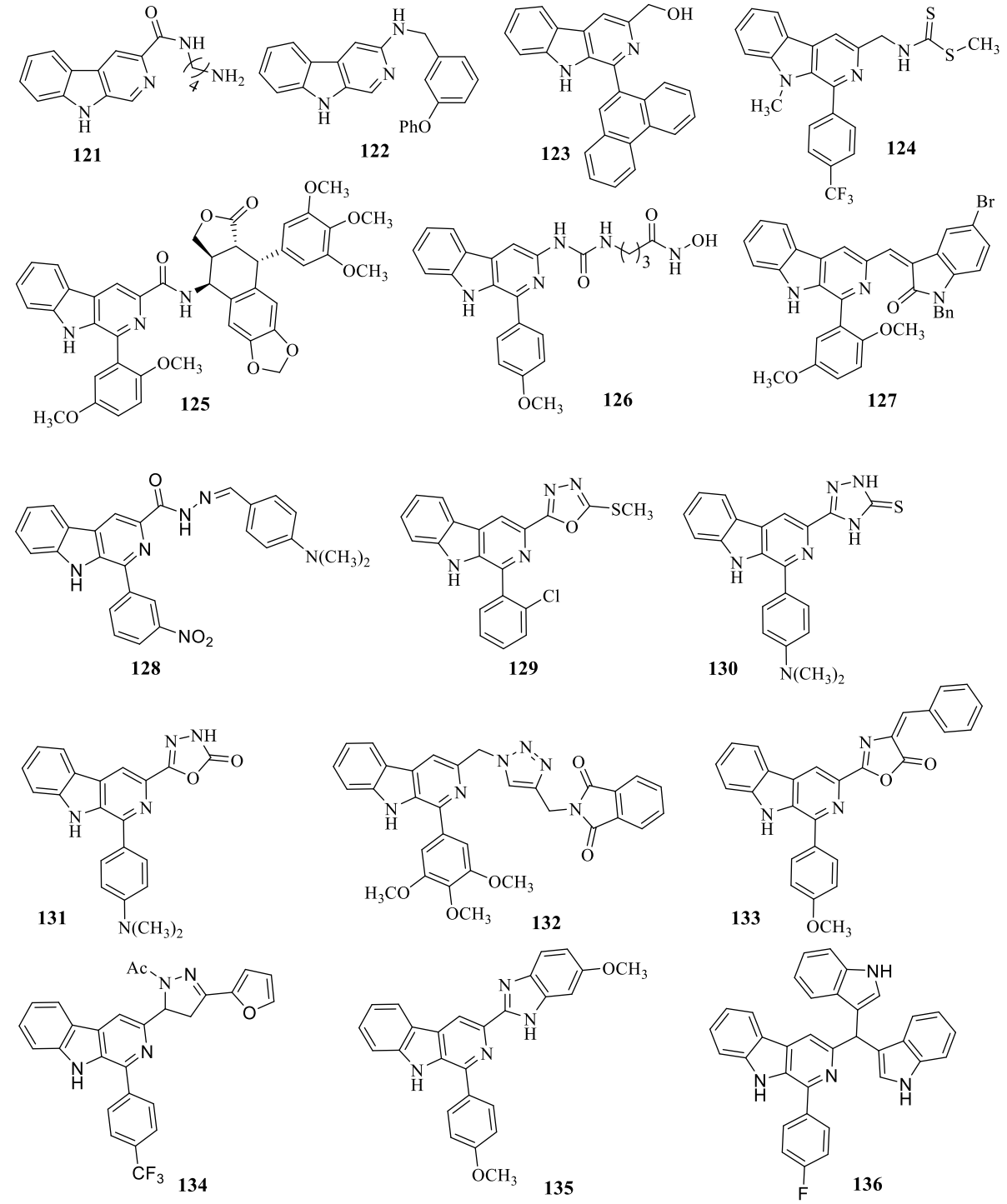


Figure 1.15 Representative examples of C-3 functionalized *β*-carbolines as anticancer agents

Formagio et al. reported 1-substituted-phenyl *β*-carbolines bearing the 1,3,4-oxadiazole and triazole groups at C-3 position to show selective cytotoxicity against tested cancer cell lines. The oxadiazole analogue **129** demonstrated high selectivity and potent anticancer activity against ovarian cell line with GI_{50} = 10 nM and triazole derivative **130** particularly showed most effectiveness on lung (GI_{50} = 60 nM), ovarian (GI_{50} = 1.09 µM) and renal (GI_{50} = 40 nM) carcinoma cells respectively.[191] The 1-(substituted phenyl)-3-(2-oxo-1,3,4-oxadiazolyl)-

β-carboline derivative **131** displayed the highest anticancer activity with GI_{50} in the range of 0.67-3.20 μM against seven cancer cell lines and also compound **131** exhibited significant DNA binding interaction. C-3-Tethered 1,2,3-triazolo-*β*-carboline hybrid **132** displayed promising cytotoxicity (IC_{50} = 3.67 μM) against the HT-29 cell line and demonstrated DNA interacting property through binding in the minor groove of DNA.[192] *β*-Carboline-3-(4-benzylidene)-*4H*-oxazol-5-one derivative **133** exhibiting a potent cytotoxic activity against glioma (U251), prostate (PC-3) and ovarian (OVCAR-03) cancer cell lines (IC_{50} 0.48-1.50 μM).[193] In 2014, Kamal et al. disclosed that C-3 pyrazolinyl linked *β*-carboline hybrid molecule **134** with antitopoisomerase I, DNA interactive and apoptosis-inducing anticancer activities (IC_{50} = 1.07 μM, MCF-7).[194] In an effort to synthesize heterocycle conjugates, Kamal group described that *β*-carboline-benzimidazole **135** with enhanced cytotoxic activity (GI_{50} = 0.3-7.1 μM) against tested cancer cell lines.[195] *β*-Carboline-bisindole analogue **136** exhibited significant antiproliferative activity against prostate cancer (DU-145) cells with IC_{50} value of 1.80 μM. Further, compound **136** presented effective inhibition of DNA topoisomerase I and plasmid DNA cleavage upon irradiation with UV light.[196]

1.4.2.2.3 N^2- and N^9-Functionalized *β*-carbolines

Any aryl/heteroaryl, linear chains or functional groups attached to *β*-carbolines through N^2 and N^9 are classified under this category. The following discussion describes anticancer activity of synthetic N^2- and N^9- substituted *β*-carbolines (Figure 1.16). For example, phenylacetyl tetrahydro-*β*-carboline **137** was identified as a novel class of transforming growth factor beta (TGF-*β*) inhibitor signaling pathway along with inhibition of cancer cell migration in the wound-healing and transwell assays.[197] In 2010, Liu *et al*. identified a series of analogues by retaining the tetrahydro-β-carboline core with a pendant 3-hydroxyphenyl moiety **138** as a potent inhibitor in a KSP ATPase assay with IC_{50} value of 0.040 μM. Also, compound **138** showed good cellular antiproliferative activities against five human tumor cell lines (IC_{50} = 0.94-8.52 μM).[198] The tetrahydro-*β*-carboline hybrid **139** containing ferulic acid and *N*-methylpiperazine ring was displayed strong antitumor activities against six human cancer cells *in vitro* with IC_{50} values of 5.93–9.14 μM. Furthermore, compound **139** could selectively inhibit tumor cells, but not non-tumor cell proliferation and significantly induce cell apoptosis in a dose dependent manner.[199] Similarly tetrahydro-*β*-carboline-triazine hybrids **140** were selectively cytotoxic towards oral cancer (IC_{50} = 0.1 μM, KB) cell line, while their enantiopure forms were less active and not selective.[200] Despite the N^2-substituted *β*-carbolines, specially N^9-substituted *β*-carbolines also found to be cytotoxic against various

cancer cell lines (Figure 1.14). For example, isoxazoline derivatives **141** were synthesized by Filali group from well-known natural product harmine and found to possess anticancer activity (IC_{50} = 0.2 μM) against MCF-7 cancer cell lines. Similarly, N^9-Substituted harmine derivatives **142** demonstrated antitumor activity with IC_{50} values <1 μM against A-549 and MCF-7 cell lines.[201]

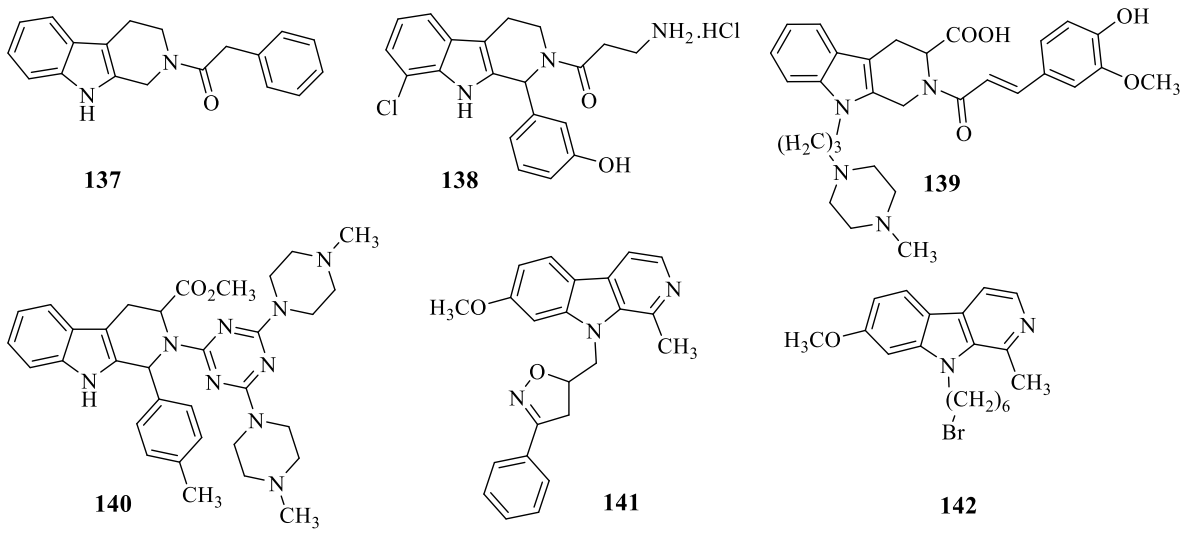


Figure 1.16 Selected cytotoxic N^2- and N^9-functionalized *β*-carbolines

1.4.2.3 Bivalent *β*-carbolines

Here, the active *β*-carboline pharmacophore has been linked to a second molecule of itself through a connecting unit (X) to provide a bivalent analogue (BC–X–BC), that may exhibit enhanced selectivity and potency relative to its monovalent ligand (BC–X) when a suitable X is employed. Such was the case with the design and synthesis of bivalent *β*-carbolines with extended antitumor activities (Figure 1.17). For example, bivalent *β*-carbolines **143** with a spacer of five methylene units between the indole nitrogen exhibited strong cytotoxic activities with IC_{50} value of 1.7 μM against SK-OV-3 cancer cells.[202] Cao et al. reported a spacer of six methylene units between the 3-carboxyl oxygens of *β*-carboline derivative **144** with good and selective cytotoxic activities against KB cell lines (IC_{50} = 15.6 μM). Also compound **144** exhibited potent antitumor activity against Lewis lung cancer in mice with a tumor inhibition rate of 64.2%. Bivalent *β*-carboline derivative **145** was found to show DNA intercalating ability and excellent cytotoxicity against panel of cancer cell lines (IC_{50} = 5.4-13.2 nM).[203] Alkyl diamine linked bivalent *β*-carbolines **146** displayed most potent antiproliferative activity with IC_{50} value of 1.06 μM against human umbilical vein (EA.HY926) cell lines.[204] Bivalent *β*-carbolines **147** with a piperazine group spacer between

β-carboline-3-methylene units demonstrated potent cytotoxicity (IC_{50} = 3-9 µM) against tumor cell lines.[205] Two *β*-carbolines linked *via* phenyl ring **148** was prepared by condensing tryptamine and teraldehyde *via* Pictet-Spengler reaction conditions and investigated to show enhanced anticancer activity (GI_{50} =1.0-7.1 µM) against sixty human cell lines at NCI.[206]

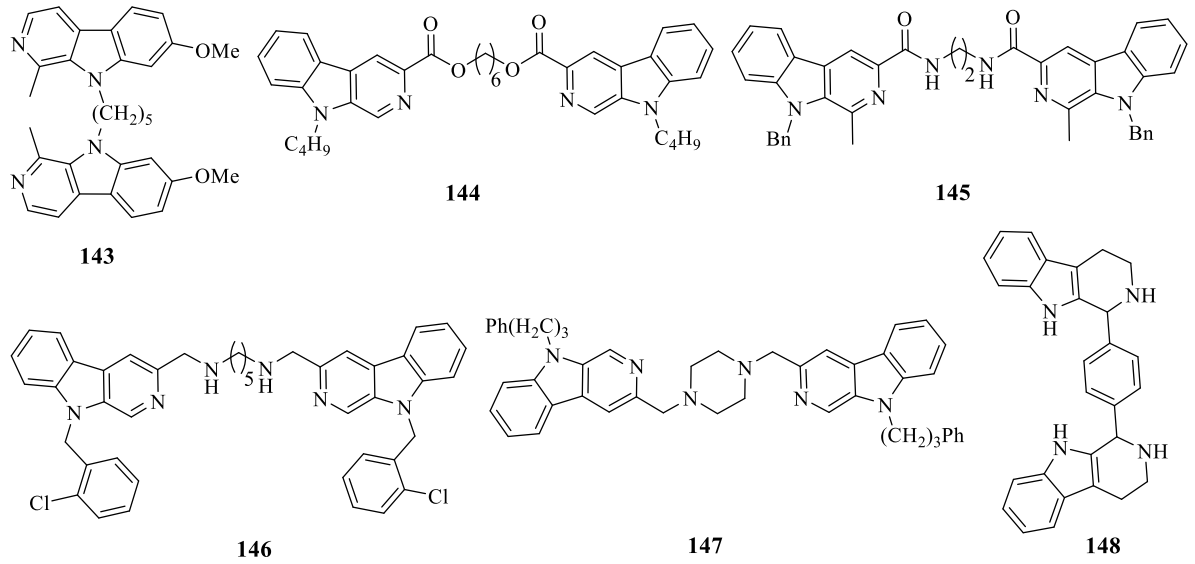


Figure 1.17 Bivalent *β*-carbolines based anticancer agents

1.4.2.4 *β*-Carbolinium salts

Quaternary *β*-carbolines are known to be *β*-carbolinium salts and these salts represented the most interesting antitumor potencies in the literature (Figure 1.18). For example, N^2-Benzylated *β*-carbolinium bromide derivative **149**, bearing a benzylidine substituent at C-1 position represented the most interesting cytotoxic activities with IC_{50} values lower than 5 µM against 10 strains of human tumor cell lines.[207] An interesting antitumor activity (IC_{50} = 1.7 µM) of N^2-benzylated *β*-carboline amino acid ester conjugates **150** against renal carcinoma (786-0) cells results confirmed that the N^2-benzyl substituent on the *β*-carboline ring played an important role in the modulation of the cytotoxic potencies.[208] A polar trisubstituted harmine derivative **151** showed high antiproliferative activity on diverse cancer cell lines (IC_{50} = 0.01-0.7 µM).[209] In 2012, Frederick and co-workers prepared the trisubstituted Harmine derivative **152** with impressive anticancer activity (IC_{50} = 0.7 µM; OE33).[210] *β*-Carbolinium bromides **153** bearing various substituents in position-1, -2, -7 and -9 of *β*-carboline ring represented most potent antitumor activity against HeLa cells (IC_{50} = 0.93 µM).[211] N^2-Benzylated quaternary *β*-carboline amino acid ester conjugate **154** was found to show most potent cytotoxicity (IC_{50} = 1.7 µM, 786-0).[208] *β*-Carboline-based *N*-heterocyclic carbene compound **155** was investigated to display significant antiproliferative and

proapoptotic effects in breast cancer (MDA-MB-231) cells with IC_{50} value 4.3 μM. Further, compound **155** was found to suppress migration and invasion capacities of highly metastatic human breast cancer MDA-MB-231 cells by decreased activities of MMPs and the down-regulation of ERK1/2 and SAPK/JNK signalling.[212] Harmine derivative **156** was cytotoxic (ED_{50} = 1.8 μM) against KB cells.[213] In 2002, Fontana et al. reported pyridazino fused *β*-carbolinium derivative **157** to exert their cytotoxic activity in nanomolar range against leukaemia (IC_{50} = 48 nM, L1210) cell line.[214]

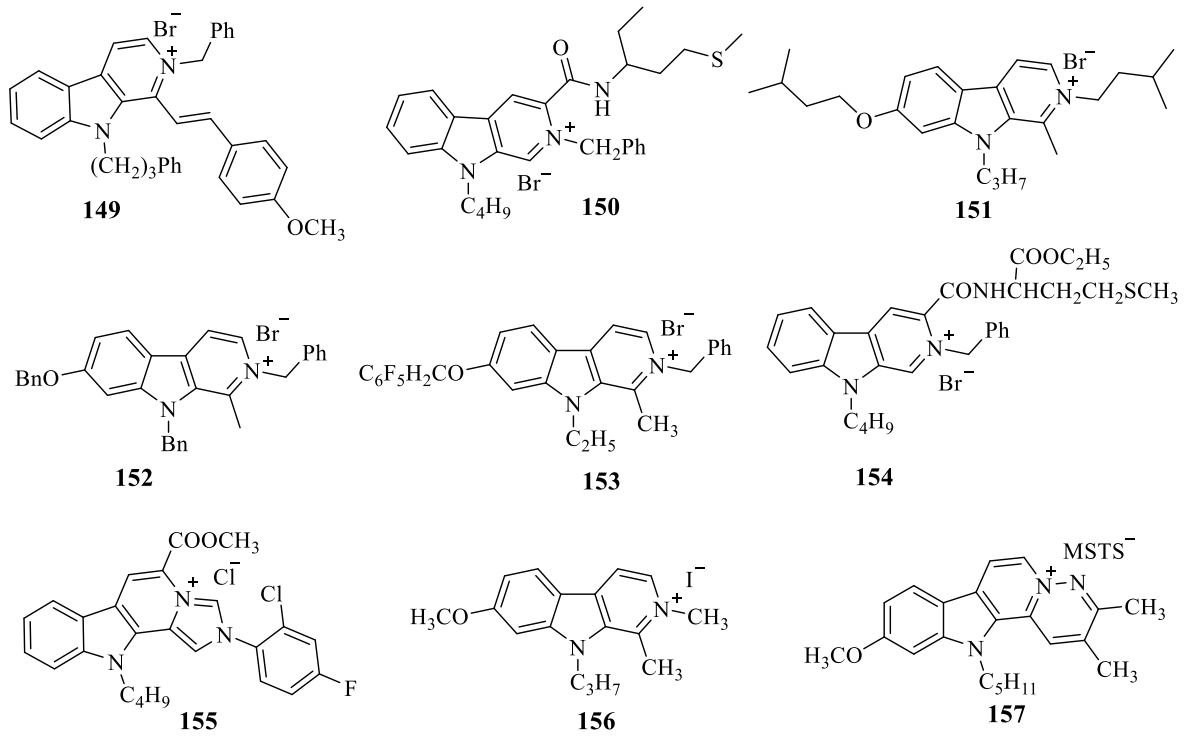


Figure 1.18 Cytotoxicity of representative *β*-carbolinium salts

1.5 Conclusions and present work

Over the years, the design of drugs for cancer chemotherapy has become increasingly sophisticated. Till now there is no cancer treatment that is 100% effective against disseminated cancer so there is a huge demand for the new chemical entities in the field of anticancer research for the development of effective anticancer drugs. Marketed drugs are suffer from various side effects including drug resistance, bioavailability and drug toxicity. Resistance to chemotherapeutics can be divided into two broad categories: intrinsic or acquired. Intrinsic resistance indicates that before receiving chemotherapy, resistance-mediating factors pre-exist in the bulk of tumor cells that make the therapy ineffective. There are limited number of carbazole and *β*-carboline based anticancer agents developed in the

literature including multi drug resistance and specific target-oriented cytotoxic agents. Further, there is a scope to develop carbazole and *β*-carboline based anticancer agents with enhanced activity.

Inspired by the literature results, in the present study, we designed and synthesized novel carbazole and *β*-carboline derivatives for their anticancer activity studies. The purpose of this study is to evaluate the antiproliferative properties of indole related compounds in order to acquire more information about the structural requirements for the possible improvement of the cytotoxic potential and to elucidate SARs between substituent properties and antitumor activities. Design, synthesis and anticancer activity evaluation against a panel of cancer cell lines of various *β*-carbolines with diversifications at C-1/C-3/N^2/N^9-H positions gave insight about their mechanism and required structural modifications.

The thesis is alienated into six chapters including a brief introduction to indole related compounds such as carbazoles and *β*-carbolines and their role in anticancer research. The first chapter introduces carbazole and *β*-carboline chemistry and clearly elaborates importance of these scaffolds in anticancer drug discovery.

The second chapter describes the syntheses and *in-vitro* anticancer activities of two series of novel *β*-carbolinium bromides. In part A, MW-assisted rapid and high yielding synthesis of phenacyl-*β*-carbolinium bromides was carried out from the reaction of *β*-carbolines with 1-aryl-2-bromoethanones in ethanol at 80 °C. All the prepared bromide salts were found to display good cytotoxicity against a panel of human cancer cell lines. The most potent *β*-carbolinium bromide unveiled broad spectrum of anticancer activity against all the tested cancer cell lines (IC_{50} = 3.16-7.93 µM) *via* apoptosis inducing pathway. Synthesis and anticancer activity studies of *β*-carboline-1-chalcones and their bromide salts are reported in Part B. The most potent compound supressed the growth of tested cancer cell lines with IC_{50} values lower than 22.5 µM by causing the apoptosis in MDA-MB-231cells.

A short and facile synthesis of *β*-carboline alkaloid pityriacitrin and its analogues is provided in the third chapter. The synthesis engages *in-situ* cyclization of bis(indolyl)ketoamides by the combination of molecular iodine and triphenylphosphine in DMSO. Generality of the protocol was demonstrated by preparing a series of pityriacitrin, alangiobussinine and their derivatives in good to excellent yields.

The fourth chapter details the high yielding synthesis of water soluble porphyrin-*β*-carboline conjugates by HATU promoted acid-amide coupling of *β*-carboline acids with 5-(4-

aminophenyl)tripyridylporphyrin followed by *N*-methylation of neutral porphyrin-*β*-carboline conjugates with excess methyl iodide under MW irradiation. The photocytotoxicity studies of cationic porphyrin-*β*-carboline conjugates revealed that all the conjugates exhibited remarkable activity (IC_{50} ~ 47-173 nM) against lung as well as colon cancer cell lines.

Chapter five deals the MW-assisted high yielding synthesis of twenty-four carbazolyl ketoamides from easily accessible carbazolyl keto acids and various aryl/heteroarylamines by using HATU as a coupling reagent. The preliminary cytotoxicity results disclosed that the most potent carbazolyl ketoamides are selectively cytotoxic against MCF-7 breast cancer cell line (IC_{50} = 9.3-9.8 μM) and induced apoptosis in Jurkat cells. In addition, four carbazolyl ketoamides were found to display prominent antibacterial activity against Gram-positive and Gram-negative bacteria (MIC = 8-16 μg/mL) by inhibiting the growth of bacteria in few hours of initial interactions.

Finally, the sixth chapter gives a comprehensive overview and concluding remarks about the research work undertaken in thesis.

1.6 References

1. Arunotayanun, W.; Gibbons, S., Natural product 'legal highs'. *Nat. Prod. Rep.,* **2012,** *29* 1304-1316.
2. Franzén, R. G., Recent advances in the preparation of heterocycles on solid support: a review of the literature. *J. Comb. Chem.,* **2000,** *2*, 195-214.
3. Bandini, M.; Eichholzer, A., Catalytic functionalization of indoles in a new dimension. *Angew. Chem. Int. Ed.,* **2009,** *48*, 9608-9644.
4. Hesse, M., *Alkaloids: nature's curse or blessing?* John Wiley & Sons: 2002.
5. Uyttenhove, C.; Pilotte, L.; Théate, I.; Stroobant, V.; Colau, D.; Parmentier, N.; Boon, T.; Van den Eynde, B. J., Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nat. Med.,* **2003,** *9*, 1269-1274.
6. Murch, S.; Krishna Raj, S.; Saxena, P., Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in-vitro* regenerated St. John's wort (*Hypericum perforatum L. cv. Anthos*) plants. *Plant Cell Rep.,* **2000,** *19*, 698-704.
7. Lancianesi, S.; Palmieri, A.; Petrini, M., Synthetic approaches to 3-(2-nitroalkyl) indoles and their use to access tryptamines and related bioactive compounds. *Chem. Rev.,* **2014,** *114*, 7108-7149.
8. O'mahony, S.; Clarke, G.; Borre, Y.; Dinan, T.; Cryan, J., Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behav. Brain Res.,* **2015,** *277*, 32-48.
9. Pevet, P.; Klosen, P.; Felder-Schmittbuhl, M. P., The hormone melatonin: Animal studies. *Best Pract. Res. Clin. Endocrinol. Metab.,* **2017,** *31*, 547-559.
10. Kumar, A.; Rani, A.; Scheinert, R. B.; Ormerod, B. K.; Foster, T. C., Nonsteroidal anti-inflammatory drug, indomethacin improves spatial memory and NMDA receptor function in aged animals. *Neurobiol. Aging,* **2018,** *70*, 184-193.
11. Hull, M.; Gardner, S.; Hawcroft, G., Activity of the non-steroidal anti-inflammatory drug indomethacin against colorectal cancer. *Cancer Treat. Rev.,* **2003,** *29*, 309-320.
12. Ferreira, S.; Moncada, S.; Vane, J., Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature New Biol.,* **1971,** *231*, 237.
13. Juruena, M. F.; Ponde00C9, E.; De Sena; Reis De Oliveira, I., Sertindole in the management of schizophrenia. *J. CNS Disease,* **2011,** *3*, 75–85.
14. Blier, P.; Bergeron, R., The use of pindolol to potentiate antidepressant medication. *J. Clin. Psychiatry,* **1998,** *59*, 16-23.

15. Bollag, G.; Hirth, P.; Tsai, J.; Zhang, J.; Ibrahim, P. N.; Cho, H.; Spevak, W.; Zhang, C.; Zhang, Y.; Habets, G.; Burton, E. A.; Wong, B.; Tsang, G.; West, B. L.; Powell, B.; Shellooe, R.; Marimuthu, A.; Nguyen, H.; Zhang, K. Y. J.; Artis, D. R.; Schlessinger, J.; Su, F.; Higgins, B.; Iyer, R.; D'Andrea, K.; Koehler, A.; Stumm, M.; Lin, P. S.; Lee, R. J.; Grippo, J.; Puzanov, I.; Kim, K. B.; Ribas, A.; McArthur, G. A.; Sosman, J. A.; Chapman, P. B.; Flaherty, K. T.; Xu, X.; Nathanson, K. L.; Nolop, K., Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature*, **2010,** *467*, 596-599.
16. Bollag, G.; Tsai, J.; Zhang, J.; Zhang, C.; Ibrahim, P.; Nolop, K.; Hirth, P., Vemurafenib: the first drug approved for BRAF-mutant cancer. *Nat. Rev. Drug Discov.,* **2012,** *11*, 873-886.
17. Diamond, E. L.; Subbiah, V.; Lockhart, A.; et al., Vemurafenib for braf v600–mutant erdheim-chester disease and langerhans cell histiocytosis: Analysis of data from the histology-independent, phase 2, open-label ve-basket study. *JAMA Oncol.,* **2018,** *4*, 384-388.
18. Balbisi, E., Frovatriptan succinate, a 5-HT1B/1D receptor agonist for migraine. *Int. J. Clin. Pract.,* **2004,** *58*, 695-705.
19. Olivier, B.; van Wijngaarden, I.; Soudijn, W., 5-HT3 receptor antagonists and anxiety; a preclinical and clinical review. *Eur. Neuropsychopharmacol.,* **2000,** *10*, 77-95.
20. Sorbera, L.; Martin, L.; Leeson, P.; Castaner, J., Monographs-IC-351. Treatment of erectile dysfunction, treatment of female sexual dysfunction, phosphodiesterase 5 inhibitor. *Drugs Fut.,* **2001,** *26*, 15-19.
21. Daugan, A.; Grondin, P.; Ruault, C.; Le Monnier de Gouville, A.-C.; Coste, H.; Linget, J. M.; Kirilovsky, J.; Hyafil, F.; Labaudinière, R., The discovery of Tadalafil: A novel and highly selective PDE5 inhibitor. 2: 2,3,6,7,12,12a-hexahydropyrazino[1',2':1,6] pyrido[3,4-*b*]indole-1,4-dione analogues. *J. Med. Chem.,* **2003,** *46*, 4533-4542.
22. Turski, L.; Stephens, D.; Jensen, L.; Petersen, E.; Meldrum, B.; Patel, S.; Hansen, J. B.; Löscher, W.; Schneider, H.; Schmiechen, R., Anticonvulsant action of the *β*-carboline abecarnil: studies in rodents and baboon, Papio papio. *J. Pharmacol. Exp. Ther.,* **1990,** *253*, 344-352.
23. Maw, G. N.; Allerton, C. M.; Gbekor, E.; Million, W. A., Design, synthesis and biological activity of *β*-carboline-based type-5 phosphodiesterase inhibitors. *Bioorganic Med. Chem. Lett.,* **2003,** *13*, 1425-1428.

24. Patil, S. A.; Patil, R.; Miller, D. D., Indole molecules as inhibitors of tubulin polymerization: potential new anticancer agents. *Future Med. Chem.,* **2012,** *4*, 2085-2115.
25. Kumar, D.; Kumar, N. M., Recent developments on synthetic indoles as potent anticancer agents. *Chemistry & Biology Interface,* **2013,** *3*, 276-303.
26. Chaniyara, R.; Tala, S.; Chen, C.-W.; Zang, X.; Kakadiya, R.; Lin, L.-F.; Chen, C.-H.; Chien, S.-I.; Chou, T.-C.; Tsai, T.-H.; Lee, T.-C.; Shah, A.; Su, T.-L., Novel antitumor indolizino[6,7-*b*]indoles with multiple modes of action: DNA cross-linking and topoisomerase I and II inhibition. *J. Med. Chem.,* **2013,** *56*, 1544-1563.
27. Conchon, E.; Anizon, F.; Aboab, B.; Golsteyn, R. M.; Léonce, S.; Pfeiffer, B.; Prudhomme, M., Synthesis, *in-vitro* antiproliferative activities, and Chk1 inhibitory properties of pyrrolo[3,4-*a*]carbazole-1,3-diones, pyrrolo[3,4-*c*]carbazole-1,3-diones, and 2-aminopyridazino[3,4-*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,7-tetraone. *Eur. J. Med. Chem.,* **2008,** *43*, 282-292.
28. Bernardo, P. H.; Chai, C. L. L.; Guen, M. L.; Smith, G. D.; Waring, P., Structure–activity delineation of quinones related to the biologically active calothrixins *Bioorganic Med. Chem. Lett.,* **2007,** *17*, 82-85.
29. Miller, C. M.; McCarthy, F. O., Isolation, biological activity and synthesis of the natural product ellipticine and related pyridocarbazoles. *RSC Adv.,* **2012,** *2*, 8883-8918.
30. Cao, R.; Fan, W.; Guo, L.; Ma, Q.; Zhang, G.; Li, J.; Chen, X.; Ren, Z.; Qiu, L., Synthesis and structure–activity relationships of harmine derivatives as potential antitumor agents. *Eur. J. Med. Chem.,* **2013,** *60*, 135-143.
31. Miller, T. A.; Vachaspati, P. R.; Labroli, M. A.; Thompson, C. D.; Bulman, A. L.; Macdonald, T. L., The synthesis and evaluation of benzannelated-azatoxins: The benzazatoxins. *Bioorganic Med. Chem. Lett.,* **1998,** *8*, 1065-1070.
32. Roy, J.; Jana, A. K.; Mal, D., Recent trends in the synthesis of carbazoles: an update. *Tetrahedron,* **2012,** *68*, 6099-6121.
33. Asche, C.; Demeunynck, M., Antitumor carbazoles. *Anticancer Agents Med. Chem.,* **2007,** *7*, 247-267.
34. Graebe, C.; Glaser, C., Ueber Carbazol. *Justus Liebigs Ann. Chem.,* **1872,** *163*, 343-360.
35. Chakraborty, D.; Barman, B.; Bose, P., Structure of girinimbine, a pyranocarbazole derivative isolated from *Murraya koenigii* Spreng. *Sci. Cult.,* **1964,** *30*, 445-448.
36. Das, K.; Chakraborty, D.; Bose, P., Antifungal activity of some constituents of *Murraya koenigii* spreng. *Cell. Mol. Life Sci.,* **1965,** *21*, 340-340.

37. Chakraborty, D. P.; Barman, B. K.; Bose, P. K., On the constitution of murrayanine, a carbazole derivative isolated from *Murraya koenigii* Spreng. *Tetrahedron,* **1965,** *21*, 681-685.
38. Schmidt, A. W.; Reddy, K. R.; Knölker, H.-J., Occurrence, biogenesis, and synthesis of biologically active carbazole alkaloids. *Chem. Rev.,* **2012,** *112*, 3193-3328.
39. Knölker, H.-J.; Reddy, K. R., Isolation and synthesis of biologically active carbazole alkaloids. *Chem. Rev.,* **2002,** *102*, 4303-4428.
40. Chakraborty, D., Chemistry and biology of carbazole alkaloids. In *The Alkaloids: Chemistry and Pharmacology*, Elsevier: 1993; Vol. 44, pp 257-364.
41. Chakraborty, D.; Roy, S., Carbazole alkaloids III. In *Fortschritte Der Chemie Organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products*, Springer: 1991; pp 71-152.
42. Chakraborty, D., Some aspects of the carbazole alkaloids. *Planta Med.,* **1980,** *39*, 97-111.
43. Głuszyńska, A., Biological potential of carbazole derivatives. *Eur. J. Med. Chem.,* **2015,** *94*, 405-426.
44. Verma, S., Overview study on *Murraya koenigii* (mitha neem): rutaceae. *J. Drug Del. Ther.,* **2018,** *8*, 90-92.
45. HUANG, L.; Zhe-Ling, F.; Yi-Tao, W.; Li-Gen, L., Anticancer carbazole alkaloids and coumarins from *Clausena* plants: A review. *Chin. J. Nat. Med.,* **2017,** *15*, 881-888.
46. Greger, H., Phytocarbazoles: alkaloids with great structural diversity and pronounced biological activities. *Phytochem. Rev.,* **2017,** *16*, 1095-1153.
47. Chauhan, B.; Dedania, J.; Mashru, R., Review on *Murraya Koenigii*: Versatile role in management of human health. *World J. Pharm. Pharm. Sci.,* **2017,** *6*, 476-493.
48. Song, J. J.; Reeves, J. T.; Fandrick, D. R.; Tan, Z.; Yee, N. K.; Senanayake, C. H., Construction of the indole nucleus through C-H functionalization reactions. *Arkivoc,* **2010,** *(i)*, 390-449.
49. Bergman, J.; Pelcman, B., Synthesis of carbazole alkaloids. *Pure Appl. Chem.,* **1990,** *62*, 1967-1976.
50. Drechsel, E., Ueber Elektrolyse des Phenols mit Wechselströmen. *J. prakt. Chem.,* **1888,** *38*, 65-74.
51. Borsche, W., Ueber Tetra- und Hexahydrocarbazolverbindungen und eine neue Carbazolsynthese. (Mitbearbeitet von. A. Witte und W. Bothe.). *Justus Liebigs Ann. Chem.,* **1908,** *359*, 49-80.

52. Graebe-Ullmann synthesis. In *comprehensive organic name reactions and reagents*.
53. Ashton, B. W.; Suschitzky, H., 916. The Graebe–Ullmann carbazole synthesis. *J. Chem. Soc.*, **1957**, 4559-4562.
54. Tsunashima, Y.; Kuroki, M., The chemistry of carbazole. VI. On the formation of *N*-ethylcarbazoles in the cadogan reaction. *J. Heterocycl. Chem.*, **1981,** *18*, 315-318.
55. JIG, C., Cameron-Wood M. Mackie RK. *Searle RJG. J. Chem. Soc.*, **1965,** *4831*.
56. Goldberg, I., Ueber phenylirungen bei gegenwart von kupfer als katalysator. *Ber. Dtsch. Chem. Ges.*, **1906,** *39*, 1691-1692.
57. Ullmann, F., Ueber eine neue darstellungsweise von phenyläthersalicylsäure. *Ber. Dtsch. Chem. Ges.*, **1904,** *37*, 853-854.
58. Schlummer, B.; Scholz, U., Palladium-catalyzed C- N and C- O coupling–a practical guide from an industrial vantage point. *Adv. Synth. Catal.*, **2004,** *346*, 1599-1626.
59. Huang, X.; Anderson, K. W.; Zim, D.; Jiang, L.; Klapars, A.; Buchwald, S. L., Expanding Pd-catalyzed C−N bond-forming processes: the first amidation of aryl sulfonates, aqueous amination, and complementarity with Cu-catalyzed reactions. *J. Am. Chem. Soc.*, **2003,** *125*, 6653-6655.
60. Ogata, T.; Hartwig, J. F., Palladium-catalyzed amination of aryl and heteroaryl tosylates at room temperature. *J. Am. Chem. Soc.*, **2008,** *130*, 13848-13849.
61. Ley, S. V.; Thomas, A. W., Modern synthetic methods for copper-mediated C(aryl)-O, C(aryl)-N, and C(aryl)-S bond formation. *Angew. Chem. Int. Ed.*, **2003,** *42*, 5400-5449.
62. Hartwig, J. F., Transition metal catalyzed synthesis of arylamines and aryl ethers from aryl halides and triflates: Scope and mechanism. *Angew. Chem. Int. Ed.*, **1998,** *37*, 2046-2067.
63. Surry, D. S.; Buchwald, S. L., Biaryl phosphane ligands in palladium-catalyzed amination. *Angew. Chem. Int. Ed.*, **2008,** *47*, 6338-6361.
64. Akermark, B.; Eberson, L.; Jonsson, E.; Pettersson, E., Palladium-promoted cyclization of diphenyl ether, diphenylamine, and related compounds. *J. Org. Chem.*, **1975,** *40*, 1365-1367.
65. Krahl, M. P.; Jäger, A.; Krause, T.; Knölker, H.-J., First total synthesis of the 7-oxygenated carbazole alkaloids clauszoline-K, 3-formyl-7-hydroxycarbazole, clausine M, clausine N and the anti-HIV active siamenol using a highly efficient palladium-catalyzed approach. *Org. Biomol. Chem.*, **2006,** *4*, 3215-3219.

66. Watanabe, T.; Ueda, S.; Inuki, S.; Oishi, S.; Fujii, N.; Ohno, H., One-pot synthesis of carbazoles by Pd-catalyzed *N*-arylation and oxidative coupling. *Chem. Commun.,* **2007**, 4516-4518.

67. Liu, Z.; Larock, R. C., Synthesis of carbazoles and dibenzofurans *via* cross-coupling of *o*-iodoanilines and *o*-iodophenols with silylaryl triflates. *Org. Lett.,* **2004,** *6*, 3739-3741.

68. Ackermann, L.; Althammer, A., Domino N-H/C-H bond activation: Palladium-catalyzed synthesis of annulated heterocycles using dichloro (hetero) arenes. *Angew. Chem. Int. Ed.,* **2007,** *46*, 1627-1629.

69. Ackermann, L.; Althammer, A.; Mayer, P., Palladium-catalyzed direct arylation-based domino synthesis of annulated *N*-heterocycles using alkenyl or (hetero) aryl 1, 2-dihalides. *Synthesis,* **2009,** *2009*, 3493-3503.

70. Neugebauer, F. A.; Fischer, H., *tert*.-Butyl-substituierte carbazole. *Chem. Ber.,* **1972,** *105*, 2686-2693.

71. Miao, F.; Song, G.; Sun, Y.; Liu, Y.; Guo, F.; Zhang, W.; Tian, M.; Yu, X., Fluorescent imaging of acidic compartments in living cells with a high selective novel one-photon ratiometric and two-photon acidic pH probe. *Biosens. Bioelectron.,* **2013,** *50*, 42-49.

72. Sengul, I. F.; Wood, K.; Kumar, N.; Black, D. S., Synthesis of macrocyclic systems derived from di-(2-indolyl) heteroarenes. *Tetrahedron*, **2012,** *68*, 9050-9055.

73. Deane, F. M.; Miller, C. M.; Maguire, A. R.; McCarthy, F. O., Modifications to the Vilsmeier-Haack formylation of 1,4-dimethylcarbazole and its application to the synthesis of ellipticines. *J. Heterocycl. Chem.,* **2011,** *48*, 814-823.

74. Sengul, I. F.; Okutan, E.; Kandemir, H.; Astarcı, E.; Çoşut, B., Carbazole substituted BODIPY dyes: synthesis, photophysical properties and antitumor activity. *Dyes Pigm.,* **2015,** *123*, 32-38.

75. Zhu, Z.; Moore, J. S., Synthesis and characterization of monodendrons based on 9-phenylcarbazole. *J. Org. Chem.,* **2000,** *65*, 116-123.

76. Sadiq, Z.; Akbar, E.; Naz, N., Synthesis and antimicrobial assessment of efficacious carbazole derivatives. *Int. J. Pharma Bio Sci.,* **2014,** *5*, P455-P464.

77. Kuroki, M.; Tsunashima, Y., The chemistry of carbazoles. VII. Syntheses of methylcarbazoles. *J. Heterocycl. Chem.,* **1981,** *18*, 709-714.

78. Venault, P.; Chapouthier, G., From the behavioral pharmacology of *β*-carbolines to seizures, anxiety, and memory. *Sci. World J.,* **2007,** *7*, 204-223.

79. Hudson, J. B., *Antiviral compounds from Plants: 0*. CRC press: 2018.

80. Castro, A. C.; Dang, L. C.; Soucy, F.; Grenier, L.; Mazdiyasni, H.; Hottelet, M.; Parent, L.; Pien, C.; Palombella, V.; Adams, J., Novel IKK inhibitors: *β*-carbolines. *Bioorganic Med. Chem. Lett.,* **2003,** *13*, 2419-2422.
81. Guan, H.; Chen, H.; Peng, W.; Ma, Y.; Cao, R.; Liu, X.; Xu, A., Design of *β*-carboline derivatives as DNA-targeting antitumor agents. *Eur. J. Med. Chem.,* **2006,** *41*, 1167-1179.
82. Deveau, A. M.; Labroli, M. A.; Dieckhaus, C. M.; Barthen, M. T.; Smith, K. S.; Macdonald, T. L., The synthesis of amino-acid functionalized *β*-carbolines as topoisomerase II inhibitors. *Bioorganic Med. Chem. Lett.,* **2001,** *11*, 1251-1255.
83. Allen, J. R.; Holmstedt, B. R., The simple *β*-carboline alkaloids. *Phytochemistry,* **1980,** *19*, 1573-1582.
84. Goebel, F., Über das harmalin. *Eur. J. Org. Chem.,* **1841,** *38*, 363-366.
85. Kodani, S.; Imoto, A.; Mitsutani, A.; Murakami, M., Isolation and identification of the antialgal compound, harmane (1-methyl-*β*-carboline), produced by the algicidal bacterium, *Pseudomonas sp*. K44-1. *J. Appl. Psychol.,* **2002,** *14*, 109-114.
86. Fattorusso, E.; Taglialatela-Scafati, O., Modern alkaloids: structure, isolation, synthesis, and biology. John Wiley & Sons: 2008.
87. Humphrey, G. R.; Kuethe, J. T., Practical methodologies for the synthesis of indoles. *Chem. Rev.,* **2006,** *106*, 2875-2911.
88. Pictet, A.; Spengler, T., Formation of isoquinoline derivatives by the action of methylal on phenylethylamine, phenylalanine and tyrosine. *Ber. Dtsch. Chem. Ges,* **1911,** *44*, 13.
89. Whaley, W. M.; Govindachari, T. R., The Pictet-Spengler synthesis of tetrahydro-isoquinolines and related compounds. *In Org. React.*, 2011; pp 151-187.
90. Tatsui, G., Preparation of 1-methyl-1,2,3,4-tetrahydro-*β*-carboline. *J. Pharm. Soc. Jpn,* **1928,** *48*, 92.
91. Rao, R. N.; Maiti, B.; Chanda, K., Application of Pictet–Spengler reaction to indole-based alkaloids containing tetrahydro-*β*-carboline scaffold in combinatorial chemistry. *ACS Comb. Sci.,* **2017,** *19*, 199-228.
92. Cox, E. D.; Cook, J. M., The Pictet-Spengler condensation: a new direction for an old reaction. *Chem. Rev.,* **1995,** *95*, 1797-1842.
93. Youn, S. W., The Pictet-Spengler reaction: Efficient carbon-carbon bond forming reaction in heterocyclic synthesis. *Org. Prep. Proced. Int.,* **2006,** *38*, 505-591.
94. Eagon, S.; Anderson, M. O., Microwave-assisted synthesis of tetrahydro-*β*-carbolines and *β*-carbolines. *Eur. J. Org. Chem.,* **2014,** *2014*, 1653-1665.

95. Desroses, M.; Koolmeister, T.; Jacques, S.; Llona-Minguez, S.; Jacques-Cordonnier, M.-C.; Cázares-Körner, A.; Helleday, T.; Scobie, M., A facile and efficient synthesis of tetrahydro-*β*-carbolines. *Tetrahedron Lett.,* **2013,** *54*, 3554-3557.
96. Srinivasan, N.; Ganesan, A., Highly efficient Lewis acid-catalysed Pictet–Spengler reactions discovered by parallel screening. *Chem. Commun.,* **2003,** *0*, 916-917.
97. Kulkarni, A.; Abid, M.; Toeroek, B.; Huang, X., A direct synthesis of *β*-carbolines via a three-step one-pot domino approach with a bifunctional Pd/C/K-10 catalyst. *Tetrahedron Lett.,* **2009,** *50*, 1791-1794.
98. Sewgobind, N. V.; Wanner, M. J.; Ingemann, S.; de Gelder, R.; van Maarseveen, J. H.; Hiemstra, H., Enantioselective binol-phosphoric acid catalyzed Pictet−Spengler reactions of *N*-benzyltryptamine. *J. Org. Chem.,* **2008,** *73*, 6405-6408.
99. Wang, L.-N.; Shen, S.-L.; Qu, J., Simple and efficient synthesis of tetrahydro-*β*-carbolines *via* the Pictet–Spengler reaction in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). *RSC Adv.,* **2014,** *4*, 30733-30741.
100. Prajapati, D.; Gohain, M., Iodine-catalyzed highly effective Pictet–Spengler condensation: An efficient synthesis of tetrahydro-*β*-carbolines. *Synth. Commun.,* **2008,** *38*, 4426-4433.
101. Kamal, A.; Tangella, Y.; Manasa, K. L.; Sathish, M.; Srinivasulu, V.; Chetna, J.; Alarifi, A., $PhI(OAc)_2$-mediated one-pot oxidative decarboxylation and aromatization of tetrahydro-*β*-carbolines: synthesis of norharmane, harmane, eudistomin U and eudistomin I. *Org. Biomol. Chem.,* **2015,** *13*, 8652-8662.
102. Kamal, A.; Sathish, M.; Prasanthi, A.; Chetna, J.; Tangella, Y.; Srinivasulu, V.; Shankaraiah, N.; Alarifi, A., An efficient one-pot decarboxylative aromatization of tetrahydro-*β*-carbolines by using *N*-chlorosuccinimide: total synthesis of norharmane, harmane and eudistomins. *RSC Adv.,* **2015,** *5*, 90121-90126.
103. Meesala, R.; Arshad, A. S. M.; Mordi, M. N.; Mansor, S. M., Iodine-catalyzed one-pot decarboxylative aromatization of tetrahydro-*β*-carbolines. *Tetrahedron,* **2016,** *72*, 8537-8541.
104. Meesala, R.; Mordi, M. N.; Mansor, S. M., Copper-catalyzed protodecarboxylation and aromatization of tetrahydro-*β*-carboline-3-carboxylic acids. *Synlett,* **2014,** *25*, 120-122.
105. Manasa, K. L.; Tangella, Y.; Ramu, G.; Nagendra Babu, B., TCCA; A mild reagent for decarboxylative/dehydrogenative aromatization of tetrahydro-*β*-carbolines: Utility in the total synthesis of norharmane, harmane, eudistomin U, I and N. *ChemistrySelect,* **2017,** *2*, 9162-9167.

106. Singh, V.; Batra, S., 1-Formyl-9*H*-*β*-carboline: A useful scaffold for synthesizing substituted-and fused *β*-carbolines. *Curr. Org. Synth.,* **2012,** *9*, 513-528.
107. Gaikwad, S.; Kamble, D.; Lokhande, P., Iodine-catalyzed chemoselective dehydrogenation and aromatization of tetrahydro-*β*-carbolines: A short synthesis of kumujian-C, eudistomin-U, norharmane, harmane harmalan and isoeudistomine-M. *Tetrahedron Lett.,* **2018,** *59*, 2387-2392.
108. Zheng, B.; Trieu, T. H.; Meng, T.-Z.; Lu, X.; Dong, J.; Zhang, Q.; Shi, X.-X., Cu-catalyzed mild and efficient oxidation of TH*β*Cs using air: application in practical total syntheses of perlolyrine and flazin. *RSC Adv.,* **2018,** *8*, 6834-6839.
109. Panarese, J. D.; Waters, S. P., Room-temperature aromatization of tetrahydro-*β*-carbolines by 2-iodoxybenzoic acid: Utility in a total synthesis of eudistomin U. *Org. Lett.,* **2010,** *12*, 4086-4089.
110. Hati, S.; Sen, S., *N*-Bromosuccinimide promoted synthesis of *β*-carbolines and 3,4-dihydro-*β*-carbolines from tetrahydro-*β*-carbolines. *Tetrahedron Lett.,* **2016,** *57*, 1040-1043.
111. Kurumanna, A.; Ashok, K.; Rambabu, S.; Sonyanaik, B.; Ravi, D.; Madhu, P.; Sakram, B., Simple and efficient method for aromatization of tetrahydro-*β*-carbolines by using $K_2S_2O_8$ as a catalyst and its antimicrobial activity comparison with molecular docking studies. *Russ. J. Gen. Chem.,* **2017,** *87*, 2703-2711.
112. Durham, S. D.; Sierra, B.; Gomez, M. J.; Tran, J. K.; Anderson, M. O.; Whittington-Davis, N. A.; Eagon, S., Synthesis of *β*-carbolines via a silver-mediated oxidation of tetrahydro-*β*-carbolines. *Tetrahedron Lett.,* **2017,** *58*, 2747-2750.
113. Bischler, A.; Napieralski, B., Zur kenntniss einer neuen isochinolinsynthese. *Chem. Ber.,* **1893,** *26*, 1903-1908.
114. Banwell, M. G.; Bissett, B. D.; Busato, S.; Cowden, C. J.; Hockless, D. C. R.; Holman, J. W.; Read, R. W.; Wu, A. W., Trifluoromethanesulfonic anhydride–4-(N,N-dimethyl amino)pyridine as a reagent combination for effecting Bischler–Napieraiski cyclisation under mild conditions: application to total syntheses of the Amaryllidaceae alkaloids *N*-methylcrinasiadine, anhydrolycorinone, hippadine and oxoassoanine. *J. Chem. Soc., Chem. Commun.,* **1995**, 2551-2553.
115. Whaley, W. M.; Govindachari, T. R., The preparation of 3, 4-dihydroisoquinolines and related compounds by the Bischler-Napieralski reaction. *Org. React.,* **2004,** *6*, 74-150.
116. Fodor, G.; Gal, J.; Phillips, B., The mechanism of the Bischler-Napieralski reaction. *Angew. Chem. Int. Ed.,* **1972,** *11*, 919-920.

117. Nagubandi, S.; Fodor, G., The mechanism of the Bischler-Napieralski reaction. *J. Heterocycl. Chem.,* **1980,** *17*, 1457-1463.
118. Movassaghi, M.; Hill, M. D., A versatile cyclodehydration reaction for the synthesis of isoquinoline and *β*-carboline derivatives. *Org. Lett.,* **2008,** *10*, 3485-3488.
119. Wu, M.; Wang, S., An alternative to Pictet-Gams reaction triggered by Hendrickson reagent: isoquinolines and *β*-carbolines from amides. *Synthesis,* **2010,** *2010*, 587-592.
120. Meruva, S. B.; Raghunadh, A.; Kamaraju, R. R.; Kumar, U. S.; Dubey, P., An oxidative amidation and heterocyclization approach for the synthesis of *β*-carbolines and dihydroeudistomin Y. *Beilstein J. Org. Chem.,* **2014,** *10*, 471.
121. Ivanov, I.; Nikolova, S.; Statkova-Abeghe, S., A simple method for the synthesis of 1-substituted-*β*-carboline derivatives from tryptamine and carboxylic acids in polyphosphoric acid. *Heterocycles,* **2005,** *65*, 2483-2492.
122. Naik, P. N.; Naik, N. H.; Khan, A.; Kusurkar, R. S., Synthesis of canthine analogues using intramolecular aza-Diels–Alder strategy and evaluation of their activity against HeLa cervical cancer cells. *Tetrahedron,* **2013,** *69*, 6545-6551.
123. Priebbenow, D. L.; Stewart, S. G.; Pfeffer, F. M., A general approach to *N*-heterocyclic scaffolds using domino Heck–aza-Michael reactions. *Org. Biomol. Chem.,* **2011,** *9*, 1508-1515.
124. Rixson, J. E.; Chaloner, T.; Heath, C. H.; Tietze, L. F.; Stewart, S. G., The development of domino reactions incorporating the Heck reaction: The formation of *N*-heterocycles. *Eur. J. Org. Chem.,* **2012,** *2012*, 544-558.
125. Wang, T.-T.; Zhang, D.; Liao, W.-W., Versatile synthesis of functionalized *β*-and *γ*-carbolines *via* Pd-catalyzed C–H addition to nitriles/cyclization sequences. *Chem. Commun.,* **2018,** *54*, 2048-2051.
126. Zhang, H.; Larock, R. C., Synthesis of *β*- and *γ*-Carbolines by the palladium-catalyzed iminoannulation of alkynes. *J. Org. Chem.,* **2002,** *67*, 9318-9330.
127. Primas, N.; El-Kashef, H.; Lancelot, J.-C.; Lesnard, A.; Dodd, R. H.; Rault, S., Triazine-fused *β*-carbolines: a facile three-step, one-pot synthesis of novel 2-alkyl (aryl) amino and 2-dialkylamino-7*H*-[1,3,5]triazino[1',2':1,6] pyrido [3, 4-*b*] indol-4-ones. *Arkivoc,* **2010,** *(xi)* 163-176
128. Domínguez, G.; Perez-Castells, J., Chemistry of *β*-carbolines as synthetic intermediates. *Eur. J. Org. Chem.,* **2011,** *2011*, 7243-7253.
129. Abranyi-Balogh, P.; Volk, B.; Milen, M., Practical synthesis of two novel series of 1, 3-disubstituted *β*-carboline derivatives. *Tetrahedron Lett.,* **2018,** *59*, 617-619.

130. S Tsutsumi, L.; Gündisch, D.; Sun, D., Carbazole scaffold in medicinal chemistry and natural products: a review from 2010-2015. *Curr. Top. Med. Chem.,* **2016,** *16*, 1290-1313.
131. Bashir, M.; Bano, A.; Ijaz, A. S.; Chaudhary, B. A., Recent developments and biological activities of *N*-substituted carbazole derivatives: A review. *Molecules,* **2015,** *20*, 13496-13517.
132. Cao, R.; Peng, W.; Wang, Z.; Xu, A., *β*-Carboline alkaloids: biochemical and pharmacological functions. *Curr. Med. Chem.,* **2007,** *14*, 479-500.
133. Hussain, H.; Al-Harrasi, A.; Al-Rawahi, A.; Green, I. R.; Gibbons, S., Fruitful decade for antileishmanial compounds from 2002 to late 2011. *Chem. Rev.,* **2014,** *114*, 10369-10428.
134. Ashok, P.; Lathiya, H.; Murugesan, S., Manzamine alkaloids as antileishmanial agents: A review. *Eur. J. Med. Chem.,* **2015,** *97*, 928-936.
135. Laine, A. E.; Lood, C.; Koskinen, A. M., Pharmacological importance of optically active tetrahydro-*β*-carbolines and synthetic approaches to create the C-1 stereocenter. *Molecules,* **2014,** *19*, 1544-1567.
136. Lan, J.-S.; Xie, S.-S.; Li, S.-Y.; Pan, L.-F.; Wang, X.-B.; Kong, L.-Y., Design, synthesis and evaluation of novel tacrine-(*β*-carboline) hybrids as multifunctional agents for the treatment of Alzheimer's disease. *Bioorganic Med. Chem.,* **2014,** *22*, 6089-6104.
137. Sherer, C.; Snape, T. J., Heterocyclic scaffolds as promising anticancer agents against tumours of the central nervous system: Exploring the scope of indole and carbazole derivatives. *Eur. J. Med. Chem.,* **2015,** *97*, 552-560.
138. Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J.; Murray, T.; Thun, M. J., Cancer statistics, 2008. *CA: Cancer J. Clin.,* **2008,** *58*, 71-96.
139. Bughani, U.; Li, S.; Joshi, H. C., Recent patents reveal microtubules as persistent promising target for novel drug development for cancers. *Recent Patents Anti-Infect. Drug Disc.,* **2009,** *4*, 164-182.
140. Nygren, P.; Larsson, R., Overview of the clinical efficacy of investigational anticancer drugs. *J. Intern. Med.,* **2003,** *253*, 46-75.
141. Bisagni, G.; Musolino, A.; Panebianco, M.; De Matteis, A.; Nuzzo, F.; Ardizzoni, A.; Gori, S.; Gamucci, T.; Passalacqua, R.; Gnoni, R., The breast avastin trial: phase II study of bevacizumab maintenance therapy after induction chemotherapy with docetaxel and capecitabine for the first-line treatment of patients with locally recurrent or metastatic breast cancer. *Cancer chemother. pharmacol.,* **2013,** *71*, 1051-1057.

142. S Shaikh, M.; Karpoormath, R.; Thapliyal, N.; A Rane, R.; B Palkar, M.; Faya, A.; M Patel, H.; S Alwan, W.; Jain, K.; A Hampannavar, G., Current perspective of natural alkaloid carbazole and its derivatives as antitumor agents. *Anticancer Agents Med. Chem.,* **2015,** *15*, 1049-1065.

143. Lin, W.; Wang, Y.; Lin, S.; Li, C.; Zhou, C.; Wang, S.; Huang, H.; Liu, P.; Ye, G.; Shen, X., Induction of cell cycle arrest by the carbazole alkaloid Clauszoline-I from *Clausena vestita* DD Tao *via* inhibition of the PKCδ phosphorylation. *Eur. J. Med. Chem.,* **2012,** *47*, 214-220.

144. Nakamura, S.; Nakashima, S.; Oda, Y.; Yokota, N.; Fujimoto, K.; Matsumoto, T.; Ohta, T.; Ogawa, K.; Maeda, S.; Nishida, S., Alkaloids from Sri Lankan curry-leaf (*Murraya koenigii*) display melanogenesis inhibitory activity: structures of karapinchamines A and B. *Bioorganic Med. Chem.,* **2013,** *21*, 1043-1049.

145. Nagappan, T.; Ramasamy, P.; Wahid, M. E. A.; Segaran, T. C.; Vairappan, C. S., Biological activity of carbazole alkaloids and essential oil of *Murraya koenigii* against antibiotic resistant microbes and cancer cell lines. *Molecules,* **2011,** *16*, 9651-9664.

146. Fabbro, D.; Buchdunger, E.; Wood, J.; Mestan, J.; Hofmann, F.; Ferrari, S.; Mett, H.; O'Reilly, T.; Meyer, T., Inhibitors of protein kinases: CGP 41251, a protein kinase inhibitor with potential as an anticancer agent. *Pharmacol. Ther.,* **1999,** *82*, 293-301.

147. Luo, W.; Sharif, T. R.; Houghton, P. J.; Sharif, M., CGP 41251 and tamoxifen selectively inhibit mitogen-activated protein kinase activation and c-Fos phosphoprotein induction by substance P in human astrocytoma cells. *Cell Growth Differ.,* **1997,** *8*, 1225-1240.

148. Songsiang, U.; Thongthoom, T.; Boonyarat, C.; Yenjai, C., Claurailas A−D, Cytotoxic carbazole alkaloids from the roots of *Clausena harmandiana*. *J. Nat. Prod.,* **2011,** *74*, 208-212.

149. Thongthoom, T.; Promsuwan, P.; Yenjai, C., Synthesis and cytotoxic activity of the heptaphylline and 7-methoxyheptaphylline series. *Eur. J. Med. Chem.,* **2011,** *46*, 3755-3761.

150. Peng, W. W.; Zeng, G. Z.; Song, W. W.; Tan, N. H., A new cytotoxic carbazole alkaloid and two new other alkaloids from *Clausena excavata. Chem. Biodivers.,* **2013,** *10*, 1317-1321.

151. Ma, Y.-L.; Liu, Y.-P.; Zhang, C.; Zhao, W.-H.; Shi, S.; He, D.-N.; Zhang, P.; Liu, X.-H.; Han, T.-T.; Fu, Y.-H., Carbazole alkaloids from *Clausena hainanensis* with their potential antiproliferative activities. *Bioorganic Chem.,* **2018,** *76*, 359-364.

152. Tsutsumi, L. S.; Gündisch, D.; Sun, D., Carbazole scaffold in medicinal chemistry and natural products: A review from 2010-2015. *Curr. Top. Med. Chem.,* **2016,** *16*, 1290-1313.
153. Caruso, A.; Iacopetta, D.; Puoci, F.; Rita Cappello, A.; Saturnino, C.; Stefania Sinicropi, M., Carbazole derivatives: A promising scenario for breast cancer treatment. *Mini-Rev. Med. Chem.,* **2016,** *16*, 630-643.
154. Jiang, H.; Zhang, W.-J.; Li, P.-H.; Wang, J.; Dong, C.-Z.; Zhang, K.; Chen, H.-X.; Du, Z.-Y., Synthesis and biological evaluation of novel carbazole-rhodanine conjugates as topoisomerase II inhibitors. *Bioorganic Med. Chem. Lett.,* **2018,** *28*, 1320-1323.
155. Li, P.-H.; Jiang, H.; Zhang, W.-J.; Li, Y.-L.; Zhao, M.-C.; Zhou, W.; Zhang, L.-Y.; Tang, Y.-D.; Dong, C.-Z.; Huang, Z.-S., Synthesis of carbazole derivatives containing chalcone analogs as non-intercalative topoisomerase II catalytic inhibitors and apoptosis inducers. *Eur. J. Med. Chem.,* **2018,** *145*, 498-510.
156. Zhang, X.-f.; Zhang, H.-j.; Xiang, J.-f.; Li, Q.; Yang, Q.-f.; Shang, Q.; Zhang, Y.-x.; Tang, Y.-l., The binding modes of carbazole derivatives with telomere G-quadruplex. *J. Mol. Struct.,* **2010,** *982*, 133-138.
157. Francisco, C. S.; Rodrigues, L. R.; Cerqueira, N. M. F. S. A.; Oliveira-Campos, A. M. F.; Esteves, A. P., Novel benzopsoralen analogues: Synthesis, biological activity and molecular docking studies. *Eur. J. Med. Chem.,* **2014,** *87*, 298-305.
158. Hou, S.; Yi, Y. W.; Kang, H. J.; Zhang, L.; Kim, H. J.; Kong, Y.; Liu, Y.; Wang, K.; Kong, H.-S.; Grindrod, S.; Bae, I.; Brown, M. L., Novel carbazole inhibits phospho-STAT3 through induction of protein–tyrosine phosphatase PTPN6. *J. Med. Chem.,* **2014,** *57*, 6342-6353.
159. Takeuchi, T.; Oishi, S.; Watanabe, T.; Ohno, H.; Sawada, J.-i.; Matsuno, K.; Asai, A.; Asada, N.; Kitaura, K.; Fujii, N., Structure–activity relationships of carboline and carbazole derivatives as a novel class of ATP-competitive kinesin spindle protein inhibitors. *J. Med. Chem.,* **2011,** *54*, 4839-4846.
160. You, X.; Zhu, D.; Lu, W.; Sun, Y.; Qiao, S.; Luo, B.; Du, Y.; Pi, R.; Hu, Y.; Huang, P., Design, synthesis and biological evaluation of *N*-arylsulfonyl carbazoles as novel anticancer agents. *RSC Adv.,* **2018,** *8*, 17183-17190.
161. Kumará Kolli, S.; VijayaáBabu, P.; AshrafáAshfaq, M.; Rameshá Raju, R., TFAA/ H_3PO_4 mediated unprecedented *N*-acylation of carbazoles leading to small molecules possessing anti-proliferative activities against cancer cells. *Org. Biomol. Chem.,* **2014,** *12*, 6080-6084.

162. Tylińska, B.; Jasztold-Howorko, R.; Mastalarz, H.; Szczaurska-Nowak, K.; Wietrzyk, J., Synthesis and anticancer activity of new 1-substituted-6*H*-pyrido[4,3-*b*]carbazole derivatives. *Arch. Pharm.,* **2008,** *341,* 351-356.
163. Li, B.; Yue, Z.-Z.; Feng, J.-M.; He, Q.; Miao, Z.-H.; Yang, C.-H., Design and synthesis of pyrido[3,2-α]carbazole derivatives and their analogues as potent antitumour agents. *Eur. J. Med. Chem.,* **2013,** *66,* 531-539.
164. Akué-Gédu, R.; Letribot, B.; Saugues, E.; Debiton, E.; Anizon, F.; Moreau, P., Kinase inhibitory potencies and *in-vitro* antiproliferative activities of *N*-10 substituted pyrrolo [2,3-*a*]carbazole derivatives. *Bioorganic Med. Chem. Lett.,* **2012,** *22,* 3807-3809.
165. Padmaja, P.; Subba Reddy, B. V.; Jain, N.; Mutheneni, S. R.; Bollepelli, P.; Polepalli, S.; Rambabu, G.; Reddy, P. N., Synthesis, molecular docking and *in-vitro* antiproliferative activity of novel pyrano[3,2-c]carbazole derivatives. *New J. Chem.,* **2016,** *40,* 8305-8315.
166. Kumar, S.; Singh, A.; Kumar, K.; Kumar, V., Recent insights into synthetic *β*-carbolines with anticancer activities. *Eur. J. Med. Chem.,* **2017,** *142,* 48-73.
167. Zhang, M.; Sun, D., Recent advances of natural and synthetic *β*-carbolines as anticancer agents. *Anticancer Agents Med. Chem.,* **2015,** *15*, 537-547.
168. Nafisi, S.; Bonsaii, M.; Maali, P.; Khalilzadeh, M. A.; Manouchehri, F., *β*-Carboline alkaloids bind DNA. *J. Photochem. Photobiol., B,* **2010,** *100,* 84-91.
169. Dong, G.; Wang, S.; Miao, Z.; Yao, J.; Zhang, Y.; Guo, Z.; Zhang, W.; Sheng, C., New tricks for an old natural product: discovery of highly potent evodiamine derivatives as novel antitumor agents by systemic structure–activity relationship analysis and biological evaluations. *J. Med. Chem.,* **2012,** *55*, 7593-7613.
170. Ibrahim, S. R. M.; Mohamed, G. A., Ingenine E, a new cytotoxic *β*-carboline alkaloid from the Indonesian sponge Acanthostrongylophora ingens. *J. Asian Nat. Prod. Res.,* **2017,** *19,* 504-509.
171. Tu, L. C.; Chen, C.-S.; Hsiao, I.-C.; Chern, J.-W.; Lin, C.-H.; Shen, Y.-C.; Yeh, S. F., The *β*-carboline analog Mana-Hox causes mitotic aberration by interacting with DNA. *Chem. Biol.,* **2005,** *12*, 1317-1324.
172. Tu, L. C.; Chou, C.-K.; Chen, C.-Y.; Chang, Y.-T.; Shen, Y.-C.; Yeh, S.-F., Characterization of the cytotoxic mechanism of Mana-Hox, an analog of manzamine alkaloids. *Biochim. Biophys. Acta,* **2004,** *1672,* 148-156.

173. Inman, W. D.; Bray, W. M.; Gassner, N. C.; Lokey, R. S.; Tenney, K.; Shen, Y. Y.; TenDyke, K.; Suh, T.; Crews, P., A *β*-carboline alkaloid from the papua new guinea marine sponge *Hyrtios reticulatus*. *J. Nat. Prod.,* **2009,** *73*, 255-257.

174. Fang, Y.; Linardic, C. M.; Richardson, D. A.; Cai, W.; Behforouz, M.; Abraham, R. T., Characterization of the cytotoxic activities of novel analogues of the antitumor agent, lavendamycin1. *Mol. Cancer Ther.,* **2003,** *2*, 517-526.

175. Cai, W.; Hassani, M.; Karki, R.; Walter, E. D.; Koelsch, K. H.; Seradj, H.; Lineswala, J. P.; Mirzaei, H.; York, J. S.; Olang, F., Synthesis, metabolism and *in-vitro* cytotoxicity studies on novel lavendamycin antitumor agents. *Bioorganic Med. Chem.,* **2010,** *18*, 1899-1909.

176. Baruah, B.; Dasu, K.; Vaitilingam, B.; Mamnoor, P.; Venkata, P. P.; Rajagopal, S.; Yeleswarapu, K. R., Synthesis and cytotoxic activity of novel quinazolino-*β*-carboline-5-one derivatives. *Bioorganic Med. Chem.,* **2004,** *12*, 1991-1994.

177. Boursereau, Y.; Coldham, I., Synthesis and biological studies of 1-amino *β*-carbolines. *Bioorganic Med. Chem. Lett.,* **2004,** *14*, 5841-5844.

178. de Oliveira Figueiredo, P.; Perdomo, R. T.; Garcez, F. R.; Matos, M. d. F. C.; de Carvalho, J. E.; Garcez, W. S., Further constituents of *Galianthe thalictroides* (Rubiaceae) and inhibition of DNA topoisomerases I and II*α* by its cytotoxic *β*-carboline alkaloids. *Bioorganic Med. Chem. Lett,* **2014,** *24*, 1358-1361.

179. Chauhan, S. S.; Singh, A. K.; Meena, S.; Lohani, M.; Singh, A.; Arya, R. K.; Cheruvu, S. H.; Sarkar, J.; Gayen, J. R.; Datta, D.; Chauhan, P. M. S., Synthesis of novel *β*-carboline based chalcones with high cytotoxic activity against breast cancer cells. *Bioorganic Med. Chem. Lett,* **2014,** *24*, 2820-2824.

180. Chen, Z.; Cao, R.; Shi, B.; Guo, L.; Sun, J.; Ma, Q.; Fan, W.; Song, H., Synthesis and biological evaluation of 1, 9-disubstituted *β*-carbolines as potent DNA intercalating and cytotoxic agents. *Eur. J. Med. Chem.,* **2011,** *46*, 5127-5137.

181. Xiao, S.; Lin, W.; Wang, C.; Yang, M., Synthesis and biological evaluation of DNA targeting flexible side-chain substituted *β*-carboline derivatives. *Bioorganic Med. Chem. Lett.,* **2001,** *11*, 437-441.

182. Ikeda, R.; Kurosawa, M.; Okabayashi, T.; Takei, A.; Yoshiwara, M.; Kumakura, T.; Sakai, N.; Funatsu, O.; Morita, A.; Ikekita, M., 3-(3-Phenoxybenzyl)amino-*β*-carboline: A novel antitumor drug targeting *α*-tubulin. *Bioorganic Med. Chem. Lett,* **2011,** *21*, 4784-4787.

183. Ikeda, R.; Kimura, T.; Tsutsumi, T.; Tamura, S.; Sakai, N.; Konakahara, T., Structure–activity relationship in the antitumor activity of 6-, 8-or 6, 8-substituted 3-benzylamino-*β*-carboline derivatives. *Bioorganic Med. Chem. Lett,* **2012,** *22*, 3506-3515.

184. Ikeda, R.; Iwaki, T.; Iida, T.; Okabayashi, T.; Nishi, E.; Kurosawa, M.; Sakai, N.; Konakahara, T., 3-Benzylamino-*β*-carboline derivatives induce apoptosis through G2/M arrest in human carcinoma cells HeLa S-3. *Eur. J. Med. Chem.,* **2011,** *46*, 636-646.

185. Bai, B.; Li, X.-Y.; Liu, L.; Li, Y.; Zhu, H.-J., Syntheses of novel *β*-carboline derivatives and the activities against five tumor-cell lines. *Bioorganic Med. Chem. Lett*, **2014,** *24*, 96-98.

186. Kamal, A.; Sathish, M.; Nayak, V. L.; Srinivasulu, V.; Kavitha, B.; Tangella, Y.; Thummuri, D.; Bagul, C.; Shankaraiah, N.; Nagesh, N., Design and synthesis of dithiocarbamate linked *β*-carboline derivatives: DNA topoisomerase II inhibition with DNA binding and apoptosis inducing ability. *Bioorganic Med. Chem.,* **2015,** *23*, 5511-5526.

187. Sathish, M.; Kavitha, B.; Nayak, V. L.; Tangella, Y.; Ajitha, A.; Nekkanti, S.; Alarifi, A.; Shankaraiah, N.; Nagesh, N.; Kamal, A., Synthesis of podophyllotoxin linked *β*-carboline congeners as potential anticancer agents and DNA topoisomerase II inhibitors. *Eur. J. Med. Chem.,* **2018,** *144*, 557-571.

188. Ling, Y.; Xu, C.; Luo, L.; Cao, J.; Feng, J.; Xue, Y.; Zhu, Q.; Ju, C.; Li, F.; Zhang, Y., Novel *β*-carboline/hydroxamic acid hybrids targeting both histone deacetylase and DNA display high anticancer activity via regulation of the p53 signaling pathway. *J. Med. Chem.,* **2015,** *58*, 9214-9227.

189. Nagula, S.; Tokala, R.; Thatikonda, S.; Sana, S.; Vanteddu, U. S.; Godugu, C., Design and synthesis of DNA-interactive *β*-carboline-oxindole hybrids as cytotoxic and apoptosis inducing agents. *ChemMedChem,* **2018,** *13*, 1-15.

190. Barbosa, V. A.; Formagio, A. S. N.; Savariz, F. C.; Foglio, M. A.; Spindola, H. M.; de Carvalho, J. E.; Meyer, E.; Sarragiotto, M. H., Synthesis and antitumor activity of *β*-carboline-3-(substituted-carbohydrazide) derivatives. *Bioorganic Med. Chem.,* **2011,** *19*, 6400-6408.

191. Formagio, A. S. N.; Tonin, L. T. D.; Foglio, M. A.; Madjarof, C.; de Carvalho, J. E.; da Costa, W. F.; Cardoso, F. P.; Sarragiotto, M. H., Synthesis and antitumoral activity of novel 3-(2-substituted-1,3,4-oxadiazol-5-yl) and 3-(5-substituted-1,2,4-triazol-3-yl) *β*-carboline derivatives. *Bioorganic Med. Chem.,* **2008,** *16*, 9660-9667.

192. Shankaraiah, N.; Jadala, C.; Nekkanti, S.; Senwar, K. R.; Nagesh, N.; Shrivastava, S.; Naidu, V.; Sathish, M.; Kamal, A., Design and synthesis of C3-tethered 1,2,3-triazolo-*β*-carboline derivatives: Anticancer activity, DNA-binding ability, viscosity and molecular modeling studies. *Bioorganic Chem.,* **2016,** *64*, 42-50.

193. Savariz, F. C.; Foglio, M. A.; De Carvalho, J. E.; Ruiz, A. L. T.; Duarte, M. C.; Da Rosa, M. F.; Meyer, E.; Sarragiotto, M. H., Synthesis and evaluation of new *β*-carboline-3-(4-benzylidene)-4*H*-oxazol-5-one derivatives as antitumor agents. *Molecules,* **2012,** *17*, 6100-6113.

194. Kamal, A.; Srinivasulu, V.; Nayak, V. L.; Sathish, M.; Shankaraiah, N.; Bagul, C.; Reddy, N. V. S.; Rangaraj, N.; Nagesh, N., Design and synthesis of C3-pyrazole/ chalcone-linked *β*-carboline hybrids: Antitopoisomerase I, DNA-interactive, and apoptosis-inducing anticancer agents. *ChemMedChem*, **2014,** *9*, 2084-2098.

195. Kamal, A.; Narasimha Rao, M. P.; Swapna, P.; Srinivasulu, V.; Bagul, C.; Shaik, A. B.; Mullagiri, K.; Kovvuri, J.; Reddy, V. S.; Vidyasagar, K.; Nagesh, N., Synthesis of *β*-carboline-benzimidazole conjugates using lanthanum nitrate as a catalyst and their biological evaluation. *Org. Biomol. Chem.,* **2014,** *12*, 2370-2387.

196. Kovvuri, J.; Nagaraju, B.; Nayak, V. L.; Akunuri, R.; Rao, M. N.; Ajitha, A.; Nagesh, N.; Kamal, A., Design, synthesis and biological evaluation of new *β*-carboline-bisindole compounds as DNA binding, photocleavage agents and topoisomerase I inhibitors. *Eur. J. Med. Chem.,* **2018,** *143*, 1563-1577.

197. Zheng, C.; Fang, Y.; Tong, W.; Li, G.; Wu, H.; Zhou, W.; Lin, Q.; Yang, F.; Yang, Z.; Wang, P., Synthesis and biological evaluation of novel tetrahydro-*β*-carboline derivatives as antitumor growth and metastasis agents through inhibiting the transforming growth factor-*β* signaling pathway. *J. Med. Chem.,* **2014,** *57*, 600-612.

198. Liu, F.; Yu, L.-Q.; Jiang, C.; Yang, L.; Wu, W.-T.; You, Q.-D., Discovery of tetrahydro-β-carbolines as inhibitors of the mitotic kinesin KSP. *Bioorganic Med. Chem.,* **2010,** *18*, 4167-4177.

199. Lin, Y.; Xia, X.; Yao, R.; Ni, L.; Hu, J.; Guo, W.; Zhu, B., Synthesis and *in vitro* biological evaluation of hybrids from tetrahydro-*β*-carboline and hydroxylcinnamic acid as antitumor carcinoma agents. *Chem. Pharm. Bull.*, **2014,** *62*, 343-349.

200. Kumar, R.; Gupta, L.; Pal, P.; Khan, S.; Singh, N.; Katiyar, S. B.; Meena, S.; Sarkar, J.; Sinha, S.; Kanaujiya, J. K., Synthesis and cytotoxicity evaluation of (tetrahydro-*β*-carboline)-1,3,5-triazine hybrids as anticancer agents. *Eur. J. Med. Chem.,* **2010,** *45*, 2265-2276.

201. Du, H.; Tian, S.; Chen, J.; Gu, H.; Li, N.; Wang, J., Synthesis and biological evaluation of N^9-substituted harmine derivatives as potential anticancer agents. *Bioorganic Med. Chem. Lett.*, **2016,** *26*, 4015-4019.
202. Shi, B.; Cao, R.; Fan, W.; Guo, L.; Ma, Q.; Chen, X.; Zhang, G.; Qiu, L.; Song, H., Design, synthesis and in vitro and in vivo antitumor activities of novel bivalent *β*-carbolines. *Eur. J. Med. Chem.*, **2013,** *60*, 10-22.
203. Guan, H.; Chen, H.; Peng, W.; Ma, Y.; Cao, R.; Liu, X.; Xu, A., Design of *β*-carboline derivatives as DNA-targeting antitumor agents. *Eur. J. Med. Chem.*, **2006,** *41*, 1167-1179.
204. Chen, Q.; Chen, W.; Fan, W.; Guo, L.; Ma, Q.; Zhang, X.; Du, R.; Cao, R., Design, synthesis and biological evaluation of novel alkyl diamine linked bivalent *β*-carbolines as angiogenesis inhibitors. *Bioorganic Med. Chem. Lett.*, **2016,** *26*, 5065-5068.
205. Sun, R.; Liu, R.; Zhou, C.; Ren, Z.; Guo, L.; Ma, Q.; Fan, W.; Qiu, L.; Yu, H.; Shao, G., Synthesis and biological evaluation of piperazine group-linked bivalent *β*-carbolines as potential antitumor agents. *MedChemComm*, **2015,** *6*, 2170-2174.
206. Samundeeswari, S.; Chougala, B.; Holiyachi, M.; Shastri, L.; Kulkarni, M.; Dodamani, S.; Jalalpur, S.; Joshi, S.; Dixit, S.; Sunagar, V., Design and synthesis of novel phenyl-1,4-*β*-carboline-hybrid molecules as potential anticancer agents. *Eur. J. Med. Chem.*, **2017,** *128*, 123-139.
207. Cao, R.; Yi, W.; Wu, Q.; Guan, X.; Feng, M.; Ma, C.; Chen, Z.; Song, H.; Peng, W., Synthesis and cytotoxic activities of 1-benzylidine substituted *β*-carboline derivatives. *Bioorganic Med. Chem. Lett.*, **2008,** *18*, 6558-6561.
208. Ma, C.; Cao, R.; Shi, B.; Li, S.; Chen, Z.; Yi, W.; Peng, W.; Ren, Z.; Song, H., Synthesis and cytotoxic evaluation of N^2-benzylated quaternary *β*-carboline amino acid ester conjugates. *Eur. J. Med. Chem.*, **2010,** *45*, 1515-1523.
209. Meinguet, C.; Bruyère, C.; Frédérick, R.; Mathieu, V.; Vancraeynest, C.; Pochet, L.; Laloy, J.; Mortier, J.; Wolber, G.; Kiss, R., 3D-QSAR, design, synthesis and characterization of trisubstituted harmine derivatives with *in-vitro* antiproliferative properties. *Eur. J. Med. Chem.*, **2015,** *94*, 45-55.
210. Frédérick, R.; Bruyère, C.; Vancraeynest, C.; Reniers, J.; Meinguet, C.; Pochet, L.; Backlund, A.; Masereel, B.; Kiss, R.; Wouters, J., Novel trisubstituted harmine derivatives with original *in-vitro* anticancer activity. *J Med Chem*, **2012,** *55*, 6489-6501.

211. Cao, R.; Guan, X.; Shi, B.; Chen, Z.; Ren, Z.; Peng, W.; Song, H., Design, synthesis and 3D-QSAR of *β*-carboline derivatives as potent antitumor agents. *Eur. J. Med. Chem.,* **2010,** *45*, 2503-2515.

212. Dighe, S. U.; Khan, S.; Soni, I.; Jain, P.; Shukla, S.; Yadav, R.; Sen, P.; Meeran, S. M.; Batra, S., Synthesis of *β*-carboline-based *N*-heterocyclic carbenes and their antiproliferative and antimetastatic activities against human breast cancer cells. *J. Med. Chem.,* **2015,** *58*, 3485-3499.

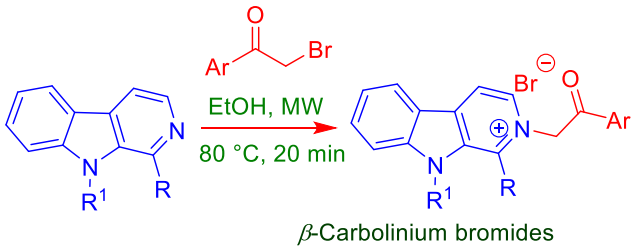
213. Ishida, J.; Wang, H.-K.; Bastow, K. F.; Hu, C.-Q.; Lee, K.-H., Antitumor agents 201.1 Cytotoxicity of harmine and *β*-carboline analogs. *Bioorganic Med. Chem. Lett.,* **1999,** *9*, 3319-3324.

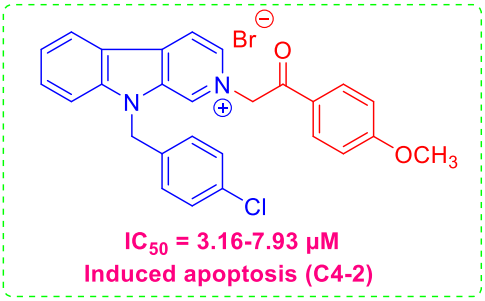
214. Fontana, A.; Benito, E. J.; Martın, M. J.; Sanchez, N.; Alajarın, R.; Vaquero, J. J.; Alvarez-Builla, J.; Lambel-Giraudet, S.; Leonce, S.; Pierré, A., Synthesis and cytotoxic activity of pyridazino[1′,6′:1,2]pyrido[3,4-*b*]indol-5-inium derivatives as anticancer agents. *Bioorganic Med. Chem. Lett.,* **2002,** *12*, 2611-2614.

Chapter 2

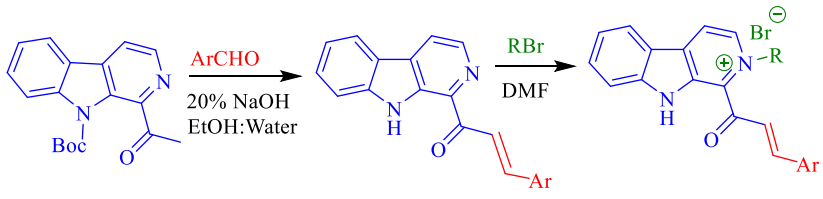
Design, Synthesis and Anticancer Activity Studies

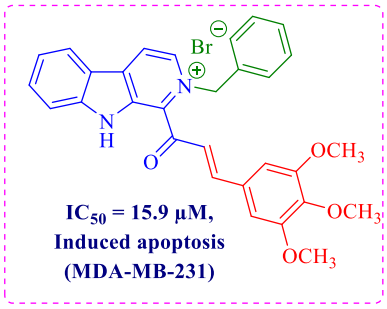
Part A: Phenacyl-*β*-carbolinium Bromides





Part B: *β*-Carboline chalcones and their Bromide Salts





2.1 Synthesis and Anticancer Activity Studies of *β*-Carbolinium Bromides

2.1.1 Introduction

β-Carbolines are an important class of nitrogen containing heterocycles due to their widespread biological and pharmacological applications.[1] Particularly, C-1 and N^2-substituted-*β*-carbolines possess remarkable antimalarial, anti-HIV, antimicrobial, antileishmania, and antitumor properties.[2-6] Recent interest in *β*-carboline alkaloids was stimulated by their potential antitumor properties through DNA intercalation, tubulin polymerization, topoisomerase and kinase inhibition (Figure 2.1.1).[7-11] For example, natural *β*-carboline alkaloids harmine **1** and fascaplysin **2** exhibited interesting antiproliferative activity through apoptosis induction, DNA intercalation and CDK inhibition.[12-23] For instance, Kobayashi et al. reported isolation and cytotoxicity of novel manzamine alkaloid xestomanzamine A **3** from Okinawan marine sponges of *Xestospongia sp*. and exhibited weak cytotoxicity against KB cell line.[24] Cardellina group isolated eudistomin T **4** from *Eudistoma olivaceum* endowed with antimicrobial and weak phototoxicity.[25-27] In 1962, Poindexter group isolated a simple *β*-carboline alkaloid, harmane **5** from tobacco and its smoke,[28] exhibited anticancer activity through DNA intercalation in addition to antibacterial activities.[29,30] Cao research group identified benzyl-*β*-carbolinium bromides **6** (IC_{50} = 0.8-8 μM), **7** (IC_{50} = 1.8-12 μM), **8** (IC_{50} = < 5 μM) and **9** (IC_{50} = 5.7 μM) as potent cytotoxic agents by modification of natural harmine and harmane.[31-35]

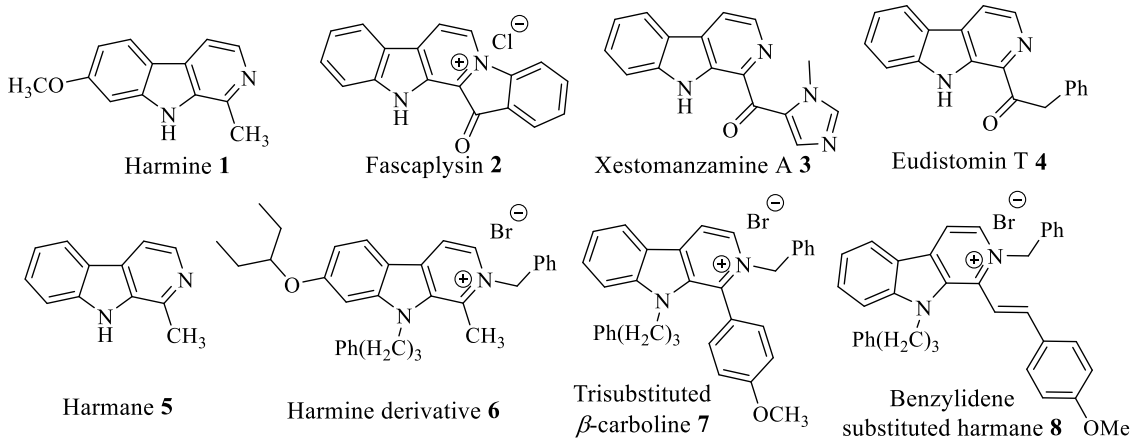


Figure 2.1.1 Representative *β*-carboline-based anticancer agents

In this chapter, synthesis and biological activity studies of two series of substituted *β*-carbolinium bromides have been discussed. Part A includes the synthesis of phenacyl-*β*-carbolinium bromides as novel cytotoxic agents. Part B deals with the synthesis and biological evaluation of *β*-carboline chalcones and their bromide salts as anticancer agents.

2.2 Part A: Phenacyl-*β*-carbolinium Bromides

2.2.1 Rational design

β-Carboline unit containing natural products and synthetic molecules often exhibit a broad spectrum of pharmacological properties including sedative, anxiolytic, hypnotic, antioxidant, anticonvulsant, antitumor, antiviral, antiparasitic and antimicrobial.[36] Particularly, *β*-carboline analogues have been reported to exhibit significant antitumor activities against several human cancer cells.[37-39] In 2012, Frederick and co-workers prepared the trisubstituted harmine derivative **10**, with impressive anticancer activity (IC_{50} = 0.7 μM; OE33).[40]

On the other hand, alkylation of azaheterocycles may lead to azolium salts with enhanced water solubility.[41,42] In the recent past, many cationic nitrogen heterocycles have emerged as potent antitumor agents.[43,44] For example, Zhang group reported imidazolium bromides as potent cytotoxic agents (IC_{50} < 5.0 μM).[45] Recently, Xu group identified a series of novel 1-((indol-3-yl)methyl)–1*H*-imidazolium salts **11** (IC_{50} = 1.89 μM; HL60) as apoptosis inducing potent anticancer agents.[46] Zeng et al. prepared 1-mesityl-3-(2-naphthoyl-methano)-1*H*-imidazolium bromide **12** (IC_{50} = 0.3-5 μM) with interesting anticancer activity *via* arresting cell cycle at G1 phase and induced apoptosis in K562 cells.[47]

In an effort to identify potent cytotoxic agents, recently, we designed and prepared indolyl heterocycles with potent anticancer activity.[48-50] Inspired by the fascinating anticancer properties of *β*-carbolines and azolium salts, in this chapter, we designed a diverse series of *β*-carbolinium salts by incorporating remarkable features of *β*-carboline and 1-aryl-2-bromoethanones in single molecule as depicted in Figure 2.2.1.

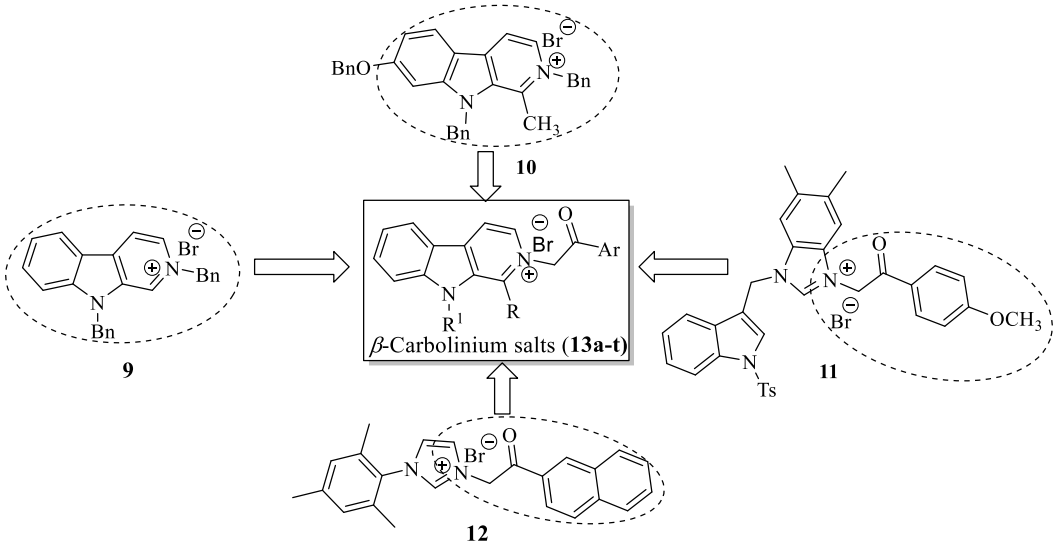
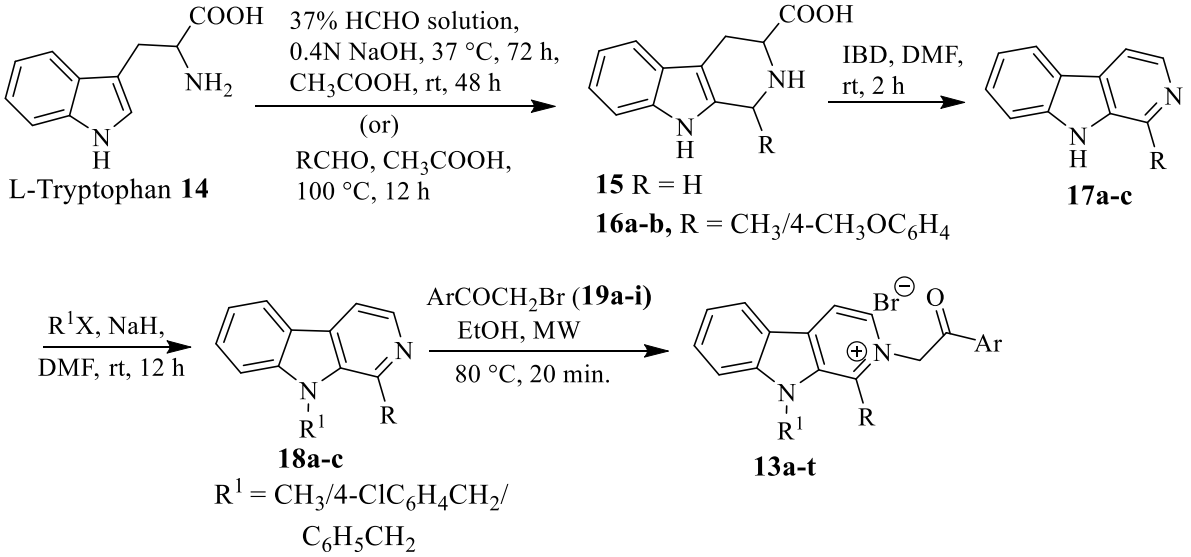


Figure 2.2.1 Rational design for *β*-carbolinium bromides **13a-t**

2.2.2 Results and Discussion

2.2.2.1 Synthesis

Substituted *β*-carboline intermediates **18** were prepared from L-tryptophan **14** as illustrated in Scheme 2.2.1.[51] Firstly, the reaction of **14** with formaldehyde solution (3.5 mL, 37%) under basic conditions produced tetrahydro-*β*-carboline-3-carboxylic acid **15**. However, under similar reaction conditions substituted tetrahydro-*β*-carboline-3-carboxylic acid **16** could not be prepared. Alternatively, carboxylic acid **16** was prepared from the reaction of **14** with aliphatic or aromatic aldehydes under acidic conditions. Next, iodobenzene diacetate-mediated decarboxylative aromatization of **15** or **16** led to *β*-carbolines **17** in good yields. Reaction of **17** with an appropriate alkyl halide and sodium hydride produced *N*-alkylated-*β*-carbolines **18** in excellent yields.



Scheme 2.2.1 Synthesis of *β*-carbolinium bromides **13a-t**

The required 1-aryl-2-bromoethanones **19** were prepared by the reaction of commercially available arylethanones with *N*-bromosuccinimide (NBS) in acetonitrile using *p*-toluene-sulfonic acid (*p*-TsOH) as a catalyst in good yields.[52] Finally, the reaction of *β*-carboline **18** and 1-aryl-2-bromoethanone **19** was performed in refluxing ethanol. After refluxing the reaction contents for 20 h, we isolated the *β*-carboline salt **13a** only in moderate yield (55%). In an attempt to enhance the reaction efficiency and product yield, we performed the N^2- alkylation of *β*-carboline **18** in focused microwave (MW). Reaction of **18** and 1-aryl-2-bromoethanone **19** in focused MW led to *β*-carbolinium bromide **13a** with improved yield and notable reduced time (from hour to min.). MW-assisted organic synthesis received

substantial attention in pharmaceutical industry due to dramatic savings of reaction times and higher product yields.[53,54] Initially, we irradiated reaction mixture in MW oven at 50 °C for 20 min and obtained **13a** in 60% yield. Notably, by increasing reaction temperature from 50 °C to 80 °C, *β*-carbolinium bromide salt **13a** was produced in 89% yield. The generality of identified reaction conditions was demonstrated by synthesizing an array of *β*-carbolinium bromides **13a-t** in good to excellent yields (75-92%). 1-Aryl-2-bromoethanones with electron-donating (CH_3 and OCH_3) and halogen (Br) groups smoothly delivered **13** in high yields.

The structures of all the synthesized *β*-carbolinium bromides **13** were confirmed by IR, NMR (^1H & ^{13}C) and mass spectral analysis. IR spectrum of **13** resonated two bands at 3418 and 1697 cm^{-1} indicated the presence of *N*-H and C=O groups respectively (Figure 2.2.2). In ^1H NMR spectrum, three characteristic singlets appeared at ~13.05, ~9.4 and ~6.6 ppm due to *N*-H, C-1 proton of *β*-carboline and CH_2- of arylacyl moiety at N^2, respectively (Figure 2.2.3). ^{13}C NMR of **13** showed a characteristic signal at ~190 ppm for the carbonyl carbon (C=O) (Figure 2.2.4). The HRMS of *β*-carboline **13b** displayed expected molecular ion (301.1335) peak in agreement with the calculated mass (301.1335) (Figure 2.2.5). The purity of *β*-carbolinium bromides **13a-t** was found to be >97% by HPLC analysis (Figure 2.2.6).

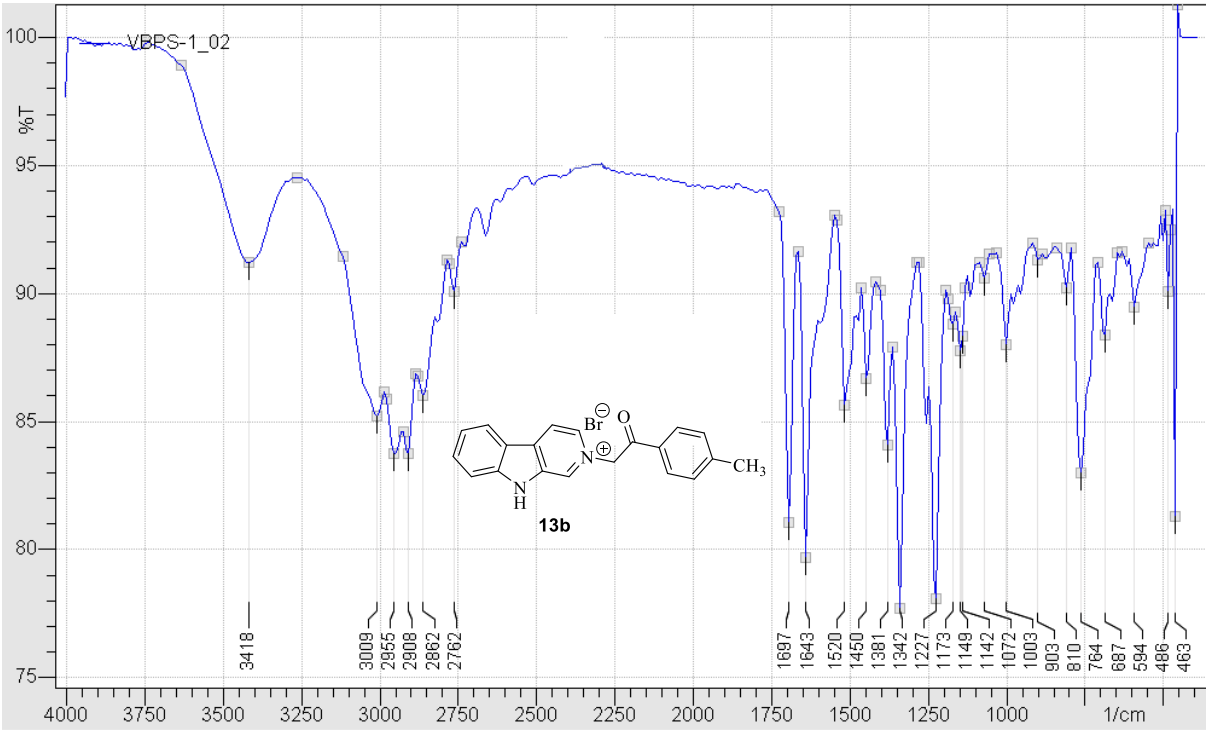


Figure 2.2.2 IR spectrum of **13b**

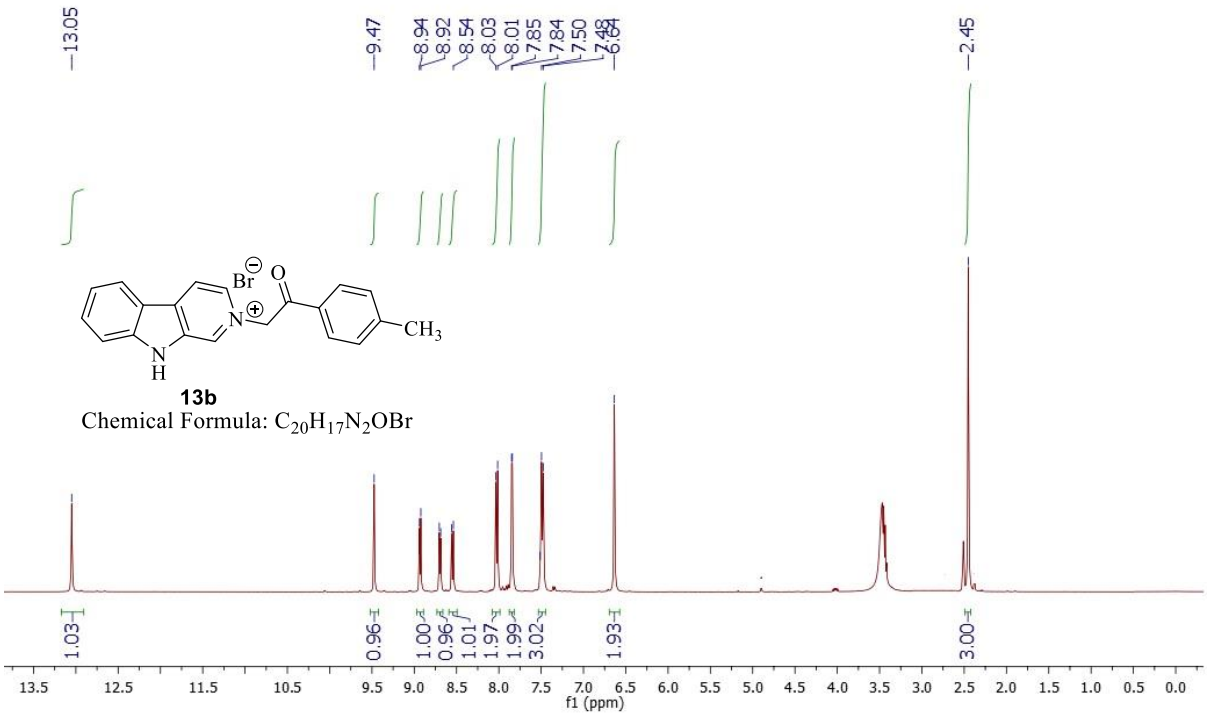


Figure 2.2.3 ^{1}H NMR spectrum of **13b**

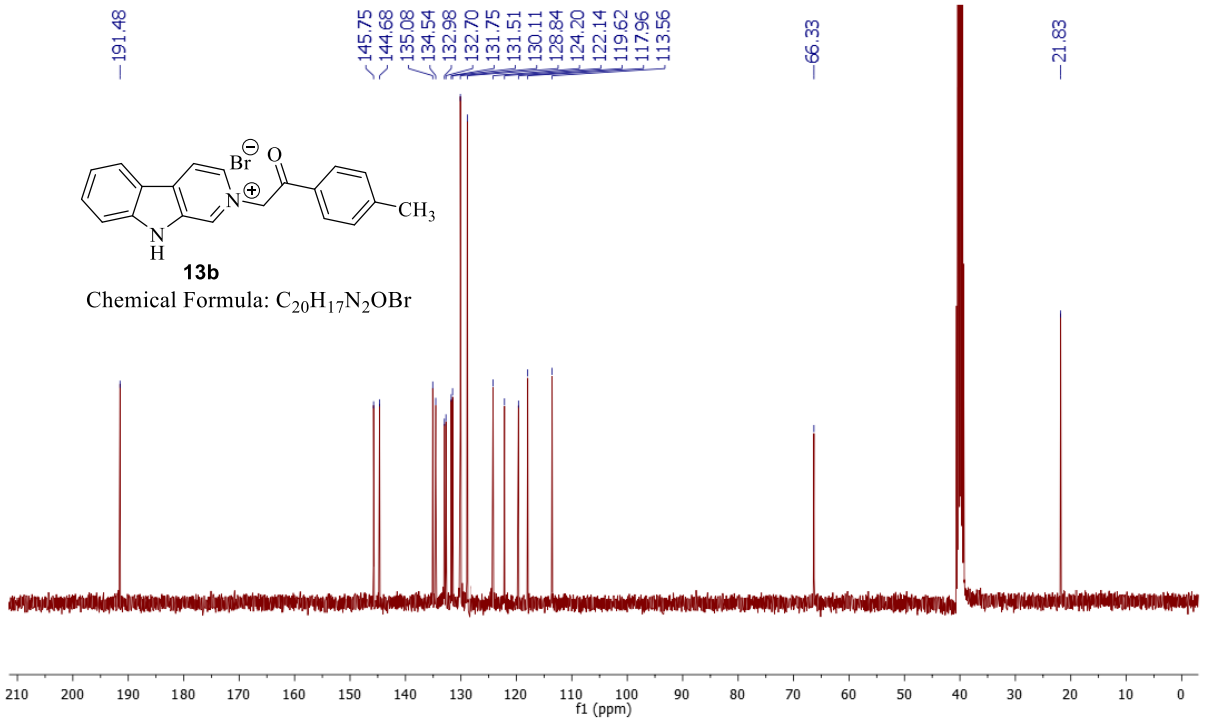
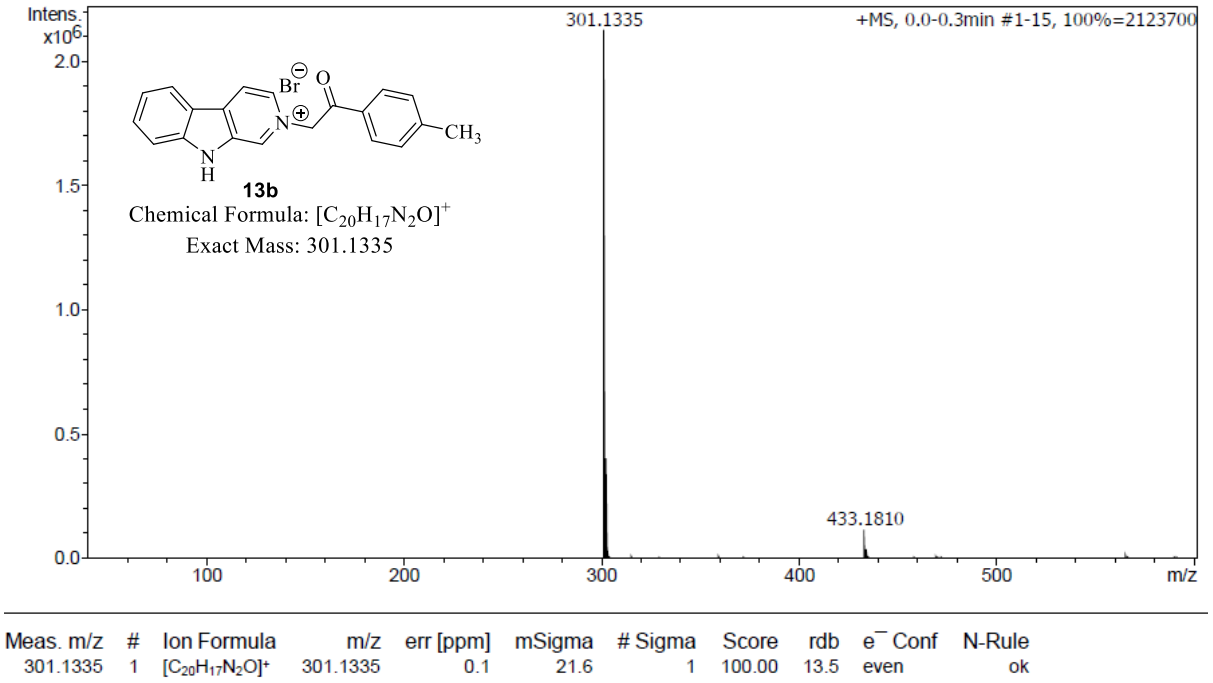
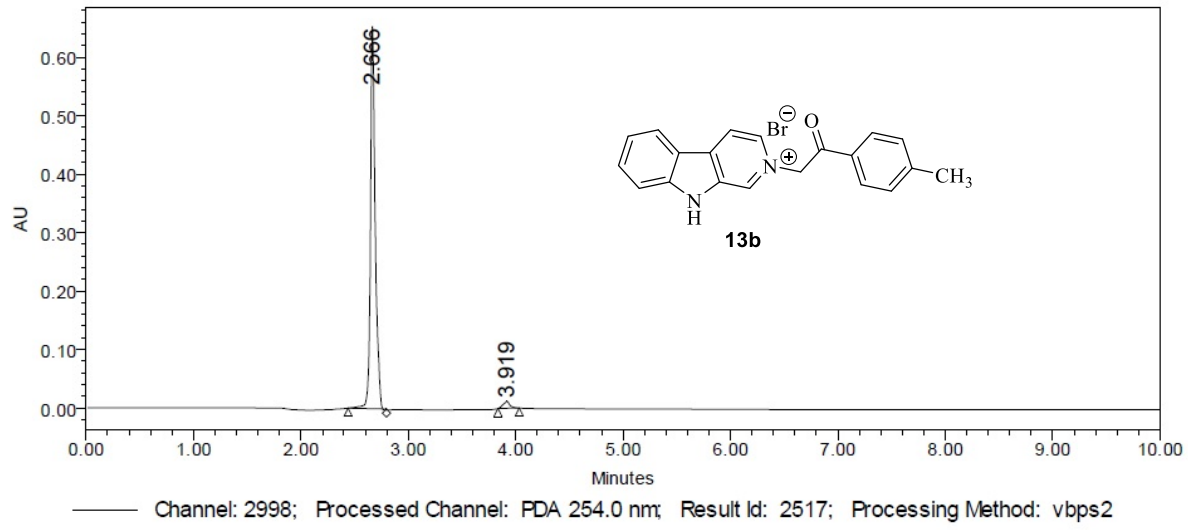


Figure 2.2.4 ^{13}C NMR spectrum of **13b**



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e¯ Conf	N-Rule
301.1335	1	$[C_{20}H_{17}N_2O]^+$	301.1335	0.1	21.6	1	100.00	13.5	even	ok

Figure 2.2.5 HRMS spectrum of **13b**



Processed Channel Descr.: PDA 254.0 nm

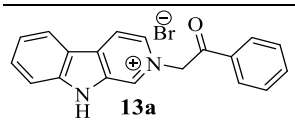
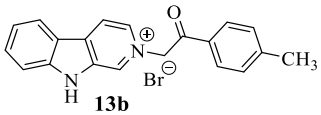
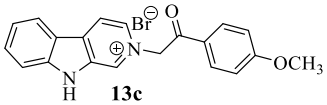
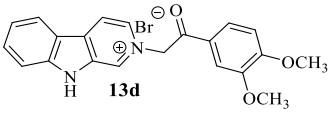
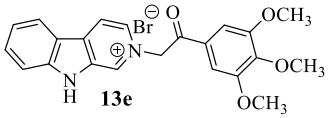
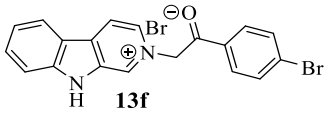
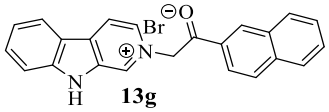
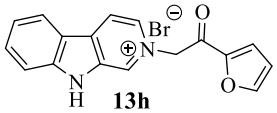
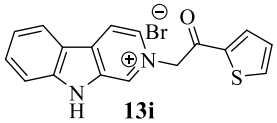
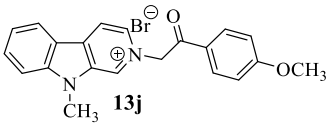
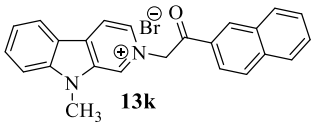
	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 254.0 nm	2.666	2024490	97.48	654522
2	PDA 254.0 nm	3.919	52426	2.52	11971

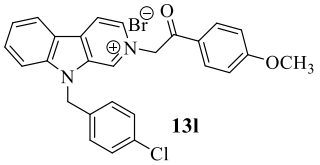
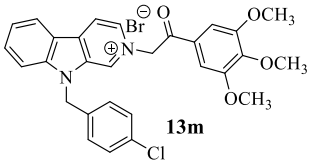
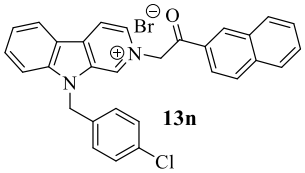
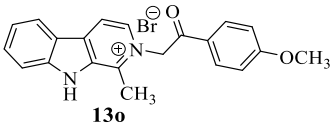
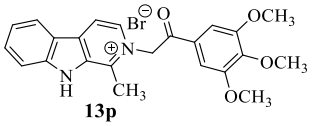
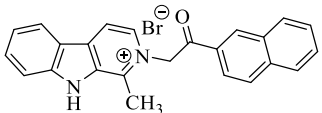
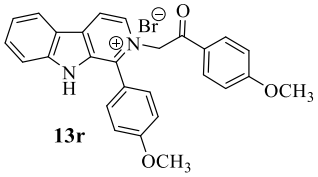
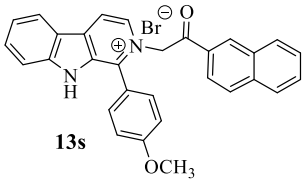
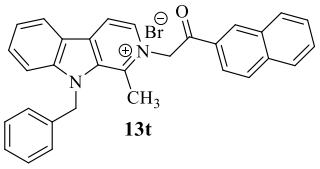
Figure 2.2.6 HPLC chromatogram of **13b**

2.2.2.2 Anticancer activity studies

In vitro cytotoxicity of *β*-carbolinium bromides **13a-t** was evaluated against pancreatic (BxPC-3), cervical cancer (HeLa), castration-resistant prostate (C4-2), human prostate (PC-3), human embryonic kidney 293 (HEK293T) and breast carcinoma (MDA-MB-231) cells by MTT assay. Doxorubicin was used as the reference drug. Activity results in terms of IC_{50} values are summarized in Table 2.2.1. Structure-activity relationship study was carried out by varying substituents on *β*-carboline (R and R^1) and 1-aryl-2-bromoethanone (Ar) moieties. Compound **13a** without any substituent on *β*-carboline and arylacyl moieties was found to be moderately active against a panel of cancer cell lines (IC_{50} = 21.6-74.9 µM). Replacement of phenyl with a *p*-tolyl ring in arylacyl part at N^2 led to **13b** with slightly improved cytotoxicity (IC_{50} = 18.4-31.9 µM, **13a** *v*s **13b**). Similarly, *p*-methoxyphenyl analogue **13c**, slightly augmented the growth inhibitory potency when compared to **13a** and **13b** (IC_{50} = 13.2-55.8 µM). Dimethoxyphenyl (**13d**) and trimethoxyphenyl (**13e**) derivatives were found to be weakly cytotoxic against kidney cells (IC_{50} = 67.8 and 28.6 µM; HEK293T) and inactive against other cell lines. *β*-Carboline **13f** with electron-withdrawing (*p*-bromophenyl) substituent displayed improved cytotoxicity (IC_{50} = 18.8-44.1 µM) when compared to compound **13a**. 2-Naphthyl analogue **13g** showed improved activity with IC_{50} values ranging 11.1-40.4 µM ((**13g** *vs* **13a).** Replacement of an aryl group with heteroaryl (furyl and thienyl) moiety led to compounds **13h-i** with weak cytotoxicity or inactive against the tested cell lines except **13i** with moderate cytotoxicity towards kidney (HEK293T) cells (IC_{50} = 18.2 µM). *N*-Methylated derivatives **13j-k** showed moderate cytotoxicity (IC_{50} = 14-65 µM) towards tested cancer cell lines. *N*-(4-Chlorobenzyl) unit is reported to be beneficial for the potency of various indole-based anticancer lead molecules.[55,56] In an effort to improve anticancer activity of the *β*-carbolinium bromides, we prepared *N*-(4-chlorobenzyl)-*β*-carbolinium bromides **13l-n,** which displayed significant enhanced cytotoxicity against the tested cancer cells compared to *β*-carbolines with free *N*-H. Notably, compound **13l** was found to be the most potent analogue of the series with broad cytotoxicity against all the tested cell lines (IC_{50} = 3.2-7.9 µM). Incorporation of methyl and *p*-anisyl substituents at position 1 (**13o-s**) of *β*-carboline further increased the growth inhibitory potency (IC_{50} = 8.7-49.2 µM) when compared to **13c**, **13e** and **13g** (IC_{50} = 11.1-116.3 µM). *N*-Benzylation of **13q** led to **13t** with moderate cytotoxicity (IC_{50} = 12.6-94.1 µM). Overall, most of the *β*-carbolinium bromides proved to be active against kidney cells (HEK293T, IC_{50} = 3.7-67.8 µM).

Table 2.2.1 *In vitro* cytotoxicity (IC_{50} in μM) of *β*-carbolinium bromides **13a-t**

***β*-Carbolinium bromides**	**BxPC-3**	**HeLa**	**C4-2**	**PC-3**	**HEK 293T**	**MDA-MB-231**
 **13a**	27.8±2.7	37.7±3.4	74.9±5.5	59.6±5.3	21.6±2.8	37.2±4.1
 **13b**	19.9±2.3	27.5±3.1	31.9±3.5	39.1±3.1	18.4±4.1	29.2±2.2
 **13c**	> 100	48.7±2.9	45.7±5.4	55.8±4.9	13.2±2.6	> 100
 **13d**	> 100	> 100	> 100	> 100	67.8±7.9	> 100
 **13e**	> 100	> 100	> 100	> 100	28.6±3.8	> 100
 **13f**	24.5±3.9	44.5±5.0	33.3±2.9	43.2±5.2	18.8±3.1	44.1±5.3
 **13g**	15.1±2.4	35.2±2.6	40.4±3.9	35.8±3.0	11.1±2.2	25.1±3.6
 **13h**	> 100	> 100	> 100	> 100	> 100	> 100
 **13i**	38.8±4.9	65.0±4.6	> 100	> 100	18.2±2.6	48.9±5.8
 **13j**	18.0±2.1	55.6±5.4	37.9±4.3	35.2±4.1	14.2±2.8	38.1±2.9
 **13k**	64.9±4.3	33±3.2	25.4±2.2	43.8±3.9	17.6±3.0	54.2±5.4

β-Carbolinium bromides	BxPC-3	HeLa	C4-2	PC-3	HEK 293T	MDA-MB-231
 **13l**	**6.3±1.0**	**3.2±0.9**	**7.4±1.2**	**5.4±0.8**	**3.8±1.1**	**7.9±1.1**
 **13m**	35.3±2.9	13.2±2.5	41.0±3.8	36.5±3.4	11.5±3.1	37.0±4.0
 **13n**	36.9±3.6	14.2±2.2	11.6±2.1	15.2±2.7	10.6±2.8	16.2±1.8
 **13o**	26.6±1.8	23.9±2.9	28.1±2.6	26.7±2.0	9.5±1.1	16.8±3.1
 **13p**	30.9±3.2	54±6.2	49.2±3.4	39.5±3.3	18.9±2.0	40.2±5.2
 **13q**	12.3±2.0	13±2.6	17.7±1.9	19.5±2.1	17.7±2.8	14.1±2.1
 **13r**	20.0±2.5	26.4±1.4	22.1±2.9	28.1±3.4	17.8±2.0	22.4±2.7
 **13s**	12.2±1.6	15.5±2.0	8.7±1.5	10.1±1.1	11.6±1.9	14.1±2.1
 **13t**	94.1±6.4	37.6±2.4	12.6±2.3	15.3±1.4	29.0±4.2	44.1±6.3
Doxorubicin	14.3	4.85	2.5	14.3±2.3	4.8±1.2	2.5±0.8

*The activity data represent mean IC_{50} values of experiments conducted in triplicates

Activity results suggest that substituents at positions 1, 2 and 9 of *β*-carboline and arylacyl part bearing 4-methoxyphenyl and 2-naphthyl groups are beneficial for the anticancer activity. Notably, the most potent compound **13l** with moderate solubility in water (86 µg/mL) was found to be 95% stable at pH 4.5 up to 24 h.

To determine the preliminary mechanism of action of *β*-carbolinium bromides, we conducted acridine orange/ethidium bromide assa. Acridine orange (AO) stains both live and dead cells, whereas ethidium bromide (EB) only stains dead cells.[57] Therefore, AO/EB staining is used to examine whether cell death occurred *via* apoptosis or necrosis. Effect of *β*-carbolinium salts on the morphological changes of C4-2 cells as illustrated in Figure 2.2.7A. Incubation of compounds **13s** (IC_{50} = 8 µM) and **13l** with C4-2 cells (IC_{50} = 7 µM) for 24 h resulted in typical nuclear fragmentation (red), whereas no visible changes in cell nucleus and cell membrane integrity was observed for the control cells.

Furthermore, PARP1 cleavage assay for the active compounds **13l** and **13s** was performed. C4-2 Cells were treated with **13l** and **13s** for 48 h, and cleaved PARP1 levels was analyzed using immunoblotting as shown in Figure 2.2.7B, exposure of C4-2 cells by either **13l** or **13s** enhanced the levels of PARP1 cleavage as indicating apoptotic induced cell death in C4-2 cells. The results of AO/EB staining revealed that compounds **13l** and **13s** trigger apoptosis in C4-2 cells, thereby confirming the PARP1 cleavage data.

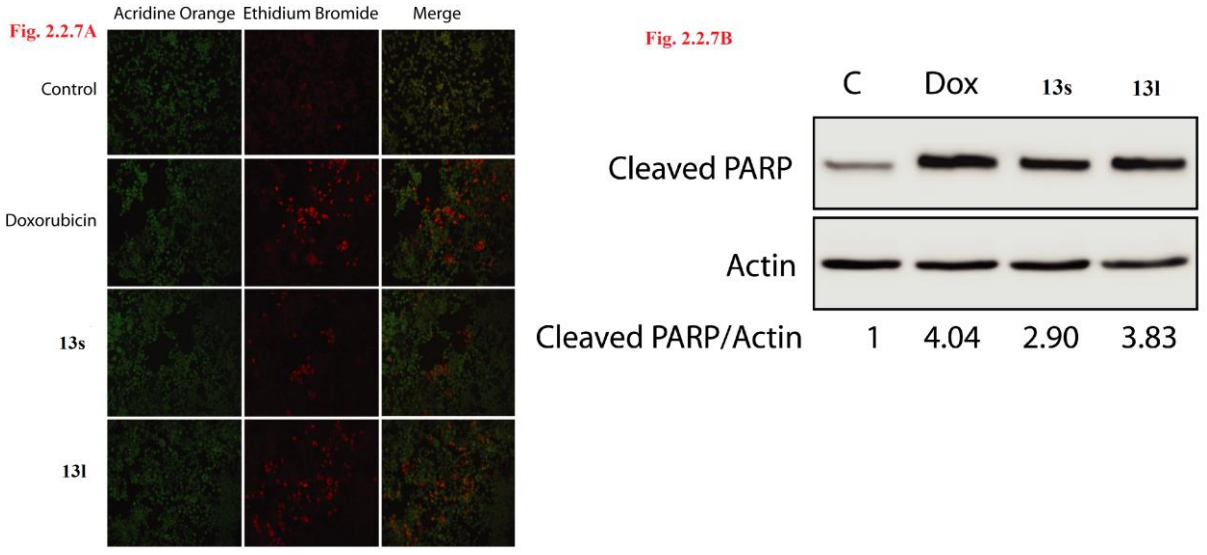


Figure 2.2.7 Apoptosis inducing effects of **13l** and **13s** in C4-2 cells.

2.2.2.3 Molecular docking studies

Recently, indole-based compounds have been reported for their significant anticancer activity through modulation of tubulin-heterodimer dynamics and binding at colchicine binding sites.[58] In order to find the theoretical binding sites of novel *β*-carboline bromides, a docking study for the identified potent compounds **13l** and **13s** was performed by Molegro Virtual docker program[59] according to reported high-resolution crystal structure of the tubulin-DAMA-colchicine (CN-2) complex (PDB ID: 1SA0).[60] Scoring functions and hydrogen bond formed with the surrounding amino acids predicted the binding affinities for **13l** and **13s** with MolDock Scores of -151.18 and -167.90, respectively. The docking poses of the molecule with the receptor are detailed in Figure 2.2.8. Binding interactions for **13l** are strongly stabilized by two hydrogen bonds; first interaction between C=O and Asp251 with hydrogen bond distance of 3.0616 Å; the second one between oxygen of 4-methoxyphenyl group and Cys241 with the bond length of 2.6103 Å.

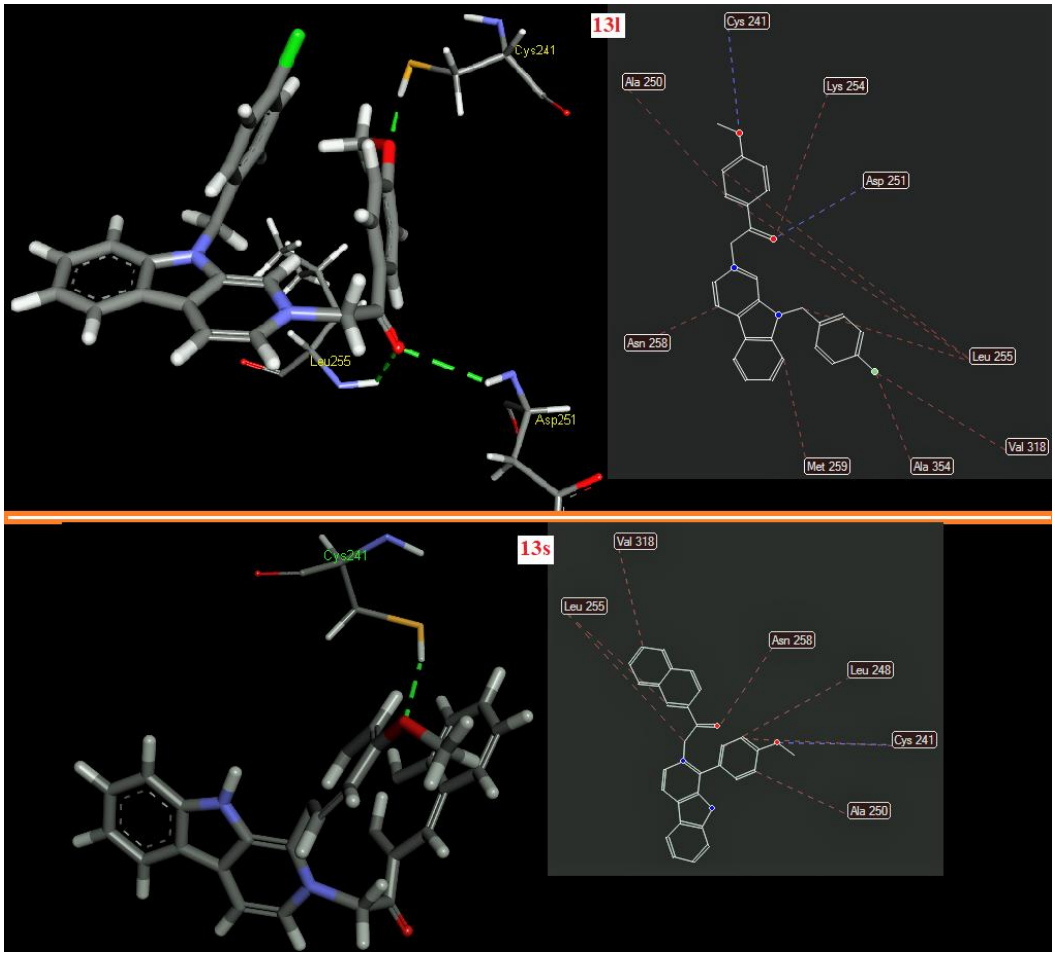


Figure 2.2.8 Binding interactions of **13l** and **13s** in the colchicine-binding site of tubulin. Hydrogen bonds (green and blue dotted lines) and steric interactions (red dotted lines) are outlined.

Similarly, compound **13s** also showed hydrogen-bonding interactions between the oxygen of 1-(4-methoxyphenyl)-*β*-carboline and Cys241 with 3.207Å distance of hydrogen bond. Apart from hydrogen bonding interactions, compounds **13l** and **13s** also strongly stabilized by steric interactions (red dotted lines) as illustrated in Figure 2.2.8. Additionally, the hydrophobic interactions in the colchicine-binding domain of the tubulin for *β*-carbolines **13l** and **13s** are shown in Figure 2.2.9.

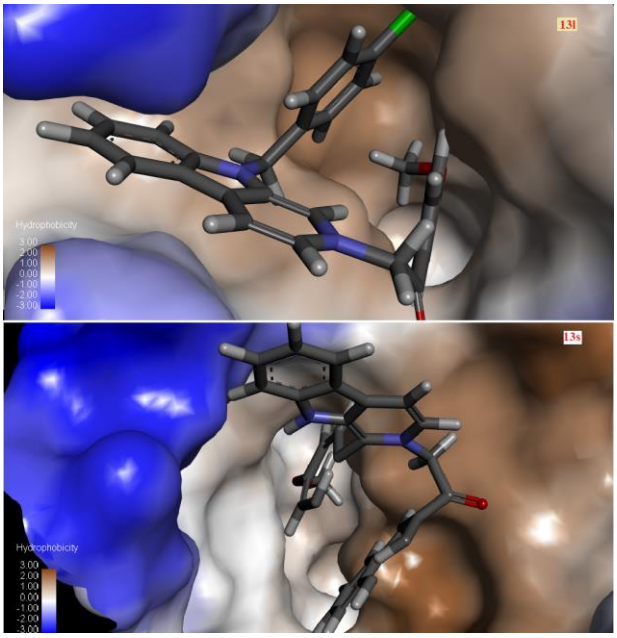


Figure 2.2.9 Hydrophobicity effect of **13l** and **13s** in the binding pocket of colchicine

2.2.2.4 Inhibition of tubulin polymerization

To validate the theoretical molecular-docking hypothesis, we examined the tubulin polymerization activity for the identified two potent compounds **13l** and **13s** in a cell free system. *β*-Carbolinium bromides **13l** and **13s** were found to inhibit tubulin polymerization at 6 µM as shown in Figure 2.2.10.

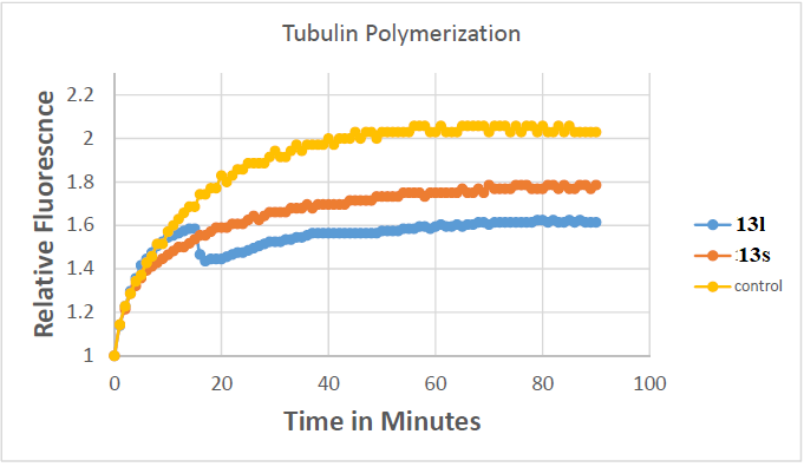


Figure 2.2.10 Effect of compounds **13l** and **13s** on *in vitro* tubulin polymerization

2.2.2.5 Calculation of Lipinski's parameters

To access the drug-likeness of *β*-carbolinium bromides **13a-t**, a theoretical study using Chem3D and DruLiTo (http://www.niper.gov.in/pi_dev_tools/DruLiToWeb/DruLiTo_index .html) was carried out for determining the Lipinski's parameters, number of rotatable bonds and total polar surface area as listed in Table 2.2.2. With the violation of more than one rule in 'Lipinski's Rule of Five' the compound may face bioavailability problem.[61] Almost all the *β*-carbolinium bromides **13a-t** follows the Lipinski's Rule of Five which indicates their more 'drug-like' nature.

Table 2.2.2 Calculated drug-like properties of *β*-carbolinium bromides **13a-t**

Compd	Lipinski's parameters				nRB	TPSA	No. of violations
	nHBA	nHBD	LogP	Molecular weight			
13a	3	1	3.13	287.12	3	32.11	0
13b	3	1	3.52	301.13	3	32.11	0
13c	4	1	2.73	317.13	4	41.34	0
13d	5	1	2.97	347.14	5	50.57	0
13e	6	1	3.21	377.15	6	59.8	0
13f	3	1	3.25	365.03	3	32.11	0
13g	3	1	4.27	337.13	3	32.11	0
13h	4	1	2.48	277.10	3	41.34	0
13i	3	1	2.84	293.07	3	32.11	0
13j	4	0	3.04	331.14	4	32.55	0
13k	3	0	4.57	351.15	3	23.32	0
13l	4	0	4.07	441.14	6	32.55	0
13m	6	0	4.55	501.16	8	51.01	1
13n	3	0	5.61	461.14	5	23.32	1
13o	4	1	2.91	331.14	4	41.34	0
13p	6	1	3.39	391.16	6	59.8	0
13q	3	1	4.45	351.15	3	32.11	0
13r	5	1	3.54	423.17	6	50.57	1
13s	4	1	5.08	443.17	5	41.34	1
13t	3	0	5.85	441.19	5	23.32	1

* LogP = Lipophilicity and nRB = Number of rotational bonds calculated using DruLiTo software

* TPSA = Topological polar surface area calculated using DruLiTo software

* nHBA & nHBD = Number of hydrogen bond acceptors (N & O) & donor present (NH & OH) in a molecule

2.2.3 Conclusions

In summary, a series of *β*-carbolinium bromides **13a-t** was prepared from easily accessible *β*-carbolines and 1-aryl-2-bromoethanones under MW irradiation. *In vitro* anticancer activity of *β*-carbolinium bromides **13a-t** was evaluated against six-human tumor cell lines. *β*-Carboline **13l** displayed most potent cytotoxicity against the tested cancer cell lines with IC_{50} values ranging 3.16-7.93 µM. The preliminary mechanism of action study revealed that compounds **13l** and **13s** induced apoptotic cell death by enhancing the level of cleaved PARP1 in C4-2 cells and exhibited their activity through the inhibition of tubulin polymerization. This class of compounds can be further exploited for obtaining potent cytotoxic agents.

2.2.4 Experimental section

2.2.4.1 General methods and materials

Reagents and solvents used in the synthesis of *β*-carbolinium bromides were procured commercially and used without further purification, unless otherwise indicated. Progress of the reaction was monitored by thin-layer chromatography (TLC) on pre-coated silica gel 60 F_{254} aluminum sheets (Merck, India), and the spots were visualized under UV light. Melting points (Mp) were recorded on an electro thermal capillary melting point apparatus (*E-Z* melting) and are uncorrected. NMR (^1H & ^{13}C) spectra were recorded on a Bruker Avance II at 400 MHz, using DMSO-d_6 and tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (ppm) and *J* values are reported in hertz (Hz). Multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), multiplet (m). IR spectra were recorded on ABB Bomen MB-3000 FT-IR spectrophotometer using KBr pellets. Mass of the prepared *β*–carboline analogues were recorded using ACQUITY LC-MS system with Tandem-Quadrupole-MS. The purity of *β*-carbolinium salts (≥97%) was quantified by WATERS 515 HPLC system with a Sunfire C-18 column (5 µm, 4.6 × 250 mm) and PDA detector using a flow rate of 1 mL/min. and a gradient of 0.05% TFA in acetonitrile.

2.2.4.2 General experimental procedures

Tetrahydro-*β*-carboline-3-carboxylic acid (15)[62]

To a stirred solution of sodium hydroxide (0.4 N, 60 mL) was added solid L-tryptophan (**14**, 5 g, 24.5 mmol) at room temperature and the contents allowed to stir until it became a clear solution. Subsequently, formaldehyde solution (37%, 3.5 mL) was added and continued

stirring at room temperature for 72 h. The solution was then acidified by slow addition of acetic acid (2.5 mL) until the pH of the mixture turned ~6. The obtained solid was filtered, washed with water and dried in the oven at 100 °C to obtain off white colored solid compound **15** in 95% yield. Mp: 304–306 °C (decomp); (Lit.[63] 302.2–304.6 °C).

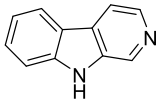
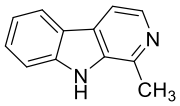
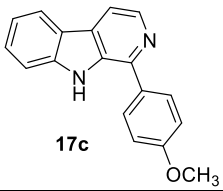
1-Substituted tetrahydro-*β*-carboline-3-carboxylic acid (16a-b)[64]

To a stirred mixture of L-tryptophan (3 mmol) in acetic acid (45 mL) was added corresponding aldehyde (3.6 mmol) at room temperature. The mixture was allowed to stir at 100 °C for 12 h. Upon completion of reaction, the mixture was cooled to room temperature. The suspension was filtered, washed with water (3×30 mL) and dried to afford **16a-b.** The crude was used without further purification for the decarboxylative aromatization step.

***β*-Carboline (17a) and 1-sustituted-*β*-carbolines (17b-c)**[65]

To a stirred solution of tetrahydro-*β*-carboline acids (**15** and **16a-b**, 10 mmol) in DMF (5 mL) was slowly added iodobenzene diacetate (6 g, 20 mmol) and the resulting mixture was stirred at room temperature for 1 h. After completion of the reaction as indicated by TLC, the contents were neutralized with saturated $NaHCO_3$ solution and extracted with dichloromethane (3×50 mL). The combined organic layer was washed with brine solution (50 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The obtained crude product was purified by column chromatography using ethyl acetate and hexane (7:3) to afford the *β*–carbolines **17a-c** in 65–72% yields.

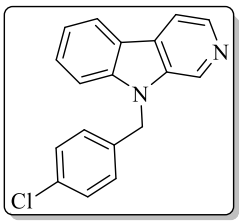
Table 2.2.3 Prepared *β*-carbolines **17a-c**

β-Carbolines	Mp (°C)	Lit. Mp (°C) [65]	Yield (%)
 **17a**	194–195	193–196	72
 **17b**	228–230	229–231	65
 **17c**	160–161	156–159	68

_N_-Substituted-_β_-carbolines (18a-c)[66,67]

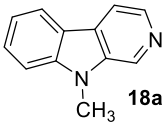
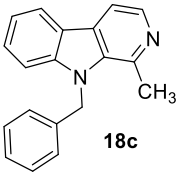
To a solution of _β_-carboline **17a-c** (1 mmol) in DMF (20 mL) was added sodium hydride (3 mmol) portion wise and the resulting reaction mixture was stirred at room temperature for 1 h. Next, a solution of alkyl halide (1.2 mmol) in DMF (2 mL) was added drop wise to reaction mixture and stirred for 12 h at room temperature. Upon completion of reaction, the contents were poured over crushed ice (100 gm) and extracted with DCM (3 × 30 mL), dried and removed the excess solvent under *vaccuo*. The residue so obtained was purified by column chromatography using ethyl acetate:hexane (1:1) as an eluent to afford *N*-alkylated *β*-carbolines **18a-c** in 77-85% yields .

9-(4-Chlorobenzyl)-9*H*-pyrido[3,4-*b*]indole (18b)



Brown solid; Yield: 85%; Mp: 138-139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.52 (d, J = 5.3 Hz, 1H), 8.20 (d, J = 7.8 Hz, 1H), 8.01 (d, J = 4.9 Hz, 1H), 7.61–7.57 (m, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.36–7.32 (m, 1H), 7.26 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 5.55 (s, 2H).

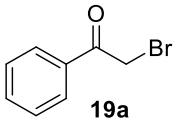
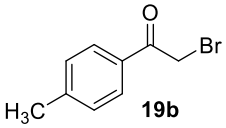
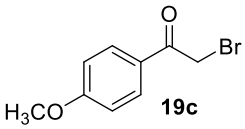
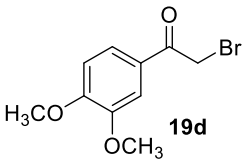
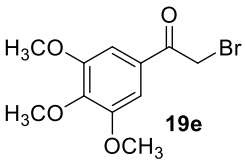
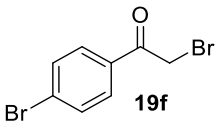
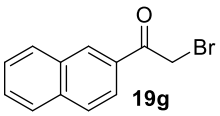
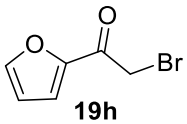
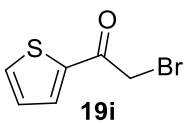
Table 2.2.4 Synthesized *N*-alkylated-*β*-carbolines **(18a & 18c)**

N-Alkylated-*β*-carbolines	Mp (°C)	Lit. Mp (°C)	Yield (%)
 **18a**	107-108	108–109[68]	80
 **18c**	113-114	111-112[29]	77

1-Aryl(heteroaryl)-2-bromoethanones (19a-i)[69]

A mixture of substituted arylethanones (10 mmol), *N*-bromosuccinimide (1.4 g, 12 mmol) and *p*-toluenesulphonic acid (2.8 g, 15 mmol) in acetonitrile (50 mL) was stirred at 85 °C for 4 h. After completion of reaction (indicated by TLC), the reaction mass was allowed to reach ambient temperature and evaporated excess of acetonitrile under reduced pressure. The residue so obtained was mixed in water, extracted with ethyl acetate (2 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuuo*. The crude product obtained was recrystallized from hexane to afford pure 1-aryl(heteroaryl)-2-bromoethanones **19a-i** in 75-84% yields.

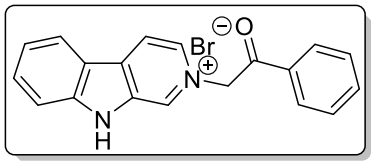
Table 2.2.5 List of prepared 1-aryl(heteroaryl)-2-bromoethanones **19a-i**

1-Aryl(heteroaryl)-2-bromoethanones	Mp (°C)	Lit. Mp (°C)[69]	Yield (%)
 **19a**	49-50	50.4 – 52.6	84
 **19b**	45-46	44.5–46.6	80
 **19c**	65-66	63.4–65.2	82
 **19d**	79-80	80-81	78
 **19e**	68-70	67-68	75
 **19f**	98-100	100.1–103.5	82
 **19g**	80-81	82-83	78
 **19h**	Semi-solid	34-36	65
 **19i**	Semi-solid	29-30	70

β-Carbolinium bromides (13a-t)

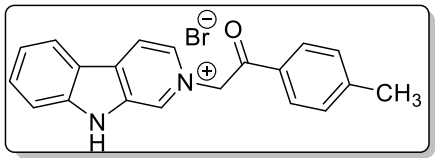
β-Carboline **17** or **18** (0.1 g, 0.6 mmol), 1-aryl-2-bromoethanone **19** (0.72 mmol) and ethanol (3 mL) were taken in 10 mL microwave (MW) vial. The resulting mixture was irradiated in focused MW oven for 20 min at 80 °C. Upon completion of reaction as indicated by TLC, ethanol was evaporated *in vaccuo* and the residue so obtained was precipitated with ethyl acetate. The obtained solid was filtered and washed with ethyl acetate (2 x 10 mL) to afford **13a-t** in 75-92% yields.

2-(2-Oxo-2-phenylethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13a)



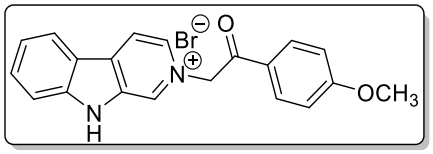
Yellow solid; Yield: 89%; Mp 225-226 °C; IR (KBr, *v*, cm^{-1}): 3418, 3009, 2955, 2908, 2862, 1697, 1643, 1520, 1450, 1381, 1342, 1227, 903, 764, 687; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H), 9.46 (s, 1H), 8.94 (d, J = 6.3 Hz, 1H), 8.69 (d, J = 6.3 Hz, 1H), 8.56 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 7.5 Hz, 2H), 7.86–7.80 (m, 3H), 7.71–7.68 (m, 2H), 7.58–7.43 (m, 1H), 6.65 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.0, 144.7, 135.1, 134.5, 134.2, 133.0, 132.7, 131.5, 129.6, 128.7, 124.2, 122.2, 119.6, 118.0, 113.6, 66.5; Anal. RP-HPLC t_R = 2.650 min, purity 98.02%; MS (ESI^+) calcd for $C_{19}H_{15}BrN_2O$ $[M + H]^+$, 367.04; found 367.03; HRMS (ESI^+) calcd for $C_{19}H_{15}N_2O^+$ $[M–Br]^+$, 287.1179; found, 287.1176.

2-(2-Oxo-2-(*p*-tolyl)ethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13b)



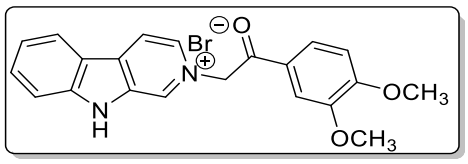
Yellow solid; Yield: 91%; Mp 236-237 °C; IR (KBr, *v*, cm^{-1}): 3425, 3078, 2924, 2854, 1697, 1643, 1589, 1504, 1458, 1420, 1381, 1342, 1257, 1126; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H), 9.47 (s, 1H), 8.93 (d, J = 6.4 Hz, 1H), 8.70 (d, J = 6.4 Hz, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.1 Hz, 2H), 7.85 (d, J = 3.7 Hz, 2H), 7.51–7.48 (m, 3H), 6.64 (s, 2H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.5, 145.7, 144.7, 135.1, 134.5, 133.0, 132.7, 131.7, 131.5, 130.1, 128.8, 124.2, 122.1, 119.6, 117.9, 113.6, 66.3, 21.8; Anal. RP-HPLC t_R = 2.666 min, purity 97.48%; MS (ESI^+) calcd for $C_{20}H_{17}BrN_2O$ $[M - Br]^+$, 301.13; found 301.15; HRMS (ESI^+) calcd for $C_{20}H_{17}N_2O^+$ $[M - Br]^+$, 301.1335; found, 301.1335.

2-(2-(4-Methoxyphenyl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13c)



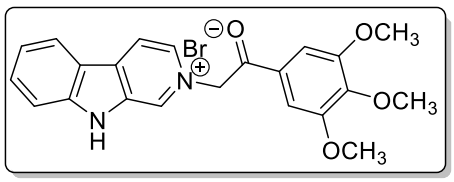
Yellow solid; Yield: 91%; Mp 233-235 °C; IR (KBr, *v*, cm^{-1}): 3433, 3063, 2962, 2924, 1690, 1643, 1587, 1455, 1342, 1257, 756, 463; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H), 9.47 (s, 1H), 8.93 (d, J = 6.3 Hz, 1H), 8.69 (d, J = 6.2 Hz, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.6 Hz, 2H), 7.85–7.84 (m, 2H), 7.51–7.47 (m, 1H), 7.20 (d, J = 8.6 Hz, 2H), 6.61 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.2, 164.6, 144.6, 135.1, 134.5, 132.9, 132.7, 131.5, 131.2, 127.0, 124.2, 122.1, 119.6, 117.9, 114.8, 113.6, 66.1, 56.3; Anal. RP-HPLC t_R = 2.672 min, purity 98.19%; MS (ESI^+) calcd for $C_{20}H_{17}BrN_2O_2$ [M - Br]$^+$, 317.12; found 317.14; HRMS (ESI^+) calcd for $C_{20}H_{17}N_2O_2^+$ [M - Br]$^+$, 317.1285; found, 317.1283.

2-(2-(3,4-Dimethoxyphenyl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-iumbromide (13d)



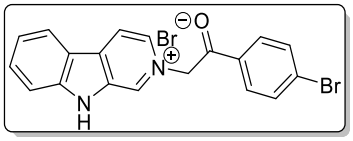
Yellow solid; Yield: 88%; Mp 263-265 °C; IR (KBr, *v*, cm^{-1}): 3433, 3055, 1682, 1643, 1589, 1520, 1474, 1342, 1211, 1142, 1016, 764, 733, 633, 463; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.04 (s, 1H), 9.47 (s, 1H), 8.93 (d, J = 6.4 Hz, 1H), 8.69 (d, J = 6.4 Hz, 1H), 8.55 (d, J = 8.1 Hz, 1H), 7.85–7.82 (m, 3H), 7.57 (d, J = 1.6 Hz, 1H), 7.52–7.48 (m, 1H), 7.25 (d, J = 8.5 Hz, 1H), 6.63 (s, 2H), 3.92 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 154.6, 149.3, 144.7, 135.1, 134.5, 132.9, 132.7, 131.5, 126.9, 124.2, 123.8, 122.2, 119.6, 117.9, 113.6, 111.7, 110.7, 66.2, 56.5, 56.2; Anal. RP-HPLC t_R = 2.665 min, purity 98.43%; MS (ESI^+) calcd for $C_{21}H_{19}BrN_2O_3$ [M - Br]$^+$, 347.13; found 347.15; HRMS (ESI^+) calcd for $C_{21}H_1N_2O_3^+$ [M - Br]$^+$, 347.1390; found, 347.1394.

2-(2-Oxo-2-(3,4,5-trimethoxyphenyl)ethyl)-9*H*-pyrido[3,4-*b*]indol-2-iumbromide (13e)



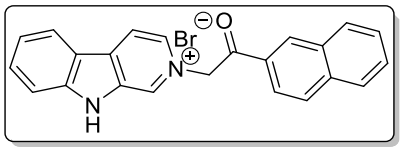
Yellow solid; Yield: 84%; Mp 211-212 °C; IR (KBr, *v*, cm^{-1}): 3410, 3055, 2947, 2839, 1682, 1643, 1597, 1512, 1342, 1242, 1173, 1011, 833, 756, 733, 463; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (s, 1H), 9.48 (s, 1H), 8.94 (d, J = 6.4 Hz, 1H), 8.71 (d, J = 6.4 Hz, 1H), 8.55 (d, J = 8.0 Hz, 1H), 7.85–7.84 (m, 2H), 7.52–7.46 (m, 1H), 7.44 (s, 2H), 6.73 (s, 2H), 3.92 (s, 6H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.0, 153.5, 144.7, 143.4, 135.1, 134.5, 133.0, 132.7, 131.4, 129.5, 124.2, 122.2, 119.6, 118.0, 113.6, 107.9, 106.5, 66.5, 60.9, 56.9; Anal. RP-HPLC t_R = 2.660 min, purity 98.01%; MS (ESI^+) calcd for $C_{22}H_{21}BrN_2O_4$ [M-Br]$^+$, 377.14; found 377.10; HRMS (ESI^+) calcd for $C_{22}H_{21}N_2O_4^+$ [M - Br]$^+$, 377.1496; found, 377.1497.

2-(2-(4-Bromophenyl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13f)



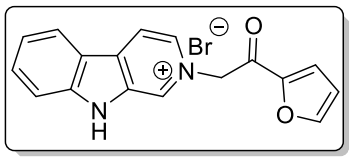
Yellow solid; Yield: 92%; Mp 183-184 °C; IR (KBr, *v*, cm^{-1}): 3433, 3055, 2947, 2839, 1690, 1647, 1595, 1341, 1242, 1153, 1031, 833, 767, 733, 463; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 (s, 1H), 9.49 (s, 1H), 8.96 (d, J = 6.4 Hz, 1H), 8.71 (d, J = 6.3 Hz, 1H), 8.56 (d, J = 8.0 Hz, 1H), 8.50 (d, J = 8.9 Hz, 2H), 8.36 (d, J = 8.8 Hz, 2H), 7.86–7.85 (m, 2H), 7.52–7.48 (m, 1H), 6.73 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.4, 151.0, 144.7, 138.9, 135.1, 134.5, 133.1, 132.8, 131.5, 130.2, 124.6, 124.2, 122.2, 119.6, 118.0, 113.6, 66.7; Anal. RP-HPLC t_R = 2.667 min, purity 98.44%; MS (ESI^+) calcd for $C_{19}H_{14}Br_2N_2O$ $[M\text{-}Br]^+$, 365.02; found 364.98; HRMS (ESI^+) calcd for $C_{19}H_{14}BrN_2O^+$ $[M\text{-}Br]^+$, 365.0284; found, 365.0282.

2-(2-(Naphthalen-2-yl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13g)



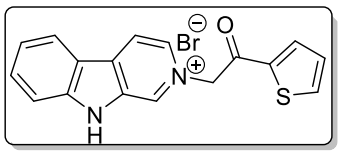
Yellow solid; Yield: 87%; Mp 225-227 °C; IR (KBr, *v*, cm^{-1}): 3441, 3009, 2924, 1697, 1643, 1520, 1466, 1342, 1265, 1188, 1126, 1003, 833, 756, 579, 432; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.17 (s, 1H), 9.55 (s, 1H), 8.95–8.94 (m, 2H), 8.77 (d, J = 6.1 Hz, 1H), 8.54 (d, J = 7.9 Hz, 1H), 8.24 (d, J = 7.8 Hz, 1H), 8.15 (d, J = 8.6 Hz, 1H), 8.09–8.07 (m, 2H), 7.85–7.83 (m, 2H), 7.77–7.68 (m, 2H), 7.52–7.46 (m, 1H), 6.84 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.0, 144.7, 136.0, 135.1, 134.5, 133.0, 132.7, 132.5, 131.5, 131.1, 130.2, 129.8, 129.2, 128.4, 127.9, 125.9, 124.2, 123.7, 122.1, 119.6, 118.0, 113.5, 66.5; Anal. RP-HPLC t_R = 2.694 min, purity 98.80%; HRMS (ESI^+) calcd for $C_{23}H_{17}N_2O^+$ $[M\text{-}Br]^+$, 337.1335; found, 337.1329.

2-(2-(Furan-2-yl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13h)



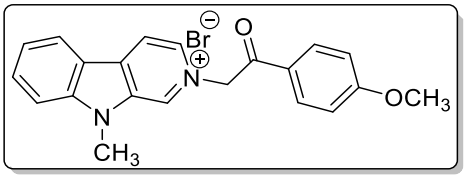
Yellow solid; Yield: 93%; Mp 174-175 °C; IR (KBr, *v*, cm^{-1}): 3294, 3109, 2993, 2939, 1674, 1643, 1520, 1458, 1396, 1335, 1265, 1149, 1034, 1003, 910, 771, 748, 594; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.04 (s, 1H), 9.45 (s, 1H), 8.92 (d, J = 6.2 Hz, 1H), 8.68 (d, J = 6.2 Hz, 1H), 8.54 (d, J = 7.9 Hz, 1H), 8.25 (s, 1H), 7.85–7.76 (m, 3H), 7.53–7.43 (m, 1H), 6.92 (s, 1H), 6.40 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.3, 149.8, 149.5, 144.7, 135.0, 134.6, 133.1, 132.8, 131.6, 124.2, 122.2, 120.6, 119.6, 117.9, 113.7, 113.6, 65.2; Anal. RP-HPLC t_R = 2.668 min, purity 98.67%. MS (ESI^+) calcd for $C_{17}H_{13}BrN_2O_2$ $[M - Br]^+$, 277.10; found 277.05.

2-(2-Oxo-2-(thiophen-2-yl)ethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13i)



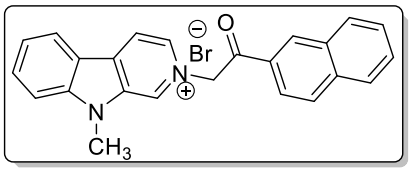
Yellow solid; Yield: 91%; Mp 228-229 °C; IR (KBr, *v*, cm^{-1}): 3394, 3009, 2924, 1697, 1643, 1520, 1466, 1342, 1265, 1219, 1188, 1126, 1003, 833, 758, 579, 432; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (s, 1H), 9.49 (s, 1H), 8.93 (d, *J* = 6.4 Hz, 1H), 8.72 (d, *J* = 6.4 Hz, 1H), 8.54 (d, *J* = 8.0 Hz, 1H), 8.30–8.25 (m, 2H), 7.85–7.84 (m, 2H), 7.51–7.47 (m, *J* = 7.9, 3.9 Hz, 1H), 7.46–7.43 (m, 1H), 6.59 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 184.9, 144.7, 140.0, 137.1, 135.4, 135.0, 134.5, 133.0, 132.7, 131.5, 129.7, 124.2, 122.2, 119.6, 118.0, 113.6, 65.8; Anal. RP-HPLC t_R = 2.679 min, purity 98.73%. MS (ESI^+) calcd for $C_{17}H_{13}BrN_2OS$ $[M - Br]^+$, 293.07; found 293.05.

2-(2-(4-Methoxyphenyl)-2-oxoethyl)-9-methyl-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13j)



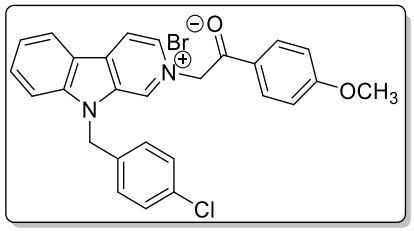
Yellow solid; Yield: 83%; Mp 237-239 °C; IR (KBr, *v*, cm^{-1}): 3022, 2986, 2873, 1692, 1645, 1579, 1520, 1459, 1367, 1286, 1192, 1113, 1004, 845, 756, 565, 438; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 8.95 (d, *J* = 6.4 Hz, 1H), 8.70 (d, *J* = 7.4 Hz, 1H), 8.58 (d, *J* = 8.0 Hz, 1H), 8.13 (d, *J* = 8.9 Hz, 2H), 8.0–7.90 (m, 2H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 2H), 6.57 (s, 2H), 4.09 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.0, 164.7, 145.4, 136.3, 134.6, 132.8, 132.4, 131.2, 130.6, 126.9, 124.3, 122.4, 119.4, 117.8, 114.9, 111.8, 66.2, 56.3, 30.8; Anal. RP-HPLC t_R = 2.667 min, purity 99.56%; MS (ESI^+) calcd for $C_{21}H_{19}BrN_2O_2$ $[M - Br]^+$, 331.14; found 331.16.

9-Methyl-2-(2-(naphthalen-2-yl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (16k)



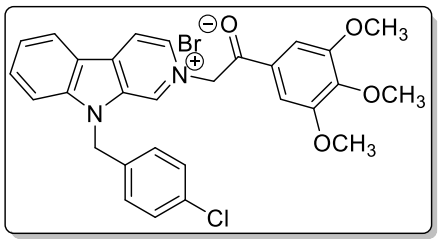
Yellow solid; Yield: 85%; Mp 243-244 °C; IR (KBr, *v*, cm^{-1}): 3079, 3001, 2913, 2844, 1697, 1643, 1568, 1456, 1370, 1321, 1215, 1117, 1023, 857, 743, 575, 456; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 1H), 8.98–8.95 (m, 2H), 8.77 (d, *J* = 4.8 Hz, 1H), 8.60 (d, *J* = 7.2 Hz, 1H), 8.26 (d, *J* = 7.0 Hz, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 8.12–8.10 (m, 2H), 7.98–7.95 (m, 2H), 7.78–7.74 (m, 2H), 7.56 (d, *J* = 5.3 Hz, 1H), 6.77 (s, 2H), 4.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.7, 145.4, 136.3, 136.1, 134.6, 132.9, 132.5, 132.4, 131.4, 131.1, 130.6, 130.2, 129.9, 129.4, 128.4, 128.0, 124.3, 123.7, 122.4, 119.4, 117.9, 111.9, 66.6, 30.8; Anal. RP-HPLC t_R = 2.667 min, purity 99.06%; MS (ESI^+) calcd for $C_{24}H_{19}BrN_2O$ $[M - Br]^+$, 351.14; found 351.12.

9-(4-Chlorobenzyl)-2-(2-(4-methoxyphenyl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13l)



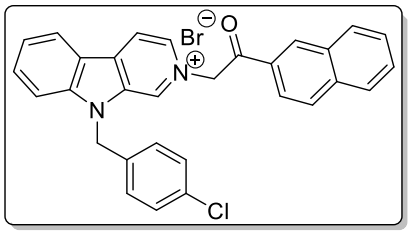
Yellow solid; Yield: 79%; Mp 132-134 °C; IR (KBr, *v*, cm^{-1}): 2955, 2839, 1682, 1643, 1597, 1512, 1466, 1335, 1242, 1173, 1095, 1011, 833, 756, 625, 579; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 9.00 (d, J = 6.4 Hz, 1H), 8.76 (d, J = 6.4 Hz, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.8 Hz, 2H), 7.99 (d, J = 8.5 Hz, 1H), 7.90 (t, J = 7.7 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 7.21 (d, J = 8.8 Hz, 2H), 6.58 (s, 2H), 5.89 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.9, 164.7, 144.7, 135.9, 135.4, 135.3, 133.1, 133.0, 132.9, 131.2, 130.7, 129.4, 129.3, 126.9, 124.6, 122.8, 119.8, 118.1, 114.9, 112.2, 66.3, 55.4, 46.6; Anal. RP-HPLC t_R = 2.683 min, purity 98.07%; MS (ESI^+) calcd for $C_{27}H_{22}BrClN_2O_2$ $[M - Br]^+$, 441.13; found 441.16.

9-(4-Chlorobenzyl)-2-(2-oxo-2-(3,4,5-trimethoxyphenyl)ethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13m)



Brown solid; Yield: 77%; Mp 185-187 °C; IR (KBr, *v*, cm^{-1}): 3077, 2981, 2873, 1690, 1643, 1512, 1466, 1342, 1165, 1126, 841, 756, 594, 455; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.02 (d, J = 6.4 Hz, 1H), 8.78 (d, J = 6.4 Hz, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 8.5 Hz, 1H), 7.90 (t, J = 7.7 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.44 (s, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.4 Hz, 2H), 6.69 (s, 2H), 5.90 (s, 2H), 3.92 (s, 6H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.7, 153.5, 144.7, 143.5, 135.9, 135.4, 135.3, 133.2, 133.1, 133.0, 130.6, 129.4, 129.3, 129.2, 124.6, 122.8, 119.8, 118.3, 112.2, 106.4, 66.7, 60.9, 56.9, 46.6; Anal. RP-HPLC t_R = 2.672 min, purity 98.49%; MS (ESI^+) calcd for $C_{29}H_{26}BrClN_2O_4$ $[M - Br]^+$, 501.15; found 501.17.

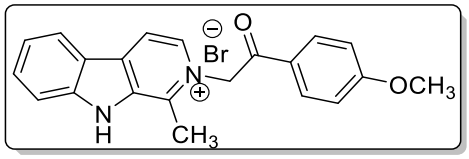
9-(4-Chlorobenzyl)-2-(2-(naphthalen-2-yl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13n)



Yellow solid; Yield: 80%; Mp 137-138 °C; IR (KBr, *v*, cm^{-1}): 3055, 1690, 1643, 1512, 1466, 1335, 1219, 1126, 1011, 825, 748, 563, 474; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 9.04 (d, J = 6.2 Hz, 1H), 8.95 (s, 1H), 8.83 (d, J = 6.3 Hz, 1H), 8.63 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 7.9 Hz, 1H), 8.17 (d, J = 8.6 Hz, 1H), 8.11–8.08 (m, 2H), 8.02 (d, J = 8.4 Hz, 1H), 7.91 (t, J = 7.7 Hz, 1H), 7.79–7.70 (m, 2H), 7.57 (t, J = 7.5 Hz, 1H), 7.40 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 6.80 (s, 2H),

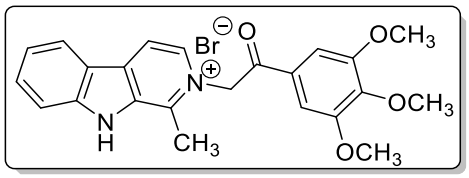
5.90 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 191.6, 144.7, 136.1, 135.9, 135.4, 135.3, 133.2, 133.1, 133.0, 132.5, 131.4, 131.2, 130.7, 130.2, 129.9, 129.4, 129.3, 129.2, 128.4, 127.9, 124.6, 123.7, 122.8, 119.8, 118.3, 112.2, 66.7, 46.7; Anal. RP-HPLC t_R = 2.659 min, purity 98.39%; MS (ESI$^+$) calcd for $C_{30}H_{22}BrClN_2O$ [M - Br]$^+$, 461.14; found 461.15.

2-(2-(4-Methoxyphenyl)-2-oxoethyl)-1-methyl-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13o)



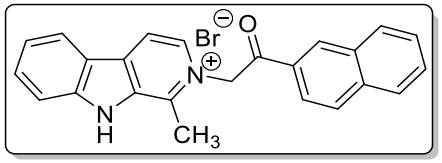
Yellow solid; Yield: 89%; Mp 182-184 °C; IR (KBr, *v*, cm^{-1}): 3418, 3079, 2980, 2893, 1690, 1643, 1605, 1520, 1335, 1242, 1173, 1023, 755, 432; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.03 (s, 1H), 8.74 (d, *J* = 6.4 Hz, 1H), 8.61 (d, *J* = 6.6 Hz, 1H), 8.51 (d, *J* = 7.5 Hz, 1H), 8.13 (d, *J* = 8.7 Hz, 2H), 7.84–7.83 (m, 2H), 7.51–7.46 (m, 1H), 7.22 (d, *J* = 8.7 Hz, 2H), 6.63 (s, 2H), 3.92 (s, 3H), 2.98 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.9, 164.9, 144.3, 141.9, 135.3, 132.4, 131.8, 131.5, 126.9, 124.1, 124.0, 122.2, 120.2, 116.1, 114.8, 113.4, 63.2, 56.3, 15.9; Anal. RP-HPLC t_R = 2.667 min, purity 98.25%; MS (ESI$^+$) calcd for $C_{21}H_{19}BrN_2O_2$ [M - Br]$^+$, 331.14; found 331.15.

1-Methyl-2-(2-oxo-2-(3,4,5-trimethoxyphenyl)ethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13p)



Yellow solid; Yield: 86%; Mp 186-187 °C; IR (KBr, *v*, cm^{-1}): 3416, 3055, 3001, 2901, 1682, 1636, 1582, 1504, 1458, 1420, 1319, 1234, 1196, 1165, 1126, 1003, 849, 748, 455; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (s, 1H), 8.75 (d, *J* = 6.5 Hz, 1H), 8.62 (d, *J* = 6.6 Hz, 1H), 8.50 (d, *J* = 7.9 Hz, 1H), 7.84–7.83 (m, 2H), 7.50-7.45 (m, 3H), 6.78 (s, 2H), 3.93 (s, 6H), 3.81 (s, 3H), 3.02 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.8, 153.4, 144.2, 143.6, 141.9, 135.3, 135.1, 132.4, 131.8, 129.3, 124.1, 122.2, 120.2, 116.2, 113.4, 106.7, 63.7, 60.8, 56.8, 16.0; Anal. RP-HPLC t_R = 2.663 min, purity 97.98%; MS (ESI$^+$) calcd for $C_{23}H_{23}BrN_2O_4$ [M - Br]$^+$, 391.16; found 391.17.

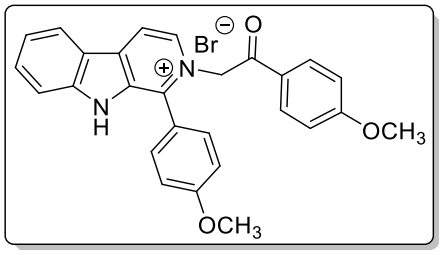
1-Methyl-2-(2-(naphthalen-2-yl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13q)



Yellow solid; Yield: 82%; Mp 143-144 °C; IR (KBr, *v*, cm^{-1}): 3406, 3058, 3012, 2920, 2880 1685, 1638, 1573, 1501, 1472, 1433, 1319, 1286, 1214, 1163, 1115, 1002, 833, 752, 576, 435; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 8.97 (s, 1H), 8.77 (d, *J* = 6.5 Hz, 1H), 8.68 (d, *J* = 6.7 Hz, 1H), 8.51 (d, *J* = 7.3 Hz, 1H), 8.44 (d, *J* = 8.5 Hz, 1H), 8.25– 8.16 (m, 2H), 8.10 (d, *J* = 7.9 Hz, 1H), 7.88–7.80 (m, 1H), 7.78–7.71 (m, 2H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.2 Hz, 1H), 6.84 (s, 2H), 2.99 (s, 3H); ^{13}C NMR (100 MHz,

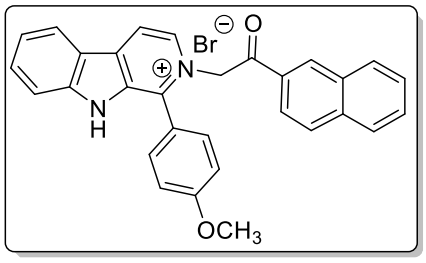
DMSO-d_6) δ 191.7, 144.3, 143.1, 142.1, 139.9, 136.1, 135.3, 132.4, 131.6, 131.2, 130.2, 129.2, 128.6, 127.9, 125.9, 124.1, 123.9, 123.6, 121.4, 116.1, 115.4, 113.1, 63.5, 16.0; Anal. RP-HPLC t_R = 2.661 min, purity 98.29%; MS (ESI^+) calcd for $C_{24}H_{19}BrN_2O$ $[M - Br]^+$, 351.14; found 351.17.

1-(4-Methoxyphenyl)-2-(2-(4-methoxyphenyl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13r)



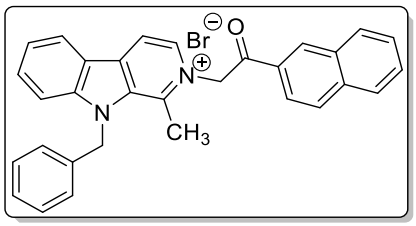
Yellow solid; Yield: 86%; Mp 217-218 °C; IR (KBr, *v*, cm^{-1}): 3441, 3053, 3009, 2911, 1682, 1628, 1605, 1597, 1512, 1327, 1250, 1180, 833, 756, 432; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 8.94 (d, *J* = 6.4 Hz, 1H), 8.79 (d, *J* = 6.6 Hz, 1H), 8.57 (d, J = 8.2 Hz, 1H), 7.94 (d, *J* = 8.7 Hz, 2H), 7.85–7.77 (m, 2H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 8.7 Hz, 2H), 6.26 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 164.8, 161.8, 145.1, 141.4, 135.7, 135.4, 133.2, 132.6, 131.6, 131.2, 126.5, 124.2, 122.3, 120.1, 117.2, 115.5, 115.4, 114.8, 113.7, 63.5, 56.3, 56.0; Anal. RP-HPLC t_R = 2.688 min, purity 99.03%; MS (ESI^+) calcd for $C_{27}H_{23}BrN_2O_3$ $[M - Br]^+$, 423.17; found 423.18.

1-(4-Methoxyphenyl)-2-(2-(naphthalen-2-yl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13s)



Yellow solid; Yield: 84%; Mp 202-204 °C; IR (KBr, *v*, cm^{-1}):3433, 3092, 3008, 2965, 2853, 1697, 1628, 1512, 1257, 1180, 756, 517, 455; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 1H), 8.99 (d, *J* = 6.5 Hz, 1H), 8.88 (d, *J* = 6.6 Hz, 1H), 8.72 (s, 1H), 8.59 (d, *J* = 7.8 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 1H), 8.10–8.06 (m, 1H), 8.05–8.00 (m, 1H), 7.94 (d, *J* = 7.0 Hz, 1H), 7.83 (t, *J* = 8.1 Hz, 1H), 7.77–7.66 (m, 3H), 7.57 (d, *J* = 8.7 Hz, 2H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 8.8 Hz, 2H), 6.49 (s, 2H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.2, 161.8, 145.2, 141.4, 136.1, 135.8, 135.4, 133.3, 132.7, 132.3, 131.9, 131.6, 131.3, 130.9, 130.2, 129.3, 128.3, 127.9, 124.2, 123.5, 122.3, 120.2, 120.1, 117.3, 115.5, 113.7, 63.8, 55.9; Anal. RP-HPLC t_R = 2.673 min, purity 99.11%; MS (ESI^+) calcd for $C_{30}H_{23}BrN_2O_2$ $[M - Br]^+$, 443.17; found 443.10.

9-Benzyl-1-methyl-2-(2-(naphthalen-2-yl)-2-oxoethyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (13t)



Brown solid; Yield: 88%; Mp 113-115 °C; IR (KBr, *v*, cm^{-1}): 3063, 3009, 2915, 2865 1688, 1647, 1575, 1513, 1467, 1419, 1345, 1291, 1219, 1171, 1125, 1006, 836, 748, 566, 433; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H), 8.79 (d, *J* = 6.2 Hz, 1H), 8.61 (d, *J* = 8.1 Hz, 1H), 8.55 (d, *J* = 6.4 Hz, 1H), 8.22–8.04 (m, 3H), 8.02–7.89 (m, 3H), 7.84–7.80 (m, 2H), 7.58–7.49 (m, 2H), 7.40–7.27 (m, 4H), 7.03 (s, 2H), 6.06 (s, 2H), 3.03 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) *δ* 191.6, 146.0, 144.6, 142.3 139.0, 138.1, 137.8, 134.3, 133.3, 132.3, 131.5, 130.3, 129.6, 129.5, 128.4, 128.1, 125.8, 124.1, 123.8, 122.4, 119.8, 119.4, 116.2, 115.4, 112.0, 111.7, 64.3, 48.1, 18.4; Anal. RP-HPLC t_R = 2.660 min, purity 97.85%. MS (ESI$^+$) calcd for $C_{31}H_{25}BrN_2O$ [M - Br]$^+$, 441.20; found 441.20.

MTT assay

MDA-MB-231, HeLa, PC-3, C4-2 and BxPC3 cancer cells were grown in RPMI 1640 media, whereas HEK293T cells were maintained in Dulbecco's modified Eagle's media (DMEM) media, both of which were supplemented with 10% fetal bovine serum and penicillin and streptomycin at 37 ºC. MTT assay was carried out as follows.[70] In brief, 4 x 10^3 cells were seeded per well in 96-well plates. After 12 h, the cells were treated with various concentrations of compounds ranging from 0.1 µM -1000 µM. The control cells were treated with 0.1% DMSO (vehicle control). The cultured cells were assayed after 48 h by adding 10 µL of 5 mg/mL MTT, followed by incubation at 37 °C for 4 h. The MTT containing media was then aspirated and 100 µL DMSO was added to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm using Tecan Spectrafluor Plus. The percentage inhibition was calculated as = 100 - [(Mean OD of treated cell X 100)/mean OD of vehicle treated cells (negative control)]. The dose response curve and IC_{50} values were obtained by nonlinear regression analysis [non-linear regression (sigmoidal dose response with variable slope)] using Graph Pad Prism, version 5.02 software (Graph Pad Software Inc., CA, USA).

Acridine orange staining

In order to check the plasma-membrane permeability, nuclear morphology and the chromatin condensation, the cells were stained with Acridine orange (AO) / Ethidium bromide (EB) dye mixture (100 µg/mL AO and 100 µg/mL EB). AO permeates all cells and makes the nucleus appear green. In contrast, EB is taken up by the cells only when the cytoplasmic membrane

integrity is lost (as in late apoptosis or in necrosis), staining the nucleus red. Briefly, cells were seeded on cover slips in 24-well plates at seeding densities of 0.5×10^5 cells and then treated with doxorubicin (IC_{50} = 2.5 μM), compounds **13l** (IC_{50} = 7μM) and **13s** (IC_{50} = 8 μM) for 24 h. After washing once with PBS, the cells were stained with 500 μL AO/EB (1:1) dye mixture dissolved in PBS solution. The cells were immediately washed with PBS, viewed under a Nikon inverted fluorescent microscope attached with a camera, and photographs were taken under fluorescent conditions.

Immunoblot analysis

PARP1 antibody was purchased from Santa Cruz Biotechnology (sc-56196). C4-2 cells were treated with doxorubicin, compound **13l** and compound **13s** for 48 h. The proteins were resolved by gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membrane, followed by blocking in 5% fat-free milk in 0.2% Tween-20. The membrane was incubated with appropriate primary antibodies (cleaved PARP and tubulin), followed by horseradish peroxidase-conjugated secondary antibody. The proteins of interest were visualized with chemiluminescence detection reagent (Pierce Biotechnology). The developed blots were subjected to densitometric analysis by Image J 1.43 software (NIH, USA) using *β*-actin as internal control.

Solubility assay

Solubility analysis of the compound **13l** was carried out by UV spectroscopy. Absorbance of 10 known concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg/L) was recorded by UV spectrophotometer. Linear regression was performed on the data and a line was fitted under the condition that passes through origin (0, 0), the result was y = 0.0281x + 0.0111. Solubility of **13l** was performed in pure water using excess amount of **13l** was added to 10 mL of volumetric flask containing 10 mL of water with vigorous shaking. Solubility of unknown concentrated **13l** in water was calculated by above line equation.

Stability assay

Stability of **13l** was performed at pH 4.5, 7.0 and 9.2 using UV absorbance method. 50 μM compounds of **13l** in pH 4.5, pH 7.0 and pH 9.2 solutions were incubated at 37 °C. Absorbance of **13l** at different pH was analyzed through UV spectrophotometer after definite intervals of time (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 24 h).

Tubulin polymerization assay

To validate the theoretical molecular-docking studies hypothesis, we examined their effect on tubulin polymerization according to the manufacturer's instructions (Tubulin Polymerization HTS Assay Kit (BK004P), Cytoskeleton, Inc.). Briefly, *β*-carbolinium bromides **13l** or **13s** (final concentration of 6μM) were added to tubulin heterodimer (final concentration of 4 mg/mL) in cold assay buffer containing 10% glycerol,80 mM PIPES, pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, and 1 mM GTP. Solutions were immediately placed in a spectrophotometer at 37 °C and absorbance at 340 nm was measured once every 1 min for 40 min.

2.3 Part B: *β*-Carboline chalcones and their Bromide Salts

2.3.1 Rational design

Compounds with enone system continue to be of great interest because of their simple chemistry, easy synthesis and biological importance and usefulness as building blocks in synthetic chemistry.[71] Particularly, wide variety of biological activities associated with chalcones have encouraged organic and medicinal chemists to undertake their structural modifications and chemistry.[72] Chalcones exhibit anti-cancer activities through various mechanisms such as inhibition of multi-drug resistance channels such as ABCG2, BCRP, *p*-glycoprotein and inhibition of protein deacetylation.[73] Also, many research groups either isolated or synthesized natural chalcones with antibacterial as well as anticancer activities. For example, Licochalcone A (**20**) isolated from *Glycyrrhiza inflate* is known to exhibit potent antibacterial activity especially towards *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus*.[74] From a series of novel *β*-carboline-based chalcones Chauhan et al. identified compound **21** with good cytotoxicity (IC_{50} =2.25 µM; MCF-7) against breast cancer cell lines.[75] In recent past, Kumar et al. reported synthesis and antitumor activity of indole-based chalcones **22** with IC_{50} values ranging 0.03-0.09 µM against human pancreatic (PaCa-2) carcinoma cells.[76]

In continuation of our efforts to develop potent anticancer agents, recently we have identified indole analogues possessing significant cytotoxicity.[77-82] Inspired by the attractive anticancer properties of *β*-carboline and chalcone units, herein, we have designed a diverse series of *β*-carboline chalcones **23a-g** and their bromide salts **24a-i** by incorporating amazing features of *β*-carboline and chalcone in a single molecule (Figure 2.3.1).

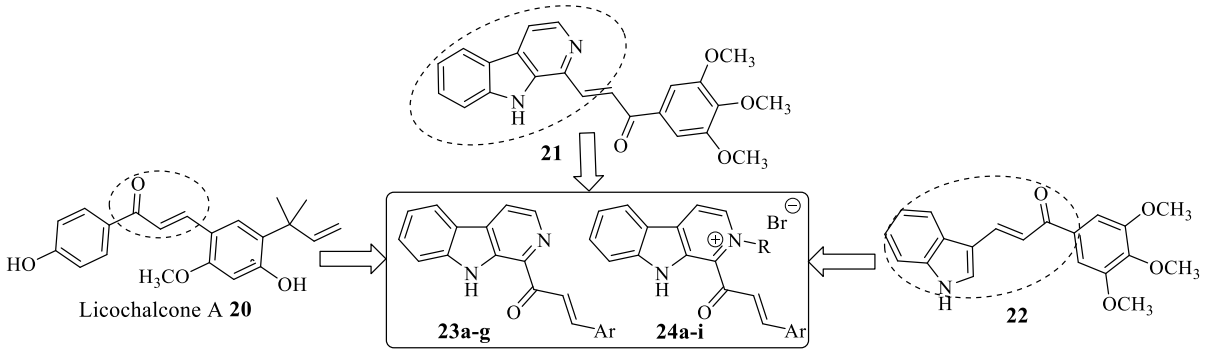
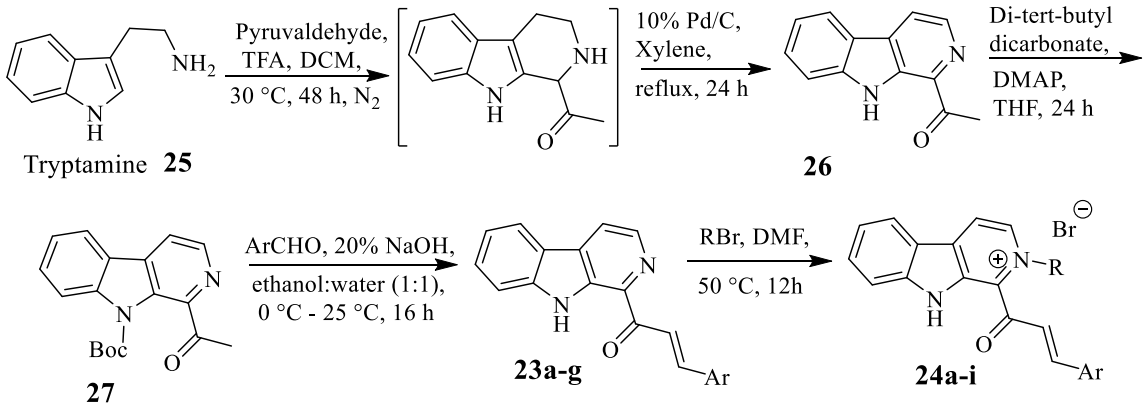


Figure 2.3.1 Rational design of *β*-carboline chalcones and their bromide salts

2.3.2 Results and discussions

2.3.2.1 Synthesis

Synthesis of novel *β*-carboline chalcone analogues **23a-g** and **24a-i** is depicted in Scheme 2.3.1.[75,83-85] Initially the 1-acetyl-*β*-carboline **26** was prepared by the Pictet–Spengler reaction of tryptamine **25** with pyruvaldehyde under acidic conditions followed by *in situ* aromatization with 10% palladium on carbon. Later the *N*-H protection of 1-acetyl-*β*-carboline with di-tert-butyldicarbonate in the presence of DMAP produced *N*-boc-1-acetyl-*β*-carboline **27**. Further, reaction of **27** with respective aldehydes in the presence of NaOH solution (20%) in aqueous ethanol for 16 h afforded *β*-carboline chalcone analogues **23a-g** in good to excellent yields. Finally, the N^2-alkylation of three *β*-carboline chalcones **23c-e** with various alkylbromides led to the corresponding bromide salts **24a-i** with more than 80% yields.



Scheme 2.3.1 Synthesis of *β*-carboline chalcones **23a-g** and their bromide salts **24a-i**.

All the prepared chalcones **23a-g** and their bromide salts **24a-i** were fully characterized using infrared (IR), NMR (^{1}H and ^{13}C) and mass spectral data (Figures 2.3.2-2.3.10). In IR spectra, two characteristic bands at ~3325 cm^{-1} (*N*-H) and ~1668 cm^{-1}(C=O) were observed for all compounds. The ^{1}H NMR spectra for chalcones **23a-g** showed broad singlet at δ ~10.5–12.0 ppm (*β*-carboline *N*-H proton) and two doublets at δ ~8.4 ppm and ~7.9 ppm (enone HC=CH protons). In the case of bromide salts **24a-i,** the ^{1}H NMR spectra demonstrated characteristic signal at δ ~6.3 ppm (CH_2 of alkyl group). In The ^{13}C NMR spectra of **23a-g** and **24a-i** exhibited signals for the carbonyl carbon at δ ~190 ppm. HRMS spectra confirmed expected mass of **23a-g** and **24a-i** in agreement with the calculated mass. HPLC analysis (Column: Waters-C18; 250×4.6 mm; condition: 0.01% TFA in acetonitrile; flow rate = 1 mL/min; and UV detector) showed the purity of the *β*-carbolines **23a-g** and **24a-i** greater than 97%.

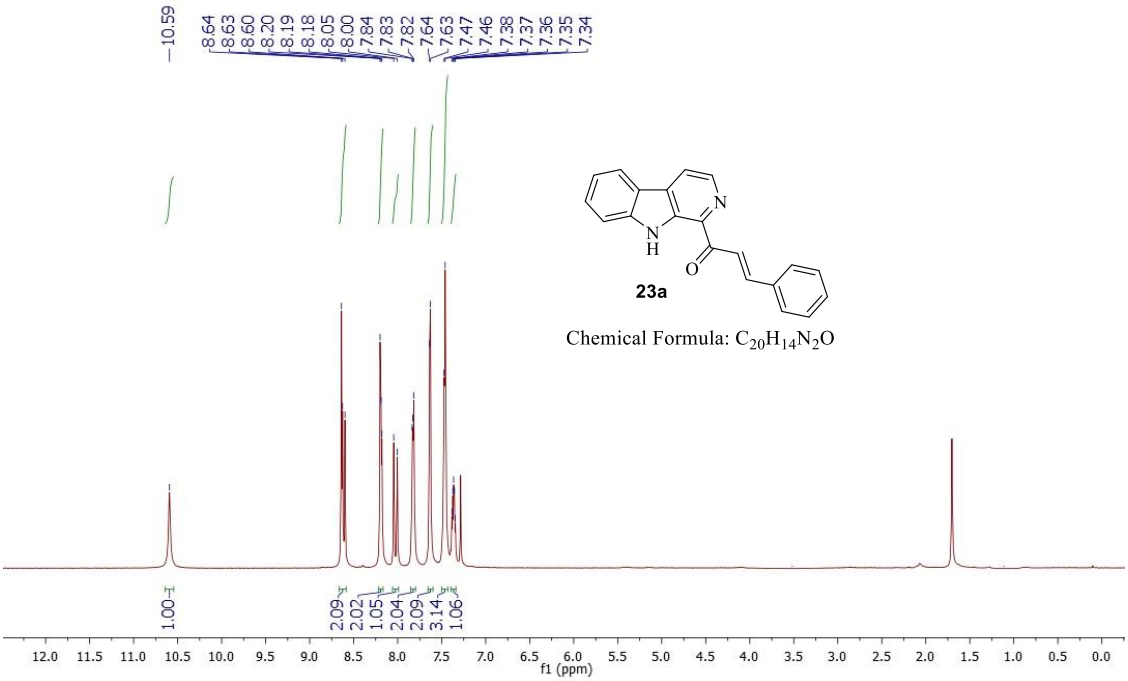


Figure 2.3.2 ^{1}H NMR spectrum of **23a**

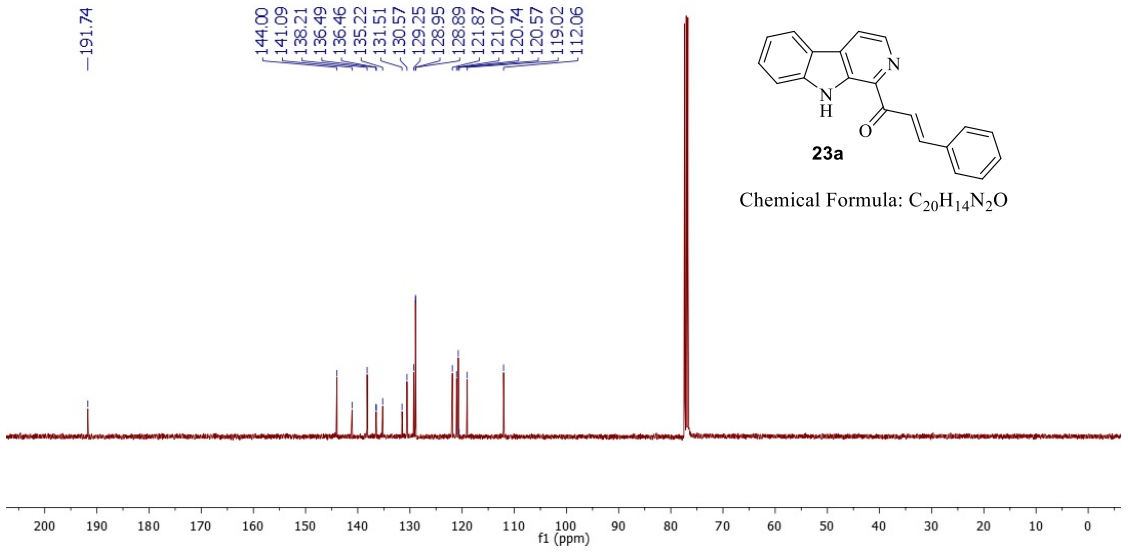


Figure 2.3.3 ^{13}C NMR spectrum of **23a**

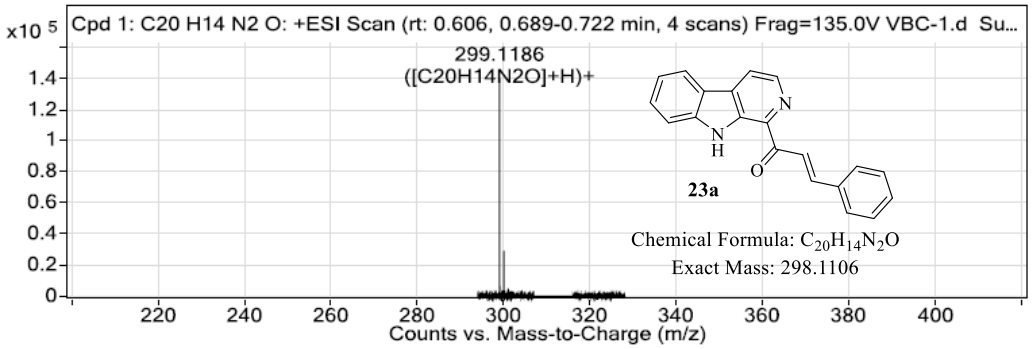


Figure 2.3.4 HRMS spectrum of **23a**

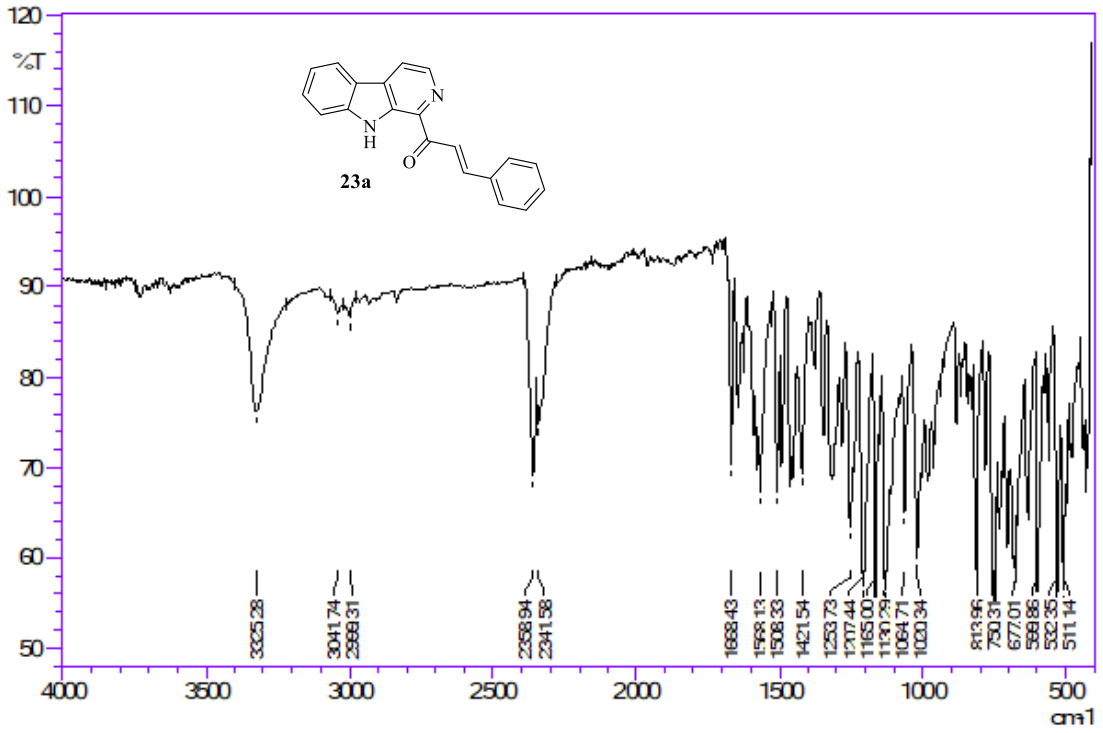
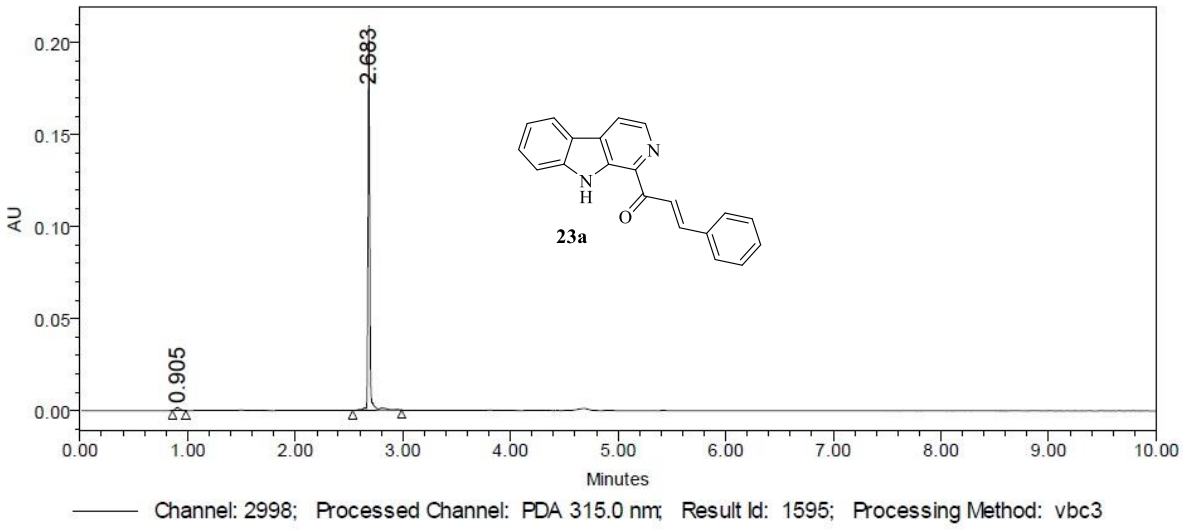


Figure 2.3.5 IR spectrum of **23a**



Processed Channel Descr.: PDA 315.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 315.0 nm	0.905	4771	1.75	1635
2	PDA 315.0 nm	2.683	267073	98.25	208849

Figure 2.3.6 HPLC chromatogram of **23a**

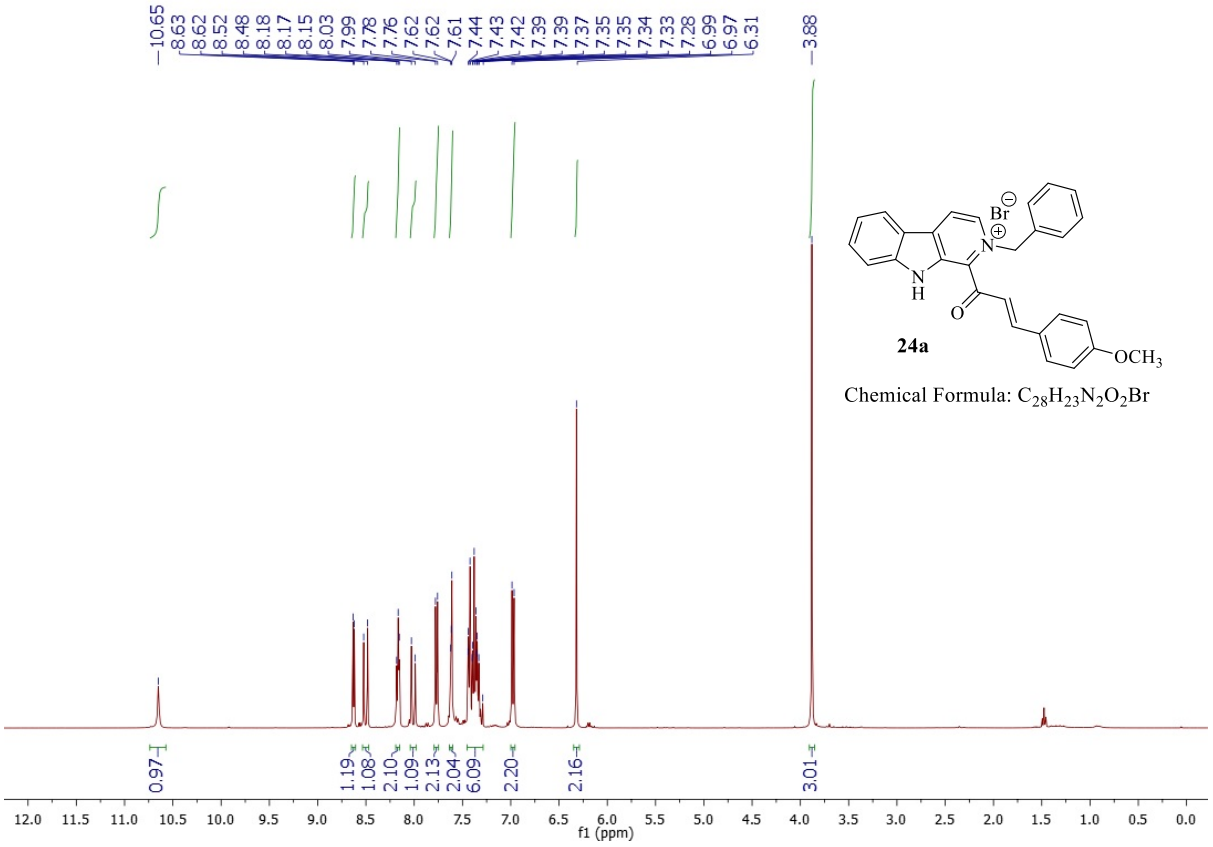


Figure 2.3.7 ^{1}H NMR spectrum of **24a**

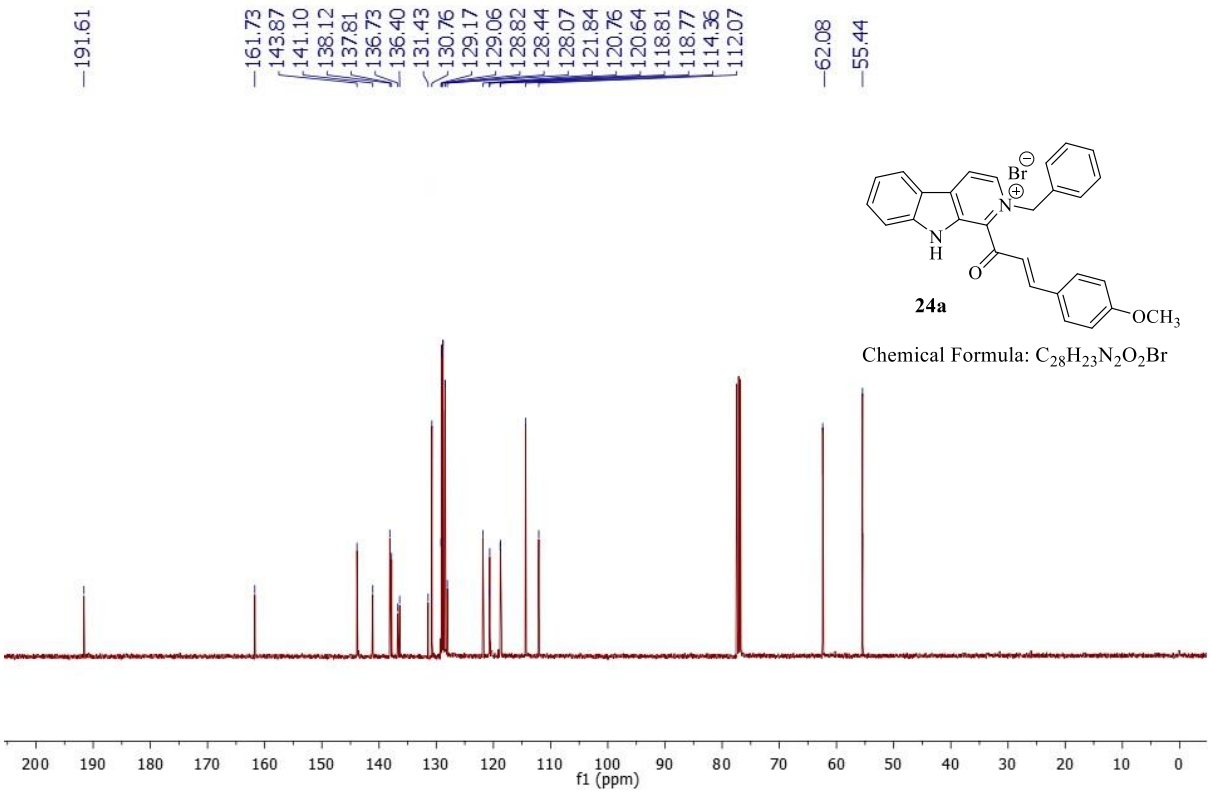


Figure 2.3.8 ^{13}C NMR spectrum of **24a**

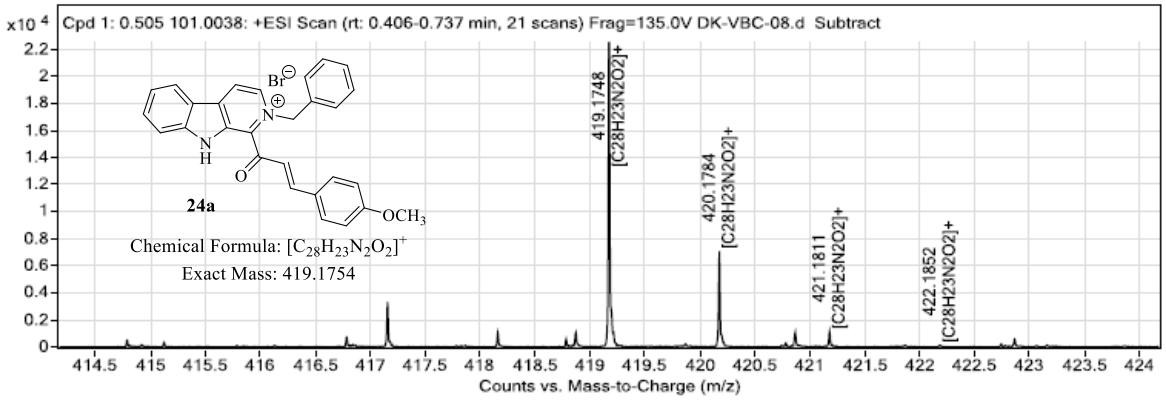
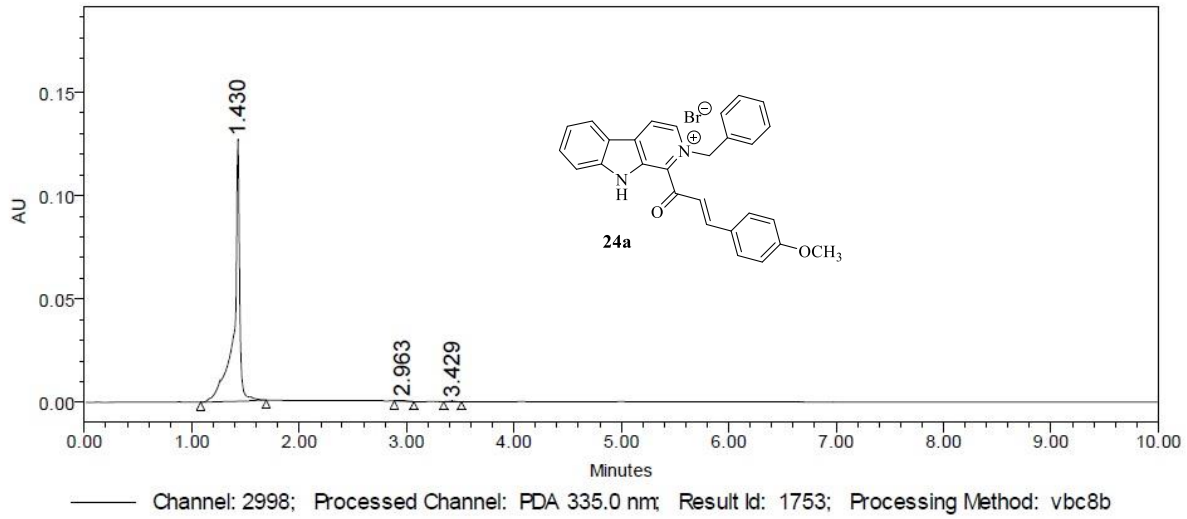


Figure 2.3.9 HRMS spectrum of **24a**



Processed Channel Descr.: PDA 335.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 335.0 nm	1.430	591259	99.58	126881
2	PDA 335.0 nm	2.963	1670	0.28	306
3	PDA 335.0 nm	3.429	794	0.13	210

Figure 2.3.10 HPLC chromatogram of **24a**

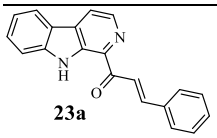
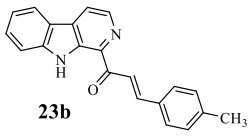
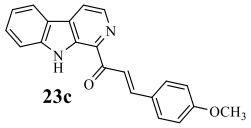
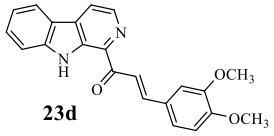
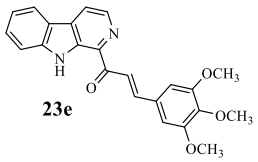
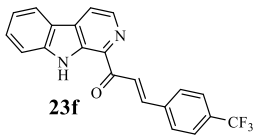
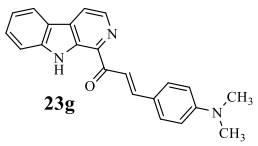
2.3.2.2 Anticancer activity studies

After synthesis and characterization, we studied the *in vitro* anticancer activity of *β*-carboline chalcones **23a-g** and their bromide salts **24a-i** against six different cancer cell lines by using MTT assay. The tumor cell line panel consisted of pancreatic cancer (BxPC-3), cervical cancer (HeLa), castration-resistant prostate cancer (C4-2), human prostate cancer (PC-3), human embryonic kidney 293 (HEK293T) and breast carcinoma (MDA-MB-231) cells. Doxorubicin was used as the reference drug. Tables 2.3.1 and 2.3.2 summarize the cytotoxicity results in terms of IC_{50} values. It is evident that most of the derivatives exhibited moderate to good cytotoxicity against the tested cancer cell lines. The structural changes by varying substituents on *β*-carboline (R) and aryl (Ar) moieties on enone part produced the sixteen compounds with a wide ranging anticancer activity with IC_{50} values ranging from 15.9 µM to >100 µM. Compound **23a** without any substituents on *β*-carboline and enone moieties displayed moderate anticancer activity against a panel of cancer cell lines (IC_{50} = 65.5-93.0 µM). Replacement of phenyl group of enone moiety with tolyl, *p*-methoxyphenyl and 3,4-dimethoxyphenyl led to inactive compounds **23b-d** (IC_{50} = >100 µM). Introduction of 3,4,5-trimethoxyphenyl moiety resulted in compound **23e** with improved activity. Cytotoxicity of compounds **23f-g** could not be tested due to solubility problem.

To improve the cytotoxic activity, N^2-alkylated derivatives of *β*-carbolines **24a-i** were prepared. Interestingly, as shown in Table 2.3.2, compared with parent *β*-carboline chalcones **23c-e** (**23c**: IC_{50} >100 µM, **23d**: IC_{50}>100 µM, **23e**: IC_{50} = 70-100 µM), the N^2-alkylated-*β*-carbolines **24a-i** showed enhanced cytotoxic activity (IC_{50} = 15-100 µM). Incorporation of benzyl at N^2 of *β*-carbolines **23c-e** yielded compounds **24a**, **24d** and **24g** shown good to moderate activity (IC_{50} = 15-88 µM). Notably, compound **24g** found to be the most potent analogue of the series with broad cytotoxicity against all the tested cell lines (IC_{50} = 15.9-22.1 µM). Introduction of propargyl moiety in *β*-carbolines **23c-e** also resulted with similar activity (**24c, 24f** and **24i,** IC_{50} = 19.1->100 µM). However, butylated derivatives **24b**, **24e** and **24h** were inferior in the activity (IC_{50} 25.1->100 µM) when compared to N^2-benzylated analogues **24g**. The activity results suggested that N^2-alkylation of *β*-carboline moiety is beneficial for the anticancer activity.

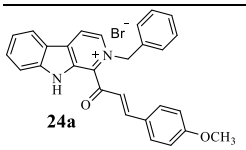
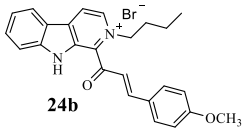
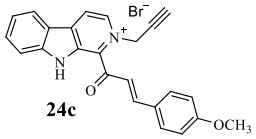
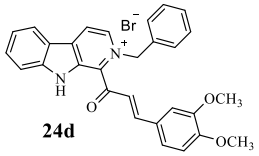
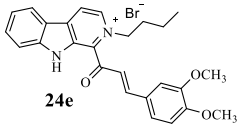
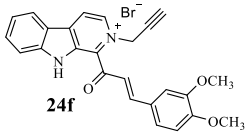
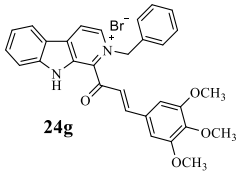
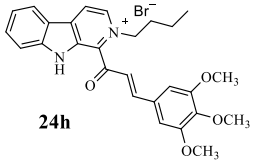
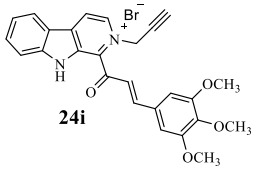
We also investigated potential toxicity of compounds **23a-g** and **24a-i** against murine fibroblast NIH3T3 cell line. The results indicate that all the tested compounds **23a-g** and **24a-i** exhibit lower toxicity than the standard drug, Doxorubicin.

Table 2.3.1 *In vitro* cytotoxicity (IC_{50} in μM) of *β*-carboline chalcones **23a-g**

***β*-Carboline chalcones**	**BxPC-3**	**HeLa**	**C4-2**	**PC-3**	**HEK 293T**	**MDA-MB-231**	**NIH3T3**
 **23a**	72.0±2.7	82.3±3.4	80.2±5.4	93.0±3.5	65.6±2.8	71.1±4.1	> 100
 **23b**	> 100	> 100	> 100	> 100	> 100	> 100	> 100
 **23c**	> 100	> 100	> 100	> 100	> 100	> 100	> 100
 **23d**	> 100	> 100	> 100	> 100	> 100	> 100	> 100
 **23e**	70.7±4.9	75.2±3.6	71.6±4.8	> 100	82.1±5.0	71.5±4.3	> 100
 **23f**	ND	ND	ND	ND	ND	ND	ND
 **23g**	ND	ND	ND	ND	ND	ND	ND
Doxorubicin	13.6±2.2	5.2±1.9	3.2±1.1	10.2±0.9	3.6±1.1	7.8±1.6	21.3 ±2.5

*The activity data represent mean values ± SD of experiments conducted in triplicates at three independent times

Table 2.3.2 *In vitro* cytotoxicity (IC_{50} in μM) of *β*-carbolinium chalcone bromides **24a-i**

***β*-Carbolinium chalcone bromides**	**BxPC-3**	**HeLa**	**C4-2**	**PC-3**	**HEK 293T**	**MDA-MB-231**	**NIH3T3**
 **24a**	50.2±4.9	60.1±4.6	65.5±5.5	88.3±7.1	41.4±3.6	52.2±4.9	85.3±5.1
 **24b**	50.7±4.9	44.9±4.3	38.7±4.9	64.9±5.5	35.1±4.1	40.9±3.9	74.6±2.5
 **24c**	> 100	> 100	> 100	> 100	> 100	> 100	> 100
 **24d**	55.3±3.4	45.2±4.4	42.0±4.3	78.9±6.4	55.1±5.8	48.2±4.6	82.2±3.3
 **24e**	> 100	> 100	> 100	> 100	> 100	> 100	> 100
 **24f**	**30.1±3.4**	**25.3±2.7**	**24.4±2.6**	**29.6±3.1**	**19.1±2.7**	**21.2±3.1**	72.1±4.1
 **24g**	**20±2.1**	**22.1±3.2**	**16.1±4.2**	**22.0±3.2**	**17.1±2.9**	**15.9±3.4**	55.2±5.8
 **24h**	**25.1±3.6**	**35.5±2.2**	**39.6±2.9**	**40.1±3.5**	**34.4±3.9**	**32.0±4.0**	68.6±6.5
 **24i**	72.0±5.3	70.1±4.9	65.5±5.7	60.9±6.1	55.1±4.4	59.6±4.8	88±3.8
Doxorubicin	13.6±2.2	5.2±1.9	3.2±1.1	10.2±0.9	3.6±1.1	7.8±1.6	21.3 ±2.5

*The activity data represent mean values ± SD of experiments conducted in triplicates at three independent times

Next, to determine the preliminary mechanism of cell death, we performed acridine orange/ethidium bromide assay.[86-88] Acridine orange is a vital dye and stains both live and dead cells. Ethidium bromide stains cells that have lost membrane integrity and tinge the nucleus red. Thus, live cells appear uniformly green in acridine orange/ethidium bromide assay. Figure 2.3.11 shows that control cells possess normal healthy morphology with intact nuclear architecture and are green in colour. Fluorescence microscopic image of MDA-MB-231 cells treated with **24g** and reference Doxorubicin clearly demonstrate morphological changes characteristic of apoptotic cells formation. This suggest that **24g** induced apoptosis in MDA-MB-231 cancer cell line.

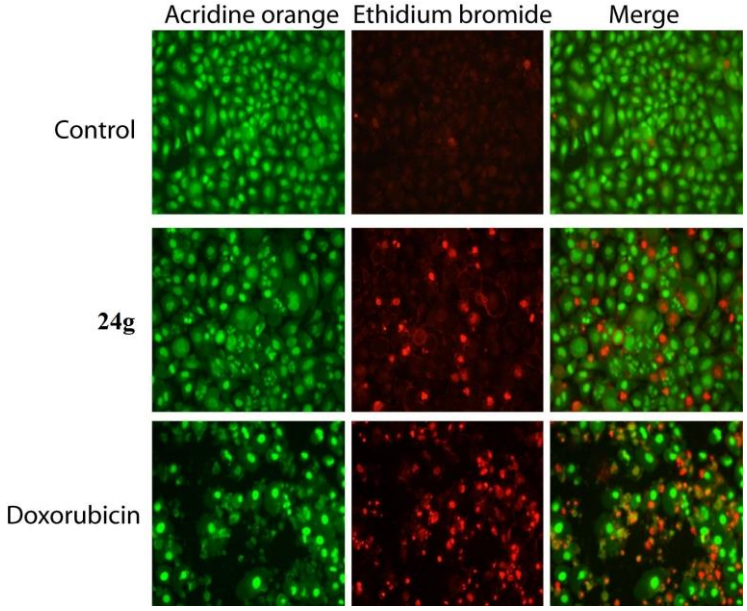


Figure 2.3.11 Morphological assessment of **24g**-treated MDA-MB-231 cells

For predicting the adsorption, distribution, metabolism and excretion (ADME) properties, computational studies for the compounds **23a-g**, and **24a-i** were performed. Lipinski's rule of five and drug likeness score were used for predicting the physiochemical properties of the molecules.[89-91] The newly prepared *β*-carboline derivatives **23a-g** and **24a-i** were evaluated for percentage absorption (% ABS) and drug-likeness score (Table 2.3.3). *β*-Carboline chalcones **23a-g** and their bromide salts **24a-i** possess lower logP values suggesting them to be better candidates for bioavailability. This was in accordance with the better cytotoxicity of *β*-carboline chalcones **23a-g** and **24a-i**. All the *β*-carbolines, **23a-g** and **24a-i** showed topological polar surface area (TPSA) less than 160 $Å^2$ but greater than 40 $Å^2$, which indicates good intestinal absorption property of these molecules than their Blood-Brain Barrier (BBB) penetration ability. Drug-likeness model score (a combined outcome of physicochemical properties, pharmacokinetics and pharmacodynamics of a compound is

represented by a numerical value) was computed by MolSoft software for the synthesized compounds. Computed drug-likeness scores are presented in Table 2.3.2. *β*-carboline chalcones, **23c-e, 24a** and **24d-i** possessed a positive score in the range of 0.06-0.48, which implies them to be good drug candidates. The most cytotoxic compound **24g** [IC_{50} = 15.9 µM against MDA-MB-231 cell line] possessed a relatively high drug score of 0.48.

Table 2.3.3 Calculated drug-like properties of *β*-carboline chalcones **23a-g** and *β*-carbolinium chalcone bromides **24a-i**

Compd	Lipinski's parameters				nRB	TPSA[b]	% ABS[c]	No. of violations	Drug-likeness Score[d]
	nHBA	nHBD	LogP[a]	Molecular weight					
23a	3	1	4.49	298.35	3	45.75	93.22	0	-0.28
23b	3	1	4.93	312.37	3	45.75	93.22	0	-0.15
23c	4	1	4.54	328.37	4	54.99	90.03	0	0.06
23d	5	1	4.13	358.40	5	64.22	86.84	0	0.40
23e	6	1	4.12	388.42	6	73.46	83.65	0	0.29
23f	3	1	5.38	366.34	4	45.75	93.22	1	-0.27
23g	4	1	4.59	341.41	4	48.99	92.10	0	-0.32
24a	4	1	1.72	419.50	6	45.98	93.13	0	0.14
24b	4	1	1.56	385.49	7	45.98	93.13	0	-0.05
24c	4	1	0.28	367.43	5	45.98	93.13	0	0.00
24d	5	1	1.31	449.53	7	55.22	89.95	0	0.33
24e	5	1	1.15	415.51	8	55.22	89.95	0	0.16
24f	5	1	-0.13	397.45	6	55.22	89.95	0	0.21
24g	6	1	1.29	479.56	8	64.45	86.76	0	0.48
24h	6	1	1.13	445.54	9	64.45	86.76	0	0.13
24i	6	1	-0.14	427.48	7	64.45	86.76	0	0.16

[a] LogP = Lipophilicity calculated using molinspiration cheminformatics software

[b] TPSA = Topological polar surface area calculated using molinspiration cheminformatics software

[c] % ABS = Percentage absorption calculated using the formula %ABS = 109 - (0.345 x TPSA).

[d] Drug-likeness Score = calculated online using Molsoft.

* nHBA = Number of hydrogen bond acceptors (N & O) present in a molecule

*nHBD = Number of hydrogen bond donors (NH & OH) present in a molecule

2.3.3 Conclusions

In conclusion, a library of sixteen simple *β*-carboline chalcones **23a-g** and bromide salts **24a-i** was prepared in good yields. *In vitro* antitumor activity of newly synthesized *β*-carbolines **23a-g** and **24a-i** was performed against a panel of cancer cell lines and compound **24g** displayed fairly good anticancer activity against all the tested cancer cells with IC_{50} value ranges 15.9 to 22.1 µM. Activity of N^2-benzylated-*β*-carboline chalcones were found to be better than that of N^2-unsubstituted-*β*-carboline chalcones Preliminary mechanism of action studies suggests that **24g** induces apoptosis in breast cancer cells and possess a relatively good drug score of 0.48.

2.3.4 Experimental section

2.3.4.1 General methods and materials

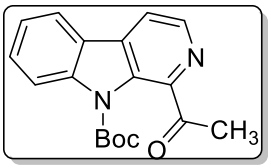
Reagents and solvents used in the synthesis of *β*-carboline chalcones and their bromides were procured commercially and used without further purification, unless otherwise indicated. Progress of the reaction was monitored by thin-layer chromatography (TLC) on pre-coated silica gel 60 F_{254} aluminum sheets (Merck, India), and the spots were visualized under UV light. Melting points (Mp) were recorded on an electro thermal capillary melting point apparatus (*E-Z* melting) and are uncorrected. NMR (^{1}H and ^{13}C) spectra were recorded on a Bruker Advance II at 400 MHz, using deuterated solvents ($CDCl_3$ and DMSO-d_6) and tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (ppm) and *J* values are reported in hertz (Hz). Multiplicities are reported as follows: singlet (s), doublet (d), triplet (t), multiplet (m). IR spectra were recorded on SHIMADZU IRAffinity-1S FT-IR spectrophotometer. HRMS of the prepared β–carboline analogues were recorded using Agilent Technologies 6545 Q-TOF HRMS instrument. The purity of *β*-carboline chalcones derivatives (≥ 97%) was quantified by WATERS 515 HPLC system with a Sunfire C-18 column (5 µm, 4.6 × 250 mm) and PDA detector using a flow rate of 1 mL/min. and a gradient of 0.01% TFA in acetonitrile.

2.3.4.2 General Experimental procedures

Synthesis of 1-acetyl-*β*-carboline (26): To a solution of tryptamine **25** (3 g, 18.75 mmol) in DCM (30 mL), pyruvic aldehyde (2 g, 27.78 mmol) was added at room temperature followed by the slow addition of trifluoro acetic acid (3 mL). After stirring the reaction at room temperature under N_2 atmosphere for 48 h, the mixture was poured into a saturated solution

of sodium hydrogen carbonate. The organic layer was extracted with DCM and dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was aromatized by treatment with 10% Pd/C (0.5 g) in xylene (25 mL) under refluxing conditions for 6 h and then filtered through a bed of celite. The organic solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (ethylacetate/hexane, 3:20) to afford 1-acetyl-*β*-carboline **26** (1.18 g, 30%) as a yellow solid. Mp 205-206°C (Lit. Mp 207–209 °C)[92,93]

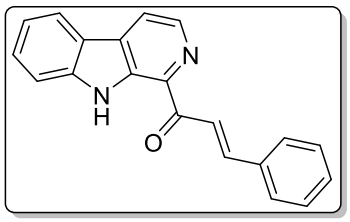
Synthesis of *N*-boc-1-acetyl-*β*-carboline (27): To a solution of 1-acetyl-*β*-carboline **26 (**0.5 g, 2.38 mmol) and DMAP (0.15 g, 1.23 mmol) in dry THF (20 mL), di-*tert*-butyldicarbonate (0.78 g, 3.58 mmol) was added slowly to the reaction mixture. After stirring the reaction mixture for 24 h at room temperature, the contents were taken into water (50 mL) and the product was extracted with ethylacetate (3×20 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethylacetate/hexane, 3:20) to afford *N*-boc protected 1-acetyl-*β*-carboline **27** (0.63 g, 85%) as a brown oily liquid.



Yield: 85%; Brown oily liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, J = 5.0 Hz, 1H), 8.12 (d, J = 8.5 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 5.0 Hz, 1H), 7.66–7.61 (m, 1H), 7.45–7.41 (m, 1H), 2.88 (s, 3H), 1.72 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.6, 154.4, 145.1, 141.4. 138.1, 135.4, 134.2, 129.2, 123.4, 121.8, 121.2, 116.3, 115.6, 85.2, 39.1, 28.2; HRMS (ESI^+) calcd for $C_{18}H_{19}N_2O_3$ $[M + H]^+$, 311.1396; found 311.1392.

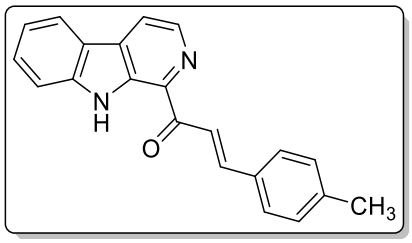
General procedure for the synthesis of *β*-carboline chalcones (23a-g): To a stirred solution of aldehyde (0.322 mmol) in methanol (5 mL) was added 20% aqueous solution of NaOH (5 mL). The resulting mixture was continued to stir at 0 °C for 15 min. and then *N*-Boc protected 1-acetyl-*β*-carboline **27** (0.1 g, 0.322 mmol) was added slowly over 20 min. After completion addition, reaction temperature was raised to room temperature and stirred for 20 h. The precipitates so formed was filtered, washed with water and dried to obtain *β*-carboline chalcones **23a-g** in 70-85% yields as yellow solids.

(E)-3-Phenyl-1-(9*H*-pyrido[3,4-*b*]indol-1-yl)prop-2-en-1-one (23a)



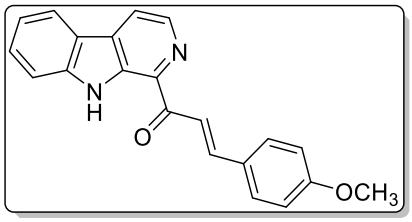
Yield: 80%; Yellow Solid; Mp 170-172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.59 (s, 1H), 8.73–8.52 (m, 2H), 8.26–8.13 (m, 2H), 8.02 (d, *J* = 16.1 Hz, 1H), 7.89–7.76 (m, 2H), 7.63 (d, *J* = 3.7 Hz, 2H), 7.47 (d, *J* = 5.1 Hz, 3H), 7.38–7.34 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 144.0, 141.1, 138.2, 136.5, 136.4, 135.2, 131.5, 130.6, 129.2, 129.0, 128.9, 121.9, 121.1, 120.7, 120.6, 119.0, 112.1; IR (ν, cm^{-1}): 3427, 3051, 3022, 1653, 1597, 1489, 1317, 1124, 1060, 981, 742, 540, 432; Anal. RP-HPLC t_R = 2.683 min, purity 98.25%; HRMS (ESI$^+$) calcd for $C_{20}H_{15}N_2O$ $[M + H]^+$, 299.1184; found 299.1186.

(*E*)-1-(9*H*-Pyrido[3,4-*b*]indol-1-yl)-3-(*p*-tolyl)prop-2-en-1-one (23b)



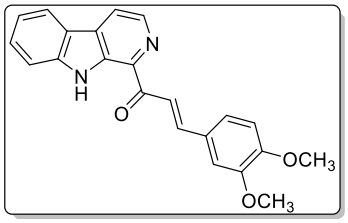
Yield: 83%; Yellow Solid; Mp 176-178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.62 (s, 1H), 8.62 (d, *J* = 4.9 Hz, 1H), 8.57 (d, *J* = 16.0 Hz, 1H), 8.17 (d, *J* = 5.5 Hz, 2H), 8.00 (d, *J* = 16.1 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 3.6 Hz, 2H), 7.38–7.33 (m, 1H), 7.26 (d, *J* = 7.9 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 144.1, 141.1, 141.0, 138.1, 136.6, 136.4, 132.5, 131.5, 129.6, 129.2, 129.0, 121.8, 120.7, 120.6, 120.0, 118.9, 112.0, 21.6; IR (ν, cm^{-1}): 3303, 3032, 2998, 1663, 1563, 1498, 1240, 1164, 1120, 1055, 810, 745, 667, 587, 510; Anal. RP-HPLC t_R = 2.669 min, purity 99.31%; HRMS (ESI$^+$) calcd for $C_{21}H_{17}N_2O$ $[M + H]^+$, 313.1341; found 313.1337.

(*E*)-3-(4-Methoxyphenyl)-1-(9*H*-pyrido[3,4-*b*]indol-1-yl)prop-2-en-1-one (23c)



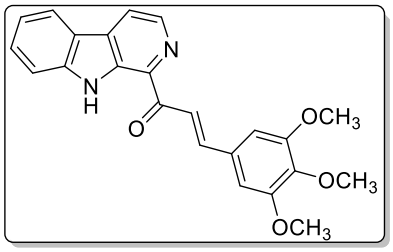
Yield: 85%; Yellow Solid; Mp 163-165 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.61 (s, 1H), 8.63 (d, *J* = 4.9 Hz, 1H), 8.49 (d, *J* = 16.0 Hz, 1H), 8.20 (d, *J* = 5.4 Hz, 2H), 8.00 (d, *J* = 16.0 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.64 (d, *J* = 3.8 Hz, 2H), 7.38–7.34 (m, 1H), 6.99 (d, *J* = 8.8 Hz, 2H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 161.7, 143.9, 141.1, 138.2, 136.7, 136.4, 131.4, 130.8, 129.2, 128.0, 121.9, 120.8, 120.7, 118.8, 118.7, 114.4, 112.0, 55.4; IR (ν, cm^{-1}): 3325, 3041, 2999, 1668, 1568, 1508, 1421, 1253, 1207, 1165, 1130, 1064, 1020, 813, 750, 677, 599, 532, 511; Anal. RP-HPLC t_R = 2.676 min, purity 99.44%; HRMS (ESI$^+$) calcd for $C_{21}H_{17}N_2O_2$ $[M + H]^+$, 329.1290; found 329.1281.

(*E*)-3-(3,4-Dimethoxyphenyl)-1-(9*H*-pyrido[3,4-*b*]indol-1-yl)prop-2-en-1-one (23d)



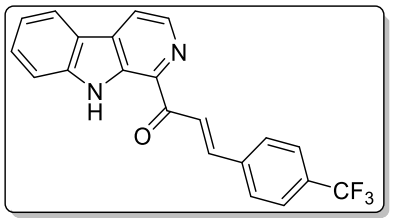
Yield: 78%; Yellow Solid; Mp 171-173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.60 (d, J = 4.9 Hz, 1H), 8.51–8.39 (m, 2H), 8.32 (d, J = 7.8 Hz, 1H), 7.95–7.83 (m, 2H), 7.61 (t, J = 8.1 Hz, 1H), 7.48–7.40 (m, 2H), 7.32 (t, J = 7.5 Hz, 1H), 7.06 (d, J = 8.2 Hz, 1H), 3.89 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 151.8, 149.5, 143.8, 142.2, 138.0, 136.9, 135.4, 131.4, 129.4, 128.1, 123.8, 122.3, 120.7, 120.4, 119.8, 119.5, 113.6, 112.2, 111.3, 56.1, 56.1, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.4; IR (v, cm^{-1}): 3415, 3049, 2994, 2833, 1651, 1575, 1506, 1417, 1253, 1128, 1018, 808, 750, 549, 522; Anal. RP-HPLC t_R = 2.702 min, purity 99.08%; HRMS (ESI$^+$) calcd for $C_{22}H_{19}N_2O_3$ [M+H]$^+$, 359.1396; found 359.1399.

(*E*)-1-(9*H*-Pyrido[3,4-*b*]indol-1-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (23e)



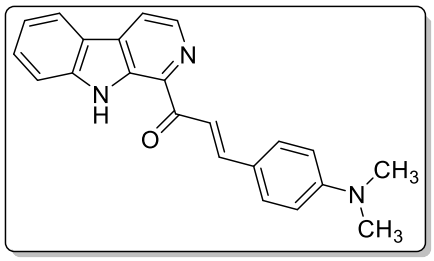
Yield: 75%; Yellow Solid; Mp 167-169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.61 (s, 1H), 8.64 (d, J = 4.9 Hz, 1H), 8.49 (d, J = 15.9 Hz, 1H), 8.24–8.17 (m, 2H), 7.95 (d, J = 16.0 Hz, 1H), 7.65 (d, J = 3.8 Hz, 2H), 7.39–7.35 (m, 1H), 7.04 (s, 2H), 3.99 (s, 6H), 3.95 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5, 153.4, 144.3, 141.1, 140.4, 138.1, 136.5, 136.5, 131.6, 130.7, 129.3, 121.9, 120.8, 120.1, 119.1, 119.0, 112.1, 106.1, 61.0, 56.3; IR (v, cm^{-1}): 3421, 1647, 1577, 1506, 1456, 1419, 1317, 1259, 1130, 987, 821, 740, 611, 432, 422; Anal. RP-HPLC t_R = 2.679 min, purity 98.83%; HRMS (ESI$^+$) calcd for $C_{23}H_{21}N_2O_4$ [M + H]$^+$, 389.1501; found 389.1483

(*E*)-1-(9*H*-Pyrido[3,4-*b*]indol-1-yl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (23f)



Yield: 70%; Yellow Solid; Mp 159-161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.53 (s, 1H), 8.67 (d, J = 16.1 Hz, 1H), 8.63 (d, J = 4.9 Hz, 1H), 8.23–8.17 (m, 2H), 7.99 (d, J = 16.1 Hz, 1H), 7.90 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.67–7.62 (m, 2H), 7.40–7.36 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.4, 141.7, 141.1, 138.6, 138.3, 136.5, 136.2, 132.0, 131.6, 129.4, 128.9, 125.9, 125.8, 125.8, 125.8, 125.3, 123.4, 122.6, 121.9, 120.9, 120.7, 119.3, 112.1; IR (v, cm^{-1}): 3441, 1654, 1600, 1317, 1161, 1107, 1060, 837, 823, 750, 734, 524; Anal. RP-HPLC t_R = 2.707 min, purity 98.54%; HRMS (ESI$^+$) calcd for $C_{21}H_{14}F_3N_2O$ [M + H]$^+$, 367.1058; found 367.1050.

(*E*)-3-(4-(Dimethylamino)phenyl)-1-(9*H*-pyrido[3,4-*b*]indol-1-yl)prop-2-en-1-one (23g)

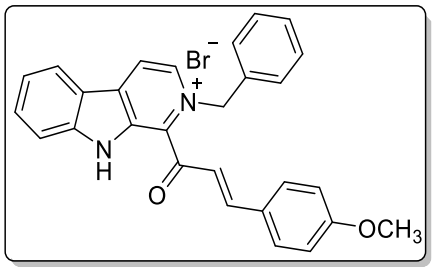


Yield: 72%; Yellow Solid; Mp 156-158 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.68 (s, 1H), 8.63 (d, *J* = 5.0 Hz, 1H), 8.40 (d, *J* = 15.8 Hz, 1H), 8.18 (t, *J* = 6.0 Hz, 2H), 8.02 (d, *J* = 15.8 Hz, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.63 (d, *J* = 3.7 Hz, 2H), 7.37–7.33 (m, 1H), 6.74 (d, *J* = 8.9 Hz, 2H), 3.09 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 191.5, 152.1, 145.2, 141.1, 138.0, 137.2, 136.4, 131.3, 131.0, 129.1, 123.1, 121.8, 120.8, 120.5, 118.5, 115.7, 112.0, 111.8, 40.2; IR (*v*, cm^{-1}): 3417, 2922, 1639, 1558, 1521, 1423, 1363, 1180, 1122, 993, 812, 750, 576, 514; Anal. RP-HPLC t_R = 2.672 min, purity 98.78%; HRMS (ESI^+) calcd for $C_{22}H_{20}N_3O$ $[M + H]^+$, 342.1606; found 342.1593.

General procedure for the synthesis of *β*-carbolinium chalcone bromides (24a-i)

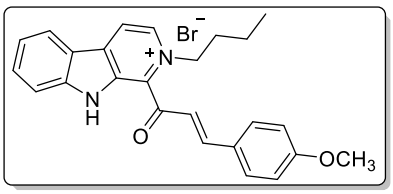
To a stirred solution of *β*-carboline chalcones **23c-e** (0.305 mmol) in *N*,*N*-dimethylformamide (2 mL), the alkylbromide (0.457 mmol) was added at room temperature. The reaction mixture was stirred at 40 °C for 12 h. After completion of the reaction as monitored by TLC, the contents were cooled and diluted with methanol (1 mL). The product was precipitated using excess of diethyl ether (30 mL) and the solid was filtered and dried in oven to obtain pure *β*-carbolinium chalcone bromides **24a-i** in 70-90% yields as brown solids.

(*E*)-2-Benzyl-1-(3-(4-methoxyphenyl)acryloyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24a)



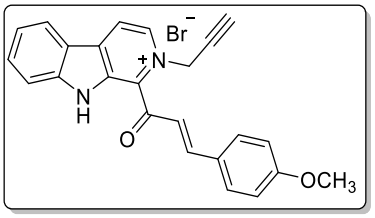
Yield: 82%; Brown Solid; Mp 255-256 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 10.65 (s, 1H), 8.63 (d, *J* = 4.9 Hz, 1H), 8.50 (d, *J* = 16.0 Hz, 1H), 8.21–8.14 (m, 2H), 8.01 (d, *J* = 16.0 Hz, 1H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.63–7.60 (m, 2H), 7.44–7.28 (m, 6H), 6.98 (d, *J* = 8.7 Hz, 2H), 6.31 (s, 2H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 191.6, 161.7, 143.9, 141.1, 138.1, 137.8, 136.7, 136.4, 131.4, 130.7, 129.2, 129.1, 128.8, 128.4, 128.1, 121.8, 120.7, 120.6, 118.8, 118.7, 114.4, 112.1, 61.8, 55.4; IR (*v*, cm^{-1}): 3331, 3052, 2998, 1670, 1560, 1425, 1250, 1170, 1067, 1020, 817, 750, 667, 583; Anal. RP-HPLC t_R = 1.430 min, purity 99.58%; HRMS (ESI^+) calcd for $C_{28}H_{23}N_2O_2^+$ $[M - Br]^+$, 419.1754; found 419.1748.

(*E*)-2-Butyl-1-(3-(4-methoxyphenyl)acryloyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24b)



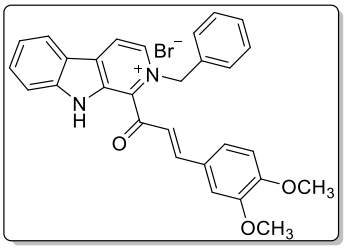
Yield: 87%; Brown Solid; Mp 243-245 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 8.59 (d, J = 4.9 Hz, 1H), 8.47 (d, J = 5.3 Hz, 1H), 8.44 (d, J = 16.1 Hz, 1H), 8.32 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 16.1 Hz, 1H), 7.86 (d, J = 6.5 Hz, 1H), 7.84 (d, J = 7.0 Hz, 2H), 7.64–7.59 (m, 1H), 7.35–7.30 (m, 1H), 7.06 (d, J = 8.8 Hz, 2H), 5.76 (t, J = 6.7 Hz, 2H), 3.84 (s, 3H), 1.80–1.73 (m, 2H), 1.44–1.35 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.2, 161.9, 143.2, 142.2, 138.0, 136.9, 135.4, 131.4, 131.1, 129.4, 127.9, 122.3, 120.7, 120.4, 119.8, 119.3, 115.1, 113.6, 60.3, 55.9, 34.7, 21.2, 13.4; IR (v, cm^{-1}): 3313, 3022, 2988, 1662, 1554, 1426, 1243, 1207, 1156, 1050, 833, 770, 663, 545, 511; Anal. RP-HPLC t_R = 1.405 min, purity 99.82%; HRMS (ESI$^+$) calcd for $C_{25}H_{25}N_2O_2^+$ [M - Br] $^+$, 385.1911; found 385.1912.

(*E*)-1-(3-(4-Methoxyphenyl)acryloyl)-2-(prop-2-yn-1-yl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24c)



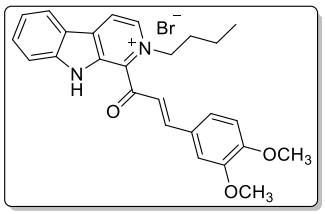
Yield: 83%; Brown Solid; Mp 118-120 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.59 (d, J = 4.8 Hz, 1H), 8.48–8.40 (m, 2H), 8.31 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 16.5 Hz, 1H), 7.86 (d, J = 9.5 Hz, 1H), 7.83 (d, J = 8.6 Hz, 2H), 7.61 (t, J = 7.6 Hz, 1H), 7.31 (t, J = 7.4 Hz, 1H), 7.04 (d, J = 8.4 Hz, 2H), 6.21 (s, 2H), 3.83 (s, 3H), 2.29 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.2, 161.9, 143.2, 142.2, 137.9, 136.9, 135.4, 131.1, 129.3, 128.6, 127.9, 125.8, 122.3, 120.4, 119.8, 119.3, 115.0, 113.6, 80.2, 78.4, 58.3, 55.8; IR (v, cm^{-1}): 3305, 3032, 2984, 2902, 2201, 1660, 1543, 1422, 1250, 1203, 1175, 1060, 813, 760, 672, 601, 540, 512; Anal. RP-HPLC t_R = 1.373 min, purity 99.07%; HRMS (ESI$^+$) calcd for $C_{24}H_{19}N_2O_2^+$ [M - Br] $^+$, 367.1441; found 367.1439.

(*E*)-2-Benzyl-1-(3-(3,4-dimethoxyphenyl)acryloyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24d)



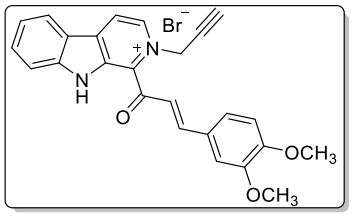
Yield: 90%; Brown Solid; Mp 269-270 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 8.60 (d, J = 4.9 Hz, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.44 (d, J = 16.1 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 16.2 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.47–7.42 (m, 3H), 7.39–7.30 (m, 3H), 7.06 (d, J = 8.2 Hz, 1H), 6.35 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 151.8, 149.5, 143.8, 142.2, 138.5, 137.9, 136.9, 135.4, 131.4, 129.7, 129.4, 129.1, 128.8, 128.1, 123.8, 122.3, 120.7, 120.4, 119.8, 119.4, 113.6, 112.2, 111.3, 62.1, 56.10, 56.0; IR (v, cm^{-1}): 3433, 3051, 2997, 1661, 1595, 1510, 1420, 1377, 1250, 1132, 1090, 809, 750, 670, 555, 512; Anal. RP-HPLC t_R = 1.408 min, purity 99.61%; HRMS (ESI$^+$) calcd for $C_{29}H_{25}N_2O_3^+$ [M - Br] $^+$, 449.1860; found 449.1870.

(*E*)-2-Butyl-1-(3-(3,4-dimethoxyphenyl)acryloyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24e)



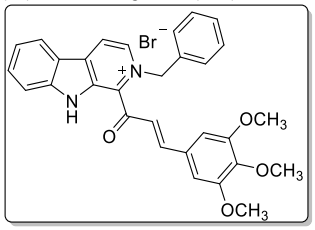
Yield: 79%; Brown Solid; Mp 247-248 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 8.60 (d, J = 4.9 Hz, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.44 (d, J = 16.0 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 16.0 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.45 (d, J = 8.9 Hz, 2H), 7.35 – 7.29 (m, 1H), 7.07 (d, J = 8.2 Hz, 1H), 5.80 (t, J = 6.7 Hz, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 1.81–1.73 (m, 2H), 1.44–1.35 (m, 2H), 0.88 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 151.8, 149.5, 143.7, 142.2, 137.9, 136.9, 135.4, 131.4, 129.3, 128.1, 123.8, 122.3, 120.6, 120.4, 119.8, 119.4, 113.6, 112.2, 111.3, 59.9, 56.1, 56.0, 34.7, 21.2, 13.4; IR (v, cm^{-1}): 3427, 3032, 2970, 1657, 1592, 1513, 1418, 1382, 1309, 1256, 1190, 1133, 1072, 810, 733, 655, 603, 560, 510; Anal. RP-HPLC t_R = 0.888 min, purity 98.73%; HRMS (ESI$^+$) calcd for $C_{26}H_{27}N_2O_3^+$ [M - Br] $^+$, 415.2016; found 415.2027.

(*E*)-1-(3-(3,4-Dimethoxyphenyl)acryloyl)-2-(prop-2-yn-1-yl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24f): Yield: 81%; Brown Solid; Mp 143-144 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ



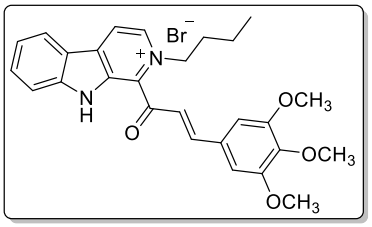
12.07 (s, 1H), 8.60 (d, J = 4.9 Hz, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.44 (d, J = 15.9 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 16.1 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.45 (d, J = 8.5 Hz, 2H), 7.32 (t, J = 7.4 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 5.79 (s, 2H), 3.90 (s, 3H), 3.85 (s, 3H), 2.30 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 151.8, 149.5, 143.8, 142.2, 137.9, 137.8, 136.9, 135.4, 131.5, 128.1, 123.8, 122.3, 120.7, 120.4, 119.8, 119.4, 113.6, 112.19, 111.3, 80.2, 78.4, 57.8, 56.1; IR (v, cm^{-1}): 3437, 3212, 3042, 3002, 2910, 2197, 1672, 1602, 1538, 1459, 1251, 1032, 799, 670, 555, 512; Anal. RP-HPLC t_R = 1.40 min, purity 98.73%; HRMS (ESI$^+$) calcd for $C_{25}H_{21}N_2O_3^+$ [M - Br] $^+$, 397.1547; found 397.1544.

(*E*)-2-Benzyl-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24g)



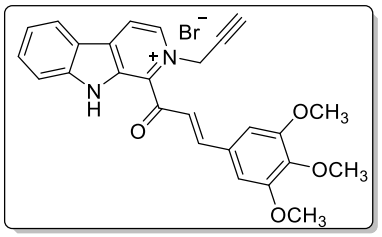
Yield: 83%; Brown Solid; Mp 223-225 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H), 8.62 (d, J = 4.7 Hz, 1H), 8.50 (d, J = 6.8 Hz, 1H), 8.48 (d, J = 3.8 Hz, 1H), 8.33 (d, J = 7.7 Hz, 1H), 7.92 (s, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.66–7.59 (m, 1H), 7.45 (d, J = 7.0 Hz, 2H), 7.38–7.31 (m, 4H), 7.21 (s, 2H), 6.21 (s, 2H), 3.91 (s, 6H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.4, 153.7, 143.7, 142.3, 140.3, 138.5, 138.0, 136.8, 135.5, 132.3, 131.5, 130.8, 129.9, 129.7, 129.4, 129.1, 128.8, 122.3, 121.1, 120.7, 120.4, 119.9, 113.6, 106.7, 60.6, 56.6, 35.0; IR (v, cm^{-1}): 3428, 3055, 2992 1652, 1587, 1522, 1462, 1322, 1263, 1140, 1087, 1003, 807, 722, 667, 533; Anal. RP-HPLC t_R = 1.341 min, purity 99.33%; HRMS (ESI$^+$) calcd for $C_{30}H_{27}N_2O_4^+$ [M - Br] $^+$, 479.1965; found 479.1969.

(*E*)-2-Butyl-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24h)



Yield: 77%; Brown Solid; Mp 196-198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09 (s, 1H), 8.61 (d, J = 3.9 Hz, 1H), 8.50–8.47 (m, 2H), 8.33 (d, J = 7.4 Hz, 1H), 7.94–7.84 (m, 2H), 7.66–7.58 (m, 1H), 7.36–7.29 (m, 1H), 7.21 (s, 2H), 5.83 (t, J = 6.1 Hz, 2H), 3.91 (s, 6H), 3.74 (s, 3H), 1.88–1.67 (m, 2H), 1.48–1.30 (m, 2H), 0.88 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 153.7, 143.7, 142.3, 140.3, 138.0, 136.8, 135.5, 131.5, 130.8, 129.4, 122.3, 121.1, 120.7, 120.4, 119.9, 113.6, 106.7,60.8, 60.6, 59.9, 56.6, 34.7, 21.2, 13.4; IR (ν, cm^{-1}): 3406, 3032, 2997 1658, 1576, 1513, 1475, 1367, 1301, 1243, 1166, 1043, 998, 798, 703, 655, 522; Anal. RP-HPLC t_R = 1.429 min, purity 98.71%; HRMS (ESI$^+$) calcd for $C_{27}H_{29}N_2O_4^+$ [M - Br] $^+$, 445.2122; found 445.2116.

(*E*)-2-(Prop-2-yn-1-yl)-1-(3-(3,4,5-trimethoxyphenyl)acryloyl)-9*H*-pyrido[3,4-*b*]indol-2-ium bromide (24i): Yield: 70%; Brown Solid; Mp 138-140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ



12.08 (s, 1H), 8.65–8.58 (m, 1H), 8.50–8.46 (m, 2H), 8.33 (d, J = 7.2 Hz, 1H), 7.94–7.82 (m, 2H), 7.66–7.58 (m, 1H), 7.35–7.30 (m, 1H), 7.20 (s, 2H), 5.88 (s, 2H), 3.90 (s, 6H), 3.74 (s, 3H), 2.30 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.3, 153.7, 143.7, 142.3, 140.3, 138.0, 136.8, 135.5, 131.5, 130.8, 129.4, 122.3, 121.1, 120.7, 120.4, 119.9, 113.6, 106.7, 80.2, 78.4, 60.6, 58.1, 56.6; IR (ν, cm^{-1}): 3428, 3226, 3013, 2983 2210, 1663, 1547, 1423, 1326, 1140, 1087, 998, 810, 702, 643, 518, 443; Anal. RP-HPLC t_R = 1.377 min, purity 98.92%; HRMS (ESI$^+$) calcd for $C_{26}H_{23}N_2O_4^+$ [M - Br] $^+$, 427.1652; found 427.1649.

MTT assay

While HeLa, MDA-MB-231, PC-3, C4-2 and BxPC3 cancer cells were grown in RPMI 1640 media, HEK293T cells were grown in Dulbecco's modified Eagle's media (DMEM) media at 37 ºC. These media were supplemented with 10% fetal bovine serum and penicillin and streptomycin. For MTT assay, 4 x 10^3 cells were seeded per well in 96-well plates. After 12 h, the cells were treated with various concentrations of compounds ranging from 0.1 µM - 1 mM. The control cells were treated with 0.1% DMSO (vehicle control). The cells were assayed after 48 h by adding 10 µL of 5 mg/mL MTT, followed by incubation at 37 °C for 4 h. The MTT containing media was aspirated and the formazan crystals were dissolved using 100µL of DMSO. The optical density (OD) was measured at 570 nm using Tecan

Spectrafluor Plus. The percentage inhibition was calculated as = 100 - [(Mean OD of treated cell X 100)/mean OD of vehicle treated cells (negative control)]. The dose response curve and IC_{50} values were obtained by nonlinear regression analysis [non-linear regression (sigmoidal dose response with variable slope)] using Graph Pad Prism, version 5.02 software (Graph Pad Software Inc., CA, USA).

Acridine orange staining

To check the plasma-membrane permeability, nuclear morphology and the chromatin condensation, the cells were stained with Acridine orange (AO) / Ethidium bromide (EB) dye mixture (100 µg/mL AO and 100 µg/mL EB). AO permeates all cells and makes the nucleus appear green. In contrast, EB is taken up by the cells only when the cytoplasmic membrane integrity is lost (as in late apoptosis or in necrosis), which stains the nucleus red. Briefly, cells were seeded on cover slips in 24-well plates at seeding densities of 0.5×10^5 cells and then treated with either doxorubicin or compound **24g** for 24 h. After washing once with PBS, the cells were stained with 500 µL AO/EB (1:1) dye mixture dissolved in PBS solution. The cells were immediately washed with PBS, viewed under a Nikon inverted fluorescent microscope attached with a camera, and photographs were taken under fluorescent conditions.

2.4 References

1. Cao, R.; Peng, W.; Wang, Z.; Xu, A., *β*-Carboline alkaloids: biochemical and pharmacological functions. *Curr. Med. Chem.,* **2007,** *14*, 479-500.
2. Laine, A. E.; Lood, C.; Koskinen, A. M., Pharmacological importance of optically active tetrahydro-*β*-carbolines and synthetic approaches to create the C-1 stereocenter. *Molecules,* **2014,** *19*, 1544-1567.
3. Lunagariya, N.; Gohil, V.; Kushwah, V.; Neelagiri, S.; Jain, S.; Singh, S.; Bhutani, K. K., Synthesis and biological evaluation of 1, 3, 6-trisubstituted *β*-carboline derivatives for cytotoxic and anti-leishmanial potential. *Planta Med.,* **2015,** *81*, PM_84.
4. Olmedo, G. M.; Cerioni, L.; González, M. M.; Cabrerizo, F. M.; Rapisarda, V. A.; Volentini, S. I., Antifungal activity of *β*-carbolines on *Penicillium digitatum* and *Botrytis cinerea*. *Food Microbiol.,* **2017,** *62*, 9-14.
5. Quintana, V. M.; Piccini, L. E.; Zénere, J. D. P.; Damonte, E. B.; Ponce, M. A.; Castilla, V., Antiviral activity of natural and synthetic *β*-carbolines against dengue virus. *Antivir. Res.,* **2016,** *134*, 26-33.
6. Lunagariya, N. A.; Gohil, V. M.; Kushwah, V.; Neelagiri, S.; Jain, S.; Singh, S.; Bhutani, K. K., Design, synthesis and biological evaluation of 1,3,6-trisubstituted *β*-carboline derivatives for cytotoxic and anti-leishmanial potential. *Bioorganic Med. Chem. Lett*, **2016,** *26*, 789-794.
7. Da Silva, C. M.; Silva, M. M.; Reis, F. S.; Ruiz, A. L. T. G.; de Carvalho, J. E.; Santos, J. C. C.; Figueiredo, I. M.; Alves, R. B.; Modolo, L. V.; de Fátima, Â., Studies on free radical scavenging, cancer cell antiproliferation, and calf thymus DNA interaction of schiff bases. *J. Photochem. Photobiol. B. Biol.,* **2017,** *172*, 129-138.
8. Kumar, S.; Singh, A.; Kumar, K.; Kumar, V., Recent insights into synthetic *β*-carbolines with anticancer activities. *Eur. J. Med. Chem.,* **2017,** *142*, 48-73.
9. Zhang, J.; Li, L.; Dan, W.; Li, J.; Zhang, Q.; Bai, H.; Wang, J., Synthesis and antimicrobial activities of 3-methyl-*β*-carboline derivatives. *Nat. Prod. Commun.,* **2015,** *10*, 899-902.
10. Samundeeswari, S.; Chougala, B.; Holiyachi, M.; Shastri, L.; Kulkarni, M.; Dodamani, S.; Jalalpur, S.; Joshi, S.; Dixit, S.; Sunagar, V.; Hunnur, R., Design and synthesis of novel phenyl-1,4-*β*-carboline-hybrid molecules as potential anticancer agents. *Eur. J. Med. Chem.,* **2017,** *128*, 123-139.
11. Zhang, M.; Sun, D., Recent advances of natural and synthetic *β*-carbolines as anticancer agents. *Anticancer Agents Med. Chem.,* **2015,** *15*, 537-547.

12. Cao, M.-R.; Li, Q.; Liu, Z.-L.; Liu, H.-H.; Wang, W.; Liao, X.-L.; Pan, Y.-L.; Jiang, J.-W., Harmine induces apoptosis in HepG2 cells *via* mitochondrial signaling pathway. *Hepatobiliary Pancreat. Dis. Int.,* **2011,** *10*, 599-604.
13. Cao, R.; Peng, W.; Chen, H.; Ma, Y.; Liu, X.; Hou, X.; Guan, H.; Xu, A., DNA binding properties of 9-substituted harmine derivatives. *Biochem. Biophys. Res. Commun.,* **2005,** *338*, 1557-1563.
14. Vignoni, M.; Erra-Balsells, R.; Epe, B.; Cabrerizo, F. M., Intra- and extra-cellular DNA damage by harmine and 9-methyl-harmine. *J. Photochem. Photobiol. B, Biol.,* **2014,** *132*, 66-71.
15. Patel, K.; Gadewar, M.; Tripathi, R.; Prasad, S. K.; Patel, D. K., A review on medicinal importance, pharmacological activity and bioanalytical aspects of *β*-carboline alkaloid "harmine". *Asian Pac. J. Trop. Biomed.,* **2012,** *2*, 660-664.
16. Al-Shamma, A.; Drake, S.; Flynn, D. L.; Mitscher, L. A.; Park, Y. H.; Rao, G. S. R.; Simpson, A.; Swayze, J. K.; Veysoglu, T.; Wu, S. T. S., Antimicrobial agents from higher plants. Antimicrobial agents from *Peganum harmala* Seeds. *J. Nat. Prod.,* **1981,** *44*, 745-747.
17. Song, Y.; Kesuma, D.; Wang, J.; Deng, Y.; Duan, J.; Wang, J. H.; Qi, R. Z., Specific inhibition of cyclin-dependent kinases and cell proliferation by harmine. *Biochem. Biophys. Res. Commun.,* **2004,** *317*, 128-132.
18. Patel, K.; Gadewar, M.; Tripathi, R.; Prasad, S. K.; Patel, D. K., A review on medicinal importance, pharmacological activity and bioanalytical aspects of *β*-carboline alkaloid "harmine". *Asian Pac. J. Trop. Biomed.,* **2012,** *2*, 660-664.
19. Song, Y.; Kesuma, D.; Wang, J.; Deng, Y.; Duan, J.; Wang, J. H.; Qi, R. Z., Specific inhibition of cyclin-dependent kinases and cell proliferation by harmine. *Biochem. Biophys. Res. Commun.,* **2004,** *317*, 128-132.
20. Cao, M.-R.; Li, Q.; Liu, Z.-L.; Liu, H.-H.; Wang, W.; Liao, X.-L.; Pan, Y.-L.; Jiang, J.-W., Harmine induces apoptosis in HepG2 cells *via* mitochondrial signaling pathway. *Hepatobiliary Pancreat. Dis. Int.,* **2011,** *10*, 599-604.
21. Vignoni, M.; Erra-Balsells, R.; Epe, B.; Cabrerizo, F. M., Intra- and extra-cellular DNA damage by harmine and 9-methylharmine. *J. Photochem. Photobiol. B, Biol.,* **2014,** *132*, 66-71.
22. Hörmann, A.; Chaudhuri, B.; Fretz, H., DNA binding properties of the marine sponge pigment fascaplysin. *Bioorganic Med. Chem.,* **2001,** *9*, 917-921.
23. Segraves, N. L.; Robinson, S. J.; Garcia, D.; Said, S. A.; Fu, X.; Schmitz, F. J.; Pietraszkiewicz, H.; Valeriote, F. A.; Crews, P., Comparison of fascaplysin and related alkaloids: A study of structures, cytotoxicities, and sources. *J. Nat. Prod.,* **2004,** *67*, 783-792.

24. Kobayashi, M.; Chen, Y.-J.; Aoki, S.; In, Y.; Ishida, T.; Kitagawa, I., Four new *β*-carboline alkaloids isolated from two Okinawan marine sponges of *Xestospongia sp*. and *Haliclona sp*. *Tetrahedron,* **1995,** *51,* 3727-3736.
25. VanWagenen, B. C.; Cardellina, J. H., Short, efficient syntheses of the antibiotic eudistomins I and T. *Tetrahedron Lett.,* **1989,** *30,* 3605-3608.
26. Kinzer, K. F.; Cardellina, J. H., Three new *β*-carbolines from the bermudian tunicate eudistoma olivaceum. *Tetrahedron Lett.,* **1987,** *28,* 925-926.
27. McNulty, J.; Still, I. W. J., Synthetic approaches to the eudistomin marine alkaloids. *Curr. Org. Chem.,* **2000,** *4,* 121-138.
28. Poindexter, E. H.; Carpenter, R. D., The isolation of harmane and norharmane from tobacco and cigarette smoke. *Phytochemistry,* **1962,** *1,* 215-221.
29. Cao, R.; Chen, H.; Peng, W.; Ma, Y.; Hou, X.; Guan, H.; Liu, X.; Xu, A., Design, synthesis and *in-vitro* and *in-vivo* antitumor activities of novel *β*-carboline derivatives. *Eur. J. Med. Chem.,* **2005,** *40,* 991-1001.
30. Kodani, S.; Imoto, A.; Mitsutani, A.; Murakami, M., Isolation and identification of the antialgal compound, harmane (1-methyl-*β*-carboline), produced by the algicidal bacterium, *Pseudomonas sp*. K44-1. *J. Appl. Phycol.,* **2002,** *14,* 109-114.
31. Cao, R.; Fan, W.; Guo, L.; Ma, Q.; Zhang, G.; Li, J.; Chen, X.; Ren, Z.; Qiu, L., Synthesis and structure–activity relationships of harmine derivatives as potential antitumor agents. *Eur. J. Med. Chem.,* **2013,** *60,* 135-143.
32. Zhang, G.; Cao, R.; Guo, L.; Ma, Q.; Fan, W.; Chen, X.; Li, J.; Shao, G.; Qiu, L.; Ren, Z., Synthesis and structure–activity relationships of N^2-alkylated quaternary *β*-carbolines as novel antitumor agents. *Eur. J. Med. Chem.,* **2013,** *65,* 21-31.
33. Cao, R.; Yi, W.; Wu, Q.; Guan, X.; Feng, M.; Ma, C.; Chen, Z.; Song, H.; Peng, W., Synthesis and cytotoxic activities of 1-benzylidine substituted *β*-carboline derivatives. *Bioorganic Med. Chem. Lett.,* **2008,** *18,* 6558-6561.
34. Cao, R.; Fan, W.; Guo, L.; Ma, Q.; Zhang, G.; Li, J.; Chen, X.; Ren, Z.; Qiu, L., Synthesis and structure–activity relationships of harmine derivatives as potential antitumor agents. *Eur. J. Med. Chem.,* **2013,** *60,* 135-143.
35. Zhang, G.; Cao, R.; Guo, L.; Ma, Q.; Fan, W.; Chen, X.; Li, J.; Shao, G.; Qiu, L.; Ren, Z., Synthesis and structure–activity relationships of N^2-alkylated quaternary *β*-carbolines as novel antitumor agents. *Eur. J. Med. Chem.,* **2013,** *65,* 21-31.
36. Rihui, C.; Wenlie, P.; Zihou, W.; Anlong, X., *β*-Carboline alkaloids: Biochemical and pharmacological functions. *Curr. Med. Chem.,* **2007,** *14,* 479-500.

37. Zhang, M.; Sun, D., Recent advances of natural and synthetic *β*-carbolines as anticancer agents. *Anticancer Agents Med. Chem.,* **2015,** *15*, 537-547.

38. Du, H.; Gu, H.; Li, N.; Wang, J., Synthesis and biological evaluation of bivalent *β*-carbolines as potential anticancer agents. *MedChemComm,* **2016,** *7*, 636-645.

39. Chauhan, S. S.; Singh, A. K.; Meena, S.; Lohani, M.; Singh, A.; Arya, R. K.; Cheruvu, S. H.; Sarkar, J.; Gayen, J. R.; Datta, D.; Chauhan, P. M. S., Synthesis of novel *β*-carboline based chalcones with high cytotoxic activity against breast cancer cells. *Bioorganic Med. Chem. Lett.,* **2014,** *24*, 2820-2824.

40. Frédérick, R.; Bruyère, C.; Vancraeynest, C.; Reniers, J.; Meinguet, C.; Pochet, L.; Backlund, A.; Masereel, B.; Kiss, R.; Wouters, J., Novel trisubstituted harmine derivatives with original *in-vitro* anticancer activity. *J. Med. Chem.,* **2012,** *55*, 6489-6501.

41. Chipon, B.; Clavé, G.; Bouteiller, C.; Massonneau, M.; Renard, P.-Y.; Romieu, A., Synthesis and post-synthetic derivatization of a cyanine-based amino acid. Application to the preparation of a novel water-soluble NIR dye. *Tetrahedron Lett.,* **2006,** *47*, 8279-8284.

42. Molina, P.; Tarraga, A.; Oton, F., Imidazole derivatives: A comprehensive survey of their recognition properties. *Org. Biomol. Chem.,* **2012,** *10*, 1711-1724.

43. Sun, C.-J.; Chen, W.; Li, Y.; Liu, L.-X.; Wang, X.-Q.; Li, L.-J.; Zhang, H.-B.; Yang, X.-D., Design, synthesis and antitumor activity of novel 8-substituted 2,3,5,6-tetrahydrobenzo[1,2-*b*:4,5-*b′*]difuran imidazolium salt derivatives. *RSC Adv.,* **2014,** *4*, 16312-16319.

44. D. Threadgill, M., 5-Aminoisoquinolin-1-one (5-AIQ), a Water-soluble inhibitor of the poly(ADP-Ribose)polymerases (PARPs). *Curr. Med. Chem.,* **2015,** *22*, 3807-3829.

45. Yang, X.-D.; Zeng, X.-H.; Zhang, Y.-L.; Qing, C.; Song, W.-J.; Li, L.; Zhang, H.-B., Synthesis and cytotoxic activities of novel phenacylimidazolium bromides. *Bioorganic Med. Chem. Lett.,* **2009,** *19*, 1892-1895.

46. Xu, X.-L.; Wang, J.; Yu, C.-L.; Chen, W.; Li, Y.-C.; Li, Y.; Zhang, H.-B.; Yang, X.-D., Synthesis and cytotoxic activity of novel 1-((indol-3-yl)methyl)–1*H*-imidazolium salts. *Bioorganic Med. Chem. Lett.,* **2014,** *24*, 4926-4930.

47. Zeng, X.; Yang, X.; Zhang, Y.; Qing, C.; Zhang, H., Synthesis and antitumor activity of 1-mesityl-3-(2-naphthoylmethano)-1*H*-imidazolium bromide. *Bioorganic Med. Chem. Lett.,* **2010,** *20*, 1844-1847.

48. Das Mukherjee, D.; Kumar, N. M.; Tantak, M. P.; Das, A.; Ganguli, A.; Datta, S.; Kumar, D.; Chakrabarti, G., Development of novel bis(indolyl)-hydrazide–hydrazone derivatives as potent microtubule-targeting cytotoxic agents against A549 lung cancer cells. *Biochemistry*, **2016,** *55*, 3020-3035.

49. Tantak, M. P.; Kumar, A.; Noel, B.; Shah, K.; Kumar, D., Synthesis and biological evaluation of 2-arylamino-5-(3′-indolyl)-1, 3, 4-oxadiazoles as potent cytotoxic agents. *ChemMedChem,* **2013,** *8*, 1468-1474.
50. Venkataramana Reddy, P. O.; Tantak, M. P.; Valdez, R.; Singh, R. P.; Singh, O. M.; Sadana, R.; Kumar, D., Synthesis and biological evaluation of novel carbazolyl glyoxamides as anticancer and antibacterial agents. *RSC Adv.,* **2016,** *6*, 9379-9386.
51. Kamal, A.; Tangella, Y.; Manasa, K. L.; Sathish, M.; Srinivasulu, V.; Chetna, J.; Alarifi, A., $PhI(OAc)_2$-mediated one-pot oxidative decarboxylation and aromatization of tetrahydro-*β*-carbolines: synthesis of norharmane, harmane, eudistomin U and eudistomin I. *Org. Biomol. Chem.,* **2015,** *13*, 8652-8662.
52. Wolf, L.; Quoos, N.; Mayer, J. C. P.; de Souza, D.; Sauer, A. C.; Meichtry, L.; Bortolotto, V.; Prigol, M.; Rodrigues, O. E. D.; Dornelles, L., Synthesis and free radical scavenging activity of 2-alkyl/arylchalcogenyl-*N*-(4-aryl-1,3-thiazol-2-yl) acetamide compounds. *Tetrahedron Lett.*, **2016,** *57*, 1031-1034.
53. Kappe, C. O.; Pieber, B.; Dallinger, D., Microwave effects in organic synthesis: Myth or reality? *Angew. Chem. Int. Ed.* **2013,** *52*, 1088-1094.
54. Bana, P.; Greiner, I., Comparison of conventional and microwave heating for evaluation of microwave effects. *Aust. J. Chem.,* **2016,** *69*, 865-871.
55. Wienecke, A.; Bacher, G., Indibulin, a novel microtubule inhibitor, discriminates between mature neuronal and nonneuronal tubulin. *Cancer Res.,* **2009,** *69*, 171-177.
56. Bacher, G.; Beckers, T.; Emig, P.; Klenner, T.; Kutscher, B.; Nickel, B., New small-molecule tubulin inhibitors. *Pure. Appl. Chem.,* **2001,** *73*, 1459-1464.
57. Pitrak, D. L.; Tsai, H. C.; Mullane, K. M.; Sutton, S. H.; Stevens, P., Accelerated neutrophil apoptosis in the acquired immunodeficiency syndrome. *J. Clin. Invest.,* **1996,** *98*, 2714-2719.
58. Patil, S. A.; Patil, R.; Miller, D. D., Indole molecules as inhibitors of tubulin polymerization: potential new anticancer agents. *Future Med. Chem.,* **2012,** *4*, 2085-2115.
59. Kusumaningrum Susi, B. E., Kosela Soleh, Sumaryono Wahono, Juniarti Fifit., The molecular docking of 1,4-naphthoquinone derivatives as inhibitors of polo-like kinase 1 using molegro virtual docker. *J. App. Pharm. Sci.,* **2014,** *4*, 47-53.
60. Ravelli, R. B.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M., Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain. *Nature,* **2004,** *428*, 198-202.
61. Shankaraiah, N.; Nekkanti, S.; Chudasama, K. J.; Senwar, K. R.; Sharma, P.; Jeengar, M. K.; Naidu, V. G. M.; Srinivasulu, V.; Srinivasulu, G.; Kamal, A., Design, synthesis and anticancer evaluation of tetrahydro-*β*-carboline-hydantoin hybrids. *Bioorganic Med. Chem. Lett.,* **2014,** *24*, 5413-5417.

62. Yin, W.; Majumder, S.; Clayton, T.; Petrou, S.; VanLinn, M. L.; Namjoshi, O. A.; Ma, C.; Cromer, B. A.; Roth, B. L.; Platt, D. M.; Cook, J. M., Design, synthesis, and subtype selectivity of 3,6-disubstituted *β*-carbolines at Bz/GABA(A) ergic receptors. SAR and studies directed toward agents for treatment of alcohol abuse. *Bioorganic Med. Chem.,* **2010,** *18*, 7548-7564.

63. Ikeda, R.; Iwaki, T.; Iida, T.; Okabayashi, T.; Nishi, E.; Kurosawa, M.; Sakai, N.; Konakahara, T., 3-Benzylamino-*β*-carboline derivatives induce apoptosis through G2/M arrest in human carcinoma cells HeLa S-3. *Eur. J. Med. Chem.,* **2011,** *46*, 636-646.

64. Drung, B.; Scholz, C.; Barbosa, V. A.; Nazari, A.; Sarragiotto, M. H.; Schmidt, B., Computational & experimental evaluation of the structure/activity relationship of *β*-carbolines as DYRK1A inhibitors. *Bioorganic Med. Chem. Lett.,* **2014,** *24*, 4854-4860.

65. Kamal, A.; Tangella, Y.; Manasa, K. L.; Sathish, M.; Srinivasulu, V.; Chetna, J.; Alarifi, A., $PhI(OAc)_2$-mediated one-pot oxidative decarboxylation and aromatization of tetrahydro-*β*-carbolines: synthesis of norharmane, harmane, eudistomin U and eudistomin I. *Org. Biomol. Chem.,* **2015,** *13*, 8652-8662.

66. Chen, Z.; Cao, R.; Shi, B.; Guo, L.; Sun, J.; Ma, Q.; Fan, W.; Song, H., Synthesis and biological evaluation of 1,9-disubstituted *β*-carbolines as potent DNA intercalating and cytotoxic agents. *Eur. J. Med. Chem.,* **2011,** *46*, 5127-5137.

67. Zhang, G.; Cao, R.; Guo, L.; Ma, Q.; Fan, W.; Chen, X.; Li, J.; Shao, G.; Qiu, L.; Ren, Z., Synthesis and structure–activity relationships of N^2-alkylated quaternary *β*-carbolines as novel antitumor agents. *Eur. J. Med. Chem.,* **2013,** *65*, 21-31.

68. Cao, R.; Chen, Q.; Hou, X.; Chen, H.; Guan, H.; Ma, Y.; Peng, W.; Xu, A., Synthesis, acute toxicities, and antitumor effects of novel 9-substituted *β*-carboline derivatives. *Bioorganic Med. Chem.,* **2004,** *12*, 4613-4623.

69. Wolf, L.; Quoos, N.; Mayer, J. C. P.; de Souza, D.; Sauer, A. C.; Meichtry, L.; Bortolotto, V.; Prigol, M.; Rodrigues, O. E. D.; Dornelles, L., Synthesis and free radical scavenging activity of 2-alkyl/arylchalcogenyl-*N*-(4-aryl-1,3-thiazol-2-yl)acetamide compounds. *Tetrahedron Lett.,* **2016,** *57*, 1031-1034.

70. Funk, D.; Schrenk, H.-H.; Frei, E., German Cancer Research Center, Heidelberg, Germany BioTechniques, *BioTechniques,* **2007,** *43*, 178-186.

71. Zhuang, C.; Zhang, W.; Sheng, C.; Zhang, W.; Xing, C.; Miao, Z., European neuropsychopharmacology chalcone: A privileged structure in medicinal chemistry. *Chem. Rev.,* **2017,** *117*, 7762-7810.

72. Singh, P.; Anand, A.; Kumar, V., Recent developments in biological activities of chalcones: A mini review. *Eur. J. Med. Chem.,* **2014,** *85*, 758-777.

73. Ciupa, A.; De Bank, P. A.; Mahon, M. F.; Wood, P. J.; Caggiano, L., Synthesis and antiproliferative activity of some 3-(pyrid-2-yl)-pyrazolines. *MedChemComm*, **2013,** *4*, 956-961.

74. Tsukiyama, R.-I.; Katsura, H.; Tokuriki, N.; Kobayashi, M., Antibacterial activity of licochalcone A against spore-forming bacteria. *Antimicrob. Agents Chemother.*, **2002,** *46*, 1226-1230.

75. Chauhan, S. S.; Singh, A. K.; Meena, S.; Lohani, M.; Singh, A.; Arya, R. K.; Cheruvu, S. H.; Sarkar, J.; Gayen, J. R.; Datta, D.; Chauhan, P. M. S., Synthesis of novel *β*-carboline based chalcones with high cytotoxic activity against breast cancer cells. *Bioorganic Med. Chem. Lett*, **2014,** *24*, 2820-2824.

76. Kumar, D.; Kumar, N. M.; Akamatsu, K.; Kusaka, E.; Harada, H.; Ito, T., Synthesis and biological evaluation of indolyl chalcones as antitumor agents. *Bioorganic Med. Chem. Lett.*, **2010,** *20*, 3916-3919.

77. Venkataramana Reddy, P. O.; Mishra, S.; Tantak, M. P.; Nikhil, K.; Sadana, R.; Shah, K.; Kumar, D., Design, synthesis and *in-vitro* cytotoxicity studies of novel *β*-carbolinium bromides. *Bioorganic Med. Chem. Lett*, **2017,** *27*, 1379-1384.

78. Tantak, M. P.; Das Mukherjee, D.; Kumar, A.; Chakrabarti, G.; Kumar, D., A facile and MW-assisted rapid synthesis of 2-arylamino-4-(3′-indolyl)-thiazoles as apoptosis inducing cytotoxic agents. *Anticancer Agents Med. Chem.*, **2017,** *17*, 442-455.

79. Tantak, M. P.; Klingler, L.; Arun, V.; Kumar, A.; Sadana, R.; Kumar, D., Design and synthesis of bis(indolyl)ketohydrazide-hydrazones: Identification of potent and selective novel tubulin inhibitors. *Eur. J. Med. Chem.*, **2017,** *136*, 184-194.

80. Venkataramana Reddy, P. O.; Tantak, M. P.; Valdez, R.; Singh, R. P.; Singh, O. M.; Sadana, R.; Kumar, D., Synthesis and biological evaluation of novel carbazolyl glyoxamides as anticancer and antibacterial agents. *RSC Adv.*, **2016,** *6*, 9379-9386.

81. Das Mukherjee, D.; Kumar, N. M.; Tantak, M. P.; Das, A.; Ganguli, A.; Datta, S.; Kumar, D.; Chakrabarti, G., Development of novel bis(indolyl)-hydrazide–hydrazone derivatives as potent microtubule-targeting cytotoxic agents against A549 lung cancer cells. *Biochemistry*, **2016,** *55*, 3020-3035.

82. Vaddula, B. R.; Tantak, M. P.; Sadana, R.; Gonzalez, M. A.; Kumar, D., One-pot synthesis and *in-vitro* anticancer evaluation of 5-(2′-indolyl)thiazoles. *Sci. Rep.*, **2016,** *6*, 23401.

83. Nemet, I.; Varga-Defterdarović, L., The role of methylglyoxal in the non-enzymatic conversion of tryptophan, its methyl ester and tryptamine to 1-acetyl-*β*-carbolines. *Bioorganic Med. Chem.*, **2008,** *16*, 4551-4562.

84. Nemet, I.; Varga-Defterdarović, L., Methylglyoxal-derived *β*-carbolines formed from tryptophan and its derivates in the Maillard reaction. *Amino Acids*, **2007,** *32*, 291-293.

85. Skouta, R.; Hayano, M.; Shimada, K.; Stockwell, B. R., Design and synthesis of Pictet–Spengler condensation products that exhibit oncogenic-RAS synthetic lethality and induce non-apoptotic cell death. *Bioorganic Med. Chem. Lett.,* **2012,** *22*, 5707-5713.

86. Kasibhatla, S.; Amarante-Mendes, G. P.; Finucane, D.; Brunner, T.; Bossy-Wetzel, E.; Green, D. R., Acridine orange/Ethidium bromide (AO/EB) staining to detect apoptosis. *Cold Spring Harb. Protoc.*, **2006,** *2006*, pdb.prot4493.

87. Ribble, D.; Goldstein, N. B.; Norris, D. A.; Shellman, Y. G., A simple technique for quantifying apoptosis in 96-well plates. *BMC Biotechnol.,* **2005,** *5*, 12.

88. Cohen, J. J., Apoptosis. *Immunol. Today*, **1993,** *14*, 126-130.

89. Tibbitts, J.; Canter, D.; Graff, R.; Smith, A.; Khawli, L. A., Key factors influencing ADME properties of therapeutic proteins: A need for ADME characterization in drug discovery and development. *MAbs,* **2016,** *8*, 229-245.

90. Roller, S. G., Chapter 8 ADME properties of peptide therapeutics in drug discovery and development. In *Peptide-based Drug Discovery: Challenges and New Therapeutics*, The Royal Society of Chemistry: 2017; pp 223-251.

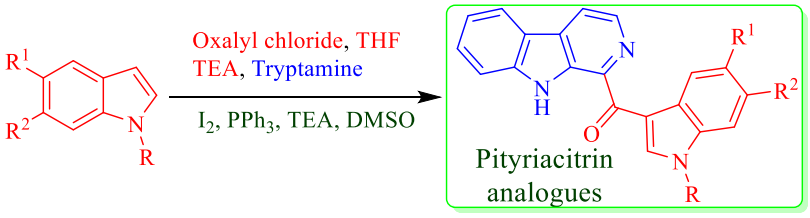
91. Zhao, Y. H.; Abraham, M. H.; Le, J.; Hersey, A.; Luscombe, C. N.; Beck, G.; Sherborne, B.; Cooper, I., Rate-limited steps of human oral absorption and QSAR studies. *Pharm. Res.*, **2002,** *19*, 1446-1457.

92. Yang, M.-L.; Kuo, P.-C.; Damu, A. G.; Chang, R.-J.; Chiou, W.-F.; Wu, T.-S., A versatile route to the synthesis of 1-substituted *β*-carbolines by a single step Pictet–Spengler cyclization. *Tetrahedron*, **2006,** *62*, 10900-10906.

93. Yang, M.-L.; Kuo, P.-C.; Hwang, T.-L.; Chiou, W.-F.; Qian, K.; Lai, C.-Y.; Lee, K.-H.; Wu, T.-S., Synthesis, *in-vitro* anti-inflammatory and cytotoxic evaluation, and mechanism of action studies of 1-benzoyl-*β*-carboline and 1-benzoyl-3-carboxy-*β*-carboline derivatives. *Bioorganic Med. Chem.,* **2011,** *19*, 1674-1682.

Chapter 3

Facile and Efficient Synthesis of Naturally Occurring *β*-Carbolines



Facile and Efficient Synthesis of Naturally Occurring *β*-Carbolines

3.1 Introduction

Marine natural products put forward an abundant source of pharmacologically active agents with enormous diversity and complexity, and the potential to produce valuable therapeutic entities.[1,2] Especially, indole alkaloids is one of the significant class of marine-derived secondary metabolites, with wide occurrence amongst variety of marine sources such as sponges, tunicates, algae, worms and microorganisms. They have been extensively investigated for their biological properties.[3] Among this chemical family, *β*-carboline alkaloids (**1-4**) exhibited a broad range of bioactivities including antimicrobial, antiviral, anti-HIV, p56 tyrosine kinase inhibition, antimalarial, anti-angiogenic and antiproliferative (Figure 3.1).[4]

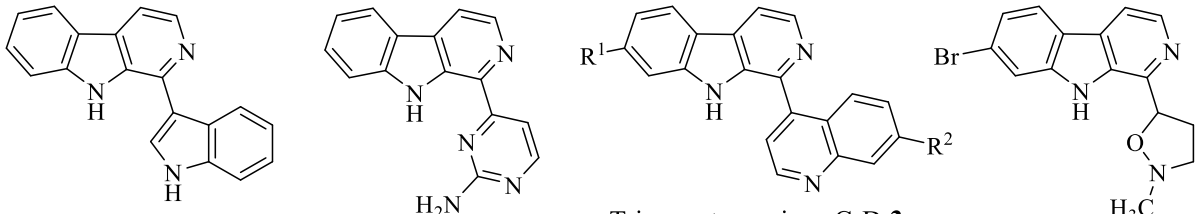


Figure 3.1 Marine natural products with *β*-carboline scaffold

Relatively rare subclass of *β*-carboline alkaloids containing a 1-acyl-linked aryl/heterocycle are well known for medicinal importance (Figure 3.2). For example, Tuboflavin **5** was isolated from *Pleiocarpa tubicina Stapf* (*P. pycnantha var. tubicina*) by Schmid et al. in 1963.[5-7] In 2008, Kang and co-workers reported the isolation of seven new *β*-carboline-based metabolites, Eudistomin Y1-Y7 from a marine tunicate or ascidian of the genus *Eudistoma* near the South Korea Sea.[8] These Eudistomin Y **6** family of compounds exhibited moderate antibacterial activity against Gram-positive bacteria (*S. epidermis* and *B. subtilis*) and growth inhibitory activity against MDA-MB-231 carcinoma cell line with IC_{50} values ranging 15-63 μM.[9,10] Cardellina II et al. reported the isolation and identification of an antimicrobial marine alkaloid Eudistomin T **7** from the extracts of active Caribbean colonial tunicate *Eudistoma olivaceum* (Polycitoridae).[11,12] Chris Ireland group identified a nitrogenous pigment, Fascaplysin **8,** from *Fijian sponge Fascaplysinopsis Bergquist sp*.[13] Fascaplysin inhibited the growth of several micro bacteria including *S. aureus* (15 mm zone at 0.1 μg/disk), and *Saccharomyces* (20 mm zone at 0.1μg/disk). Fascaplysin also displayed an IC_{50}

of 0.2 μg/mL against leukemia L1210 cancer cells.[14,15] Two new biologically active *β*-carboline alkaloids with an imidazole, xestomanzamine A **9** and xestomanzamine B **10**, were isolated from an Okinawan marine sponge of *Xestospongia sp.* and inhibited cytotoxicity against human epidermoid carcinoma (KB) cells.[16,17] In 2005, another imidazole containing *β*-carboline alkaloid, gesashidine A **11**, was identified as antibacterial activity against *Micrococcus luteus* (MIC, 16.6 μg/mL) from an Okinawan marine sponge of the family *Thorectidae* (SS-1035).[18] Hyrtiomanzamine **12** possessing immunosuppressive activity was isolated by Guyot et al. from the marine sponge *Hyrtios erecta* collected in the Red Sea.[19,20] A DNA grove binding natural *β*-carboline alkaloid, maxonine **13**, with naphthyridine skeleton, was obtained from the roots of a plant *Simira maxonii*.[21,22]

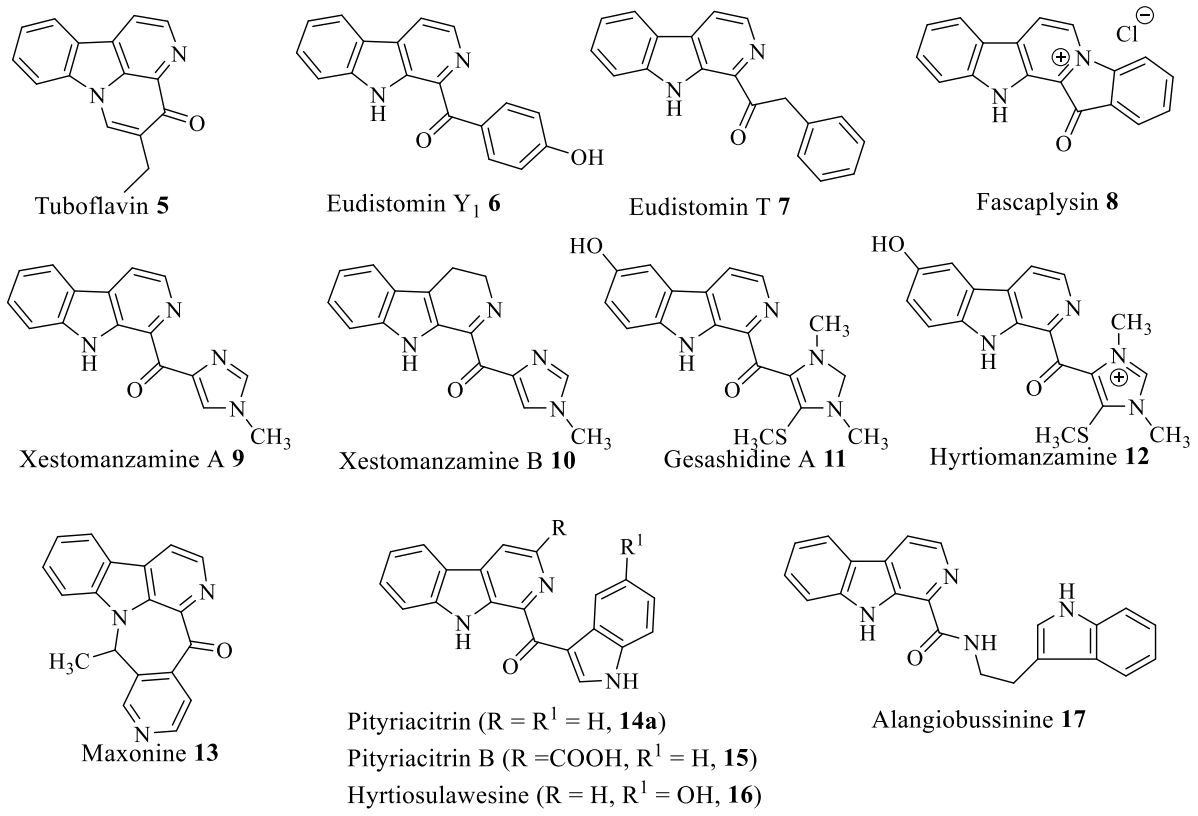
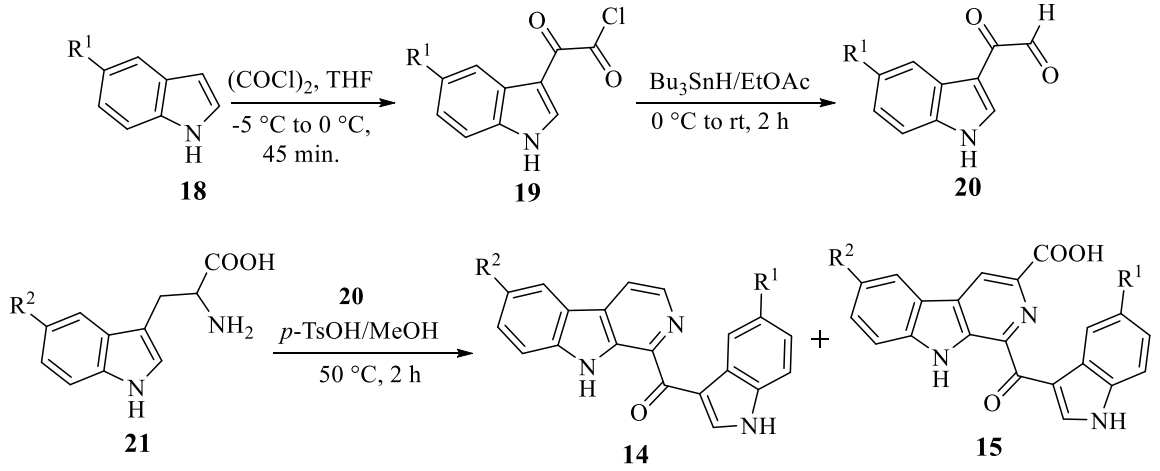


Figure 3.2 Representative natural alkaloids of 1-acyl-*β*-carboline

Pityriacitrin **14a**, extracted from marine bacterium *Paracoccus* sp., was found to exhibit a broad spectrum of pharmaceutical and biological activities including antibacterial, anti-inflammatory, anticancer, antidiabetic, antiviral, and antifungal properties.[23,24] Later, the Pityriacitrin **14a** and Pityriacitrin B **15** were isolated from cultures of the human pathogenic yeast *Malasszia furfur* and these two *β*-carboline natural products are known as a very rare potential UV filters because of their broad UV absorption spectra.[23,25] In 2002, Braekman group found a new 5-hydroxy-tryptamine-derived *β*-carboline alkaloid, hyrtiosulawesine **16**

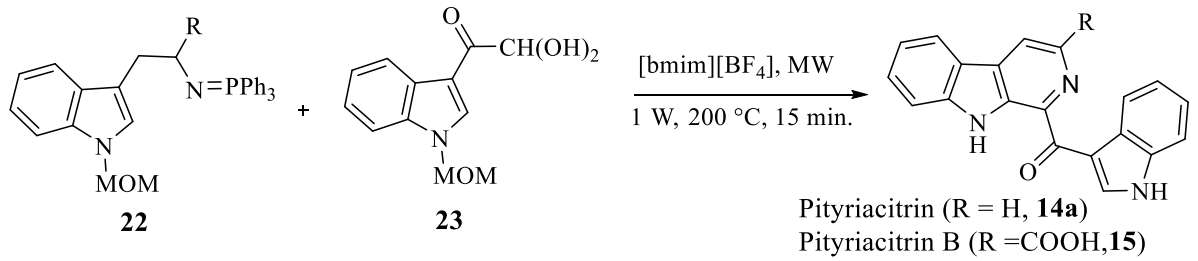
in Indonesian specimens of the marine sponges *Hyrtios erectus* and investigated for their secondary metabolite contents.[26]

Despite the immense usefulness of *β*-carboline alkaloids in medicinal chemistry, the limited synthetic methods for the preparation of *β*-carboline natural products especially, pityriacitrin analogues are reported (Scheme 3.1-3.4).[27] For example, Jiang and coworkers prepared pityriacitrin and its derivatives in 2011 by using *p*-TsOH catalyzed Pictet-Spengler cyclization of L-tryptophan and indole-3-glyoxal and evaluated for antiproliferative activity against a panel of breast and prostate cancer cells (Scheme 3.1).[28]



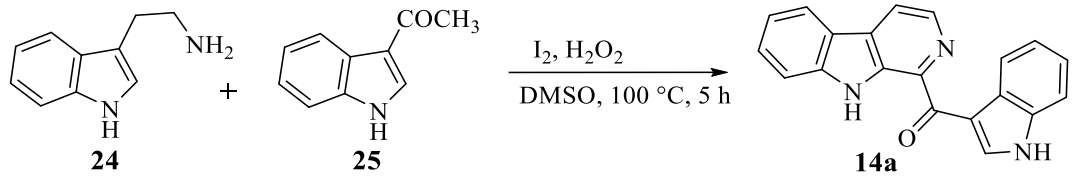
Scheme 3.1 Synthesis of pityriacitrin and its analogues

Fresneda group developed a microwave assisted method for the synthesis of 1-aryl/aroyl-*β*-carbolines through the tandem aza-Wittig/electrocyclic ring-closure reaction by a pyridoannulation process involving the simultaneous deprotection of *N*-methoxymethyl group using ionic liquid in short reaction times with 65–90% yields (Scheme 3.2).[29]



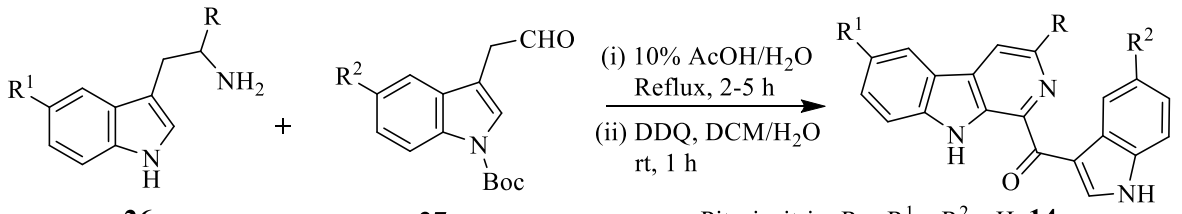
Scheme 3.2. Synthesis of pityriacitrins *via* aza-Wittig/electrocyclic ring-closure reaction

In the recent past, Naveed Ahmed group and Wu et al. reported molecular iodine in DMSO promoted synthesis of pityriacitrin involving 3-acetylindole and tryptamine precursors as shown in Scheme 3.3.[30,31]



Scheme 3.3 A cascade strategy for the synthesis of pityriacitrin

Liew et al. demonstrated the synthesis of pityriacitrins involving substituted tryptamine and indole-3-acetaldehydes in the presence of acetic acid followed by oxidation with DDQ as delineated in Scheme 3.4. These compounds exhibited activity towards a chloroquine-resistant strain (FcB1) of *Plasmodium falciparum* (IC_{50} 1.0-23 µM).[32]



Pityriacitrin, R = R^1 = R^2 = H, **14a**
Pityriacitrin B, R =COOH, R^1 = R^2 = H, **15**
Hyrtiosulawesine, R = R^1 = H, R^2 = OH, **16**

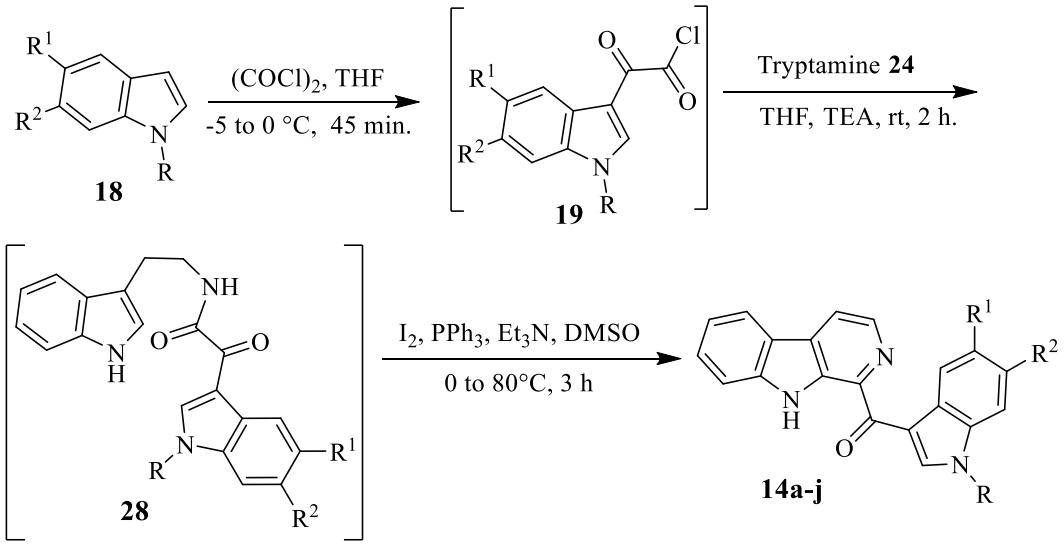
Scheme 3.4 Synthesis of pityriacitrin, pityriacitrin B and hyrtiosulawesine

Literature procedures revealed that existing reports involved the use of inaccessible precursors such as acetylindole, indole-3-glyoxal and indole acetaldehyde etc. to access the pityriacitrin and its analogues in lower yields with limited substrate scope. Therefore, a general and eco-friendly protocol to construct a library of pityriacitrin and its analogues from easily available starting materials is highly desirable. In the pursuit of our interest to access indoles and their analogues for biological assays, we have developed a sequential one-pot strategy for the preparation of pityriacitrin analogues from commercially available indole and tryptamines using molecular iodine and triphenylphosphine.

In recent years, the use of molecular iodine is very attractive for organic synthesis due to its readily available, non-toxicity, mild Lewis acidity and inexpensive nature.[33,34] The mild Lewis acidity associated with iodine enhanced its usage in organic synthesis to realize numerous organic transformations employing stoichiometric levels to catalytic amounts.[35-37]

3.2 Results and discussion

Recently, we developed a novel and efficient sequential one-pot synthesis for the construction of *β*-carboline natural product analogues from easily available indoles and tryptamines (Scheme 3.5). This approach involves the *in-situ* generation of bis(indolyl)glyoxamides **28** followed by oxidative cyclization to afford *β*-carbolines in a sequential one-pot manner. Initially we adopted our previous strategy to synthesize bis(indolyl)glyoxamide **28**, from the reaction of indole **18** with tryptamine **19** in the presence of oxalyl chloride in tetrahydrofuran.[38] Further intermediate **28** undergoes oxidative intramolecular cyclodehydration to furnish *β*-carboline derivatives **14a-j**.

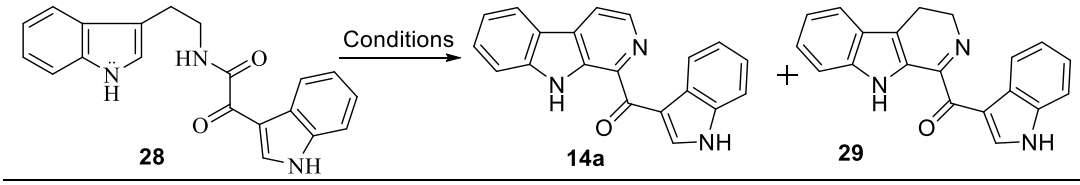


Scheme 3.5 General synthesis of *β*-carboline derivatives

Therefore, the main task is to find out the optimized reaction conditions for the oxidative cyclization step, and it should be compatible with the previous step to make it sequential one-pot approach. To optimize this cyclization strategy, a series of parameters (solvents, reagents, time and temperature) were explored using bis(indolyl)glyoxamide **28** as model substrate (Table 3.1). Firstly, a variety of reagents such as $POCl_3$, *p*-TsOH and triflic anhydride in presence of 2-chloropyridine were screened, but in all cases either failed to produce **14a** or resulted in poor yield (entries 1-7, Table 3.1). Furthermore, a combination of iodobenzene diacetate and copper triflate was also unsuccessful to afford **14a** (entries 9-11, Table 3.1). Next, we envisaged the use of Lewis acid, molecular iodine likely to generate *β*-carboline **14a**/**29** more efficiently.[39,40] Unfortunately, molecular iodine mediated dehydrative cyclization of **28** in DMSO also failed to deliver **14a** at room temperature as well as at 80 °C (entries 12-13, Table 3.1). Even, the addition of bases such as potassium carbonate or

DBU along with iodine in DMSO could not enhance the efficacy of this transformation (entries 14-17, Table 3.1). While searching for conditions, we found I_2/PPh_3 mediated-dehydrative cyclization has been reported to afford oxazoles/oxadiazoles.[41,42] Inspired by this report, we switched to I_2/PPh_3 system for our strategy and obtained **14a** with improved yield (entry 19, Table 3.1). Then, a variety of solvents like DMSO, DCE, PEG and DMF (entries 21-23, Table 3.1) were tested, but DMSO was found as optimal choice to produce **14a** in 70% yield (entry 20, Table 3.1). Finally, based on these results we successfully developed sequential one-pot method to access *β*-carbolines **14a-j** from easily available precursors.

Table 3.1 Cyclization of bis(indolyl)ketoamide **28**

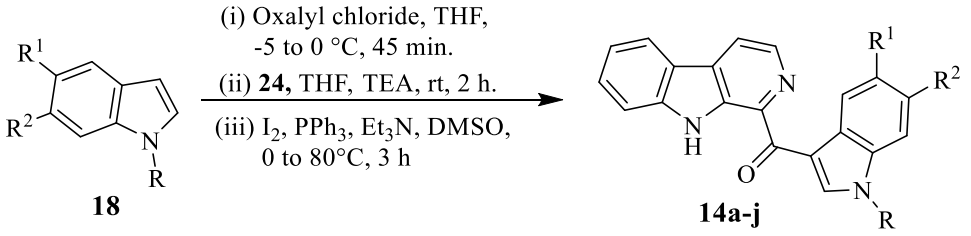


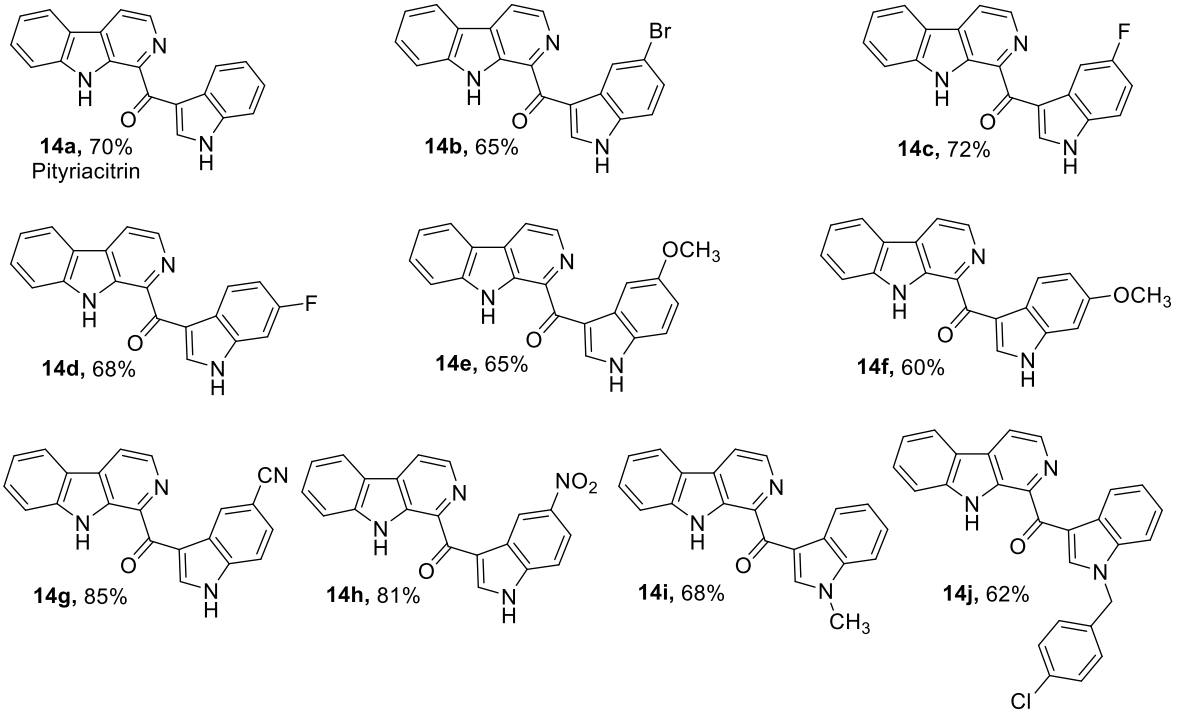
Entry	Conditions	Temp. (°C)	Time (h)	Yield (%)[a]	
				14a	**29**
1	$POCl_3$	rt	36	0	0
2	$POCl_3$	80	16	5	20
3	$POCl_3$, benzene	80	8	10	35
4	$POCl_3$, toluene	110	6	10	30
5	*p*-TsOH, methanol	65	10	5	40
6	*p*-TsOH, ethanol	80	7	5	25
7	*p*-TsOH, PEG	120	5	0	0
8	Tf_2O, 2-ClPy, DCM,	-78 to 25	2	trace	trace
9	DIB, MeCN	rt	24	0	0
10	DIB, MeCN	70	24	0	0
11	$Cu(OTf)_2$(10 mol%), DIB, DCE	85	24	0	0
12	I_2, DMSO	rt	32	0	0
13	I_2, DMSO	80	18	5	10
14	I_2, K_2CO_3, DMSO	80	6	10	15
15	I_2, K_2CO_3, DMSO	120	4	10	0
16	I_2, DBU, DMSO	80	5	15	0
17	I_2, DBU, DMSO	100	5	20	0
18	I_2, PPh_3, DMSO	0 to 80	2	trace	trace
19	I_2, PPh_3, TEA, DCM	0 to 40	12	40	0
20	I_2, PPh_3, TEA, DMSO	0 to 40	3	70	0
21	I_2, PPh_3, TEA, DCE	0 to 80	12	0	0
22	I_2, PPh_3, TEA, PEG	0 to 80	12	0	0
23	I_2, PPh_3, TEA, DMF	0 to 80	15	0	0

*rt means room temperature. [a]Isolated yield

With these optimized conditions, we turned our attention to the scope and limitations of the present methodology by employing tryptamine and indole bearing different substituents. Gratifyingly, the electronic nature and position of the substituents on indole had significant influence on the product yields. Initially, the effect of substitution on C-5 and C-6 position of indole was studied. The reaction furnished the expected C-5 and C-6 substituted indole derivatives **14a-h** in moderate to good yields. We observed that the indoles bearing electron-withdrawing groups (F, CN, and NO_2) on C-5 delivered the respective products **14c** and **14g-h** in superior yields when compared with electron-donating group (OCH_3). The C-6 substituted indoles also led to products **14d** (68%) and **14f** (60%) in good yields. Next, we examined the performance of *N*-alkylated indoles such as *N*-methyl and *N*-4-chlorobenzyl and obtained **14i** and **14j** in 68% and 62% yields, respectively (Table 3.2**)**.

Table 3.2 Sequential one-pot synthesis of *β*-carbolines **14a-j**





*Reaction conditions: Indole (2.564 mmol), tryptamine (2.564 mmol), oxalyl chloride (2.821 mmol), Ph_3P (3.846 mmol), I_2 (3.846 mmol), triethyl amine (12.82 mmol), DMSO (2 mL), 0 to 80 ºC, 3 h.

Structures of the prepared *β*-carbolines **14a-j** were unambiguously assigned by NMR (^{1}H and ^{13}C) and HRMS spectral data (Figures 3.3-3.5). In ^{1}H NMR spectrum, two broad singlets at *δ* ~12.2 ppm and *δ* ~12.0 ppm for *N*-H proton of *β*-carboline and indole were observed. ^{13}C NMR spectrum of **14a-j** showed a characteristic signal for the carbonyl carbon at *δ* ~188 ppm. The HRMS of *β*-carbolines **14a-j** displayed expected molecular ion peak in agreement with the calculated mass.

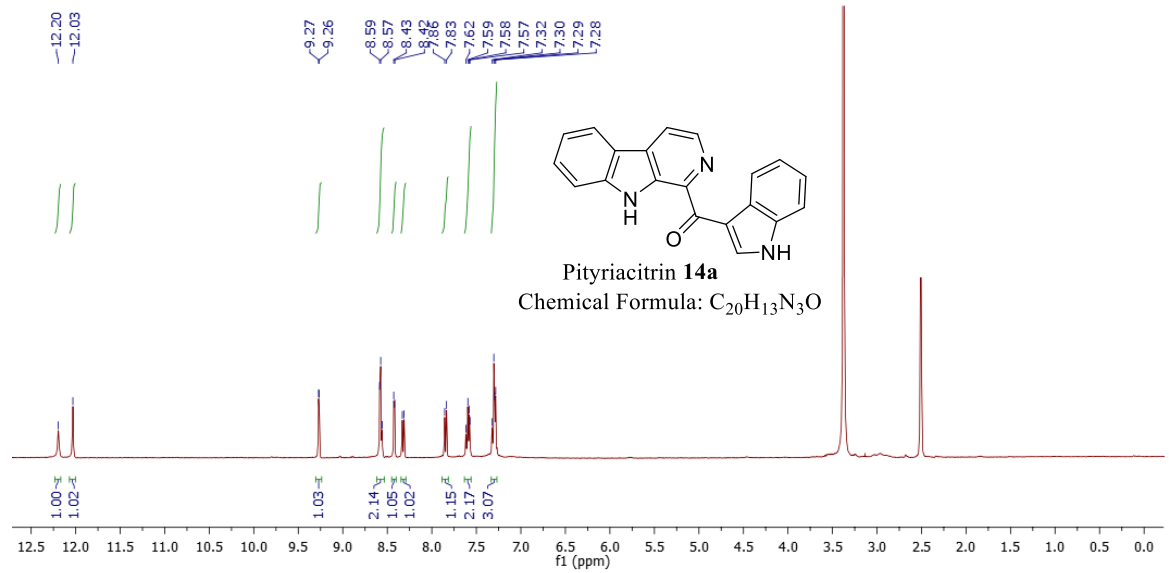


Figure 3.4 ^{1}H NMR spectrum of **14a**

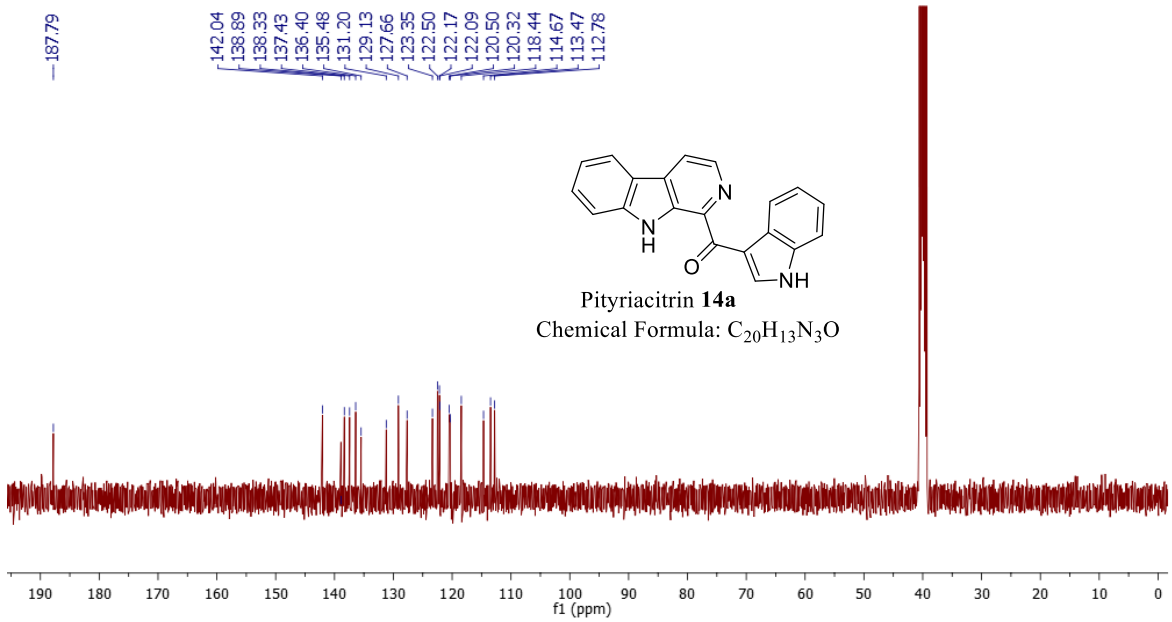


Figure 3.5 ^{13}C NMR spectrum of **14a**

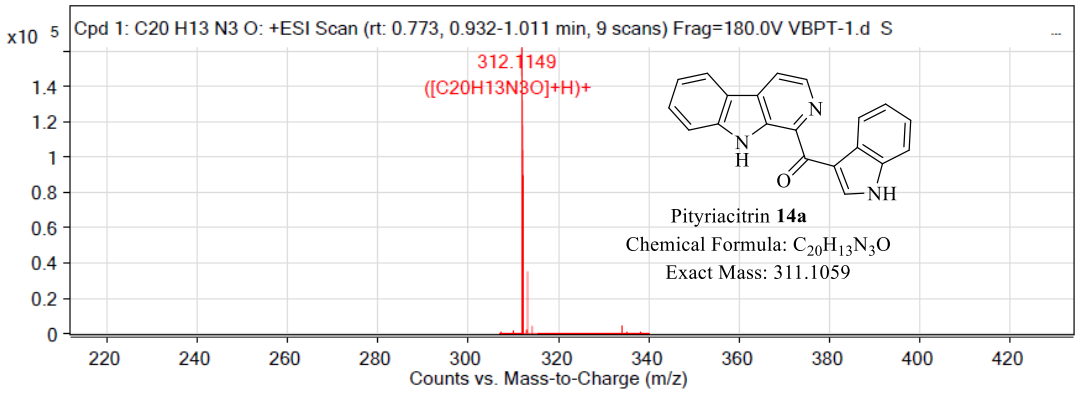
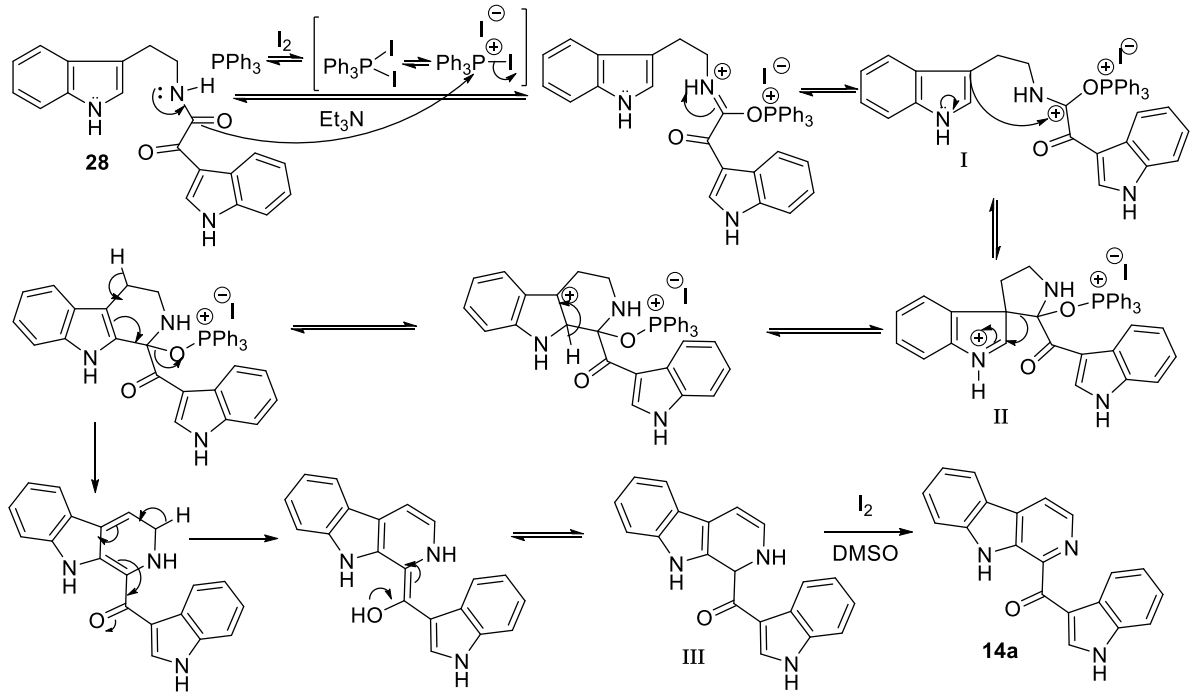


Figure 3.3 HRMS spectrum of **14a**

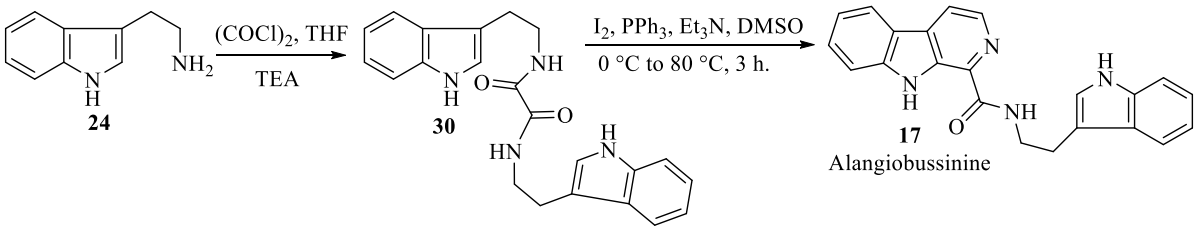
Based on our experimental results and literature reports,[31,43-45] a plausible mechanism for the dehydrative cyclization is proposed as depicted in Scheme 3.6. It is believed that initially triphenylphosphine coordinates with molecular iodine to generate reactive triphenylphosphonium iodide, which in the presence of triethylamine activates the ketoamide **28** to generate species **I**. Intramolecular cyclization of **I** *via* spirocyclic intermediate **II,** and subsequent rearrangement and aromatization believed to produce carboline **14a**.



Scheme 3.6 Proposed mechanism for the dehydrative cyclization of ketoamide **28**

3.3 Synthesis of Alangiobussinine

Next, we extended the optimized conditions to prepare Alangiobussinine **17**; a biologically interesting *β*-carboline alkaloid that is isolated from the leaves of *Alangium bussyanum*.[46] Recently, Sarpong group has reported the functionalization of Alangiobussinine using carboline-directed C-2 alkynylations.[47] In 2011, Baiget et al. disclosed the multi-step synthesis of Alangiobussinine **17** from the coupling reaction of *β*-carboline ester with tryptamine **24** in 67% yield.[48] Our alternative and convenient method to achieve Alangiobussinine **17** involves the reaction of tryptamine **24** with oxalyl chloride to *in-situ* generate glyoxamide **30** which upon treatment with molecular iodine and triphenylphosphine in the presence of triethylamine successfully afforded **17** in good yield (Scheme 3.7).



Scheme 3.7 Synthesis of Alangiobussinine **17**

Structure of the natural *β*-carboline, Alangiobussinine **17** was confirmed by NMR (^{1}H & ^{13}C) and mass spectral data (Figures 3.6-3.8).

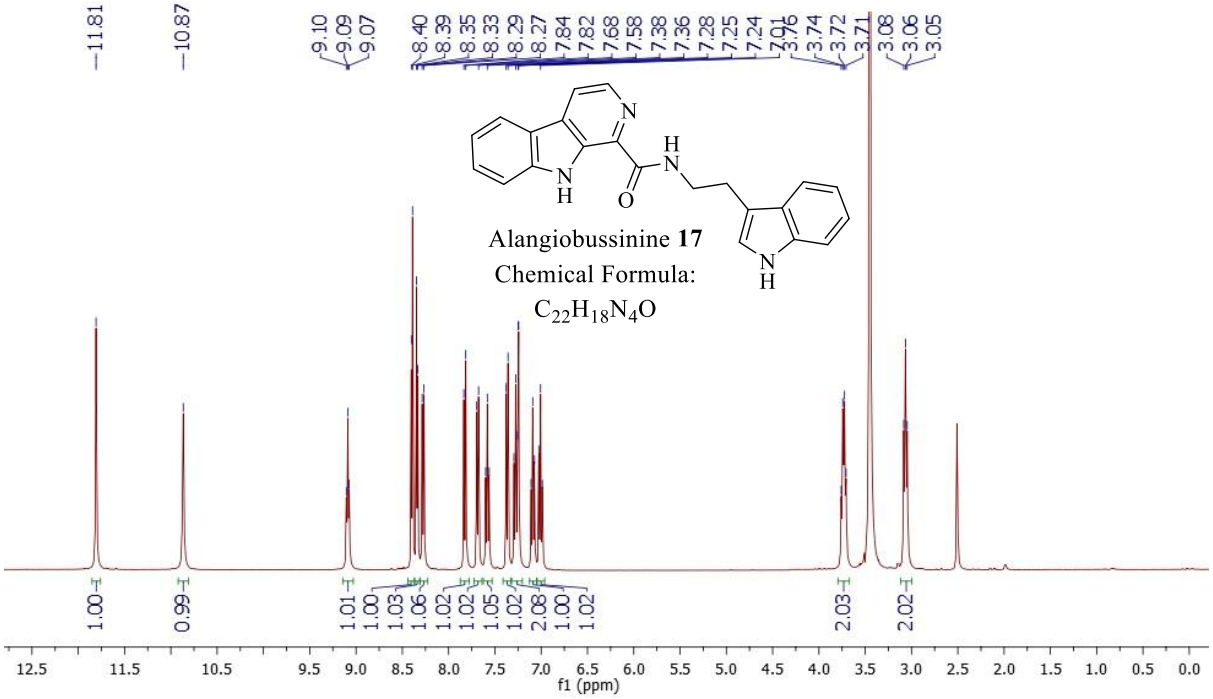


Figure 3.6 ^{1}H NMR spectrum of **17**

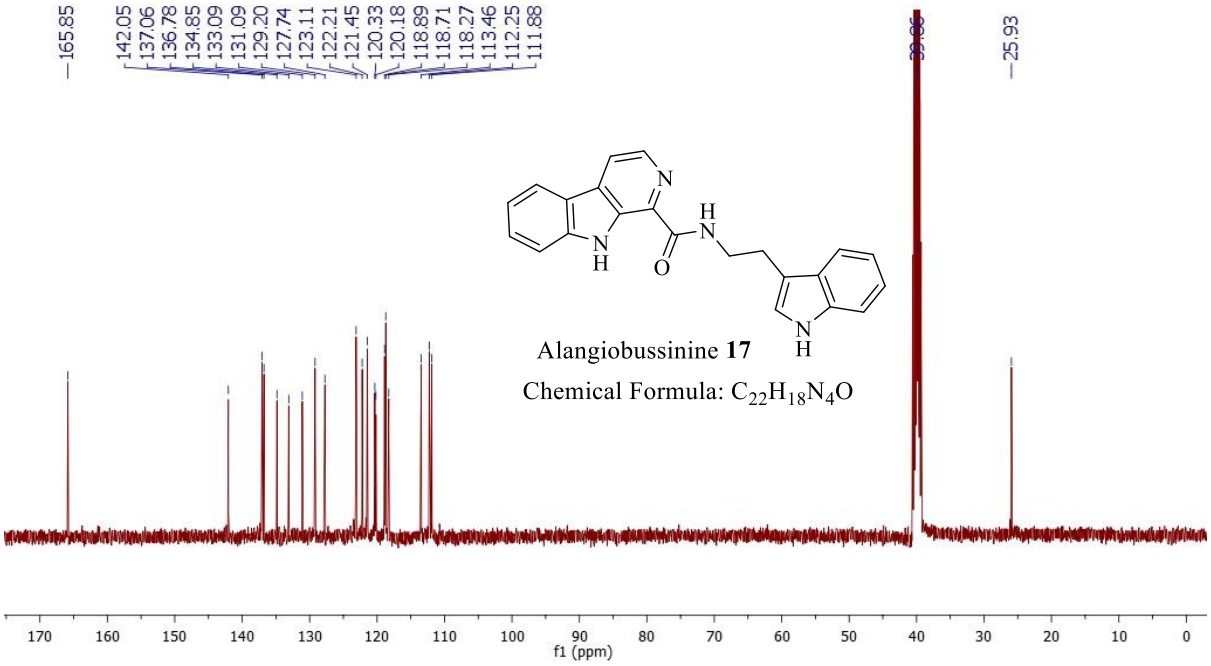


Figure 3.7 ^{13}C NMR spectrum of **17**

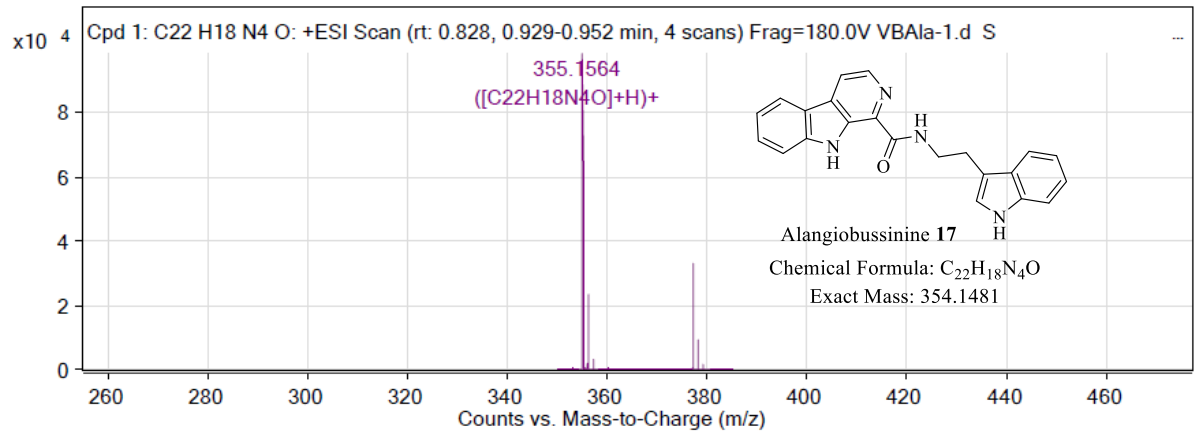


Figure 3.8 HRMS spectrum of **17**

3.4 Conclusions

In summary, we have developed a novel sequential one-pot strategy for the synthesis of natural *β*-carboline analogues **14a-j** from different indoles **18** and tryptamine **24** involving *in-situ* dehydrative cyclization of key intermediate ketoamide **28** by using triphenylphosphine and molecular iodine. The developed high yielding approach is general and operationally simple and expands the synthetic utility of various indoles for the construction of 1-indoloyl-*β*-carbolines. The methodology was successfully applied for the preparation of naturally occurring *β*-carbolines like pityriacitrin, hyrtiosulawesine and alangiobussinine.

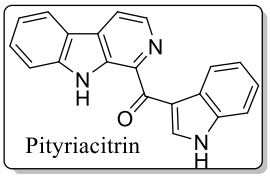
3.5 Experimental section

All the reagents and solvents were procured commercially and purified according to common methods. NMR (^{1}H and ^{13}C) spectra were recorded on a Bruker Avance at 400 MHz, using deuterated solvents ($CDCl_3$ and DMSO-d_6), chemical shift values are listed in parts per million (ppm) downfield from TMS as the internal standard. Data reported as follows: chemical shift (ppm on the δ scale), multiplicity (s: singlet, d: doublet, t: triplet, m: multiplet), and coupling constant J (Hz). High resolution mass spectra (HRMS) were recorded by Agilent Technologies 6545 mass spectrometer with an electron spray ionization time-of-flight (ESI-TOF). Reactions were controlled by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated plates, and the spots were visualized under UV light. Melting points were recorded on an electro thermal capillary melting point apparatus (*E-Z* melting) and are uncorrected. Reaction products were purified by column chromatography using silica gel 100–200 mesh with methanol and dichloromethane (1:99) as eluents.

3.5.1 General experimental procedure for synthesis of *β*-carboline analogues (14a-j)

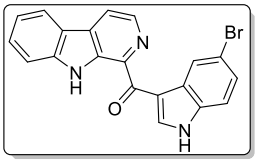
To a stirred solution of indole **18** (0.3 g, 2.564 mmol) in tetrahydrofuran (3 mL), oxalyl chloride (3.077 mmol.) was added slowly while maintaining the temperature below 0 °C. After 1 h, tryptamine **24** (2.564 mmol) and triethylamine (0.5 mL) were added and the mixture was continued to stir for another 2 h. After completion of the reaction as monitored by TLC, removed the solvent and the intermediate **28** was taken in DMSO (2 mL) and added a cold solution of molecular iodine (3.846 mmol) and triphenylphosphine (3.846 mmol) at 0 ºC. Subsequently, triethylamine (12.82 mmol) was added and the reaction temperature was slowly raised to 80 °C and continued stirring for another 3 h. Upon completion of reaction as confirmed by TLC, the contents were cooled to room temperature and poured into 10% hypo solution and extracted with dichloromethane (3×10 mL). The combined organic extracts were dried with anhydrous sodium sulfate and concentrated under reduced pressure. The obtained crude product was purified by column chromatography (methanol and dichloromethane 1:99) to afford the *β*-carboline analogues **14a-j** in good to excellent yields (60-85%).

(1*H*-Indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14a): Yellow solid; Yield 70%; Mp 239-240 °C (lit.[32] 241-242 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 12.03 (s, 1H), 9.27 (d, J = 3.1 Hz, 1H), 8.59–8.56 (m, 2H), 8.42 (d, J = 4.9 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.62–7.58 (m, 2H), 7.32–7.28 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.8, 142.0, 138.9, 138.3, 137.4, 136.4, 135.5, 131.2, 129.1, 127.6, 123.3, 122.5, 122.2, 122.1, 120.5, 120.3, 118.4, 114.7, 113.5, 112.8. HRMS (ESI^{+}) calcd for $C_{20}H_{14}N_3O$ $[M + H]^{+}$, 312.1137; found 312.1149.

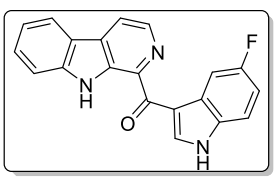


Pityriacitrin

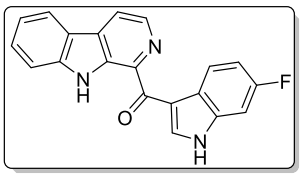
(5-Bromo-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14b): Yellow solid; Yield, 65%; Mp 264-265 °C (lit.[28] 266–268 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 12.05 (s, 1H), 9.33 (d, J = 3.6 Hz, 1H), 8.77 (d, J = 2.0 Hz, 1H), 8.57 (d, J = 4.9 Hz, 1H), 8.41 (d, J = 5.0 Hz, 1H), 8.30 (d, J = 7.8 Hz, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.66–7.52 (m, 2H), 7.49–7.41(m, 1H), 7.30 (t, J = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.7, 142.1, 139.2, 138.4, 137.5, 135.9, 135.6, 135.1, 131.3, 129.5, 129.2, 125.8, 124.5, 122.2, 120.5, 120.4, 118.7, 115.3, 114.8, 113.5; HRMS (ESI$^+$) calcd for $C_{20}H_{13}BrN_3O$ [M + H]$^+$, 390.0242; found 390.0237; [M+H+2]$^+$ 392.0222, found 392.0218.



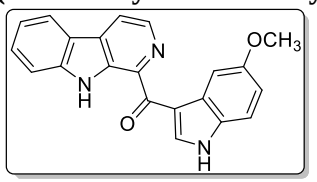
(5-Fluoro-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14c): Yellow solid; Yield 72%; Mp 249-251 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (s, 1H), 12.04 (s, 1H), 9.34 (d, J = 3.2 Hz, 1H), 8.58 (d, J = 4.9 Hz, 1H), 8.43 (d, J = 4.9 Hz, 1H), 8.32 (d, J = 7.9 Hz, 1H), 8.25–8.22 (m, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.64–7.57 (m, 2H), 7.30 (t, J = 7.8 Hz, 1H), 7.18–7.12 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.7, 160.5, 158.1, 142.1, 139.7, 138.5, 137.5, 135.5, 133.0, 131.3, 129.2, 122.2, 120.5, 120.4, 118.6, 114.8, 114.7, 114.1, 113.9, 113.5, 111.5, 111.3, 107.1, 106.8, 103.3. HRMS (ESI$^+$) calcd for $C_{20}H_{13}FN_3O$ [M + H]$^+$, 330.1043; found 330.1040.



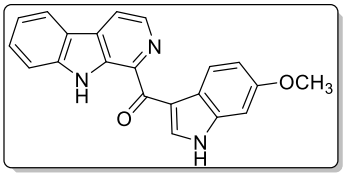
(6-Fluoro-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14d): Yellow solid; Yield 68%; Mp 258-260 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 12.04 (s, 1H), 9.28 (d, J = 1.6 Hz, 1H), 8.58 (d, J = 4.9 Hz, 1H), 8.56–8.53 (m, 1H), 8.42 (d, J = 4.9 Hz, 1H), 8.32 (d, J = 7.9 Hz, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.41–7.38 (m, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.19–7.14 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.7, 160.9, 158.6, 142.1, 139.0, 138.9, 138.6, 137.5, 136.6, 136.5, 135.5, 131.3, 129.2, 124.3, 123.2, 123.1, 122.2, 120.5, 120.4, 118.6, 114.6, 113.5, 110.8, 110.6, 99.2, 99.0; HRMS (ESI$^+$) calcd for $C_{20}H_{13}FN_3O$ [M + H]$^+$, 330.1043; found 330.1034.



(5-Methoxy-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14e): Yellow solid; Yield 65%; Mp 213-215 °C (lit.[28] 214-216 °C); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (s, 2H), 9.24 (d, J = 3.2 Hz, 1H), 8.57 (d, J = 4.9 Hz, 1H), 8.41 (d, J = 4.9 Hz, 1H), 8.31 (d, J = 7.8 Hz, 1H), 8.12 (d, J = 2.5 Hz, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.59 (t, J = 8.1 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 6.93–6.90 (m, 1H), 3.86 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.7, 156.1, 142.0, 138.9, 138.4, 137.4, 135.5, 131.2, 131.1, 129.1, 128.5, 122.1, 120.5, 120.3, 118.4, 114.6, 113.5, 113.4, 113.2, 103.8, 55.7; HRMS (ESI$^+$) calcd for $C_{21}H_{16}N_3O_2$ [M+H]$^+$, 342.1243; found 342.1243.

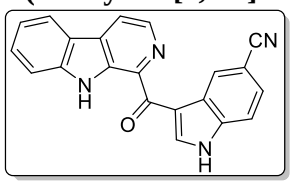


(6-Methoxy-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14f): Yellow solid; Yield



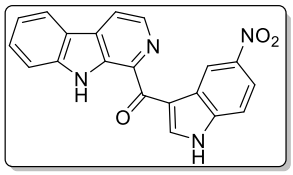
60%; Mp 213-215 °C (lit. 214-216 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.76 (s, 1H), 11.58 (s, 1H), 8.50 (d, J = 3.8 Hz, 1H), 8.41 (d, J = 4.3 Hz, 1H), 8.24 (d, J = 8.6 Hz, 1H), 8.20 (s, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.2 Hz, 1H), 7.58–7.50 (m, 1H), 7.26–7.21 (m, 1H), 6.91 (s, 1H), 6.82 (d, J = 8.5 Hz, 1H), 3.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 156.8, 143.0, 139.1, 137.7, 137.4, 130.6, 130.5, 122.5, 122.1, 121.0, 120.7, 119.9, 117.4, 114.9, 114.0, 112.9, 112.5, 112.3, 95.7, 55.5; HRMS (ESI^+) calcd for $C_{21}H_{16}N_3O_2$ $[M + H]^+$, 342.1243; found 342.1246.

3-(9*H*-Pyrido[3,4-*b*]indole-1-carbonyl)-1*H*-indole-5-carbonitrile (14g): Yellow solid; Yield



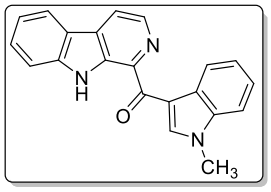
85%; Mp 260-263 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (s, 1H), 12.07 (s, 1H), 9.46 (d, J = 2.8 Hz, 1H), 8.93 (s, 1H), 8.60 (d, J = 4.9 Hz, 1H), 8.45 (d, J = 4.9 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.31 (t, J = 7.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.8, 142.1, 140.3, 138.3, 138.1, 137.6, 135.6, 131.4, 129.3, 127.5, 127.1, 126.3, 122.2, 120.8, 120.5, 120.4, 118.9, 114.8, 114.3, 113.5, 104.6; HRMS (ESI^+) calcd for $C_{21}H_{14}N_4O$ $[M + H]^+$, 337.1089; found 337.1096.

(5'-Nitro-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14h): Yellow solid; Yield



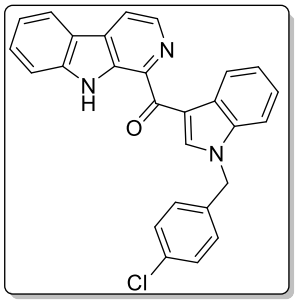
81%; Mp 275-276 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 12.15 (s, 1H), 9.54 (s, 1H), 9.52 (d, J = 1.8 Hz, 1H), 8.61 (d, J = 4.8 Hz, 1H), 8.46 (d, J = 4.6 Hz, 1H), 8.33 (d, J = 7.8 Hz, 1H), 8.19 (d, J = 8.9 Hz, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 8.9 Hz, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.32 (t, J = 7.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.7, 143.4, 142.2, 141.3, 139.6, 137.9, 137.6, 135.6, 131.5, 129.3, 127.2, 122.3, 120.5, 120.4, 119.1, 118.8, 118.7, 115.9, 113.6, 113.5; HRMS (ESI^+) calcd for $C_{20}H_{13}N_4O_3$ $[M + H]^+$, 357.0988; found 357.0992.

(1-Methyl-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14i): Yellow solid; Yield

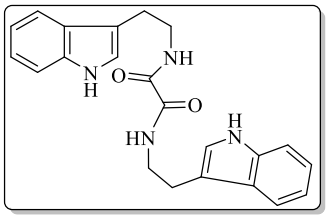


68%; Mp 175-176 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.01 (s, 1H), 8.65 (d, J = 7.3 Hz, 1H), 8.45 (d, J = 5.0 Hz, 1H), 8.39 (d, J = 5.0 Hz, 1H), 8.30 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.47 (d, J = 7.7 Hz, 1H), 7.31–7.25 (m, 1H), 7.20–7.15 (m, 2H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.5, 146.2, 142.0, 136.9, 135.3, 133.1, 130.9, 129.3, 126.4, 124.0, 123.2, 122.3, 121.1, 120.4, 120.2, 118.4, 114.9, 113.4, 111.8, 108.3, 55.8; HRMS (ESI^+) calcd for $C_{21}H_{16}N_3O$ $[M + H]^+$, 326.1293; found 326.1289.

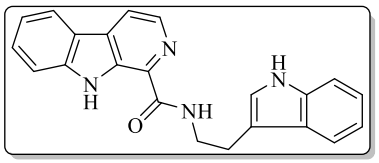
(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)(9*H*-pyrido[3,4-*b*]indol-1-yl)methanone (14j): Yellow solid; Yield 62%; Mp 157-159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.81 (s, 1H), 9.47 (s, 1H), 8.76 (d, J = 7.9 Hz, 1H), 8.58 (d, J = 4.9 Hz, 1H), 8.20 (d, J = 7.8 Hz, 1H), 8.16 (d, J = 4.9 Hz, 1H), 7.64–7.63 (m, 2H), 7.44–7.40 (m, 1H), 7.38–7.34 (m, 1H), 7.36–7.30 (m, 4H), 7.15 (d, J = 8.5 Hz, 2H), 5.46 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.5, 141.0, 140.4, 138.3, 137.5, 136.6, 136.1, 134.6, 133.9, 131.4, 129.2, 129.0, 128.7, 128.1, 123.6, 123.1, 123.0, 121.7, 120.8, 120.4, 117.8, 114.9, 111.98, 110.3, 50.4; HRMS (ESI$^+$) calcd for $C_{27}H_{19}ClN_3O$ $[M + H]^+$, 436.1219; found 436.1208.



3.5.2 *N*^{1}, *N*^{2}-bis(2-(1*H*-indol-3-yl)ethyl)oxalamide (30): To a cold solution of tryptamine **24** (0.5 g, 3.125 mmol.) in tetrahydrofuran (20 mL), oxalyl chloride (0.2 g, 1.562 mmol.) was added slowly at below 0 °C and then triethylamine (0.5 mL). After completion of the reaction, filtered the reaction mixture and concentrated under vacuum. The residue obtained was washed with hexane to furnish bis(indolyloxalamide) **30**. Brown solid; Yield 90%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.87 (t, J = 5.7 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.18 (s, 1H), 7.08 (t, J = 7.4 Hz, 1H), 6.99 (t, J = 7.3 Hz, 1H), 3.44 (q, J = 6.9 Hz, 2H), 2.90 (t, J = 7.3 Hz, 2H).



3.5.3 *N*-(2-(1*H*-indol-3-yl)ethyl)-9*H*-pyrido[3,4-*b*]indole-1-carboxamide (Alangiobussinine) (17): Alangiobussinine **17** was prepared by following the procedure described for **5a-j.** Intermediate **30** (0.2 g, 0.535 mmol) was dissolved in DMSO (2 mL) and added a cold solution of molecular iodine (0.535 mmol) and triphenylphosphine (0.535 mmol) in DMSO while maintaining 0 ºC temperature. Subsequently, triethylamine (1.34 mmol) was added, slowly raised the reaction temperature to 80 °C and allowed to stir for 3 h. Pale yellow solid; Yield 62%; Mp 196-198 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 10.86 (s, 1H), 9.08 (t, J = 5.9 Hz, 1H), 8.40 (d, J = 5.0 Hz, 1H), 8.34 (d, J = 5.0 Hz, 1H), 8.28 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.29–7.24 (m, 2H), 7.09 (t, J = 7.2 Hz, 1H), 7.01 (t, J = 7.3 Hz, 1H), 3.76-3.71 (m, 2H), 3.06 (t, J = 7.4 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.8, 142.0, 137.0, 136.8, 134.8, 133.1, 131.1, 129.2, 127.7, 123.1, 122.2, 121.4, 120.3, 120.2, 118.9, 118.7, 118.3, 113.4, 112.2, 111.9, 39.8, 25.9; HRMS (ESI$^+$) calcd for $C_{22}H_{19}N_4O$ $[M + H]^+$, 355.1559; found 355.1564.



3.6 References

1. Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P., Drug development from marine natural products. *Nat. Rev. Drug Discov.,* **2009,** *8*, 69-85.
2. Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H.; Prinsep, M. R., Marine natural products. *Nat. Prod. Rep.,* **2015,** *32*, 116-211.
3. Ndagijimana, A.; Wang, X.; Pan, G.; Zhang, F.; Feng, H.; Olaleye, O., A review on indole alkaloids isolated from *Uncaria Rhynchophylla* and their pharmacological studies. *Fitoterapia,* **2013,** *86*, 35-47.
4. Cao, R.; Peng, W.; Wang, Z.; Xu, A., *β*-Carboline alkaloids: biochemical and pharmacological functions. *Curr. Med. Chem.,* **2007,** *14*, 479-500.
5. Kump, C.; Seibl, J.; Schmid, H., Über die Struktur des tuboflavins. 4. Mitteilung über Pleiocarpa-Alkaloide. *Helv. Chim. Acta,* **1963,** *46*, 498-505.
6. Rosenkranz, H.; Schmid, H., Notiz über die Synthese von iso-, noriso-und desäthyl-tuboflavin. *Helv. Chim. Acta,* **1968,** *51*, 565-568.
7. Rosenkranz, H. J.; Botyos, G.; Schmid, H., Synthese von tuboflavin, 4-athyl-canthin-6-on und canthin-6-on. *Eur. J. Org. Chem.,* **1966,** *691*, 159-164.
8. Wang, W.; Nam, S.-J.; Lee, B.-C.; Kang, H., *β*-Carboline alkaloids from a Korean tunicate *Eudistoma sp*. *J. Nat. Prod.,* **2008,** *71*, 163-166.
9. Jin, H.; Zhang, P.; Bijian, K.; Ren, S.; Wan, S.; Alaoui-Jamali, M.; Jiang, T., Total synthesis and biological activity of marine alkaloid eudistomins Y1–Y7 and their analogues. *Mar. Drugs,* **2013,** *11*, 1427.
10. Phillip Kennedy, J.; Breininger, M. L.; Lindsley, C. W., Total synthesis of eudistomins Y1–Y6. *Tetrahedron Lett.,* **2009,** *50*, 7067-7069.
11. Kinzer, K. F.; Cardellina Ii, J. H., Three new *β*-carbolines from the Bermudian tunicate *Eudistoma olivaceum*. *Tetrahedron Lett.,* **1987,** *28*, 925-926.
12. Bracher, F.; Hildebrand, D.; Ernst, L., *β*-Carboline alkaloids, V: Total synthesis of the antimicrobial marine alkaloid eudistomin T. *β*-carbolin-alkaloide, 5. Mitt.: Total synthese des marinen alkaloides eudistomin T. *Arch. Pharm.,* **1994,** *327*, 121-122.
13. Roll, D. M.; Ireland, C. M.; Lu, H. S. M.; Clardy, J., Fascaplysin, an unusual antimicrobial pigment from the marine sponge *Fascaplysinopsis sp*. *J. Org. Chem.,* **1988,** *53*, 3276-3278.
14. Khokhar, S.; Feng, Y.; Campitelli, M. R.; Ekins, M. G.; Hooper, J. N. A.; Beattie, K. D.; Sadowski, M. C.; Nelson, C. C.; Davis, R. A., Isolation, structure determination and cytotoxicity studies of tryptophan alkaloids from an Australian marine sponge *Hyrtios sp*. *Bioorganic Med. Chem. Lett.,* **2014,** *24*, 3329-3332.

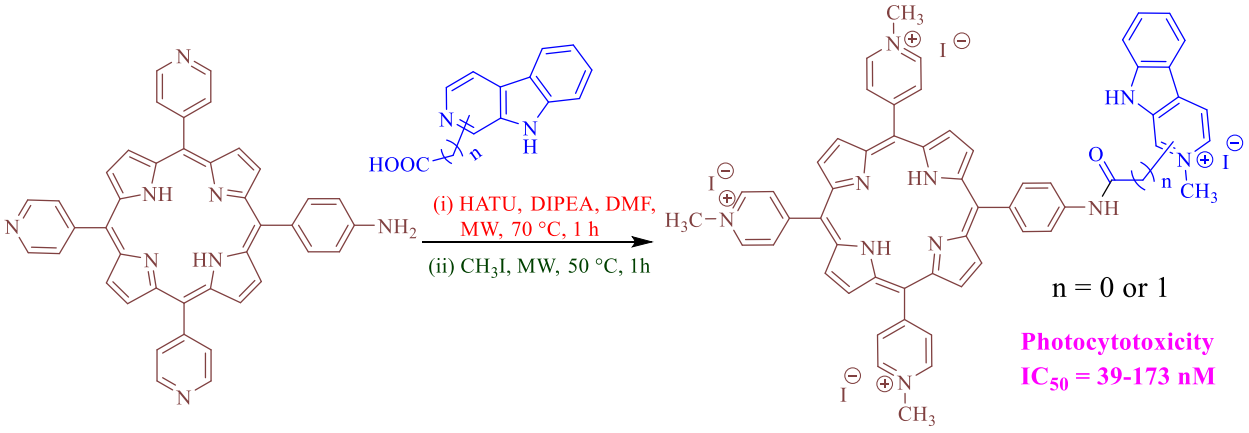
15. Soni, R.; Muller, L.; Furet, P.; Schoepfer, J.; Stephan, C.; Zumstein-Mecker, S.; Fretz, H.; Chaudhuri, B., Inhibition of cyclin-dependent kinase 4 (Cdk4) by Fascaplysin, a marine natural product. *Biochem. Biophys. Res. Commun.,* **2000,** *275*, 877-884.
16. Kobayashi, M.; Chen, Y.-J.; Aoki, S.; In, Y.; Ishida, T.; Kitagawa, I., Four new *β*-carboline alkaloids isolated from two Okinawan marine sponges of *Xestospongia sp*. and *Haliclona sp*.). *Tetrahedron,* **1995,** *51*, 3727-3736.
17. Burm, B. E.; Jongmans, E.; van Kampen, E.; Wanner, M.; Koomen, G.; Blokker, P., Synthesis of xestomanzamines A and B. *Heterocycles,* **2001,** *55*.
18. Iinuma, Y.; Kozawa, S.; Ishiyama, H.; Tsuda, M.; Fukushi, E.; Kawabata, J.; Fromont, J.; Kobayashi, J. i., Gesashidine A, a *β*-Carboline alkaloid with an imidazole ring from a *Thorectidae Sponge*. *J. Nat. Prod.,* **2005,** *68*, 1109-1110.
19. Abdjul, D.; Yamazaki, H.; Ukai, K.; Namikoshi, M., Two new indole derivatives from a marine sponge *Ircinia sp*. collected at Iriomote Island. *J. Nat. Med.,* **2015,** *69*, 416-420.
20. Bourguet-Kondracki, M. L.; Martin, M. T.; Guyot, M., A new *β*-carboline alkaloid isolated from the marine sponge *Hyrtios erecta*. *Tetrahedron Lett.,* **1996,** *37*, 3457-3460.
21. Hasbun, C. P.; Calderon, M.; Castro, O.; Gács-Baitz, E.; Delle Monache, G.; Delle Monache, F., Maxonine, a novel alkaloid from *Simira maxonii*. *Tetrahedron Lett.,* **1989,** *30*, 6199-6202.
22. Kelly, T. R.; Wei, X.; Sundaresan, J., Maxonine: Structure correction and synthesis. *Tetrahedron Lett.,* **1993,** *34*, 6173-6176.
23. Mayser, P.; Schäfer, U.; Krämer, H.-J.; Irlinger, B.; Steglich, W., Pityriacitrin–an ultraviolet-absorbing indole alkaloid from the yeast *Malassezia furfur*. *Arch. Dermatol. Res.,* **2002,** *294*, 131-134.
24. Machowinski, A.; Krämer, H.-J.; Hort, W.; Mayser, P., Pityriacitrin – a potent UV filter produced by *Malassezia furfur* and its effect on human skin microflora. *Mycoses,* **2006,** *49*, 388-392.
25. Irlinger, B.; Bartsch, A.; Krämer, H.-J.; Mayser, P.; Steglich, W., New tryptophan metabolites from cultures of the lipophilic yeast *Malassezia furfur*. *Helv. Chim. Acta,* **2005,** *88*, 1472-1485.
26. Salmoun, M.; Devijver, C.; Daloze, D.; Braekman, J.-C.; van Soest, R. W. M., 5-Hydroxytryptamine-derived alkaloids from two marine sponges of the *Genus Hyrtios*. *J. Nat. Prod.,* **2002,** *65*, 1173-1176.
27. Zhang, P. Y.; Wan, S. B.; Ren, S. M.; Jiang, T., First total synthesis of marine alkaloid Hyrtiosulawesine. *Chin. Chem. Lett.,* **2010,** *21*, 1307-1309.

28. Zhang, P.; Sun, X.; Xu, B.; Bijian, K.; Wan, S.; Li, G.; Alaoui-Jamali, M.; Jiang, T., Total synthesis and bioactivity of the marine alkaloid pityriacitrin and some of its derivatives. *Eur. J. Med. Chem.,* **2011,** *46*, 6089-6097.
29. Fresneda, P. M.; Blázquez, J. A., Synthesis of *β*-carbolines using microwave-assisted aza-Wittig methodology in ionic liquids. *Tetrahedron Lett.,* **2012,** *53*, 2618-2621.
30. Battini, N.; Padala, A. K.; Mupparapu, N.; Vishwakarma, R. A.; Ahmed, Q. N., Unexplored reactivity of 2-oxoaldehydes towards Pictet-Spengler conditions: concise approach to *β*-carboline based marine natural products. *RSC Adv.,* **2014,** *4*, 26258-26263.
31. Zhu, Y.-P.; Liu, M.-C.; Cai, Q.; Jia, F.-C.; Wu, A.-X., A cascade coupling strategy for one-pot total synthesis of *β*-carboline and isoquinoline-containing natural products and derivatives. *Chem. Eur. J.,* **2013,** *19*, 10132-10137.
32. Liew, L. P. P.; Fleming, J. M.; Longeon, A.; Mouray, E.; Florent, I.; Bourguet-Kondracki, M.-L.; Copp, B. R., Synthesis of 1-indolyl substituted *β*-carboline natural products and discovery of antimalarial and cytotoxic activities. *Tetrahedron,* **2014,** *70*, 4910-4920.
33. Bandgar, B. P.; Shaikh, K. A., Molecular iodine-catalyzed efficient and highly rapid synthesis of bis(indolyl)methanes under mild conditions. *Tetrahedron Lett.,* **2003,** *44*, 1959-1961.
34. Veisi, H., Molecular iodine: Recent application in heterocyclic synthesis. *Curr. Org. Chem.,* **2011,** *15*, 2438-2468.
35. More, S. V.; Sastry, M. N. V.; Wang, C.-C.; Yao, C.-F., Molecular iodine: a powerful catalyst for the easy and efficient synthesis of quinoxalines. *Tetrahedron Lett.,* **2005,** *46*, 6345-6348.
36. Togo, H.; Iida, S., Synthetic use of molecular iodine for organic synthesis. *Synlett,* **2006,** *2006*, 2159-2175.
37. Hojat, V., Molecular iodine: Recent application in heterocyclic synthesis. *Curr. Org. Chem.,* **2011,** *15*, 2438-2468.
38. Tantak, M. P.; Gupta, V.; Nikhil, K.; Arun, V.; Singh, R. P.; Jha, P. N.; Shah, K.; Kumar, D., Sequential one-pot synthesis of bis(indolyl)glyoxylamides: Evaluation of antibacterial and anticancer activities. *Bioorganic Med. Chem. Lett,* **2016,** *26*, 3167-3171.
39. French, A. N.; Bissmire, S.; Wirth, T., Iodine electrophiles in stereoselective reactions: recent developments and synthetic applications. *Chem. Soc. Rev.,* **2004,** *33*, 354-362.
40. Parvatkar, P. T.; Parameswaran, P. S.; Tilve, S. G., Recent developments in the synthesis of five- and six-membered heterocycles using molecular iodine. *Chem. Eur. J.,* **2012,** *18*, 5460-5489.
41. Wipf, P.; Miller, C. P., A new synthesis of highly functionalized oxazoles. *J. Org. Chem.,* **1993,** *58*, 3604-3606.

42. Johns, B. A.; Weatherhead, J. G.; Allen, S. H.; Thompson, J. B.; Garvey, E. P.; Foster, S. A.; Jeffrey, J. L.; Miller, W. H., 1,3,4-Oxadiazole substituted naphthyridines as HIV-1 integrase inhibitors. Part 2: SAR of the C5 position. *Bioorganic Med. Chem. Lett.*, **2009,** *19*, 1807-1810.
43. Phakhodee, W.; Wangngae, S.; Wiriya, N.; Pattarawarapan, M., Ph_3P/I_2-mediated synthesis of *N,N′*-disubstituted and *N,N,N′*-trisubstituted amidines. *Tetrahedron Lett.*, **2016,** *57*, 5351-5354.
44. Meruva, S. B.; Raghunadh, A.; Kamaraju, R. R.; Kumar, U. K. S.; Dubey, P. K., An oxidative amidation and heterocyclization approach for the synthesis of *β*-carbolines and dihydroeudistomin Y. *Beilstein J. Org. Chem.*, **2014,** *10*, 471-480.
45. Cotton, F. A.; Kibala, P. A., Reactions of iodine with triphenylphosphine and triphenylarsine. *J. Am. Chem. Soc.*, **1987,** *109*, 3308-3312.
46. Diallo, A.; Mehri, H.; Iouzalen, L.; Plat, M., Alkaloids from leaves of *Alangium bussyanum*. *Phytochemistry*, **1995,** *40*, 975-977.
47. Viart, H. M. F.; Bachmann, A.; Kayitare, W.; Sarpong, R., *β*-Carboline amides as intrinsic directing groups for C(sp2)–H functionalization. *J. Am. Chem. Soc.*, **2017,** *139*, 1325-1329.
48. Baiget, J.; Llona-Minguez, S.; Lang, S.; MacKay, S. P.; Suckling, C. J.; Sutcliffe, O. B., Manganese dioxide mediated one-pot synthesis of methyl 9*H*-pyrido[3, 4-*b*]indole-1-carboxylate: Concise synthesis of alangiobussinine. *Beilstein J. Org. Chem.*, **2011,** *7*, 1407.

Chapter 4

Design and Synthesis of Water-soluble Cationic Porphyrin-*β*-carboline Conjugates as Potent Photocytotoxic Agents



Design and Synthesis of Water-soluble Cationic Porphyrin-*β*-carboline Conjugates as Potent Photocytotoxic Agents

4.1 Introduction

Porphyrins are highly intensely coloured compounds, containing a core macrocycle of twenty carbon atoms and four nitrogen atoms which is surrounded by metal cation or, two protons (*N*H) in the case of a free-base porphyrin. The fundamental structure of porphine **1** ring is shown in Figure 4.1. Due to the high symmetry of porphyrins, there are only two types of carbon atoms along the ring that can undergo substitution. The bridging of methine carbons (positions 5, 10, 15, and 20 in Figure 4.1) are designated the *meso*-positions and the pyrrolic carbons (positions 2, 3, 7, 8, 12, 13, 17, and 18) are defined as the *β*-positions.[1] If the substituents present either at *meso* or *β*-positions of porphine **1** then the corresponding structure is termed as porphyrin **2**.

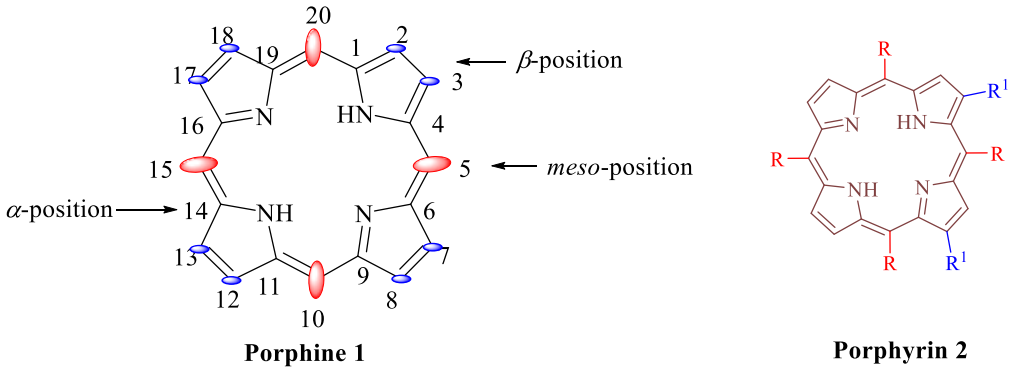


Figure 4.1 Numbering of tetrapyrrolic ring system **1**

W. Kuster first proposed the structure of porphyrins in 1912 and received with skepticism at that time, because the macrocycle seems to be intrinsically unstable. Later, Hans Fischer successfully achieved the synthesis of heme (iron porphyrin in hemoproteins) from pyrrolic starting materials in 1929 opened an era of captivating research of the tetrapyrrolic macrocycles.[2]

Porphyrins are tetrapyrrole macrocycles that are ubiquitous in nature.[3] They play fundamental and useful roles in a variety of biological processes, such as oxygen transport in hemoglobin and myoglobin, cytochrome P-450 enzyme catalyzed oxidations, electrical conduits or shuttles in cytochromes C, bacterial and plant photosynthesis and as reducing agents in methyl-coenzyme-M reductase.[4] Here, the iron-containing heme **4**, found in hemoglobin, is responsible for the binding and transportation of oxygen within blood cells.[5] The natural porphyrins such as cobalt-containing cobyrinic amide **5**, the central component of

vitamin B-12, plays a key role in cellular metabolism. Chlorophyll **6**, a magnesium-metallated porphyrin-like chromophore acts as the light-harvesting agent in photosynthetic organisms. Interestingly, these three natural porphyrins share a common biosynthetic precursor, uroporphyrinogen III **3** (Figure 4.2).[6]

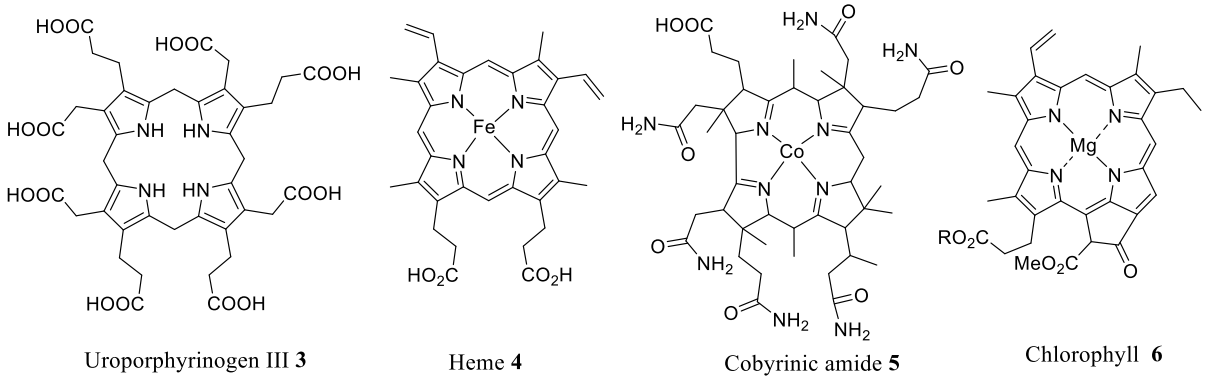


Figure 4.2 Structures of natural porphyrins

4.2 Porphyrin as a photosensitizer in PDT

Porphyrins have found several applications as luminescent markers,[7] oxygen sensors,[8] photosensitizers in medical applications such as photodynamic therapy (PDT),[9] protein cross-linking agents,[10] optical imaging agents in photodynamic diagnosis (PDD),[11] radiation therapy[12] and fluorescent labeling in flow cytometry.[13] Each of these applications depends on the ability of the chromophore to be excited by incident light to an excited state and to decay to a lower energy state with the consequent energy emission.

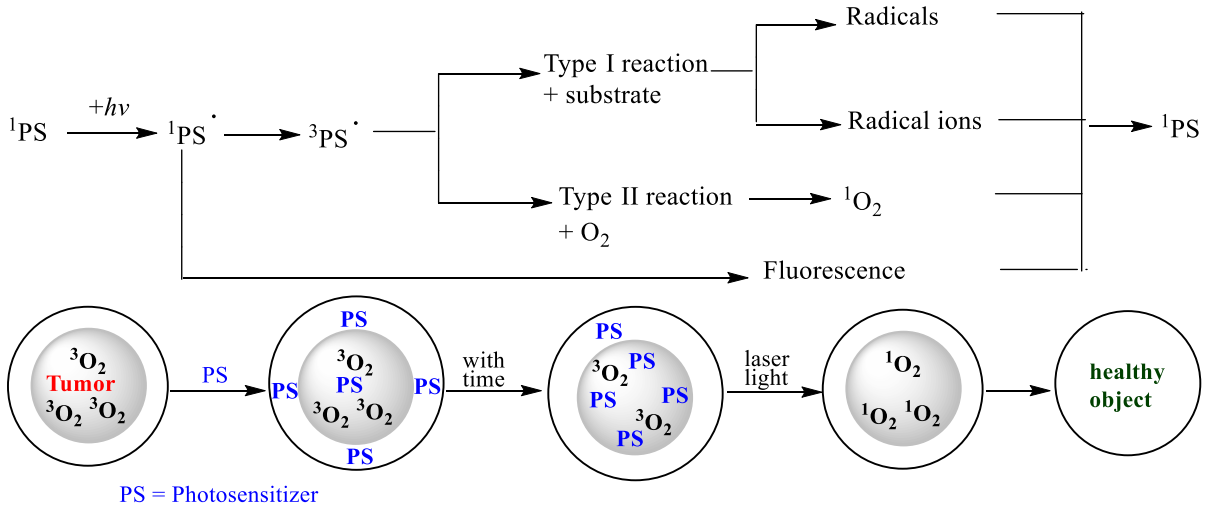


Figure 4.3 Principle of photodynamic therapy

Importantly, the utilization of porphyrin in PDT is one of the focused areas of research wherein a photosensitizer is introduced into the body and then it accumulates preferentially in

tumor tissues. Later, exposure to visible light irradiation at a specific wavelength or by white light, photosensitizers are activated to their triplet states.[14] The triplet photosensitizers then interact with molecular oxygen to result in the photosensitizer ground states and highly reactive singlet oxygen (Figure 4.3). Cell or tissue death is caused by necrosis and/or apoptosis due to singlet oxygen reacts with various cellular components.[15,16]

As porphyrins need to be applicable for PDT and other biological studies, a certain level of water solubility is desired. The inclusion of highly polar groups such as amine, hydroxyl, and carboxylic acid is one of the ways to achieve water solubility. Alternatively, ionized (cationic and anionic) macrocycles also help to impart water solubility. In the recent past, various cationic porphyrins have emerged as potent photosensitizers (Figure 4.4).[17,18]

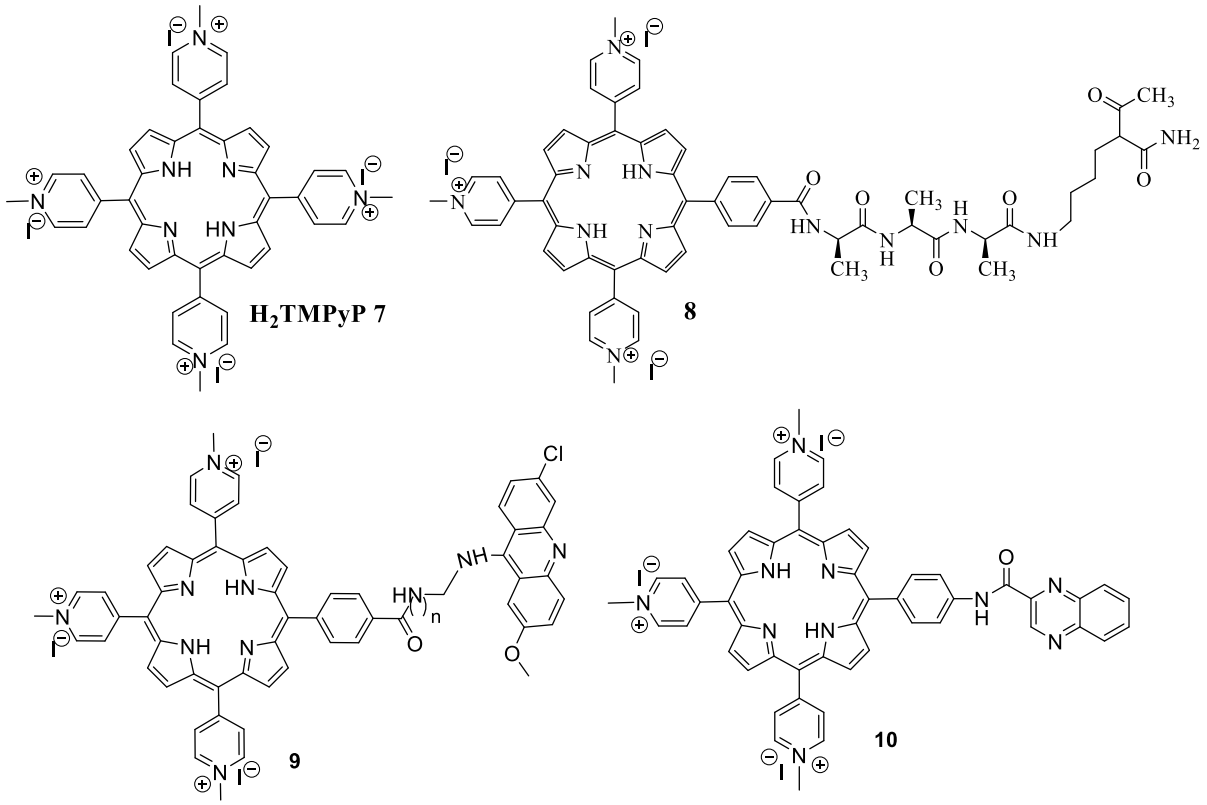


Figure 4.4 Cationic porphyrins as photocytotoxic agents

One of the most explored cationic porphyrins is *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (H_2TMPyP) **7**. Cationic porphyrin **7** intercalates into DNA and causes DNA cleavage upon irradiation, partly because of the DNA nuclease activity. H_2TMPyP is also an efficient photosensitizer for PDT, where it targets mitochondrial membranes.[19,20] The ability of **7** to directly damage DNA *via* intercalation sets it apart from the anionic and liposome-delivered PDT agents, which damage malignant cells through the destruction of the tumor vasculature.[21] Villanueva *et al.* showed that **7** localizes to tumor tissue with high selectivity,

and little accumulation of porphyrin occurred in the skin or the brains of subject mice.[22] Therefore, the skin sensitization is a side effect of Photofrin treatment and is unlikely to be a side effect of H_2TMPyP-mediated photosensitization as porphyrin is cleared quickly from tissues. Unlike many of the current porphyrinic photosensitizers, H_2TMPyP is water soluble and requires no vehicle for administration to *in vivo*.[23]

Orosz and coworkers demonstrated the interactions of cationic porphyrin-tetrapeptide conjugates **8** with encapsidated DNA in T7 phage nucleoprotein (NP) complex as therapeutic targets are nucleoprotein and not naked DNA. The porphyrin-peptide conjugate **8** showed notable red shifts and hypochromicity of the Soret band in absorption spectrum which was accompanied by a negative and positive induced band in the circular dichroism spectra suggesting both intercalation and external binding to encapsidated T7 DNA. Further, DNA melting experiments revealed that bound porphyrins do not influence the capsid stability or protein–DNA interactions, but efficiently stabilize the double helical structure of DNA without respect to binding form.[24] Ishikawa group synthesized porphyrin-acridine conjugate **9** and reported to exhibit the more enhanced photocleavage activity of pUC18 plasmid DNA than H_2TMPyP which is well known to bind to DNA tightly and to cleave DNA effectively.[25] Kumar et al. identified a novel porphyrin–quinoxaline conjugate **10** as photocytotoxic agent (IC_{50} = 60 nM) against A549 cancer cells. Interaction study of conjugate **10** with ctDNA showed two distinct binding modes suggesting intercalation followed by self-stacking along the DNA surface.[26]

The lipophilicity of cationic porphyrins may play a vital role in their efficacy as PDT agents, as cancer cells require greater amounts of lipoproteins to proliferate, and lipoproteins will carry lipophilic porphyrins in the bloodstream.[27,28] The self-aggregation of cationic pyridyl-porphyrins, especially those substituted with long-chain alkyl groups, contribute to PDT damage through changes in their absorption spectra upon aggregation.[29]

Conjugation of porphyrinic macrocycle to naturally occurring or synthetic biologically active motifs has emerged as a promising approach to identify new lead compounds for the development of more efficient and selective photosensitizers. Further, the chemistry involved in the preparation of porphyrin conjugates with natural products, synthetic bioactive heterocycles, or peptides has made tremendous progress in PDT. It appears that porphyrin conjugated to bioactive heterocycles, peptides, carbohydrates, nucleic acids, antibodies, and endogenous ligands exhibits significant photocytotoxicity and has led to photosensitizers with improved efficacy, preferential localization in vital cell organelles and better stability.[30-32]

4.3 *β*-Carbolines as photo-induced DNA intercalators

β-Carboline and related derivatives have been explored as DNA photocleaving agents due to the photochemical reaction of *β*-carboline upon light irradiation as represented in Figure 4.5.[33-36] Firstly, Toshima et al. proposed that under light irradiation, conjugated carbon-nitrogen (C=N) double bond of *β*-carboline may facilitate the generation of photo-excited $^3(n\text{-}\pi^*)$ or $^3(\pi\text{-}\pi^*)$ state(s) or electron transfer. Upon photo-irradiation, electron present in the stimulated state is activated to escape from the molecular orbital and exchange energy with surroundings *via* a Type I or Type II photochemical reaction.[37] In the Type I reaction, the escaped electron binds to another *β*-carboline molecule that loses an electron without destroying the structure of the compound. While in the Type II reaction in the presence of O_2, the escaped electron binds to O_2 to yield the $O_2^{\bullet}$, and the left *β*-carboline ring without one electron is prone to be attacked by other electron-providing molecules, which leads to the structural destruction of the compound. In succession, the $O_2^{\bullet}$ undergoes a Harber–Weiss reaction to form the $^{\bullet}OH$ in a water solution, and all these free radicals can bring up comprehensive biological effects to tumor cells.[38]

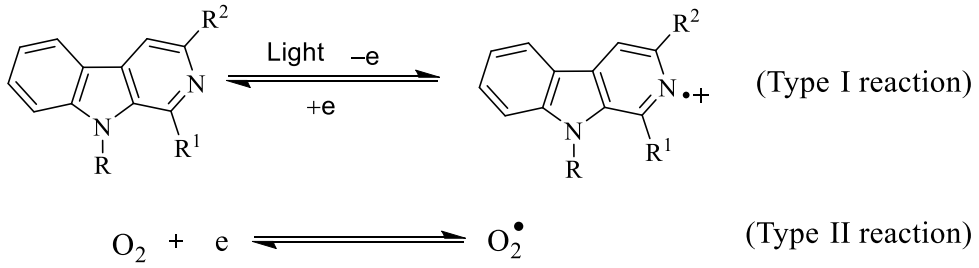


Figure 4.5 Photochemical reaction mechanism of *β*-carboline derivatives

Significantly, *β*-carboline derivatives have been found in several marine natural products and pharmaceutically relevant molecules (Figure 4.6).[39] Numerous investigations focusing on the effects of *β*-carboline alkaloids on the central nervous system, such as their affinity with a benzodiazepine, 5-hydroxytryptamine, dopamine, and imidazoline receptors have also been documented.[40,41] However, considerable recent interests in these alkaloids were stimulated by their potential antitumor activities.[42] For example, *β*-carboline-1-carboxamide **11** was found to show antitumor activity against HTC 116 cancer cells (IC_{50}= 6.7 μM).[43] Biologically active natural *β*-carboline amides **12** and alangiobussinine **13** were isolated from marine sources are known as DNA intercalators.[44,45] Also, Toshima group found *β*-carboline-carbohydrate derivative **14** as an effective DNA cleaving agent at the guanine site upon UV irradiation.[37] Naturally occurring fascaplysin **5** is known to possess high cytotoxic activity

(IC_{50} = 0.2 μg/mL).[46,47] Synthetic analogue of harmine **16** exhibited significant antitumor activities *in vitro*.[48]

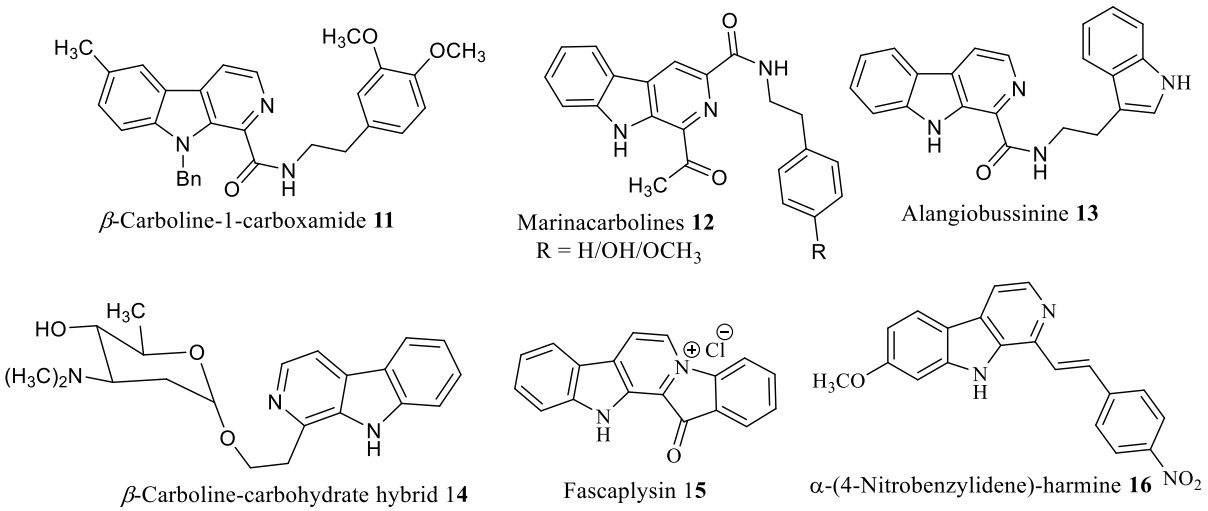


Figure 4.6 Selected examples of bioactive *β*-carbolines

In continuation of our efforts to investigate water-soluble porphyrins as photocytotoxic agents, recently we have identified various porphyrin conjugates and tricationic *meso*-(4′-cyano-phenyl)porphyrin with improved efficacy and selectivity.[49-52] Encouraged by the results of previously identified porphyrin conjugates and important biological properties and photo-induced DNA cleavage associated with *β*-carbolines, herein we designed porphyrin-*β*-carboline conjugates by incorporating both the important structural features in a single entity (Figure 4.7). The amide linkage is present in various drug molecules and also the key linking moiety in proteins and peptide drug products.[53]

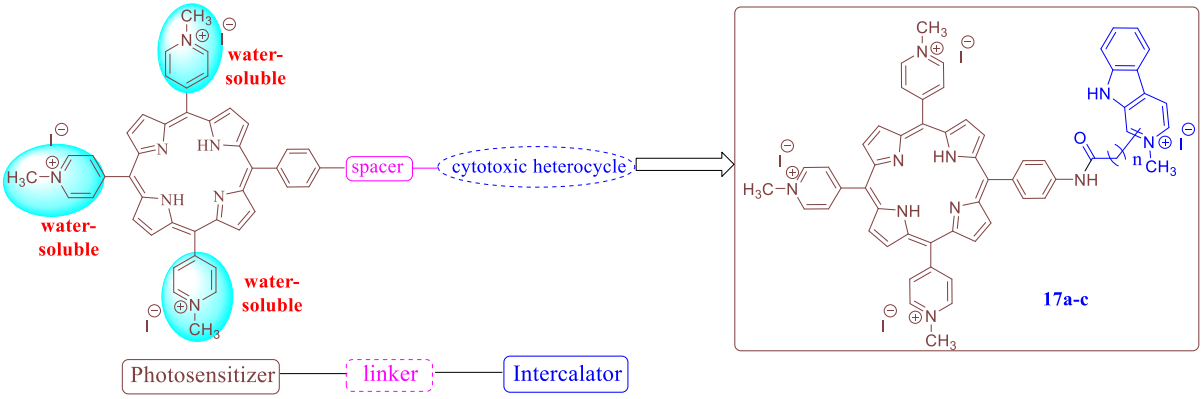
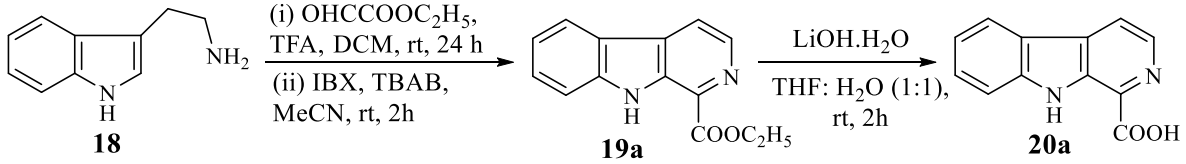


Figure 4.7 Design of water-soluble porphyrin-*β*-carboline conjugates

4.4 Results and discussions

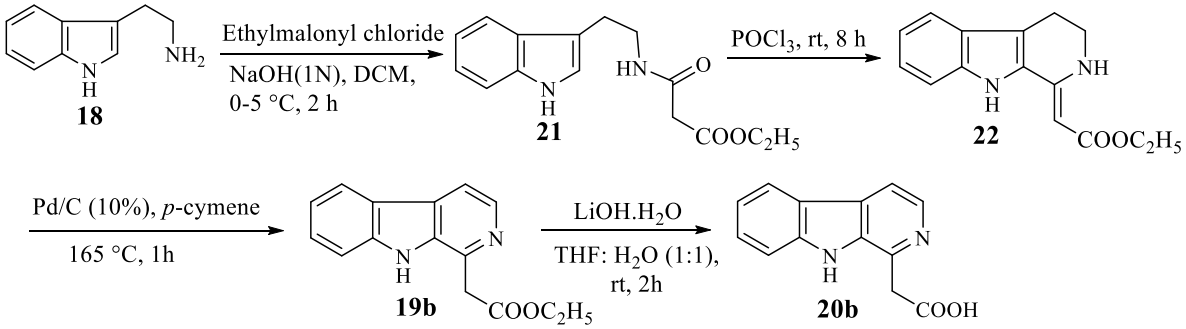
4.4.1 Chemistry

The porphyrin-*β*-carboline conjugates **17a-c** were achieved from the coupling reaction of *β*-carboline acids **20a-c** with 5-(4-amino-phenyl)tripyridylporphyrin **31** followed by *N*-methylation of porphyrin **32a-c**. The required *β*-carboline acids **20a-c** were prepared from the reported procedures with necessary modifications.[54-58] Initially, the synthesis of *β*-carboline acid **20a** was carried out from the reaction of readily available tryptamine with ethyl glyoxylate under Pictet–Spengler conditions followed by *in-situ* IBX-promoted aromatization afforded *β*-carboline ester **19a**. Finally, hydrolysis of **19a** in the presence of lithium hydroxide monohydrate ($LiOH.H_2O$) produced *β*-carboline-1-carboxylic acid **20a** in good yield (Scheme 4.1).



Scheme 4.1 Preparation of *β*-carboline-1-carboxylic acid **20a**

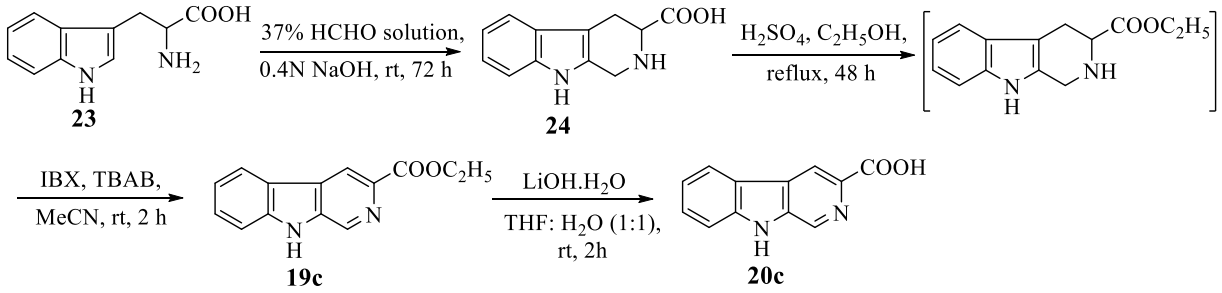
The *β*-carboline acid **20b** was synthesized from the reaction of tryptamine **18** with ethyl malonyl chloride resulted amidoester **21.** Bischler–Napieralski cyclization of **21** in $POCl_3$ at room temperature to give enaminoester **22**. Further, the reaction of **22** with 10% Pd/C led to **19b** which was subjected to hydrolysis using lithium hydroxide to furnish **20b** in excellent yield (Scheme 4.2).



Scheme 4.2 Preparation of *β*-carboline-1-acetic acid **20b**

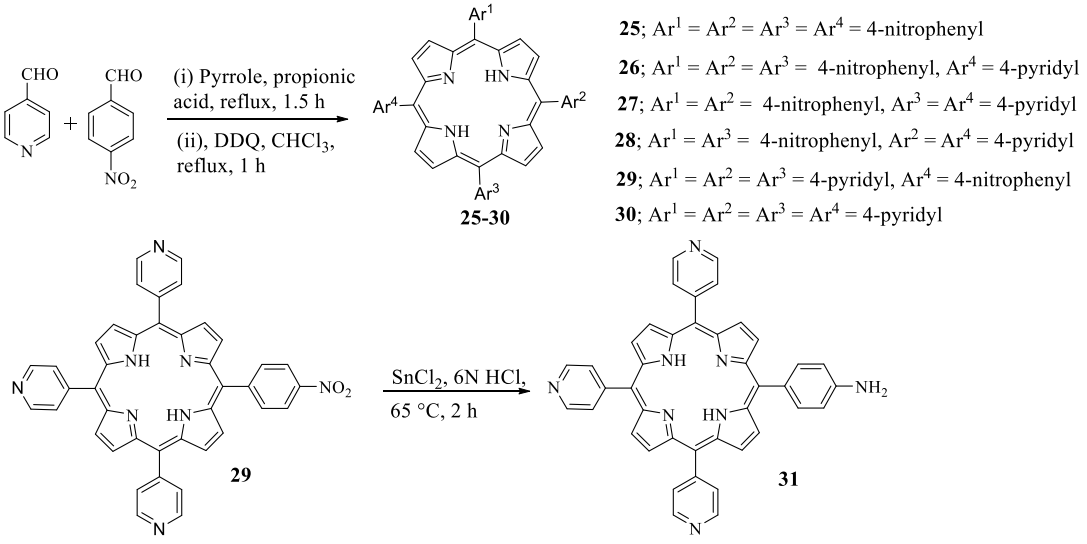
The *β*-carboline acid **20c** was achieved as illustrated in Scheme 4.3. The reaction of commercially available L-tryptophan **23** with formaldehyde solution under basic conditions produced tetrahydro-*β*-carboline-3-carboxylic acid **24**. Subsequently, esterification of **24**

followed by IBX-promoted aromatization generated **19c**. Finally, hydrolysis of ester **19c** using lithium hydroxide produced **20c** in good yield.



Scheme 4.3 Preparation of *β*-carboline-3-carboxylic acid **20c**

The intermediate 5-(4-nitrophenyl)tripyridylporphyrin **29** was prepared by the acid-catalyzed condensation reaction of pyrrole with a specific aldehyde, followed by oxidation of the resulting colourless porphyrinogen. This procedure, initially developed by Rothemund and Menotti,[59] was improved by Adler and Longo.[60] A mixture of 4-nitrobenzaldehyde (1 equiv), 4-pyridine carboxaldehyde (3 equiv) and pyrrole (4 equiv) was refluxed in excess of propionic acid and the residue was treated with DDQ to afford a mixture of porphyrins (Scheme 4.4). The solution was stored at 0 ºC for overnight to allow the porphyrins to precipitate. The resulting porphyrins were filtered and washed repeatedly with methanol. The desired porphyrin **29** was then separated by repeated silica gel column chromatography (3 times) by using chloroform/hexane for elution. Porphyrins were eluted from the column in sequence from **25**, **26**, **27**, **28, 29** and then **30.** Later, the reduction of separated nitroporphyrin **29** with stannous chloride in 6N HCl afforded the corresponding aminoporphyrin **31** in 70% yield.[61]



Scheme 4.4 Synthesis of 5-(4-aminophenyl)tripyridylporphyrin **31**

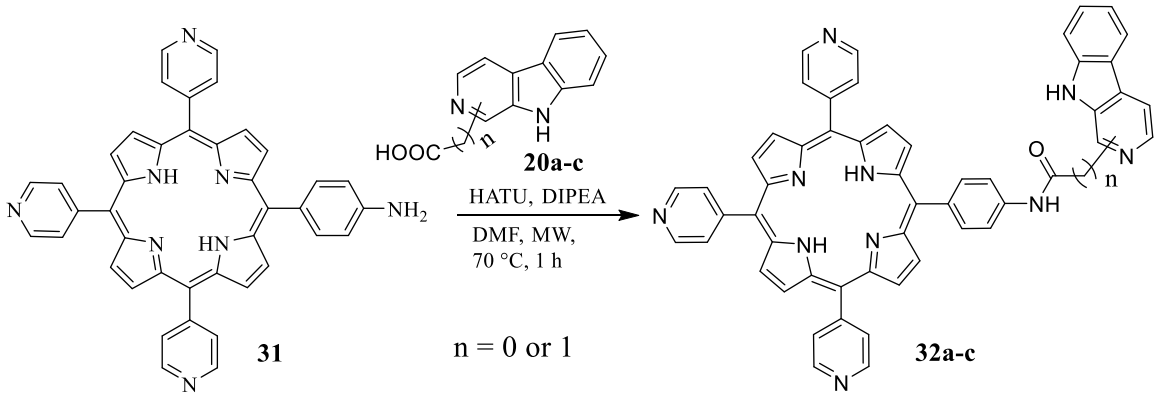
Next, to prepare the porphyrin-*β*-carboline conjugate **32a**, we optimized the reaction conditions by selecting 5-(4-aminophenyl)tripyridylporphyrin **31** and *β*-carboline-1-carboxylic acid **20a** as model substrates. The efficiencies of various reagents like $SOCl_2$, oxalyl chloride, ethylchloroformate and carbodiimide reagents (CDI, EDCI.HCl) were first examined under conventional heating, but it failed to produce **32a** (entry 1-6, Table 4.1). However, the use of HATU [O-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium-hexafluorophosphate] as coupling reagent afforded **32a** in 30% yield (entry 9, Table 4.1).

Table 4.1 Optimization of the reaction conditions for conjugate **32a**

Entry	Conditions	Conventional heating		Microwave Irradiation	
		Time (h)	Yield (%)[a]	Time (h)	Yield (%)[a]
1	$SOCl_2$, DCM, TEA, rt	12	trace	NA	NA
2	$(COCl)_2$, DCM, TEA, rt	15	trace	NA	NA
3	$ClCO_2Et$, DCM, TEA, rt	6	trace	NA	NA
4	CDI, THF, TEA, rt	20	trace	NA	NA
5	EDCI/HOBt, THF, DIPEA, rt	14	trace	1	trace
6	EDCI/HOBt, DMF, TEA, 70 °C	12	trace	1	trace
7	HATU, THF, DIPEA, 60 °C	15	trace	1	trace
8	HATU, DMF, DIPEA, rt	24	trace	1	0
9	**HATU, DMF, DIPEA, 70** °C	**12**	**30**	**1**	**80**
10	HATU, DMF, TEA, 70 °C	12	trace	1	40

*NA indicates not attempted. [a]Isolated yield

Next, we explored the use of microwave (MW) activation to enhance reaction efficiency and product yield.[62] Under MW irradiation conditions, the coupling of 5-(4-aminophenyl)-tripyridylporphyrin **31** and *β*-carboline acid **20a** in the presence of HATU and DIPEA in DMF at 70 °C smoothly proceeded to afford **32a** in 80% yield (entry 9, Table 4.1). Using optimal conditions, porphyrin-*β*-carboline conjugates **32b-c** were obtained in 65-80% yields as delineated in Scheme 4.5. The structures of **32a-c** were confirmed by MALDI-TOF mass and NMR (^1H & ^{13}C) spectral data (Figures 4.8-4.10).



Scheme 4.5. MW-assisted synthesis of porphyrin conjugates **32a-c**

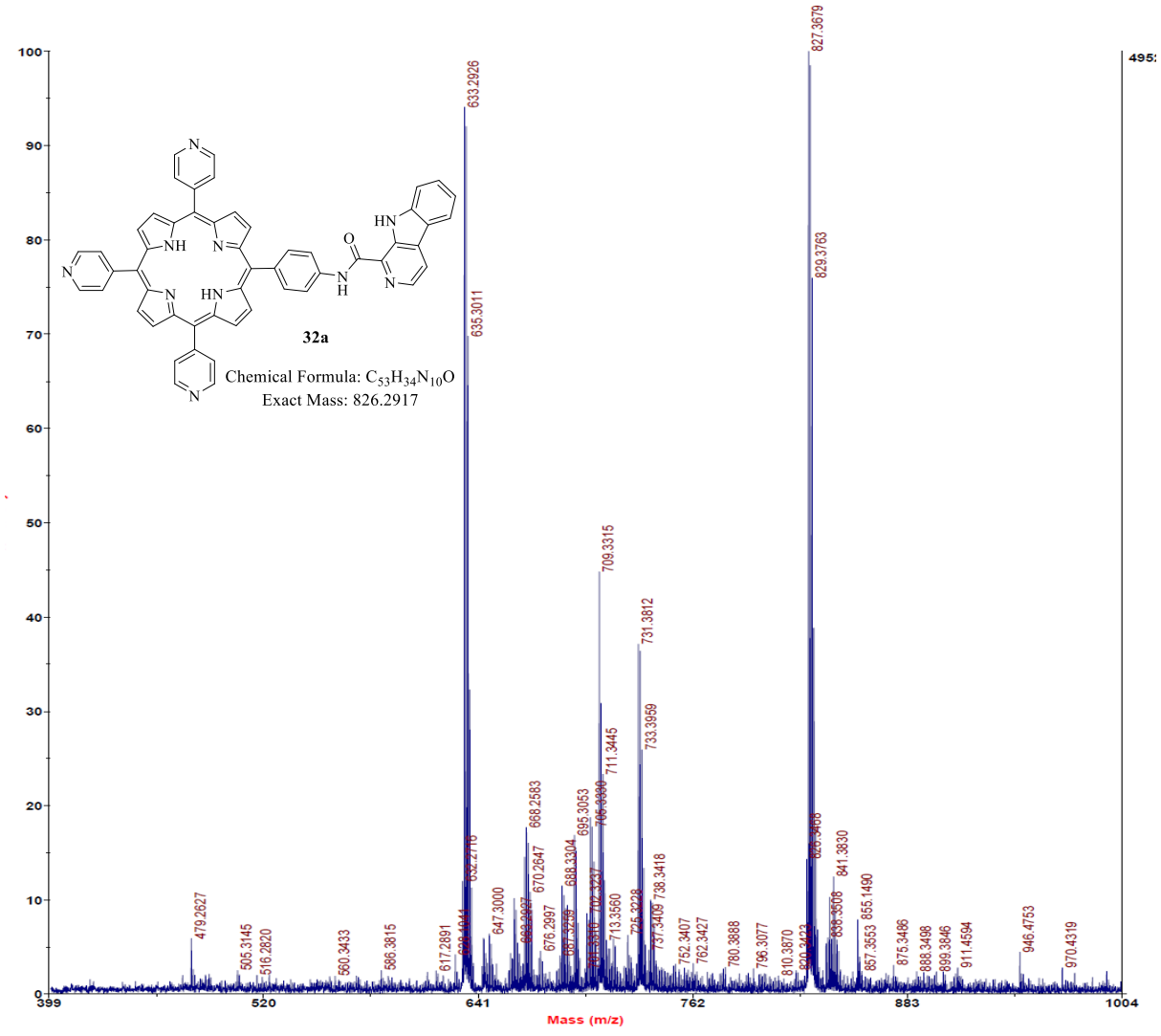


Figure 4.8 MALDI-TOF mass spectrum of **32a**

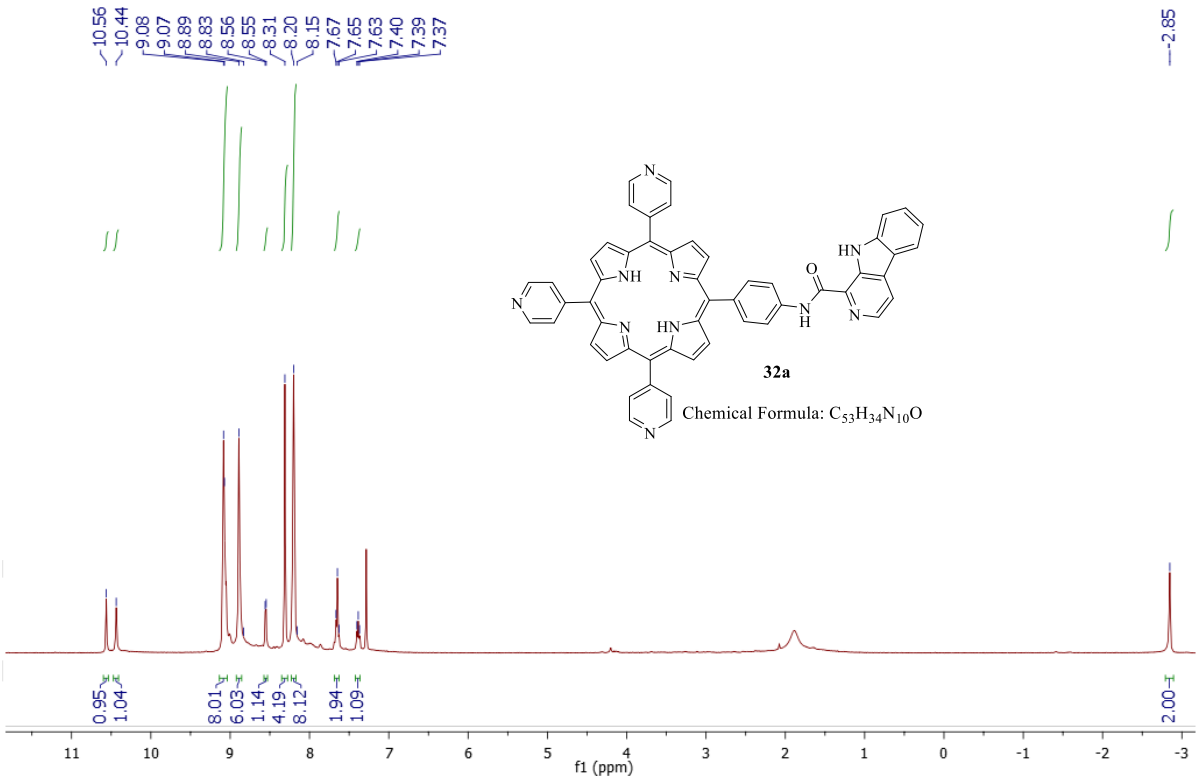


Figure 4.9 ^{1}H NMR spectrum of **32a**

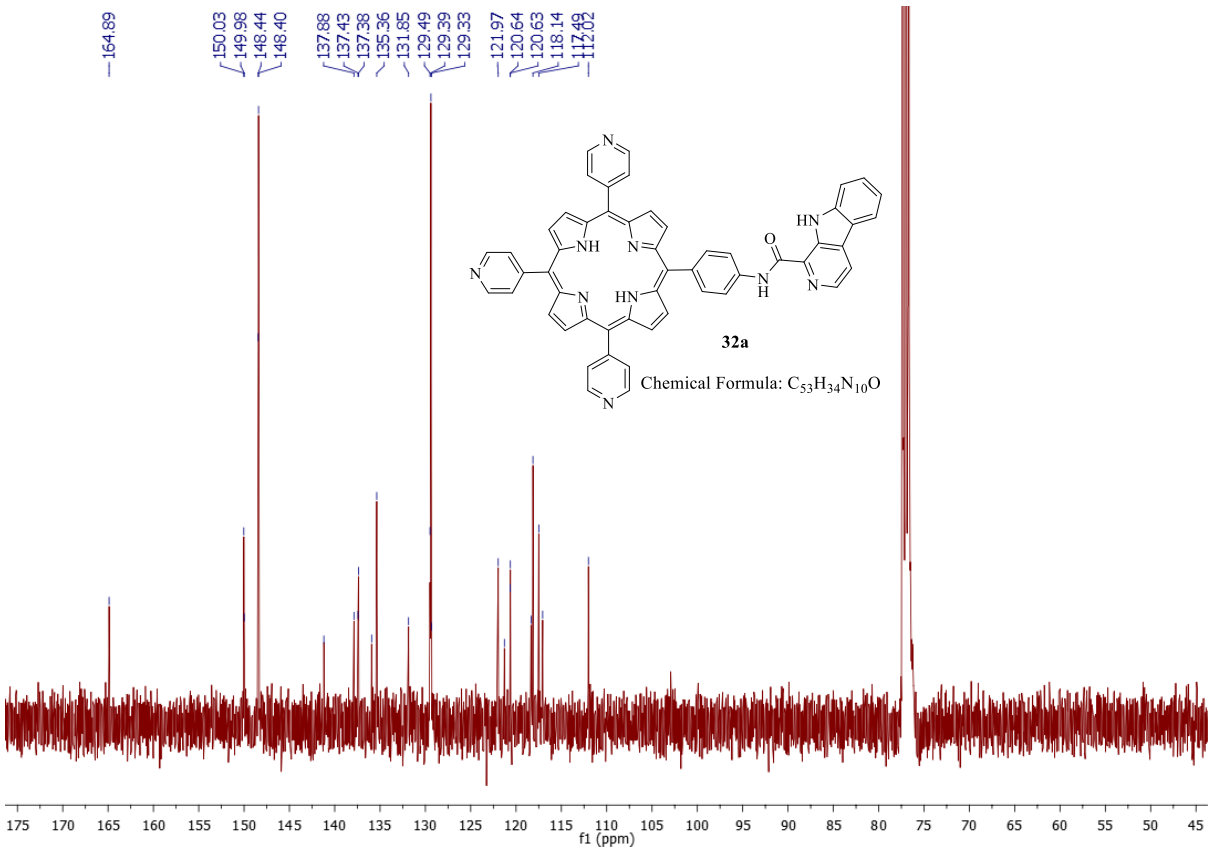
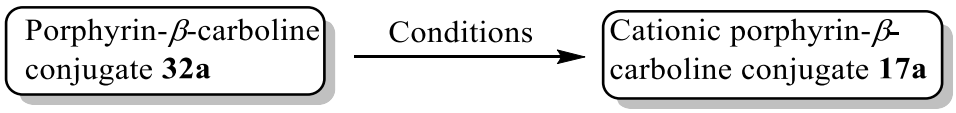


Figure 4.10 ^{13}C NMR spectrum of **32a**

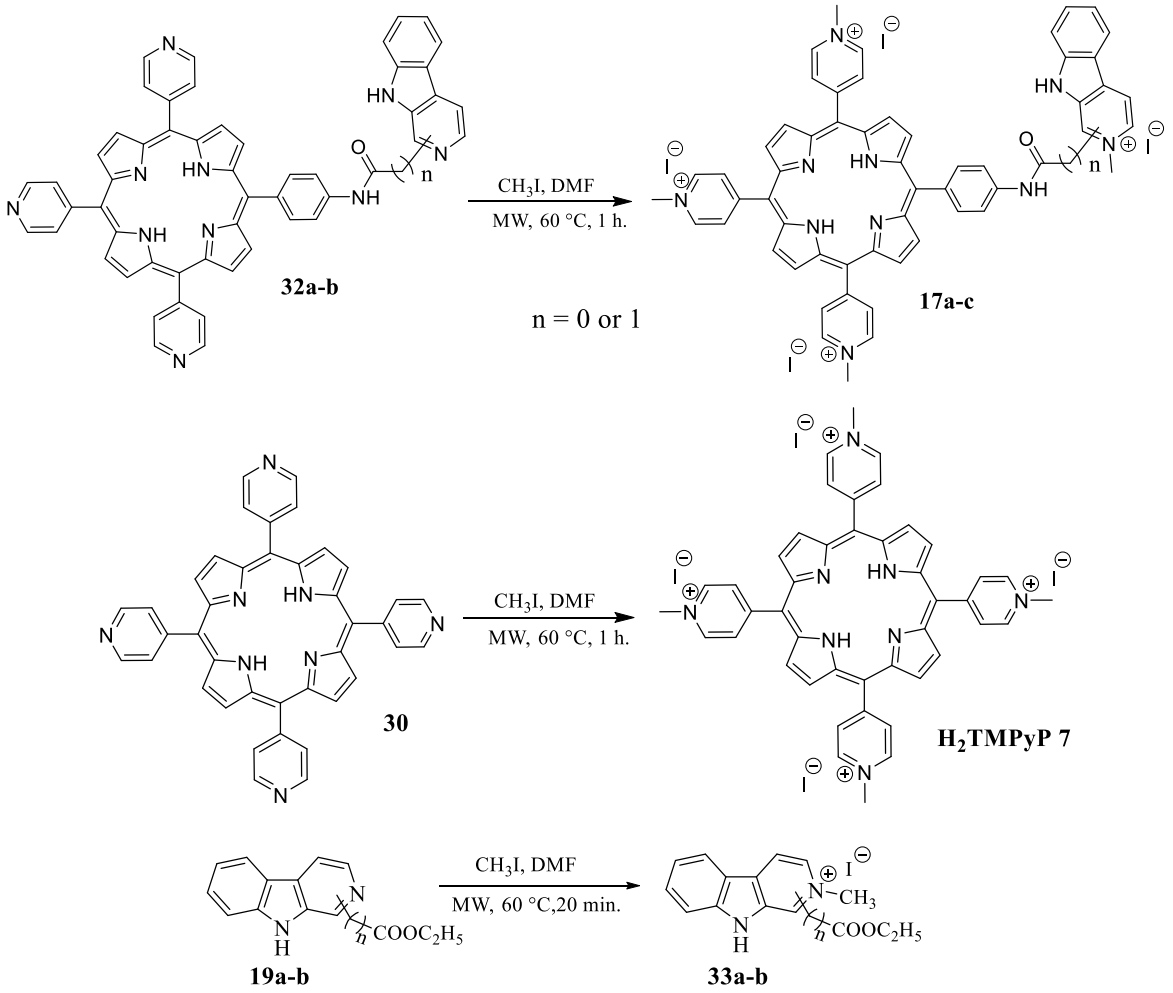
Finally, reaction of porphyrin-*β*-carboline conjugate **32** with methyl iodide was explored to prepare cationic porphyrin conjugate **17**.[49,61] The trial reaction of **32a** with excess of methyl iodide (200 mmol) under conventional heating in DCM, chloroform and THF produced trace amount of cationic porphyrin **32a** (entries 1-3, Table 4.2) however, in DMF (300 h) **32a** resulted in 40% yield (entry 5, Table 4.2). Under conventional heating in oil bath, methylation of **32a** resulted in low product yield and required longer reaction times. Thus, we conducted the *N*-methylation under MW irradiation. Interestingly, MW drastically improved the efficiency of reaction with higher product yield (>90%) and comparatively lesser use of methyl iodide (40 mmol) in a shorter time (entry 8, Table 4.2). Similarly, using MW irradiation, cationic porphyrin-*β*-carboline conjugates **17b-c, H_2TMPyP 7** and 2-methyl-*β*-carbolinium iodides **33a-c** were prepared in >90% yields as illustrated in Scheme 4.6.

Table 4.2 *N*-Methylation of conjugate **32a**



Entry	CH_3I (mmol)	Solvent	Temp (°C)	Time (h)	Yield (%)[a]	
					Conventional	MW
1	200	DCM	40	100	trace	NA
2	200	$CHCl_3$	60	100	trace	NA
3	200	THF	65	120	trace	NA
4	200	DMF	60	100	10	NA
5	200	DMF	60	300	40	NA
6	10	DMF	60	1	NA	30
7	20	DMF	60	1	NA	60
8	40	DMF	60	1	NA	92
9	40	DMF	80	1	NA	70
10	40	DMF	100	1	NA	40
11	40	DMF	60	0.5	NA	44

*NA indicates not attempted. [a]Isolated yield



Scheme 4.6 MW-assisted *N*-methylation of porphyrins and *β*-carbolines

The structures of **17a-c** were confirmed by UV, NMR (^{1}H & ^{13}C) and MALDI-TOF mass spectral data. In proton NMR, a set of twelve *N*-methyl protons of porphyrin and *β*-carboline moieties were observed at *δ* ~4.63 ppm. The internal pyrrolic *N*-H protons were resonated in the upfield region *δ* ~ –3 ppm (Figure 4.11). The carbon NMR of **17a-c** displayed characteristic signals due to amidic carbon (~165 ppm) and *N*-methyl carbons of porphyrin (~48 ppm) and *β*-carboline (~53 ppm) units (Figure 4.12). The MALDI-TOF spectra of **17a-c** displayed corresponding molecular ion in agreement with the calculated mass (Figure 4.13). HPLC analysis indicated the purity of conjugates **17a-c** greater than 98% (Figure 4.14).

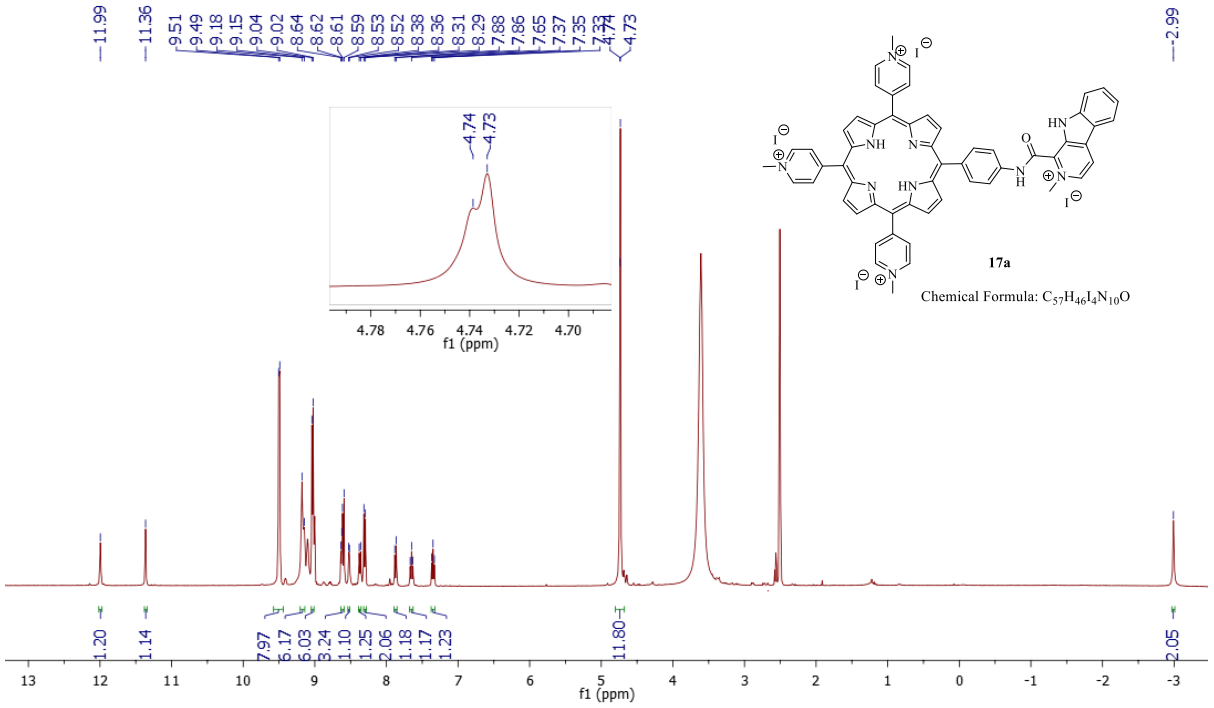


Figure 4.11 ^{1}H NMR spectrum of **17a**

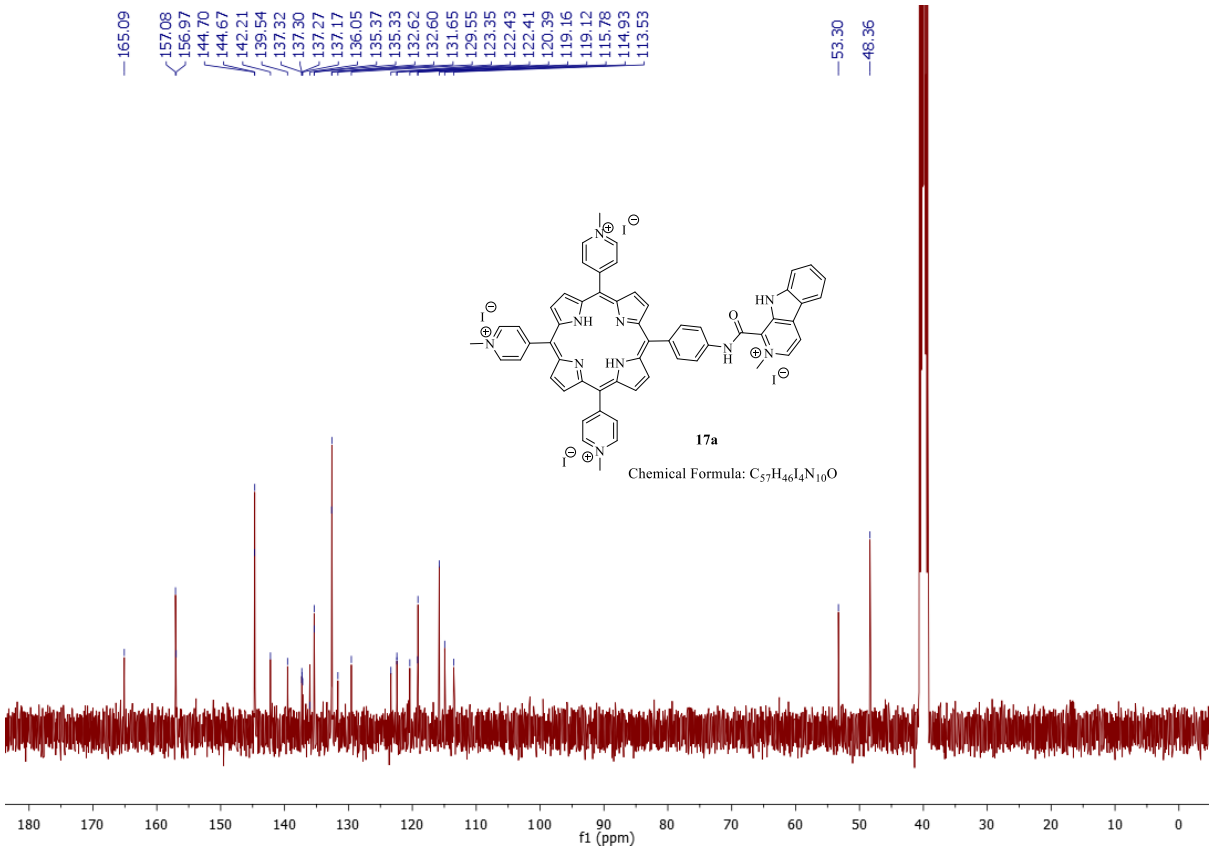


Figure 4.12 ^{13}C NMR spectrum of **17a**

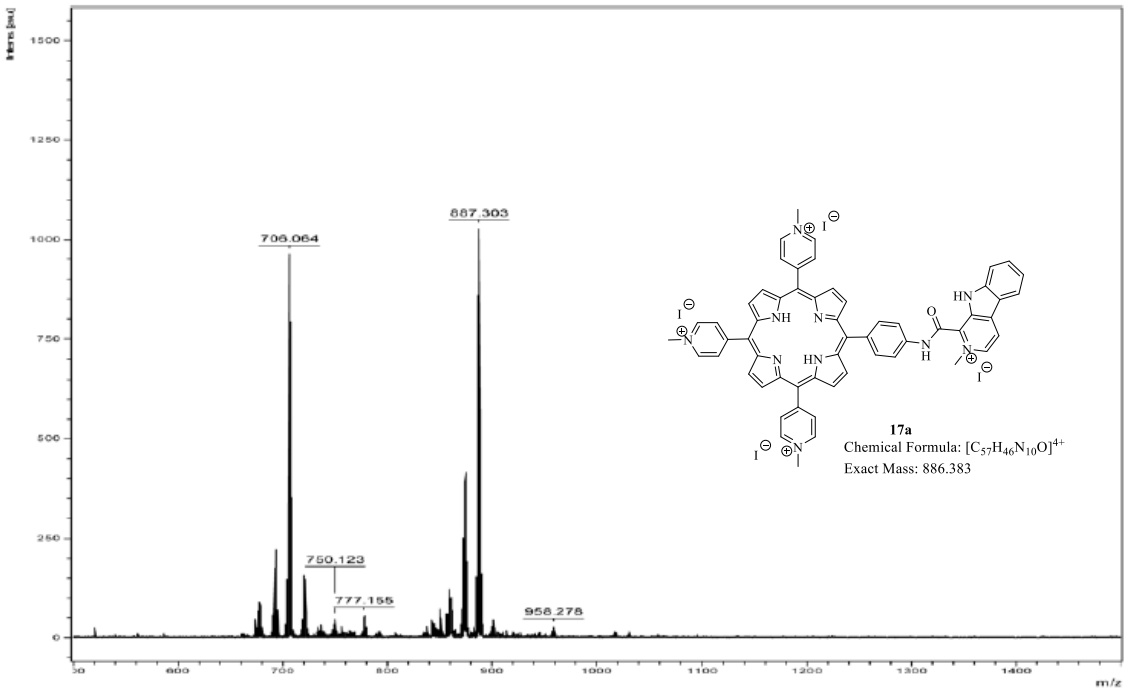
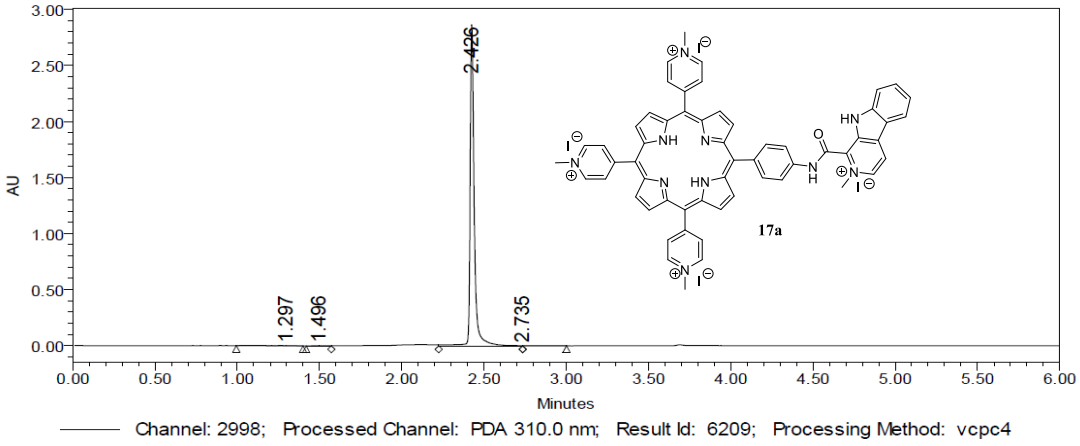


Figure 4.13 MALDI-TOF Mass spectrum of **17a**



Processed Channel Descr.: PDA 310.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 310.0 nm	1.297	25771	0.52	2146
2	PDA 310.0 nm	1.496	12669	0.25	2088
3	PDA 310.0 nm	2.426	4943319	99.07	2874066
4	PDA 310.0 nm	2.735	8170	0.16	1496

Figure 4.14 HPLC chromatogram of **17a**

4.4.2 Photocytotoxicity studies

Porphyrin-*β*-carboline conjugates **32** and **17** were evaluated for their photocytotoxicity towards lung (A549) and, colon (Colon-26) carcinoma cells using WST method. H_2TMPyP was used as a positive control. Under identical conditions, porphyrins **32a-c, 17a-c,** and **H_2TMPyP** inhibited 50% cell proliferation (IC_{50}) as described in Figure 4.15.

The photocytotoxicity study revealed that the cationic porphyrins **17a-c** are more potent than their neutral counterparts **32a-c**. Interestingly, neutral conjugate **32b** linking porphyrin and carboline moieties through methylene unit showed significant photocytotoxicity against A549 cell line with the IC_{50} value of 66 nM, but **32a** and **32c** are not photocytotoxic against tested cancer cells. The cationic porphyrin-*β*-carboline conjugates linking through C-1 (**17a**) and C-3 (**17c**) of carboline unit exhibited considerable photocytotoxicity (IC_{50} = 144-173 nM). Methylation of conjugate **32b** led to **17b** with 1.7-fold enhanced photocytotoxicity against A549 (IC_{50} = 39 nM) cancer cells. Conjugate **17b** showed better photocytotoxicity when compared to **H_2TMPyP** (Figure 4.15**)**. In addition, *β*-carbolinium salts **33a-c** did not show any photocytotoxicity under identical conditions. The identified potent conjugate **17b** demonstrated synergistic effect and substantially enhanced photocytotoxicity when compared with free **H_2TMPyP 7** and *β*-carbolinium salts **33a-c**.

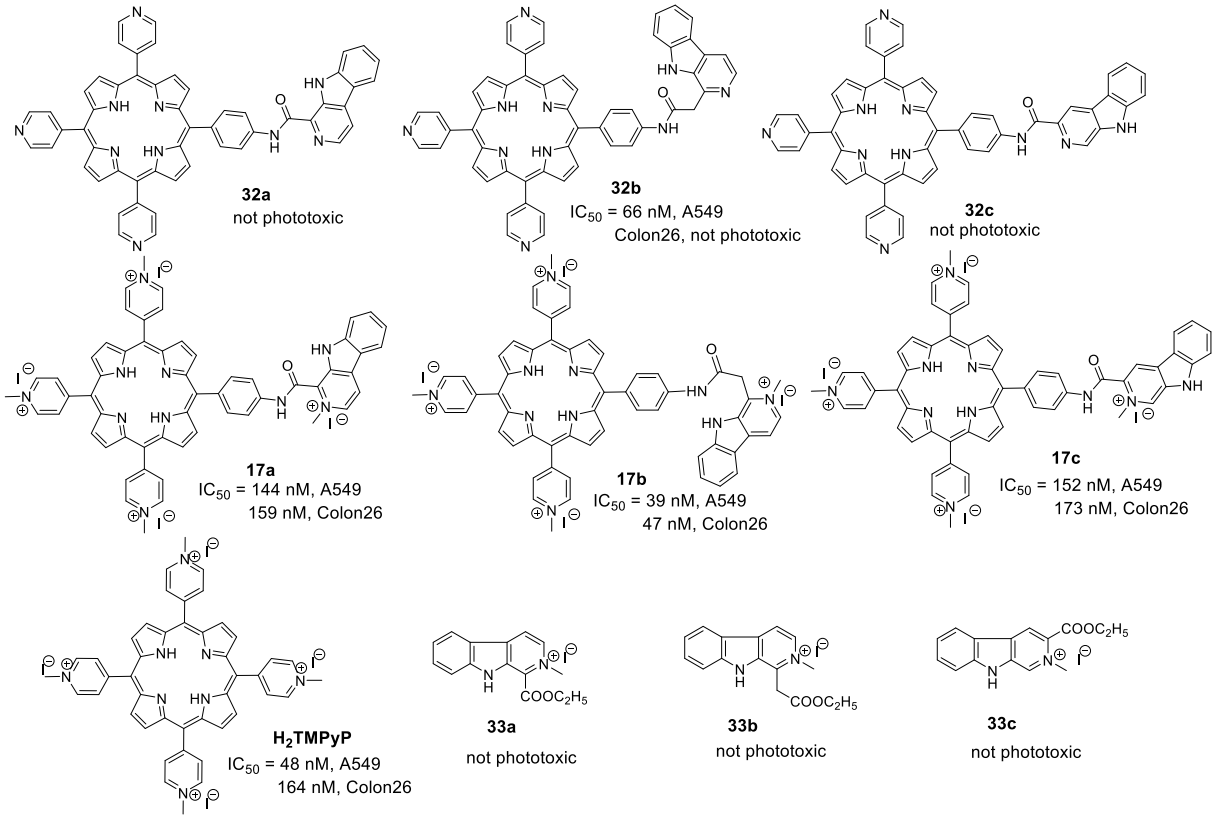


Figure 4.15 Photocytotoxicity of porphyrin and *β*–carboline analogues

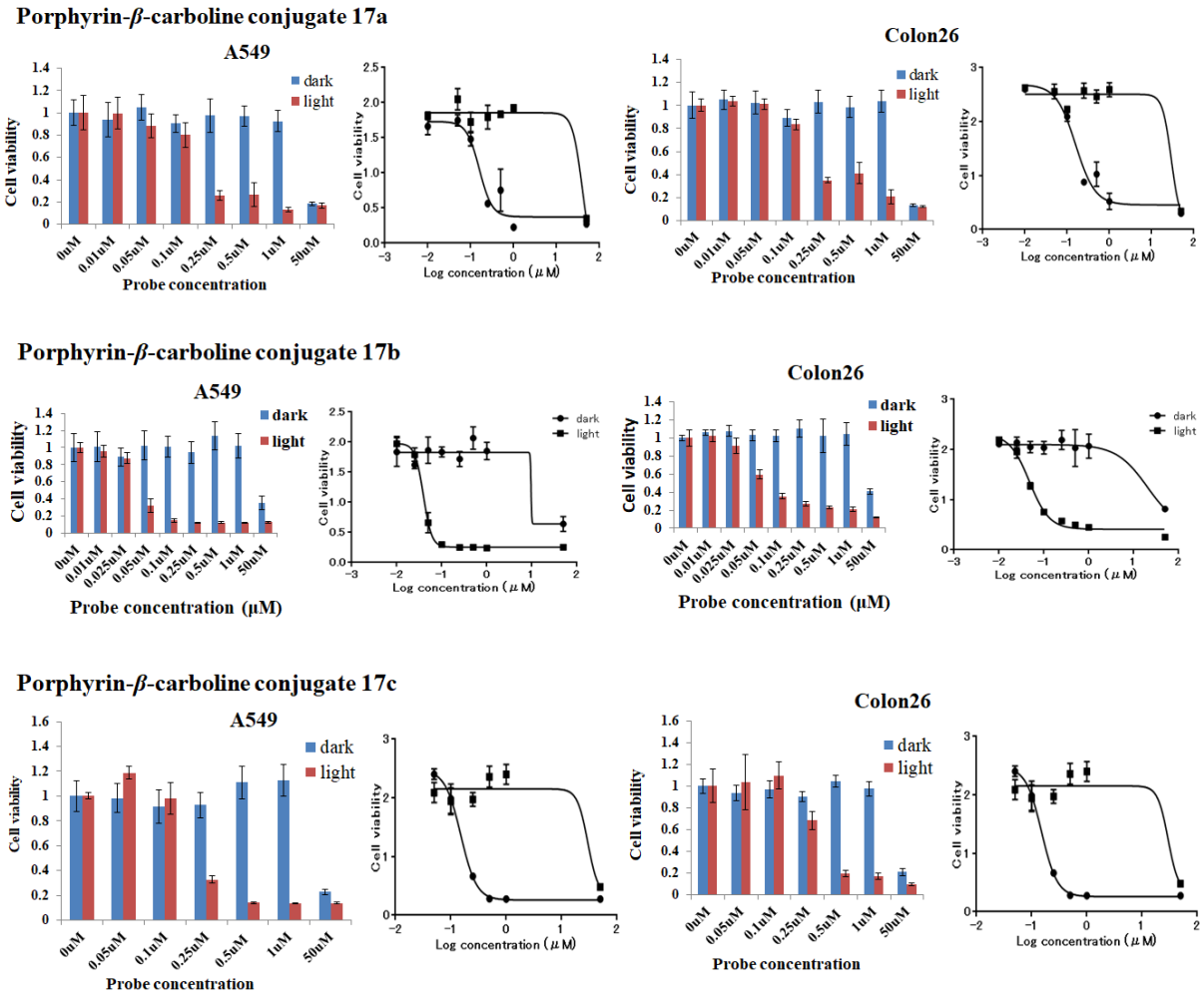


Figure 4.16 Determination of IC_{50} for conjugates **17a-c**

Synthesis and biological studies of conjugate **17b** (preliminary mechanism of cell death, interaction with ctDNA and DNA photocleavage activity) are well presented in the thesis submitted by Dr. K. P. Chandra Shekar thesis. Briefly, synthesis and activity results of **17b** have also described in this chapter.

4.5 Conclusions

In summary, the MW-assisted approach has been developed to access novel porphyrin-*β*-carboline conjugates **32a-c** in good yields. *N*-Methylation of porphyrin-*β*-carboline conjugates under MW irradiation rapidly proceeded to afford cationic porphyrin-*β*-carboline conjugates **17a-c** in excellent yields. Prepared cationic porphyrin-*β*-carboline conjugates, **17a-c** displayed significant photocytotoxicity towards lung (A549, IC_{50} = 39-152 nM) as well as colon (Colon-26, IC_{50} = 47-173 nM) cancer cell lines. Also, the conjugate **17b** was found to be relatively more potent than **H_2TMPyP 7** and thus proving to be a novel and potent photosensitizing agent that likely to be a potential candidate for PDT.

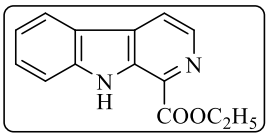
4.6 Experimental procedures

4.6.1 General methods and materials

Used chemicals were procured from Sigma-Aldrich, India and Spectrochem Pvt Ltd., and were of analytical grade. Synthesized compounds were purified by column chromatography with 100-200 mesh silica gel. IR spectra of the compounds were recorded on a FT-IR Shimadzu spectrophotometer using KBr discs. NMR (^{1}H & ^{13}C) spectra were recorded in DMSO-d_6 and $CDCl_3$ on a Bruker-avance 400 MHz instrument using TMS as an internal standard. Chemical shifts are stated in ppm and multiplicities are designated by s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Coupling constants, *J*, are shown in hertz (Hz). Mass spectra were obtained on a Bruker ProFLEX III MALDI-TOF mass spectrometer using DHB (2,5-dihydroxybenzoic acid) as the matrix. The UV–visible spectroscopy was carried out on Hitachi U-2900 spectrophotometer and fluorescence spectra were recorded on Horiba Jobin Yvon Fluoro max-4-scanning fluorimeter. Quartz cuvettes were used with 1 cm path length. The purity of all the synthesized compounds was > 98% as determined by WATERS 515 HPLC system with a Sunfire C-18 column (5 µm, 4.6 × 250 mm) and PDA detector using a flow rate of 1 mL/min and a gradient of isocratic (0.05% TFA)/acetonitrile.

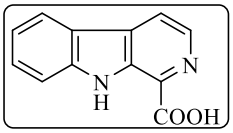
4.6.2 Synthesis of *β*–Carboline acids (20a-c)

***β*–Carboline-1-carboxylic acid ester (19a):** Tryptamine **18** (6 g, 37.5 mmol),



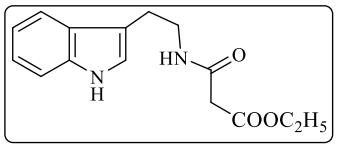
ethylglyoxalate (6 mL, 56.25 mmol) and DCM (60 mL) were taken into round-bottomed flask and the resulting solution was stirred at room temperature for 4 h. The reaction mixture was cooled to -5 °C and trifluoroacetic acid (1mL) was added slowly. The contents were allowed to reach room temperature and stirred for 24 h. The reaction mixture was then slowly poured into saturated $NaHCO_3$ (50 mL), the DCM layer was separated, and the remaining aqueous layer was extracted with DCM (2×50 mL). The combined organic layers were concentrated in *vacuo.* The obtained residue was dissolved in acetonitrile (60 mL), TBAB (6.1 g, 18.75 mmol) and IBX (21 g, 75.0 mmol) were added and the reaction mixture was continued to stir for 2 h at room temperature. Upon completion of the reaction, the reaction mass was quenched with saturated hypo (50 mL) and ammonia (5 mL) solutions and extracted with chloroform (3×30 mL). The chloroform was removed in *vacuo* and purified by silica gel column chromatography (hexane/ethylacetate = 7/3) to afford *β*-carboline-1-carboxylic acid ester **19a** as yellow solid. Yield 65%; Mp 144-145 °C (lit. 145-146 °C).[63]

***β*–Carboline-1–carboxylic acid 20a:** To a solution of *β*–carboline ester **19a** (8.34 mmol) in



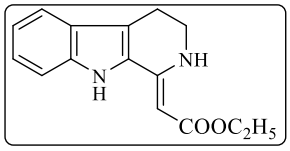
THF (5 mL) and water (5 mL) was added $LiOH.H_2O$(1.75 g, 41.67 mmol) in portion wise. The reaction mixture was stirred at room temperature for 2 h. Excess THF was removed, the obtained solution was acidified to pH~6 with acetic acid and filtered the solid to obtain **20a** in excellent yield. Yellow solid; Yield 92%; Mp 238-239 °C (lit. 237-238 °C).[64]

***N*–(Indol–3–yl–ethyl)–2–ethoxycarbonylacetamide (21)[56,65]:** To a stirred solution of



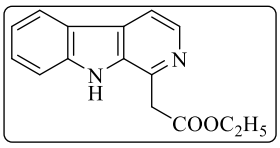
tryptamine **18** (5 g, 31.25 mmol) at 0 °C was simultaneously added a solution of ethylmalonyl chloride (5.5 g, 36.5 mmol) in dichloromethane (75 mL) and aqueous NaOH solution (3 g, 75 mL) over a period of 30 min with vigorous stirring. The reaction mixture was stirred at room temperature for another 1 h and then diluted with DCM (50 mL) and added HCl (5%, 10 mL). Organic phase was washed with saturated $NaHCO_3$ (2×10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The yellow oil so obtained was washed with a mixture of DCM: hexane (1:9) to afford **21** (7.5 g, 88 %). IR (KBr) 3410, 3315, 1735, 1667, 1559 cm^{-1}.

1–(Ethoxycarbonylmethylidene)tetrahydro–*β*–carboline (22)[56]: The amide ester **21** (7.0 g,



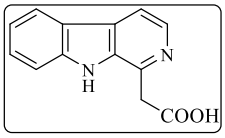
25.5 mmol) was taken in phosphorous oxychloride (30 mL) and stirred the contents at room temperature for 7 h. After completion of the reaction as indicated by thin layer chromatography, excess of $POCl_3$ was distilled off in *vacuo* and the residue so obtained was basified with saturated sodium bicarbonate and extracted with DCM. After removal of DCM, the resulting crude was washed with a mixture of dichloromethane-hexane to obtain **22** as yellow oil (3.7 g, 57 %). IR (KBr) 3348, 1631, 1607, 1538 cm^{-1}.

1–(Ethoxycarbonylmethyl)–*β*–carboline (19b)[56]: To a solution of the enamino ester **22** (3.5

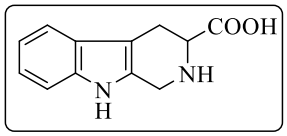


g, 13.7 mmol) in *p*-cymene (180 mL) at 40 °C was added 10% Pd/C (6.0 g) in one portion and the resulting mixture was immediately kept in a preheated oil bath at 165 °C for 15 min. After completion of the reaction, the mixture was diluted with ethyl acetate (100 mL), filtered and distilled off under *vacuo*. The crude product so obtained was purified by column chromatography using ethyl acetate: hexane (1:5) as eluent to afford *β*-carboline ester **19b** (1.6 g, 45 %). IR (KBr) 3507, 1738, 1635 cm^{-1}.

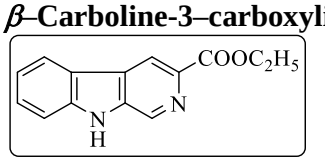
1–(Carboxymethyl)–*β*–carboline (20b): *β*-Carboline acid **20b** was prepared by using the procedure described for **20a.** Brown solid; Yield 85%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 8.00 (d, J = 6.2 Hz, 1H), 7.86 (t, J = 7.3 Hz, 2H), 7.74 (d, J = 8.3 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.23 (d, J = 7.5 Hz, 1H), 4.62 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.5, 144.3, 136.2, 136.1, 135.7, 132.6, 132.5, 124.1, 122.3, 120.0, 117.2, 113.4, 45.4.



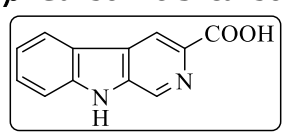
Tetrahydro-*β*–carboline-3–carboxylic acid (24): To a stirred aqueous solution of NaOH (60 mL, 0.4 N) at room temperature was added L-tryptophan **23** (5 g, 24.5 mmol). The contents were continued to stir until the mixture became a clear solution. To this, a formaldehyde solution (37%, 2.8 mL, 34.3 mmol) was added and the mixture was stirred at 37 °C for 72 h. Adjusted the pH of the mixture to 6 by the slow addition of acetic acid (2.5 mL). The solid sample was filtered and then washed with water (4 × 50 mL) and dried in an oven (100 °C) to get off white colored compound **24** in 95% yield. Mp 303-305 °C (lit. 302-304 °C (decomp)).[66]



***β*–Carboline-3–carboxylic acid ester (19c):** The acid **24** (4.5 g, 20.8 mmol) was dissolved in dry ethanol (90 mL) and conc. H_2SO_4 (2.2 mL, 41.7 mmol) under N_2 atmosphere and the resulting mixture was heated to reflux for 48 h. After cooling, the ethanol was evaporated; the obtained crude was diluted with water (30 mL), $CHCl_3$ (30 mL) and basified by ammonia solution to get pH = 8. The phases were separated, and the aqueous phase was extracted with $CHCl_3$ (4×30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated in *vacuo* to afford the crude tetrahydro-*β*–carboline-3–carboxylic acid ester intermediate. The crude intermediate was taken in acetonitrile, added TBAB (3.4 g, 10.4 mmol) followed by IBX (11.7 g, 41.7 mmol). The reaction mixture was reacted at room temperature for 2 h. After completion of the reaction, the reaction mixture was quenched with hypo (50 mL) and ammonia (5 mL) solution. The aqueous phase was extracted with $CHCl_3$ (3 × 30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated in *vacuo* to afford brown solid. Purification by column chromatography [ethylacetate:hexane (4:1)] afforded the brown solid **19c** in 70% yield. Mp 224-225°C (lit.[55] 225-227 °C).



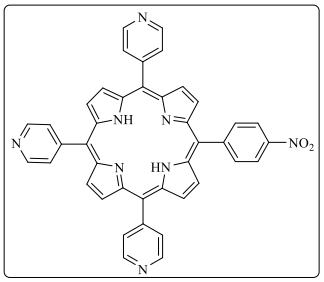
***β*–Carboline-3–carboxylic acid (20c) :** By using procedure similar to the preparation of **20a,** the *β*–carboline acid **20c** was prepared. Yellow solid; Yield 90%; Mp 223-224°C (lit. 220-221 °C);[55] ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 8.97–8.93 (m, 2H), 8.40 (d, J = 7.7 Hz, 1H), 7.73 – 7.53 (m, 2H), 7.32 (t, J = 7.3 Hz, 1H).



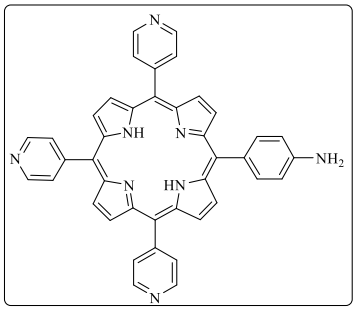
4.6.3. Preparation of 5-(4-Aminophenyl)–10,15,20–tripyridylporphyrin 31

Porphyrin **31** was synthesized from **29** as detailed below

5-(4-Nitrophenyl)–10,15,20–tripyridylporphyrin (29): *p*-Nitrobenzaldehyde (10 g, 66.2 mmol) and 4-formylpyridine (18.7 mL, 198.7 mmol) were taken into round bottom flask containing propionic acid (750 mL) at 110 °C. Pyrrole (18.3 mL, 264.9 mmol) was then added portion wise and the reaction mixture was refluxed for 1.5 h. Removed the propionic acid by distillation under reduced pressure. The residue thus obtained was neutralized with 1N NaOH (100 mL) in an ice bath, extracted the aqueous layer with chloroform (3×60 mL). The combined organic extract was washed with brine (2×50 mL), dried over anhydrous sodium sulfate and removed the excess of solvent. The obtained crude brown solid was dissolved in chloroform (200 mL) and followed by the addition of DDQ (21 g, 92.5 mmol) at room temperature. The reaction contents were refluxed for 1 h. After completion of the reaction, the chloroform was distilled off from reaction mixture and the crude material was adsorbed over silica gel (100-200 mesh) with 90% chloroform/hexane and purified by repeated column chromatography to afford nitro compound **29** as purple solid in 8% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34–8.77 (m, 14H), 8.27 (s, 6H), 7.89 (d, J = 7.8 Hz, 2H), 7.88–7.55 (m, 2H), –2.92 (s, 2H).



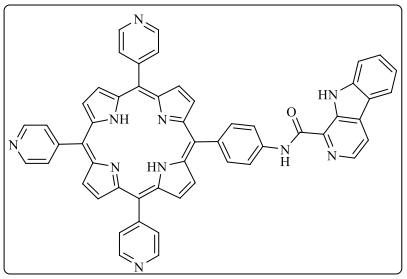
5-(4-Aminophenyl)–10,15,20–tripyridylporphyrin 31: To a solution of above 5-(4-nitrophenyl)-10,15,20-tripyridyl porphyrin **29** (1 g, 1.51 mmol) in 6N HCl (30 mL) was added stannous chloride (0.52 g, 2.27 mmol) and heated to 65 °C for 2 h. It was then basified with sodium carbonate to pH ~ 8 and extracted with chloroform (3×50 mL). The solvent was evaporated under vacuum and subjected to column chromatography over silica gel (100-200 mesh) in chloroform to give 0.67 g (70 %) of 5-(4-aminophenyl)–10,15,20–tripyridyl porphyrin **31.** ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31–8.78 (m, 14H), 8.30 (s, 6H), 7.92 (d, J = 7.8 Hz, 2H), 7.86–7.48 (m, 2H), –2.94 (s, 2H). ESIMS *m/z*: calcd for $C_{41}H_{29}N_8$ $[M+H]^+$ 633.2, found: 633.4.



4.6.4. Preparation of porphyrin–*β*–carboline conjugates 32a-b

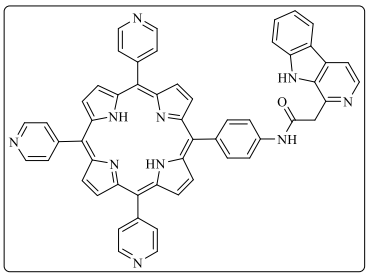
5-(4-Aminophenyl)-10,15,20-tripyridylporphyrin **31** (0.1g, 0.158 mmol), *β*–carboline carboxylic acids **20a-b** (0.24 mmol), HATU (0.09 g, 0.24 mmol), DIPEA (0.15 mL, 0.79 mmol) and DMF (1 mL) were taken into a MW glass tube (10 mL) equipped with a magnetic stir bar. Sealed the tube with a cap and irradiated the mixture in focused MW (40W, 70 °C) for 60 min. After completion of the reaction (followed by TLC), the contents were poured into cold water (30 mL) extracted with chloroform (3×25 mL). The solvent was evaporated, and the crude product obtained was purified by column chromatography to furnish purple compounds **32a-c** in good yields.

***N*-(4-(10,15,20-tri(pyridin-4-yl)porphyrin-5-yl)phenyl)-9*H*-pyrido[3,4-*b*]indole-1-carboxamide (32a):** Yield 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.56 (s, 1H), 10.44 (s, 1H),



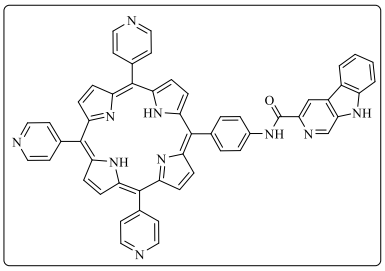
9.08 (d, *J* = 4.3 Hz, 8H), 8.89 (d, *J* = 8.1 Hz, 6H), 8.55 (d, *J* = 5.0 Hz, 1H), 8.31 (s, 4H), 8.20 (d, *J* = 4.2 Hz, 8H), 7.65 (t, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 6.5 Hz, 1H), -2.85 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 150.0, 149.9, 148.5, 148.4, 141.2, 137.9, 137.5, 137.4, 135.4, 131.8, 129.5, 129.4, 129.3, 121.9, 121.3, 120.7, 120.6, 118.3, 118.1, 117.5, 117.0, 112.0. MALDI-TOF *m/z*: calcd for $C_{53}H_{35}N_{10}O$: 827.2995 $[M+H]^+$; found: 827.3679 $[M+H]^+$.

2-(9*H*-pyrido[3,4-b]indol-1-yl)-*N*-(4-(10,15,20-tri(pyridin-4-yl)porphyrin-5-yl)phenyl) acetamide (32b): Yield 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1H), 9.27 (s, 1H), 9.08



(d, *J* = 3.9 Hz, 6H), 8.99 (d, *J* = 4.7 Hz, 3H), 8.87 (d, *J* = 4.8 Hz, 6H), 8.60 (d, *J* = 7.8 Hz, 1H), 8.27 (d, *J* = 8.3 Hz, 2H), 8.19 (d, *J* = 3.5 Hz, 8H), 7.38 (s, 2H), 7.20 (d, *J* = 8.1 Hz, 2H), 3.14 (s, 2H), -2.84 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.5, 150.0, 149.8, 148.9, 148.3, 141.7, 138.1, 137.0, 136.4, 135.3, 134.4, 133.7, 129.4, 128.4, 128.0, 124.5, 124.0, 122.9, 120.9, 118.3, 117.5, 117.1, 112.3, 110.6, 45.8; MALDI-TOF *m/z*: calcd for $C_{54}H_{37}N_{10}O$:841.3152 $[M+H]^+$; found: 841.3146 $[M+H]^+$.

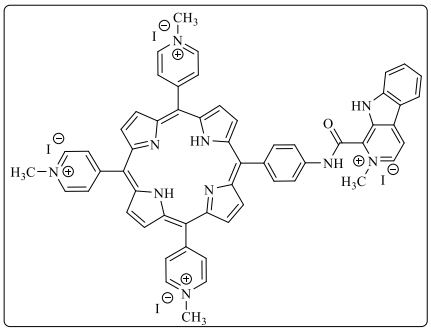
***N*-(4-(10,15,20-tri(pyridin-4-yl)porphyrin-5-yl)phenyl)-9*H*-pyrido[3,4-b]indole-3-carboxamide (32c):** Yield 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.64 (s, 1H), 9.96 (s, 1H), 9.14 (s, 1H), 9.07 (d, J = 5.0 Hz, 8H), 8.99 (s, 1H), 8.87 (d, J = 8.5 Hz, 6H), 8.31-8.27 (m, 5H), 8.19 (d, J = 4.4 Hz, 6H), 7.66 (t, J = 8.8 Hz, 2H), 7.40 (t, J = 7.3 Hz, 1H), -2.86 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9, 150.2, 150.1, 148.3, 148.3, 141.1, 139.8, 138.5, 137.6, 136.8, 135.3, 132.0, 129.7, 129.4, 129.0, 122.1, 121.7, 121.6, 120.8, 118.1, 117.4, 116.9, 114.9, 112.2. MALDI-TOF *m/z*: calcd for $C_{53}H_{35}N_{10}O$: 827.2995 $[M+H]^+$; found: 827.2496 $[M+H]^+$.



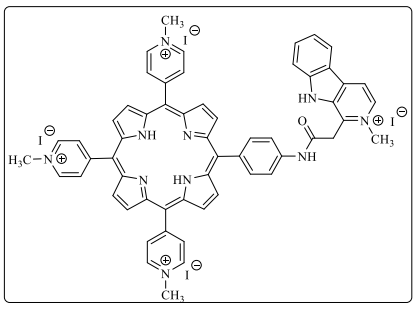
4.6.5 Preparation of cationic porphyrin-*β*–carboline conjugates 17a-c

An appropriate porphyrin-*β*–carboline conjugate **32** (0.11 mmol) in DMF (2 mL) and methyl iodide (2 mL, 32.1 mmol) were taken into a 10 mL MW tube equipped with a magnetic stir bar. The vial was sealed with a cap and the contents were irradiated in a CEM microwave for 60 min at 60 °C (~1-2 bar). Reaction contents were cooled to 50 °C by compressed air and decapped. Diluted with methanol (3 mL) and then precipitated with excess of diethyl ether. The solid product was filtered and washed well with methanol to afford cationic porphyrins **17a-c** as purple solids.

4,4',4''-(15-(4-(2-Methyl-9*H*-pyrido[3,4-*b*]indol-2-ium-1-carboxamido)phenyl)porphyrin-5,10,20-tris(1-methylpyridin-1-ium)) iodide (17a): Yield 94%; Mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 11.36 (s, 1H), 9.50 (d, J = 6.5 Hz, 8H), 9.18-9.15 (m, 6H), 9.04-9.02 (m, 6H), 8.64-8.59 (m, 3H), 8.52 (d, J = 3.9 Hz, 1H), 8.37 (d, J = 7.9 Hz, 1H), 8.30 (d, J = 8.4 Hz, 2H), 7.87 (d, J = 8.1 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 4.74 (s, 12H), -2.99 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.1, 157.1, 157.0, 144.7, 144.6, 142.2, 139.5, 137.4, 137.3, 137.2, 137.1, 136.0, 135.4, 135.3, 132.7, 132.6, 131.6, 129.5, 123.3, 122.5, 122.4, 120.4, 119.2, 119.1, 115.8, 114.9, 113.5, 53.3, 48.4. MALDI-TOF *m/z*: calcd for $C_{57}H_{46}N_{10}O^{4+}$:887.391 $[M-4I+H]^+$; found: 887.303 $[M-4I+H]^+$; HPLC purity: 99.07%.

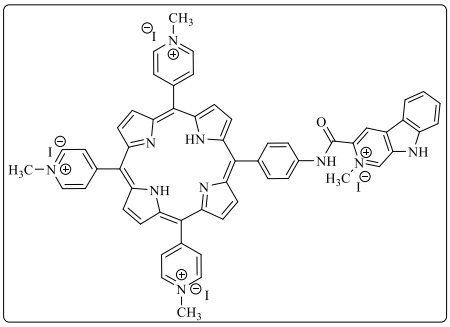


4,4',4''-(20-(4-(2-(2-Methyl-9*H*-pyrido[3,4-*b*]indol-2-ium-1-yl)acetamido)phenyl) porphyrin-5,10,15-tris(1-methylpyridin-1-ium)) iodide (17b): Yield 91%; Mp >300 °C;



^{1}H NMR (400 MHz, $CDCl_3$ & DMSO-d_6) δ 9.38–9.33 (m, 7H), 9.30–9.27 (m, 1H), 8.97–8.88 (m, 7H), 8.80 (d, J = 4.4 Hz, 2H), 8.74–8.68 (m, 8H), 8.35–8.31 (m, 3H), 8.26 (d, J = 8.9 Hz, 2H), 4.63 (s, 12H), 3.09 (s, 2H), -3.19 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.4, 160.8, 157.9, 157.1, 156.9, 156.0, 148.7, 147.9, 145.8, 144.7, 144.6, 143.5, 139.9, 136.1, 134.5, 132.6, 131.4, 130.2, 126.2, 124.4, 122.9, 116.9, 115.9, 115.8, 114.9, 114.7, 114.2, 107.8, 58.9, 48.4, 46.5. MALDI-TOF *m/z*: calcd for $C_{58}H_{48}N_{10}O^{4+}$: 900.4013 $[M-4I]^+$; found: 900.3967 $[M-4I]^+$; HPLC purity: 99.20%.

4,4',4''-(15-(4-(2-Methyl-9*H*-pyrido[3,4-*b*]indol-2-ium-3-carboxamido)phenyl)porphyrin -5,10,20-tris(1-methylpyridin-1-ium)) iodide (17c): Yield 90%; Mp >300 °C; ^{1}H NMR

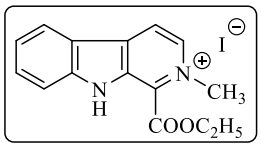


(400 MHz, DMSO-d_6) δ 12.29 (s, 1H), 11.25 (s, 1H), 9.49 (d, J = 6.6 Hz, 6H), 9.23 (s, 1H), 9.18–9.10 (m, 8H), 9.03 (d, J = 6.7 Hz, 6H), 9.00 (s, 1H), 8.53 (d, J = 8.5 Hz, 3H), 8.28 (d, J = 8.5 Hz, 2H), 7.77 (d, J = 8.3 Hz, 1H), 7.73–7.68 (m, 1H), 7.41 (t, J = 7.4 Hz, 1H), 4.73 (s,12H), -2.99 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0, 157.1, 156.9, 144.8, 144.7, 144.6, 141.9, 140.8, 139.8, 139.7, 137.8, 135.8, 135.4, 132.6, 132.5, 129.3, 123.4, 123.0, 121.3, 120.9, 119.1, 119.0, 115.8, 115.7, 115.6, 114.9, 113.0, 53.3, 48.3; MALDI-TOF *m/z*: calcd for $C_{57}H_{46}N_{10}O^{4+}$:886.383 $[M-4I]^+$; found: 886.289 $[M-4I]^+$; HPLC purity: 98.48%.

4.6.6. Preparation of 2-methyl-*β*–carbolinium iodides 33a-c and H_2TMPyP 7

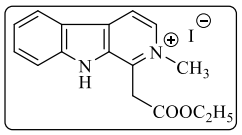
β-Carbolinium iodides **33a-c** and **H_2TMPyP 7** were prepared from *β*-carboline esters **(19a-c)** and tetrapyridylporphyrin **30** by following the procedure described for the preparation of **17a-c.** But reaction proceeded in 20 min.

1-(Ethoxycarbonyl)-2-methyl-9*H*-pyrido[3,4-*b*]indol-2-ium iodide (33a): Yellow solid;



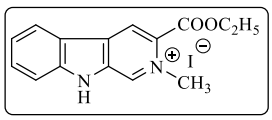
Yield 96%; Mp 172-173 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s, 1H), 9.01 (d, J = 6.3 Hz, 1H), 8.92 (d, J = 6.4 Hz, 1H), 8.53 (d, J = 8.0 Hz, 1H), 7.90–7.84 (m, 2H), 7.52–7.48 (m, 1H), 4.69 (q, J = 7.1 Hz, 2H), 4.64 (s, 3H), 1.50 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.2, 145.2, 137.7, 136.3, 135.6, 133.5, 126.8, 124.3, 122.7, 119.8, 119.3, 113.9, 64.6, 48.8, 14.4.

1-(2-Ethoxy-2-oxoethyl)-2-methyl-9*H*-pyrido[3,4-*b*]indol-2-ium iodide (33b): Yellow solid;



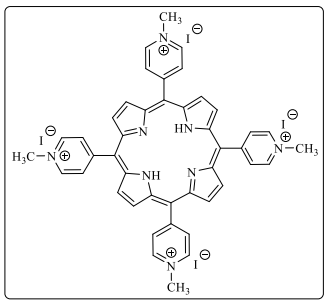
Yield 92%; Mp 220-221 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s, 1H), 8.00 (d, J = 6.2 Hz, 1H), 7.86 (t, J = 7.3 Hz, 2H), 7.74 (d, J = 8.3 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.23 (d, J = 7.5 Hz, 1H), 4.62 (s, 2H), 4.24 (s, 3H), 4.13 (q, J = 6.9 Hz, 2H), 1.20 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.5, 144.3, 136.2, 136.1, 135.7, 132.6, 132.6, 124.1, 122.3, 120.0, 117.2, 113.4, 62.4, 53.3, 45.4, 14.4.

3-(Ethoxycarbonyl)-2-methyl-9H-pyrido[3,4-b]indol-2-ium iodide (33c): Yellow solid; Yield



95%; Mp 186-187 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 9.40 (s, 1H), 8.65 (d, J = 8.0 Hz, 1H), 7.87 (s, 2H), 7.53 (s, 1H), 4.66 (s, 3H), 4.52 (q, J = 7.0 Hz, 2H), 1.46 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.2, 144.6, 135.4, 134.9, 131.5, 131.4, 124.4, 121.9, 120.2, 113.9, 63.5, 49.0, 14.4.

***meso*-Tetrakis(*N*-methylpyridinium-4-yl)porphyrin (7):** Purple solid; Yield 95%; Mp >300



°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.51 (d, J = 6.0 Hz, 8H), 9.23 (s, 8H), 9.02 (d, J = 6.0 Hz, 8H), 4.74 (s, 12H), -3.09 (s, 2H).

4.6.7 WST Assay

A549 and Colon26, a human epithelial cell lines derived from a lung and colon carcinoma (doubling time; 20–24 h), was obtained from American Type Culture Collection. A549 and Colon26 were grown in Dulbecco's modified Eagle medium with high concentrations of glucose (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg streptomycin. Cell line was incubated at 37°C in a humidified atmosphere consisting of 5% CO_2/air. Porphyrins **32a-c**, **17a-c** and H_2TMPyP **7** were dissolved in DMSO (final concentration: 0.1% DMSO in culture medium). A549 and Colon26 cells were seeded (5000 cells/well) on a 96 well plates the day before chemicals treatment. After 24 h of incubation in the presence of the porphyrins (0–5 µM), cells were washed with PBS, and incubated in new PBS for 10 min at 37°C. Cells were exposed to UV–A or visible light for 10 min, and then PBS was replaced to FBS containing medium. After 48 h incubation in the dark, cell viability was determined by the Cell Counting Kit-8 (Dojin, Kumamoto, Japan) using a spectrophotometer (xMark; Bio-Rad, Hercules, CA, USA). IC_{50} values were determined using 4-parameter equations.

4.7 References

1. Kadish, K. M.; Smith, K. M.; Guilard, R., *The porphyrin handbook*. Academic Press, New York: 2000; Vol. 1-10.
2. Milgrom, L. R., *The colours of life*. Oxford University Press: 1997.
3. Mauzerall, D. C., Evolution of porphyrins. *Clin. Dermatol.,* **1998,** *16*, 195-201.
4. Ungashe, S. B.; Groves, J. T., Porphyrins and metalloporphyrins in synthetic bilayer membranes. *Adv. Inorg. Biochem.,* **1994,** *9*, 317-351.
5. Falk, J. E., *Porphyrins and Metalloporphyrins*. Elsevier scientific publishing company: Amsterdam, 1975; Vol. 399-514.
6. Mitzi, D. B., Thin-film deposition of organic−inorganic hybrid materials. *Chem.Mater.,* **2001,** *13*, 3283-3298.
7. Agarwal, N., The synthesis and characterization of photonic materials composed of substituted fluorene donors and a porphyrin acceptor. *Dyes Pigm.,* **2009,** *83*, 328-333.
8. Amao, Y.; Okura, I., Optical oxygen sensor devices using metalloporphyrins. *J. Porphyrins Phthalocyanines,* **2009,** *13*, 1111-1122.
9. Alvarez, M. G.; Milanesio, M.; Rivarola, V.; Durantini, E.; Batlle, A.; Fukuda, H., Endogenous and exogenous porphyrins as photosensitizers in the Hep-2 human carcinoma cell line. *Cell Mol. Biol.,* **2009,** *55*, 8-14.
10. Arja, K.; Sjolander, D.; Aslund, A.; Prokop, S.; Heppner, F. L.; Konradsson, P.; Lindgren, M.; Hammarstrom, P.; Aslund, K. O. A.; Nilsson, K. P. R., Enhanced fluorescent assignment of protein aggregates by an oligothiophene-porphyrin-based amyloid ligand. *Macromol. Rapid. Comm.,* **2013,** *34*, 723-730.
11. Celli, J. P.; Spring, B. Q.; Rizvi, I.; Evans, C. L.; Samkoe, K. S.; Verma, S.; Pogue, B. W.; Hasan, T., Imaging and photodynamic therapy: Mechanisms, monitoring, and optimization. *Chem. Rev.,* **2010,** *110*, 2795-2838.
12. Allen, B. J., Internal high linear energy transfer (LET) targeted radiotherapy for cancer. *Phys. Med. Biol.,* **2006,** *51*, R327-R341.
13. Borbas, K. E.; Mroz, P.; Hamblin, M. R.; Lindsey, J. S., Bioconjugatable porphyrins bearing a compact swallowtail motif for water solubility. *Bioconjugate Chem.,* **2006,** *17*, 638-653.
14. Pandey, R. K.; Zheng, G., Porphyrins as photosensitizers in photodynamic therapy. *The porphyrin handbook,* **2000,** *6*, 157-230.
15. Lu, Z.; Hornia, A.; Joseph, T.; Sukezane, T.; Frankel, P.; Zhong, M.; Bychenok, S.; Xu, L.; Feig, L. A.; Foster, D. A., Phospholipase D and RalA cooperate with the epidermal growth factor receptor to transform 3Y1 rat fibroblasts. *Mol. Cell Biol.,* **2000,** *20*, 462-467.

16. Rangasamy, S.; Ju, H.; Um, S.; Oh, D.-C.; Song, J. M., Mitochondria and DNA targeting of 5,10,15,20-tetrakis(7-sulfonatobenzo[*b*]thiophene) porphyrin-induced photodynamic therapy *via* intrinsic and extrinsic apoptotic cell death. *J. Med. Chem.,* **2015,** *58*, 6864-6874.
17. Bárbara, P. P. M.; Pansa, M. F.; Laura, N. M. S.; Carla, M. B. C.; Faustino, M. A. F.; Maria Graça, P. M. S. N.; José, A. S. C.; Natalia, B. R. V.; Viviana, A. R., Cationic porphyrin derivatives for application in photodynamic therapy of cancer. *Laser Phys.,* **2014,** *24*, 045603.
18. Yu, J. H.; Weng, Y. X.; Wang, X. S.; Zhang, L.; Zhang, B. W.; Cao, Y., The triplet excited state changes of amphiphilic porphyrins with different side-chain length in AOT reverse micelles. *J. Photochem. Photobiol. A: Chemistry,* **2003,** *156*, 139-144.
19. Fujiwara, N.; Mazzola, M.; Cai, E.; Wang, M.; Cave, J. W., TMPyP4, a stabilizer of nucleic acid secondary structure, is a novel acetylcholinesterase inhibitor. *PloS one,* **2015,** *10*, e0139167.
20. Praseuth, D. l.; Gaudemer, A.; Verlhac, J.-B.; Kraljic, I.; Sissoëff, I.; Guillé, E., Photocleavage of DNA in the presence of synthetic water-soluble porphyrins. *Photochem. Photobiol.,* **1986,** *44*, 717-724.
21. Fiel, R. J., Porphyrin—nucleic acid interactions: A review. *J. Biomol. Struct. Dyn.,* **1989,** *6*, 1259-1274.
22. Villanueva, A.; Caggiari, L.; Jori, G.; Milanesi, C., Morphological aspects of an experimental tumour photosensitized with a meso-substituted cationic porphyrin. *J. Photochem. Photobiol. B: Biol.,* **1994,** *23*, 49-56.
23. Villanueva, A.; Jori, G., Pharmacokinetic and tumour-photosensitizing properties of the cationic porphyrin *meso*-tetra(4*N*-methylpyridyl)porphine. *Cancer Lett.,* **1993,** *73*, 59-64.
24. Orosz, A.; Mezo, G.; Herenyi, L.; Habdas, J.; Majer, Z.; Liwa-Kurdziel, B. M.; Toth, K.; Csik, G., Binding of new cationic porphyrin-tetrapeptide conjugates to nucleoprotein complexes. *Biophys Chem,* **2013,** *177*, 14-23.
25. Ishikawa, Y.; Yamashita, A.; Uno, T., Efficient photocleavage of DNA by cationic porphyrin & acridine hybrids with the effective length of diamino alkyl linkage. *Chem. Pharm. Bull.,* **2001,** *49*, 287-293.
26. Kumar, D.; Shekar, K. C.; Mishra, B.; Kurihara, R.; Ogura, M.; Ito, T., Cationic porphyrin–quinoxaline conjugate as a photochemically triggered novel cytotoxic agent. *Bioorganic Med. Chem. Lett.,* **2013,** *23*, 3221-3224.
27. Hamblin, M. R.; Luke Newman, E., New trends in photobiology: On the mechanism of the tumour-localising effect in photodynamic therapy. *J. Photochem. Photobiol B: Biol.,* **1994,** *23*, 3-8.

28. Reddi, E., Role of delivery vehicles for photosensitizers in the photodynamic therapy of tumours. *J. Photochem. Photobiol. B: Biol.,* **1997,** *37*, 189-195.
29. Borissevitch, I. E.; Tominaga, T. T.; Schmitt, C. C., Photophysical studies on the interaction of two water-soluble porphyrins with bovine serum albumin. Effects upon the porphyrin triplet state characteristics. *J. Photochem. Photobiol. A - Chem.,* **1998,** *114*, 201-207.
30. Onyeibor, O.; Croft, S. L.; Dodson, H. I.; Feiz-Haddad, M.; Kendrick, H.; Millington, N. J.; Parapini, S.; Phillips, R. M.; Seville, S.; Shnyder, S. D.; Taramelli, D.; Wright, C. W., Synthesis of some cryptolepine analogues, assessment of their antimalarial and cytotoxic activities, and consideration of their antimalarial mode of action. *J. Med. Chem.,* **2005,** *48*, 2701-2709.
31. Bierer, D. E.; Dubenko, L. G.; Zhang, P.; Lu, Q.; Imbach, P. A.; Garofalo, A. W.; Phuan, P.-W.; Fort, D. M.; Litvak, J.; Gerber, R. E.; Sloan, B.; Luo, J.; Cooper, R.; Reaven, G. M., Antihyperglycemic activities of cryptolepine analogues: An ethnobotanical lead structure isolated from *Cryptolepis sanguinolenta*. *J. Med. Chem.,* **1998,** *41*, 2754-2764.
32. S. M. S. Chauhan, B. G., T. Bisht., Electrostatic interaction between cationic calix [4] pyrroles and anionic porphyrins in water. *J. Incl. Phenom. Macrocycl. Chem.* , **2010,** *67*, 241-246.
33. Xiao, S.; Lin, W.; Wang, C.; Yang, M., Synthesis and biological evaluation of DNA targeting flexible side-chain substituted *β*-carboline derivatives. *Bioorganic Med. Chem. Lett.,* **2001,** *11*, 437-441.
34. Guan, H.; Liu, X.; Peng, W.; Cao, R.; Ma, Y.; Chen, H.; Xu, A., *β*-Carboline derivatives: Novel photosensitizers that intercalate into DNA to cause direct DNA damage in photodynamic therapy. *Biochem. Biophys. Res. Commun.,* **2006,** *342*, 894-901.
35. Vignoni, M.; Rasse-Suriani, F. A.; Butzbach, K.; Erra-Balsells, R.; Epe, B.; Cabrerizo, F. M., Mechanisms of DNA damage by photoexcited 9-methyl-*β*-carbolines. *Org. Biomol. Chem.,* **2013,** *11*, 5300-5309.
36. Dias, A.; Varela, A.; Miguel, M. d. G.; Macanita, A.; Becker, R. S., *β*.-Carboline photosensitizers. Photophysics, kinetics and excited-state equilibria in organic solvents, and theoretical calculations. *J. Phys. Chem. A,* **1992,** *96*, 10290-10296.
37. Toshima, K.; Okuno, Y.; Nakajima, Y.; Matsumura, S., *β*-Carboline–carbohydrate hybrids: molecular design, chemical synthesis and evaluation of novel DNA photocleavers. *Bioorganic Med. Chem. Lett.,* **2002,** *12*, 671-673.
38. Armitage, B., Photocleavage of nucleic acids. *Chem. Rev.,* **1998,** *98*, 1171-1200.

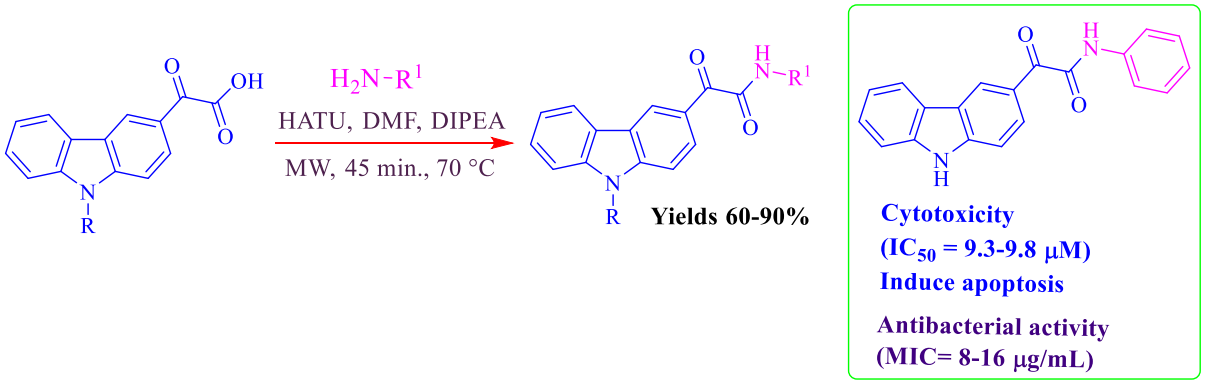
39. Viart, H. l. n. M.-F.; Bachmann, A.; Kayitare, W.; Sarpong, R., *β*-Carboline amides as intrinsic directing groups for C(sp2)–H functionalization. *J. Am. Chem. Soc.*, **2017,** *139*, 1325-1329.
40. Glennon, R. A.; Grella, B.; Tyacke, R. J.; Lau, A.; Westaway, J.; Hudson, A. L., Binding of *β*-carbolines at imidazoline I2 receptors: a structure–affinity investigation. *Bioorganic Med. Chem. Lett.*, **2004,** *14*, 999-1002.
41. Grella, B.; Teitler, M.; Smith, C.; Herrick-Davis, K.; Glennon, R. A., Binding of *β*-carbolines at 5-HT2 serotonin receptors. *Bioorganic Med. Chem. Lett.*, **2003,** *13*, 4421-4425.
42. Kumar, S.; Singh, A.; Kumar, K.; Kumar, V., Recent insights into synthetic *β*-carbolines with anti-cancer activities. *Eur. J. Med. Chem.*, **2017,** *142*, 48-73.
43. Xiao, Z. L., Z.; Lei, C.; Wang, J.; Chen, Z.; Han, G.; Xiao, Y.; He, H.; Chen, S., 1,3,6,9-substitution *β*-carboline compound and preparation method thereof. In China Pat. Appl. CN103130800A, June 05, 2013.
44. Huang, H.; Yao, Y.; He, Z.; Yang, T.; Ma, J.; Tian, X.; Li, Y.; Huang, C.; Chen, X.; Li, W.; Zhang, S.; Zhang, C.; Ju, J., Antimalarial *β*-carboline and indolactam alkaloids from *Marinactinospora thermotolerans*, a deep sea isolate. *J. Nat. Prod.*, **2011,** *74*, 2122-2127.
45. Baiget, J.; Llona-Minguez, S.; Lang, S.; MacKay, S. P.; Suckling, C. J.; Sutcliffe, O. B., MnO_2 mediated one-pot synthesis of methyl 9*H*-pyrido[3,4-*b*]indole-1-carboxylate: Concise synthesis of alangiobussinine. *Beilstein J. Org. Chem.*, **2011,** *7*, 1407.
46. Roll, D. M.; Ireland, C. M.; Lu, H. S. M.; Clardy, J., Fascaplysin, an unusual antimicrobial pigment from the marine sponge *Fascaplysinopsis sp*., *J. Org. Chem.*, **1988,** *53*, 3276-3278.
47. Khokhar, S.; Feng, Y.; Campitelli, M. R.; Ekins, M. G.; Hooper, J. N. A.; Beattie, K. D.; Sadowski, M. C.; Nelson, C. C.; Davis, R. A., Isolation, structure determination and cytotoxicity studies of tryptophan alkaloids from an Australian marine sponge *Hyrtios sp*., *Bioorganic Med. Chem. Lett.*, **2014,** *24*, 3329-3332.
48. Ishida, J.; Wang, H.-K.; Bastow, K. F.; Hu, C.-Q.; Lee, K.-H., Antitumor agents 201.1 Cytotoxicity of harmine and *β*-carboline analogs. *Bioorganic Med. Chem. Lett.*, **1999,** *9*, 3319-3324.
49. Kumar, D.; Mishra, B. A.; Shekar, K. C.; Kumar, A.; Akamatsu, K.; Kusaka, E.; Ito, T., Remarkable photocytotoxicity of a novel triazole-linked cationic porphyrin–*β*-carboline conjugate. *Chem. Commun.*, **2013,** *49*, 683-685.
50. Kumar, D.; Mishra, B. A.; Chandra Shekar, K. P.; Kumar, A.; Akamatsu, K.; Kurihara, R.; Ito, T., Novel porphyrin-psoralen conjugates: synthesis, DNA interaction and cytotoxicity studies. *Org. Biomol. Chem*, **2013,** *11*, 6675-6679.

51. Kumar, D.; Chandra Shekar, K. P.; Mishra, B.; Kurihara, R.; Ogura, M.; Ito, T., Cationic porphyrin–quinoxaline conjugate as a photochemically triggered novel cytotoxic agent. *Bioorganic Med. Chem. Lett.,* **2013,** *23*, 3221-3224.
52. Kumar, D.; Mishra, B.; Chandrashekar, K. P.; Khandagale, S. B.; Tantak, M. P.; Kumar, A.; Akamatsu, K.; Kusaka, E.; Tanabe, K.; Ito, T., Synthesis of *meso*-(4′-cyanophenyl) porphyrins: efficient photocytotoxicity against A549 cancer cells and their DNA interactions. *RSC Adv.*, **2015,** *5*, 53618-53622.
53. Brown, D. G.; Boström, J., Analysis of past and present synthetic methodologies on medicinal chemistry: Where have all the new reactions gone? *J. Med. Chem.,* **2016,** *59*, 4443-4458.
54. Takasu, K.; Shimogama, T.; Saiin, C.; Kim, H.-S.; Wataya, Y.; Brun, R.; Ihara, M., Synthesis and evaluation of *β*-carbolinium cations as new antimalarial agents based on *π*-delocalized lipophilic cation hypothesis. *Chem. Pharm. Bull.*, **2005,** *53*, 653-661.
55. Yin, W.; Majumder, S.; Clayton, T.; Petrou, S.; VanLinn, M. L.; Namjoshi, O. A.; Ma, C.; Cromer, B. A.; Roth, B. L.; Platt, D. M.; Cook, J. M., Design, synthesis, and subtype selectivity of 3,6-disubstituted *β*-carbolines at Bz/GABA(A)ergic receptors. SAR and studies directed toward agents for treatment of alcohol abuse. *Bioorganic Med. Chem.,* **2010,** *18*, 7548-7564.
56. Torisawa, Y.; Hashimoto, A.; Nakagawa, M.; Seki, H.; Hara, R.; Hino, T., A total synthesis of manzamine C and its geometrical isomer. *Tetrahedron,* **1991,** *47*, 8067-8078.
57. Panarese, J. D.; Waters, S. P., Room-temperature aromatization of tetrahydro-*β*-carbolines by 2-iodoxybenzoic acid: Utility in a total synthesis of eudistomin U. *Org. Lett.,* **2010,** *12*, 4086-4089.
58. Skouta, R.; Hayano, M.; Shimada, K.; Stockwell, B. R., Design and synthesis of Pictet–Spengler condensation products that exhibit oncogenic-RAS synthetic lethality and induce non-apoptotic cell death. *Bioorganic Med. Chem. Lett.*, **2012,** *22*, 5707-5713.
59. Rothemund, P., Formation of porphyrins from pyrrole and aldehydes. *J. Am. Chem. Soc*., **1935,** *57*, 2010-2011.
60. Adler, A. D.; Longo, F. R.; Finarelli, J. D.; Goldmacher, J.; Assour, J.; Korsakoff, L., A simplified synthesis for meso-tetraphenylporphine. *J. Org. Chem.,* **1967,** *32*, 476-476.
61. Biron, E.; Voyer, N., Synthesis of cationic porphyrin modified amino acids. *Chem. Commun.,* **2005,** *0*, 4652-4654.
62. Polshettiwar, V.; Varma, R. S., MW-assisted organic synthesis and transformations using benign reaction media. *Acc. Chem. Res.,* **2008,** *41*, 629-639.

63. Benson, S. C.; Gross, J. L.; Snyder, J. K., Indole as a dienophile in inverse electron demand Diels-Alder reactions: reactions with 1,2,4-triazines and 1,2-diazines. *J. Org. Chem.,* **1990,** *55*, 3257-3269.
64. Haynes, H.; Nelson, E.; Price, J., Alkaloids of the Australian *Rutaceae*: Pentaceras australis; Hook. F. I. Isolation of the alkaloids and identification of canthin-6-one. *Aust. J. Chem.,* **1952,** *5*, 387-400.
65. Muratore, M. E.; Shi, L.; Pilling, A. W.; Storer, R. I.; Dixon, D. J., Exploiting a novel size exclusion phenomenon for enantioselective acid/base cascade catalysis. *Chem. Commun.,* **2012,** *48*, 6351-6353.
66. Ikeda, R.; Iwaki, T.; Iida, T.; Okabayashi, T.; Nishi, E.; Kurosawa, M.; Sakai, N.; Konakahara, T., 3-Benzylamino-*β*-carboline derivatives induce apoptosis through G2/M arrest in human carcinoma cells HeLa S-3. *Eur. J. Med. Chem.*, **2011,** *46*, 636-646.

Chapter 5

Efficient Synthesis of Carbazolylglyoxamides as Anticancer and Antibacterial agents



Efficient Synthesis of Carbazolylglyoxamides as Anticancer and Antibacterial Agents

5.1 Introduction

Indole alkaloids are an important class of natural products and medicinally significant molecules, which elicit a wide range of biological activities through diverse mechanisms.[1,2] Among the indole-derived compounds, carbazole commonly found in a wide variety of natural products and pharmaceuticals is endowed with antitumor, psychotropic, antimicrobial, antiviral, anti-inflammatory, antihistaminic, anti-alzheimer's, antidiabetic, antibiotic and anti-oxidative properties.[3-5] In particular, carbazole derivatives have been reported as potential anticancer and/or antibacterial properties (Figure 5.1).[6-12]

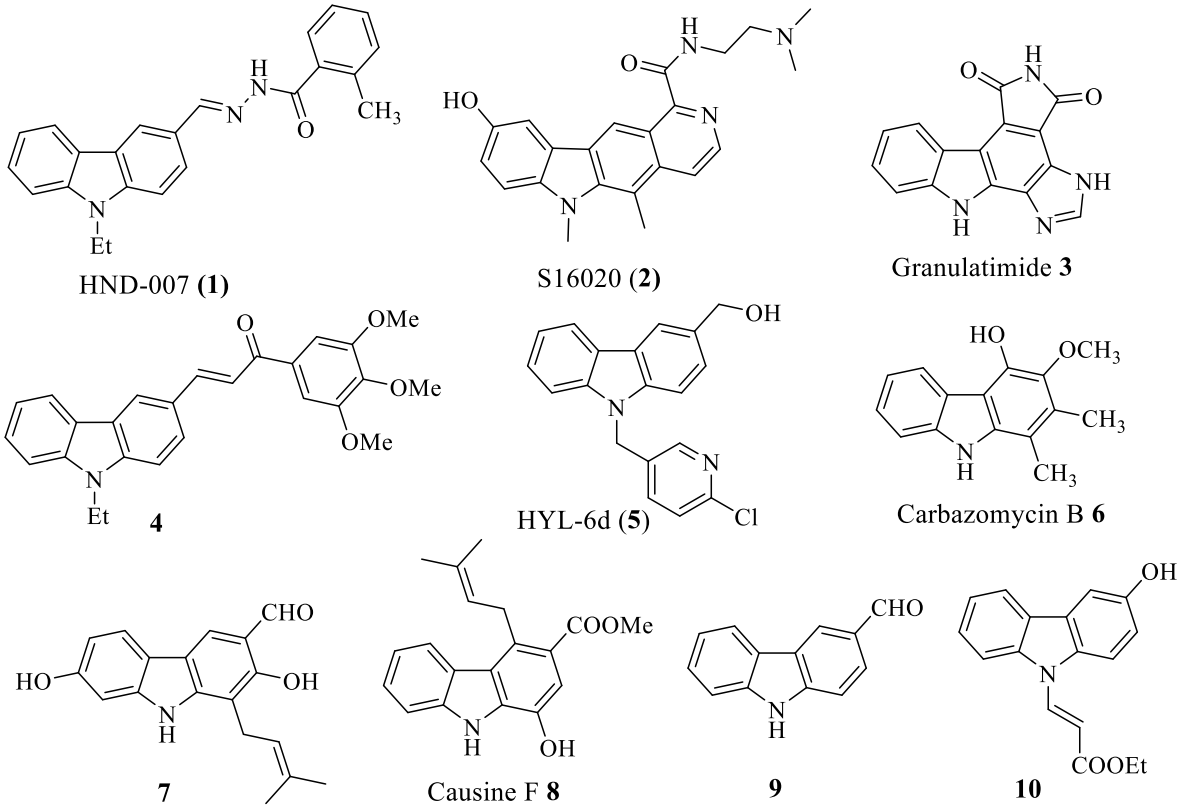


Figure 5.1 Biologically active carbazole analogues

In 2015, Fujita et al. investigated carbazole-based hydrazone, HND-007 (**1**) and related compounds for their *in vivo* antitumor activity suppressing the growth of various cancer cell lines (IC_{50} ~ 1.3–4.6 μM).[13] Pyridine fused carbazole derivative, S16020 (**2**) exhibited potent cytotoxic effects (IC_{50} = 27.5 nM) by virtue of its DNA intercalative and topoisomerase inhibition properties.[14-16] Multidrug-resistant tumor cell lines are sensitive to S16020 and it is currently being evaluated in the clinical stages.[17,18] Naturally occurring carbazole derivative,

granulatimide **3** was isolated from the ascidian *Didemnum granulatum* and found to display anticancer and antibacterial activities.[19] Caulfield and co-workers had patented carbazolyl chalcone **4** for its potential as a tubulin polymerization inhibitor (IC_{50} = 2 μM).[20] Further, a novel synthetic carbazole derivative, HYL-6d (9-[(6-chloropyridin-4-yl)methyl]-9*H*-carbazole-3-carbinol) **5** was found to suppress MCF-7 cell growth after 48 h with IC_{50} = 20–30 μM by inducing cell arrest at the S phase and triggering cell apoptosis. Compound **5** was also shown to inhibit cell proliferation, cell migration, and VEGF- or bFGF induced tube formation in human umbilical vein endothelial cells (HUVECs) under pathological angiogenic conditions, thus indicating *in-vitro* anti-angiogenic activity.[21] Nakamura and co-workers reported the isolation of the carbazomycin B (**6**) from *Streptoverticillium ehimense* and these carbazole-based congeners were found to possess promising antibacterial and antifungal activities.[22-24] In 2012, Laphookhieo group isolated 3-formylcarbazole derivative **7** from the roots of *Clausena wallichii* and reported to display significant antibacterial activity against methicillin-resistance *S. aureus* (MRSA) with a MIC of 4 μg/mL.[25] Another antibacterial carbazole alkaloid, clausine F **8** was isolated from a related plant, *Clausena harmandiana*, and it exhibited excellent activity against MRSA (SK1) and *S. aureus* (TISTR 1466), with a MIC of 4 μg/mL for both test organisms.[26] Jiang et al. reported the isolation of cytotoxic carbazole derivatives from *Clausena lansium* with 3-formylcarbazole **9** displaying IC_{50} values of 6.19-37.64 μg/mL against tested cancer cell lines.[27] In 2014, Esteves and colleagues reported the synthesis and evaluation of a simple carbazole derivative **10** for its anticancer activity against MDA-MB231 and TCC-SUP tumor cell lines with GI_{50} values of 0.198 and 0.025 μM, respectively.[28] Another synthetic derivative of carbazole sulfonamide **11**, exhibited highest cytotoxicity against leukemia cells (IC_{50} = 19 nM).[29]

The current interest in carbazoles for clinical applications arises mainly due to their high efficiencies against several types of diseases, limited toxic side effects, and complete lack of hematological toxicity.[30] In addition to interesting and useful biological applications, carbazole derivatives are also used as organic materials due to their photorefractive, photoconductive, hole transporting and light-emitting properties.[31]

5.2 Rational design

Glyoxamide is a vital structural unit found in many biologically active compounds and synthetic drug candidates,[32,33] especially those with anticancer and antibacterial properties (Figure 5.3). Indibulin **12** destabilizes tubulin polymerization by arresting tumor cell growth at the G2/M phase. Indibulin is also active in multidrug resistant tumor cell lines and its oral formulation is currently being examined in clinical trials.[34] Structurally similar, conscinamides A-C (**13**) containing an indolic enamide fragment, were isolated from marine sponge *Coscinoderma sp* and found to display antitumor activity against a human prostate cancer cell line (IC_{50} = 7.6 µg/mL)[35] and partial cytoprotection against HIV.[36] More recently, Singh et al. have described the synthesis of different bis(indole)glyoxamides **14** as potent antibacterial candidates.[37]

In an effort to find out biologically active indole-based molecules, recently, we have identified various indole analogues as potent anticancer agents.[38-43] Encouraging anticancer and antibacterial activities of glyoxamides and pivotal roles of carbazole scaffold in bioactive compounds prompted us to investigate their new analogues. Herein, we report a series of 24 carbazolyl glyoxamides **15a–x** by incorporating important scaffolds, glyoxamide and carbazole in a single molecule (Figure 5.2)

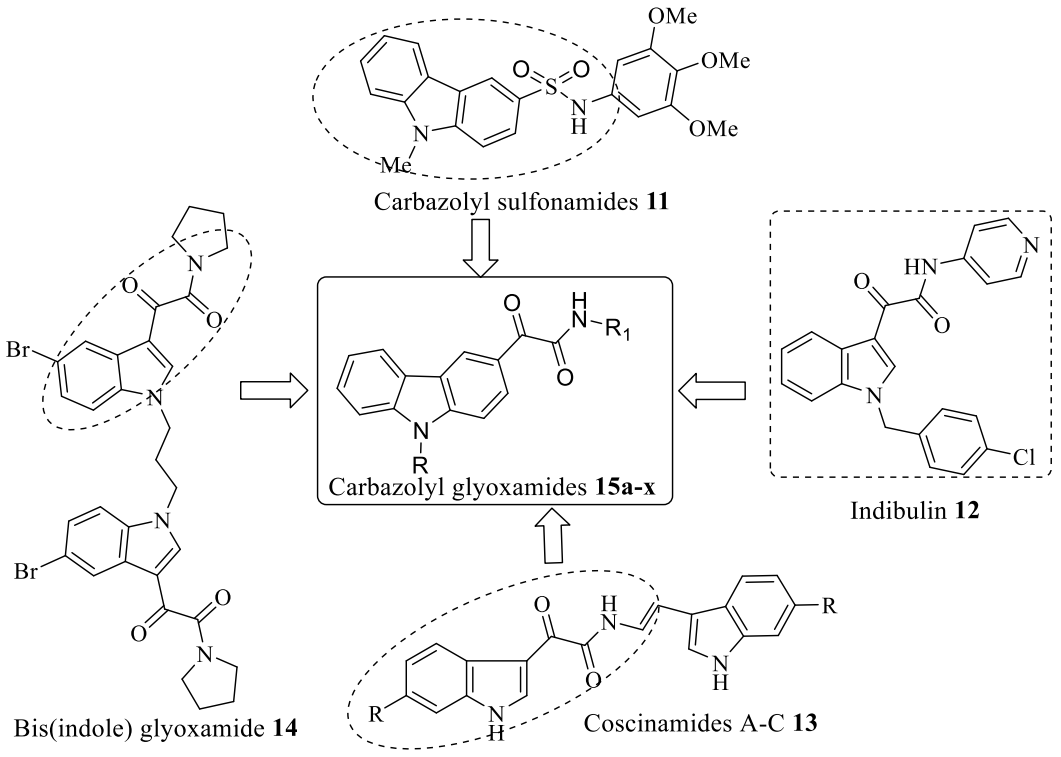
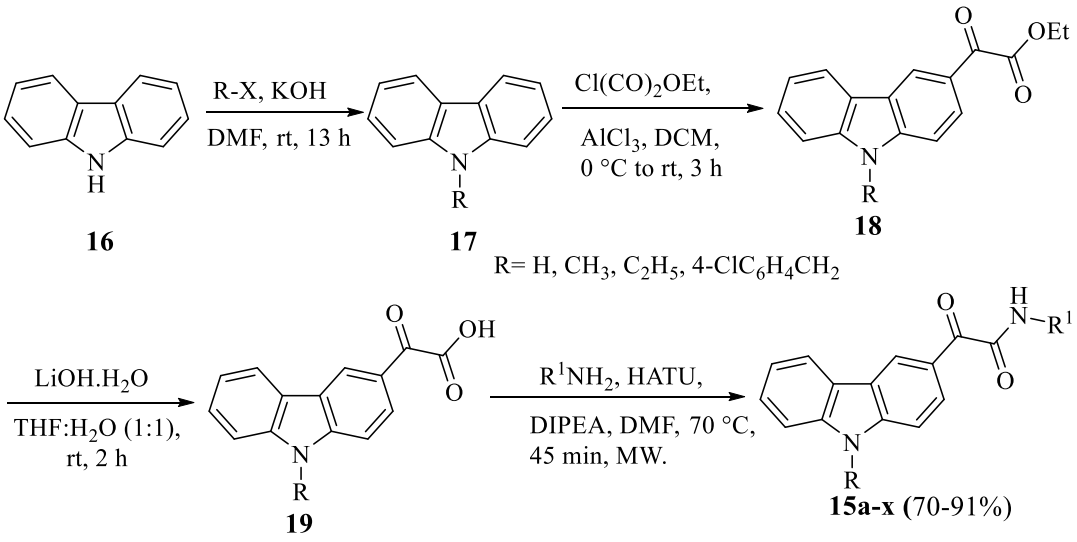


Figure 5.2 Design of carbazolyl glyoxamides **15a–x**

5.3 Results and discussion

5.3.1 Synthesis and characterization

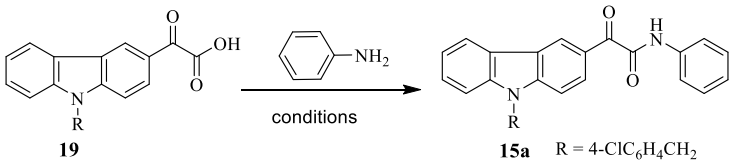
Carbazolyl glyoxamides **15a-x** were synthesized from the reaction of carbazole **17** with ethylchlorooxoacetate in the presence of anhydrous $AlCl_3$ followed by ester hydrolysis using LiOH to afford glyoxalic acid **19** in good yield (Scheme 5.1**)**.



Scheme 5.1 Synthesis of carbazolyl glyoxamides **15a-x**

For the coupling of **19** with aryl/heteroaryl amines, we optimized the reaction conditions by varying temperatures, solvents, and reagents as illustrated in Table 5.1. Initial efforts by using thionyl chloride or oxalyl chloride failed to produce **15a** (Table 5.1, entries 1-2). Subsequent attempts by employing well-known carbodiimides including DCC, CDI, and EDCI.HCl as coupling reagents under conventional as well as microwave (MW) irradiation conditions generated **15a** in low yield (40%, Table 5.1, entries 3-6). Finally, the reaction of **19a** and aryl/heteroarylamines in the presence of O-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) at 70 °C under MW irradiation led to **15a** in 70% yield (Table 5.1, entry 8). Scope and generality of this developed protocol were further demonstrated by coupling glyoxalic acid **19** with various arylamines and a series of carbazolyl glyoxamides **15a-x** was prepared in 70-91% yields.

Table 5.1 Optimization of reaction conditions for the synthesis of carbazolyl glyoxamide **15a**



19 **15a** R = 4-ClC$_6$H$_4$CH$_2$

Entry	Reagent	Solvent	Base	Temp (°C)	Conventional heating		Microwave irradiation	
					Time (h)	Yield (%)	Time (min.)	Yield (%)
1	$SOCl_2$	DCM	TEA	RT	12	Trace	NA	NA
2	$(COCl)_2$	DCM	TEA	RT	15	Trace	NA	NA
3	DCC	THF	TEA	RT	30	Trace	NA	NA
4	CDI	THF	TEA	RT	20	Trace	NA	NA
5	EDCI.HCl/HOBt	THF	DIPEA	RT	14	30	60	40
6	EDCI.HCl/HOBt	DMF	TEA	70	12	Trace	60	Trace
7	HATU	THF	DIPEA	60	15	Trace	60	Trace
8	HATU	DMF	DIPEA	70	12	40	45	70
9	HATU	DMF	TEA	70	12	20	45	40

RT: room temperature; NA: not attempted

Structures of the newly synthesized glyoxamides were well characterized by using spectroscopic techniques including HRMS, NMR (^{1}H and ^{13}C) and IR (Figure 5.3-5.7). The HRMS spectra disclosed expected mass of **15a-x** in agreement with the calculated mass. In ^{1}H NMR spectra of **15a-x,** three characteristic singlets resonated at ~9.4, ~9.1 and ~5.5 ppm due to *N*-H of amide, the C-4 proton of carbazole and CH_2 of 4-chlorobenzyl at *N*[9], respectively. Further, in ^{13}C NMR spectra of **15a-x**, the carbons of carbonyl and amide functionalities were resonated at ~180 (CO) and ~160 ppm (NHCO). In IR spectra of **15a-x**, characteristic peaks at ~1690 and 1655 cm^{-1} were assigned to the carbonyl and amide functionalities, respectively. HPLC analysis of synthesized carbazolyl glyoxamides **15a-x** indicated the purity of all the compounds was greater than 97%.

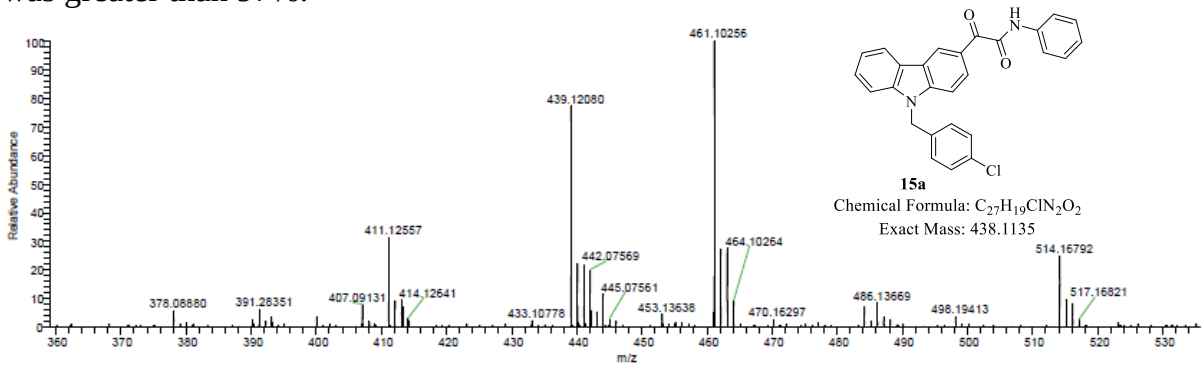


Figure 5.3 HRMS spectrum of **15a**

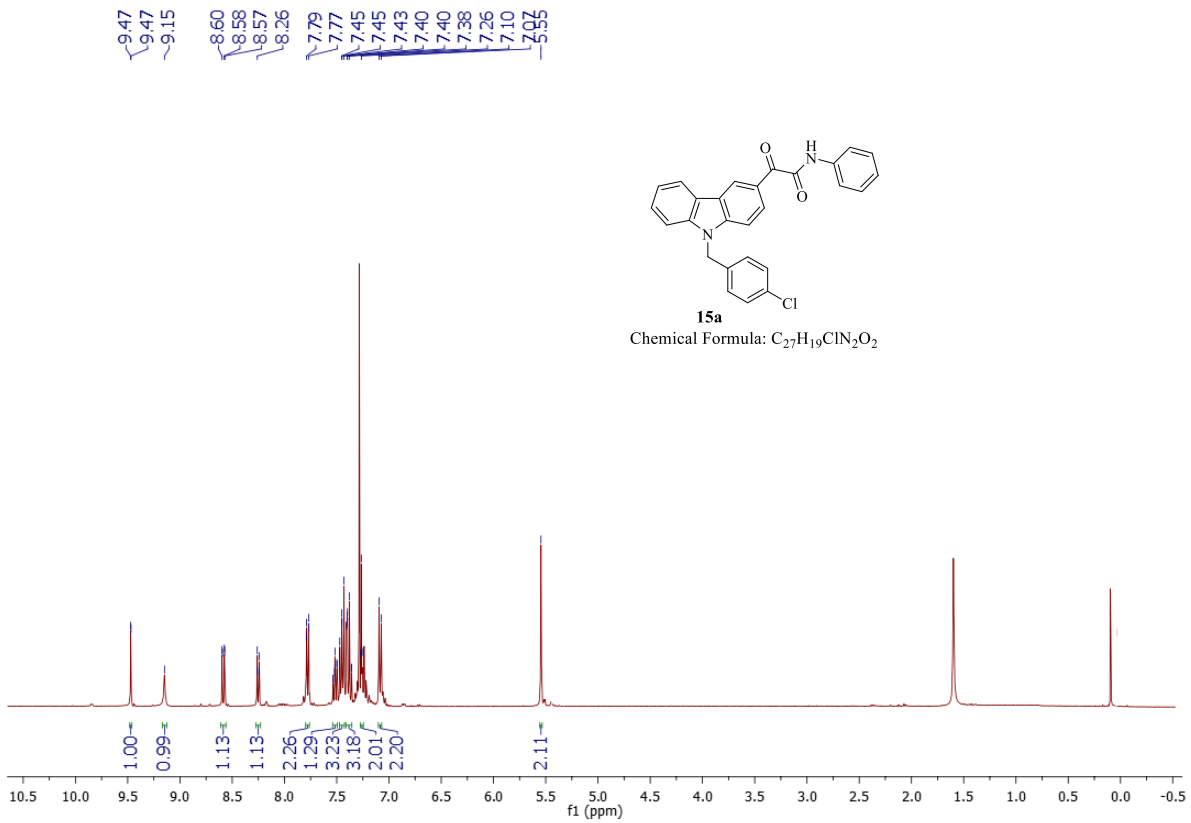


Figure 5.4 ^{1}H NMR spectrum of **15a**

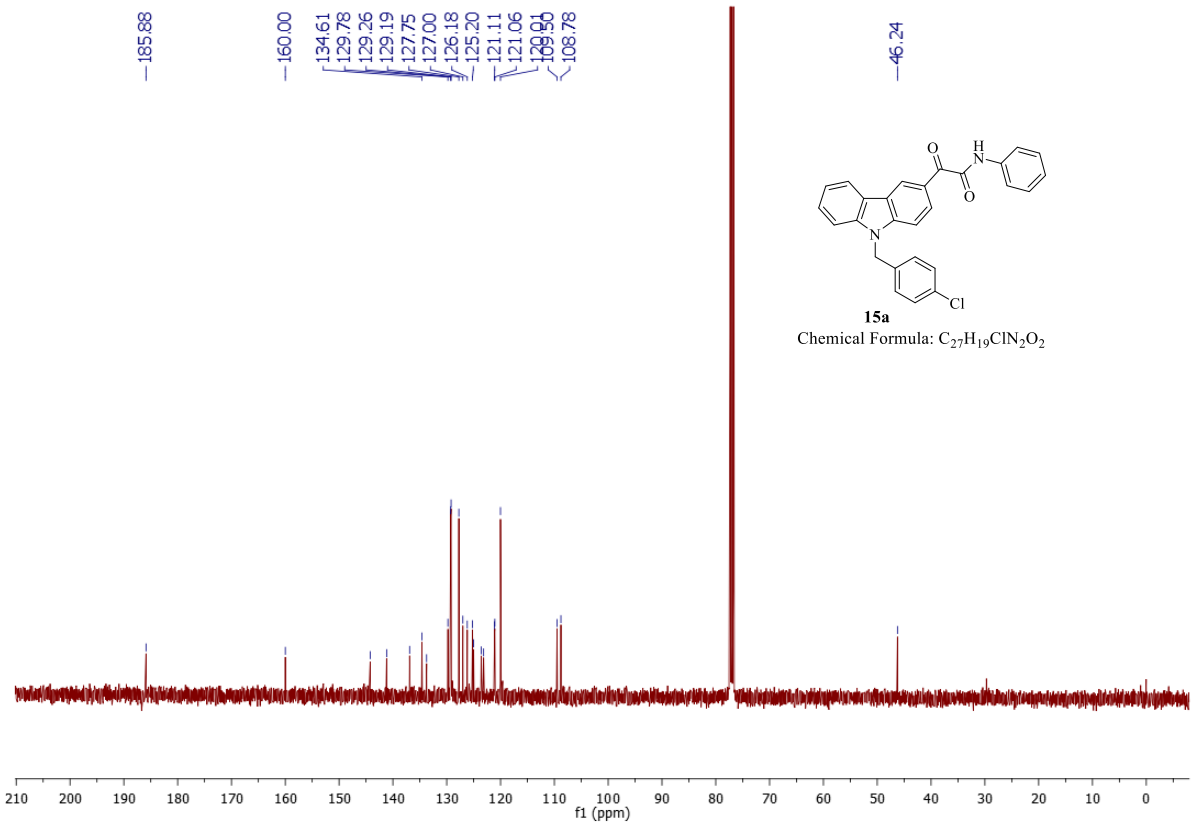


Figure 5.5 ^{13}C NMR spectrum of **15a**

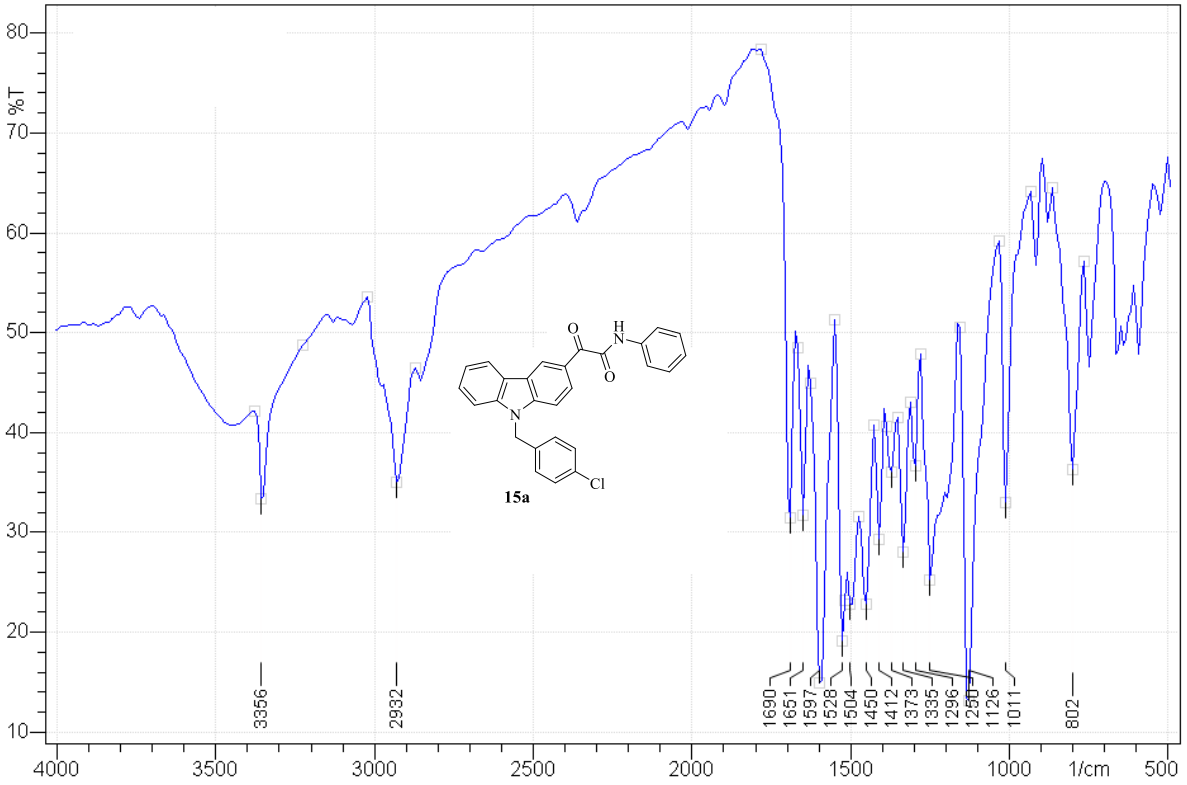
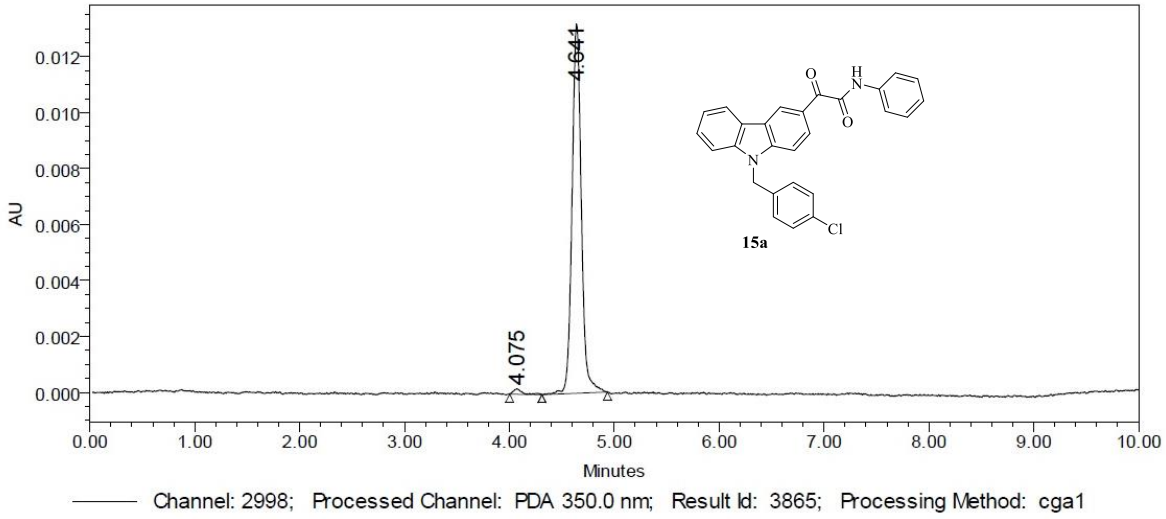


Figure 5.6 IR spectrum of **15a**



Processed Channel Descr.: PDA 350.0 nm

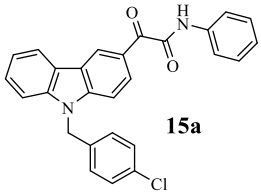
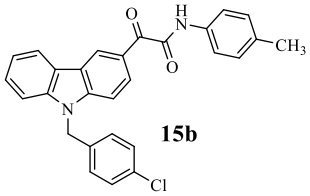
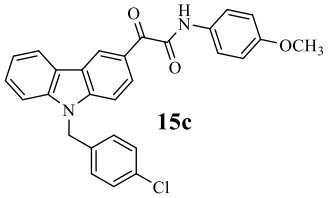
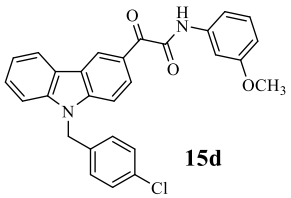
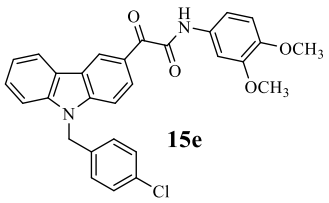
	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 350.0 nm	4.075	1194	1.45	194
2	PDA 350.0 nm	4.641	81123	98.55	13204

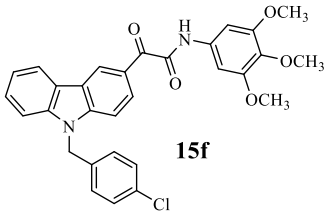
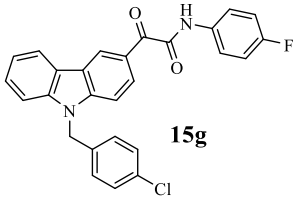
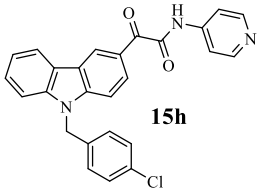
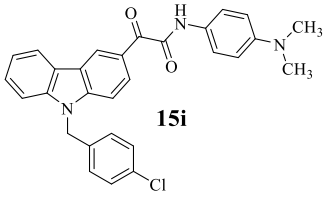
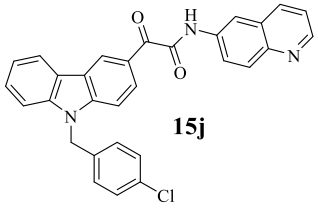
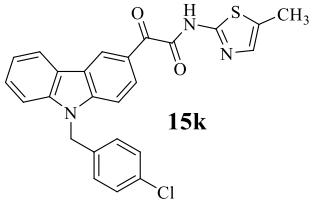
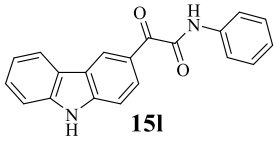
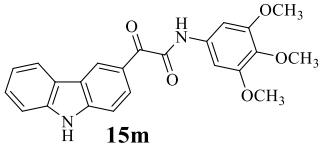
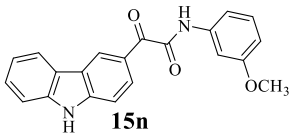
Figure 5.7 HPLC chromatogram of **15a**

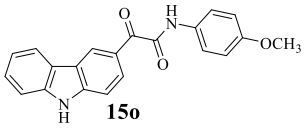
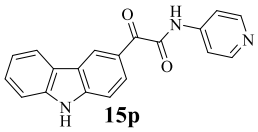
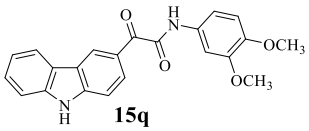
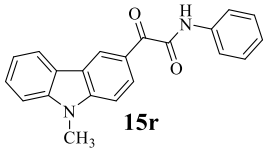
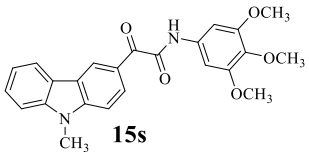
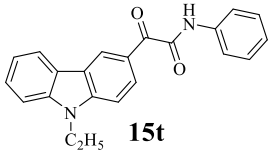
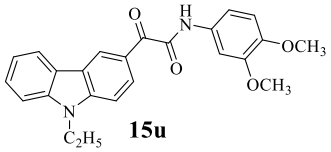
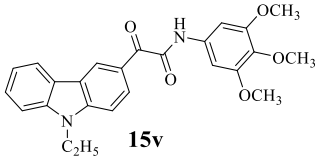
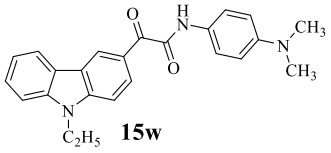
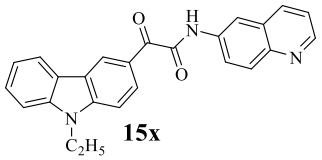
5.3.2 Anticancer activity

Twenty-four synthesized glyoxamides were evaluated for their anticancer activities towards human T lymphocyte (Jurkat), histiocytic lymphoma (U937), and breast (MCF-7 and MDA-MB-231) cancer cell lines (Table 5.2). Structure-activity relationship (SAR) studies of carbazolyl glyoxamides **15a-x** were demonstrated by varying aryl/heteroarylamines and substitution at the 9-*NH* position of carbazoles. Initial screening of compounds **15a-x** at 10 μM concentration indicated that compounds **15i-m** and **15q** displayed about 50% cell survival (Table 5.2). *N*-Methyl and *N*-ethyl congeners of the carbazole were reported to possess significant cytotoxicity, therefore, derivatives **15r-x** were synthesized.[13,29] Unfortunately, analogues **15r-x** exhibited low activity against the tested tumor cell lines.

Table 5.2 *In vitro* cytotoxicity of carbazolyl glyoxamides **15a-x**

	Percentage cell survival (@ 10 μM)			
Carbazolyl glyoxamides	**Jurkat**	**U937**	**MCF-7**	**MDA-MB-231**
 **15a**	71.5±10.7	88.5±5.2	69.5±7.8	82.3±7.5
 **15b**	70.2±0.8	79.5±3.4	67.1±7.4	83.5±6.8
 **15c**	71.3±26.7	82.1±2.2	74.6±0.9	80.5±5.5
 **15d**	71.3±14.4	67.2±8.5	70.9±10.4	78.6±6.8
 **15e**	69.8±14.7	85.5±5.3	67.3±4.6	72.5±2.2

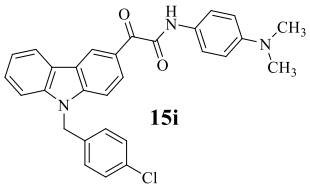
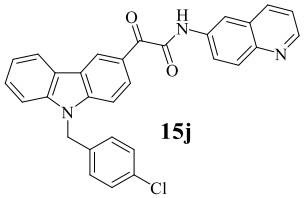
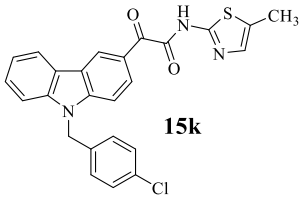
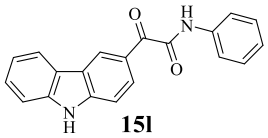
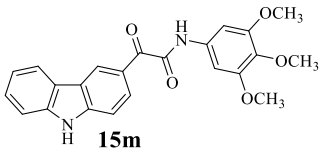
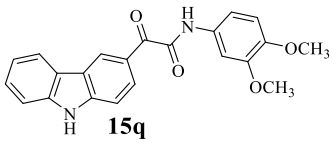
Carbazolyl glyoxamides	Jurkat	U937	MCF-7	MDA-MB-231
 **15f**	64.5±1.4	98.8±2.9	73.7±19.9	70.9±5.2
 **15g**	118.4±26.4	83.2±6.7	93.3±5.6	88.9±6.4
 **15h**	83.1±19.6	59.1±14.8	66.3±5.4	90.2±2.2
 **15i**	**56.5±6.9**	78.8±1.2	70.5±5.8	**62.8±3.5**
 **15j**	**56.5±1.6**	**60.3±17.4**	68.0±7.0	**63.5±2.9**
 **15k**	**51.3±0.5**	**62.2±5.8**	58.4±6.6	**64.8±1.9**
 **15l**	83.4±41.0	**64.4±8.7**	**48.5±4.0**	75.2±2.4
 **15m**	**52.4±5.9**	67.4±5.8	**59.1±13.3**	61.3±2.0
 **15n**	60.7±0.7	72.1±6.9	59.8±19.9	72.6±8.3

Carbazolyl glyoxamides	Jurkat	U937	MCF-7	MDA-MB-231
 **15o**	77.9±9.4	85.6±6.5	89.4±17.3	84.9±10.5
 **15p**	94.9±6.3	92.8±5.2	60.5±10.6	98.2±5.4
 **15q**	60.5±6.2	65.8±8.5	**50.0±7.3**	65.2±4.5
 **15r**	95.31±7.2	109.7±10.4	104.2±6.7	94.7±6.2
 **15s**	99.77±6.6	107.5±9.3	72.1±5.4	92.3±3.9
 **15t**	93.7±5.3	91.3±10.9	114.2±11.3	94.5±5.2
 **15u**	92.3±2.2	104.7±9.5	106.9±12.1	96.5±6.6
 **15v**	100.4±10.8	106.9±7.4	78.1±8.8	88.8±7.1
 **15w**	102.3±9.7	93.6±4.2	79.7±7.6	89.9±9.2
 **15x**	100.4±10.8	95.7±7.2	73.8±8.5	83.9±8.9
Control (negative)	100±5.9	101.5±9.5	100±3.2	100±2.2
Doxorubicin (positive)	**21.2±5.6**	**31.8±1.1**	**38.2±10.6**	**40.2±3.5**

*The activity data represent mean values ± SD of experiments conducted in triplicates at three independent times

The IC_{50} values of selected carbazolyl glyoxamides **15i-m** and **15q** are summarized in Table 5.3. Compound **15i**, bearing 4-chlorobenzyl and *N,N′*-dimethylaminophenyl moieties was found to show moderate activity. Cytotoxicity was retained by the replacement of a phenyl ring in **15a** with heteroaryl moieties such as 6-quinolyl (**15j**) and 2-(5-methyl)thiazolyl (**15k**). Compound **15l** with *N*-H free carbazole and *N*-phenyl glyoxamide was identified as the most active member of the series with IC_{50} values between 9.3 to 31.2 µM. Also, compound **15l** was found to be 2-3-fold more cytotoxic towards MCF-7 (IC_{50} = 9.3 µM) and Jurkat (IC_{50} = 11.8 µM) cells. No significant change in activity was observed by the replacement of a phenyl group in compound **15l** with a trimethoxyphenyl (**15m**) and dimethoxyphenyl (**15q**) groups except for **15q** (IC_{50} = 9.8 µM; MCF-7).

Table 5.3 IC_{50} values (µM) of selected carbazolyl glyoxamides

Carbazolyl glyoxamides	Jurkat	U937	MCF-7	MDA-MB-231
 **15i**	10.5 ± 2.1	29.2 ± 3.9	23.5 ± 8.2	17.9 ± 5.7
 **15j**	11.3 ± 3.6	15.1 ± 4.8	18.9 ± 3.8	18.7 ± 7.7
 **15k**	10.2 ± 2.9	17.5 ± 8.2	12.2 ± 5.4	20.1 ± 8.4
 **15l**	11.8 ± 3.3	18.3 ± 7.8	**9.3 ± 4.3**	31.2±11.4
 **15m**	12.1 ± 1.8	23.3 ± 10	11.5 ± 5.5	14.7 ± 5.4
 **15q**	17.8 ± 3.2	29.2 ± 9.2	**9.8 ± 2.8**	18.5 ± 5.4
Doxorubicin	0.25 ± .11	0.15 ± .05	0.35 ± .15	0.5 ± .20

*Bold value indicates IC_{50} > 10 µM

However, compound **15q** was found to be equipotent (compounds **15l** vs **15q**) against MCF-7 cell line (IC_{50} = 9.8 µM). From the structural variation, it was realized that carbazole-*N*-H with its appended glyoxamide bearing phenyl, dimethoxyphenyl, and trimethoxyphenyl units increase cytotoxicity.

To further characterize the mode of cellular death by carbazolyl glyoxamides, apoptosis induction studies for the selected compounds **15i-m** and **15q** were performed on Jurkat cells by the caspases 3/7 activation method. Caspases belonging to a family of cysteine proteases are known to play an essential role in apoptosis.[44] Out of these caspases, caspase-3 is an effector caspase that cleaves multiple proteins in cells leading to apoptotic cell death. Therefore, activation of caspase 3 pathway is a hallmark of apoptosis and can be used in a cellular assay to quantify activator. Of the carbazolyl glyoxamides tested, compounds **15i**, **15k**, **15l** and **15q** showed 4-5-fold enhancement in caspase level compared to the control (Figure 5.8). These results imply that carbazolyl glyoxamides induced apoptosis in Jurkat cells *via* caspase-3-dependent pathway.

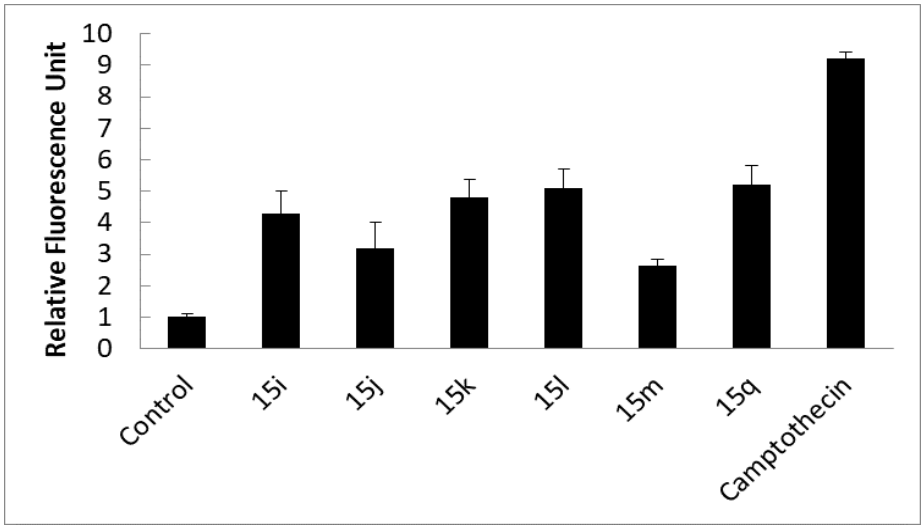


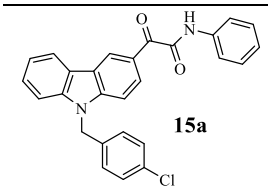
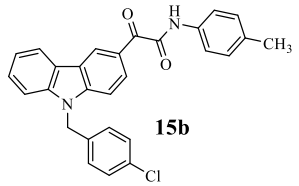
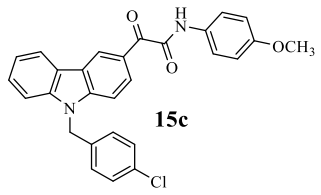
Figure 5.8 Compounds **15i-m** and **15q** induced caspase activation in Jurkat cells

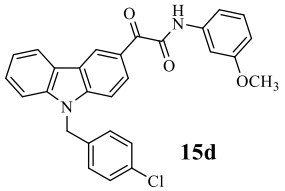
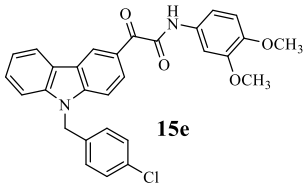
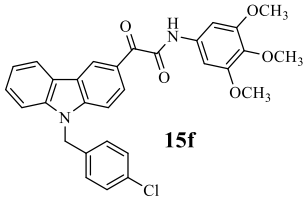
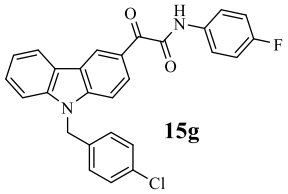
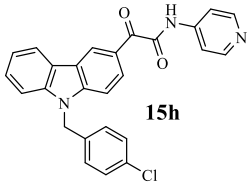
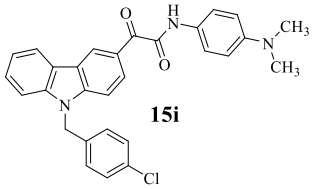
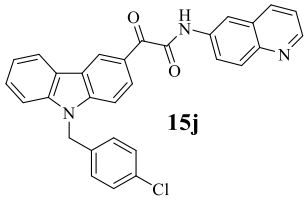
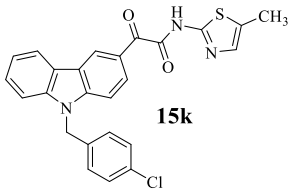
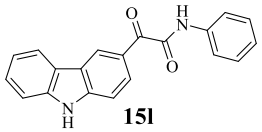
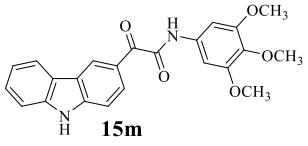
5.3.3 Antibacterial activity

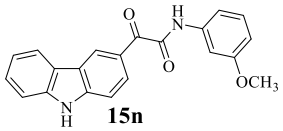
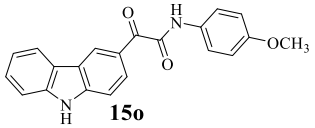
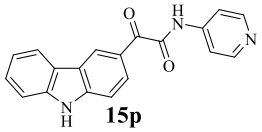
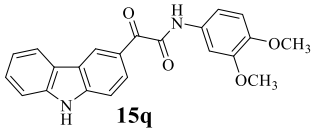
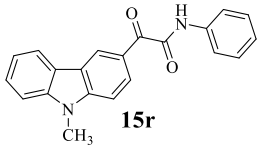
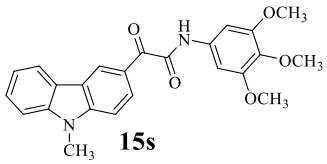
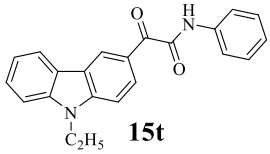
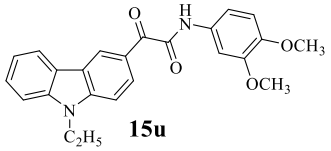
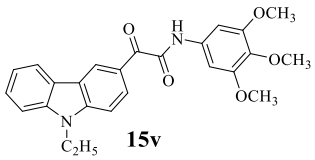
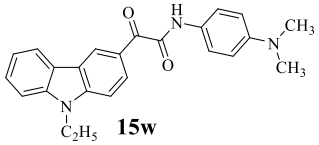
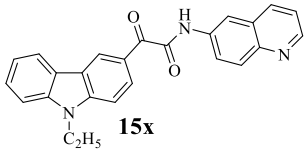
In light of interesting antibacterial activities of many carbazole containing natural and synthetic compounds, the newly synthesized carbazolyl glyoxamides **15a-x** were screened for their antibacterial activity.[27] All the compounds were tested for their *in vitro* antibacterial activities against Gram-positive bacteria including *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121), and Gram-negative bacteria including *Escherichia coli* (MTCC 1652) and *Pseudomonas putida* (MTCC102) with

respect to chloramphenicol, a standard drug. The Minimum Inhibitory Concentrations (MICs) and Zone of Inhibition (ZOI) for compounds **15a-x** were determined *in vitro* by the modified broth micro-dilution values method as given in Table 5.4. Compounds containing *N*-chlorobenzyl carbazole and C_6H_5 (**15a**), $CH_3C_6H_4$ (**15b**) $CH_3OC_6H_4$ (**15c**), $(CH_3O)_3C_6H_2$ (**15f**) and *N,N′*-$(CH_3)_2C_6H_4$ (**15i**) substituents in the glyoxamide fragment were found to display moderate activity against the tested bacterial strains. Interestingly, the introduction of an electron-withdrawing fluoro group in the phenyl ring resulted in **15g** endowed with potent antibacterial activity against all tested bacterial strains with MIC values ranging between 8 to16 µg/mL. Replacement of a *N*-phenyl ring in **15a** with heteroaryl groups such as 4-pyridyl (**15h**) and 6-quinolyl (**15j**) led to inactive derivatives; except **15k** bearing 2-(5-methyl)thiazolyl) moiety exhibited comparable antibacterial activity against *B. cereus* and *E. coli* bacterial strains (ZOI = 16-19 mm; MIC = 16 µg/mL). Compound **15l** with carbazole *N*-H and a phenyl moiety on the amide part displayed improved activity when compared to the corresponding *N*-substituted carbazole **15a**. Replacement of a phenyl ring in **15l** by methoxyphenyl (**15m-o** and **15q**) or 4-pyridyl (**15p**) moiety led to a decrease in the activity except for **15n** (MIC = 8-16 µg/mL). Alkylation of carbazole *N*-H resulted in **15r-x** with moderate activity against the tested bacterial strains.

Table 5.4 *In vitro* antibacterial activities of carbazolyl glyoxamides **15a-x**

Carbazolylglyoxamides	Gram-positive bacteria				Gram-negative bacteria			
	B. cereus		***S. aureus***		***E. coli***		***P.putida***	
	ZOI	MIC	ZOI	MIC	ZOI	MIC	ZOI	MIC
 **15a**	16	32	16	>32	14	64	15	32
 **15b**	15	>32	-	-	16	64	-	-
 **15c**	16	32	15	32	17	>16	15	>16

 **15d**	17	16	-	-	14	>16	-	-
 **15e**	15	>64	17	64	15	32	16	>16
 **15f**	15	>32	17	>64	18	32	16	32
 **15g**	**18**	**16**	**18**	**>8**	**20**	**8**	**19**	**16**
 **15h**	15	128	17	>32	16	32	16	32
 **15i**	16	64	18	>32	14	32	16	64
 **15j**	15	>32	16	>128	15	32	18	64
 **15k**	16	**16**	**19**	**>8**	**19**	**16**	**18**	>16
 **15l**	**17**	**16**	**18**	**>8**	**19**	>8	**17**	**8**
 **15m**	15	>16	17	>16	16	32	16	64

 **15n**	**18**	**>8**	**19**	**16**	16	**16**	**18**	**8**
 **15o**	14	64	14	>64	17	128	15	>64
 **15p**	15	32	16	>64	18	32	16	>32
 **15q**	16	128	18	>32	17	>16	14	>64
 **15r**	13	>32	13	64	15	32	14	32
 **15s**	14	64	13	32	17	32	15	>32
 **15t**	15	>32	14	64	16	>16	17	>16
 **15u**	14	64	-	-	16	>32	16	>16
 **15v**	15	>16	15	>32	14	>64	-	-
 **15w**	15	>32	14	>64	16	32	16	32
 **15x**	12	32	14	32	13	>32	14	64
Chloramphenicol	21	32	21	16	22	16	21	16

*ZOI (in mm) and MIC (in µg/mL) values, bold values indicate comparable or even better antibacterial activity than chloramphenicol.

Although antibacterial assays (ZOI & MIC) might show a potential to kill pathogenic micro-organisms, concentration vs time curves provide more insight about the rate of antibacterial activity. To determine the rate of bactericidal activity of the two most active compounds **15g** and **15l**, time-kill studies were performed. The interactive time and the change in the number of microorganisms for compounds **15g** and **15l** is presented in Figure 5.9A-D. The percent population reduction at different time interval was calculated to demonstrate the change of the population of microorganism respect to concentration dependent dose. The compound **15g** showed high bacteriostatic effect against *E. coli* in the first hours of incubation. Moreover, the highest reduction (85%) in *E. coli* population was observed at 8 h of incubation with compound **15g** (Figure 5.9A). Compound **15g** was also showed about >70% reduction in viable cells of *S. aureus* (Figure 5.9B). Compound **15l** with free carbazole *N*-H and a phenyl moiety in amidic part led to significant inhibition of bacterial growth after 4 h of incubation. It is evident that within 8 h, compound **15l** exhibited almost 80% reduction in the viability of *P. Putida* (Figure 5.9C). Similarly, bacteriostatic effect of compound **15l** against *S. aureus* reveals about 70% reduction of the viable cell at 8 h of incubation (Figure 5.9D). Thus, the results of present study revealed that **15g** and **15l** were capable of inhibiting the bacterial growth within a few hours of initial interactions.

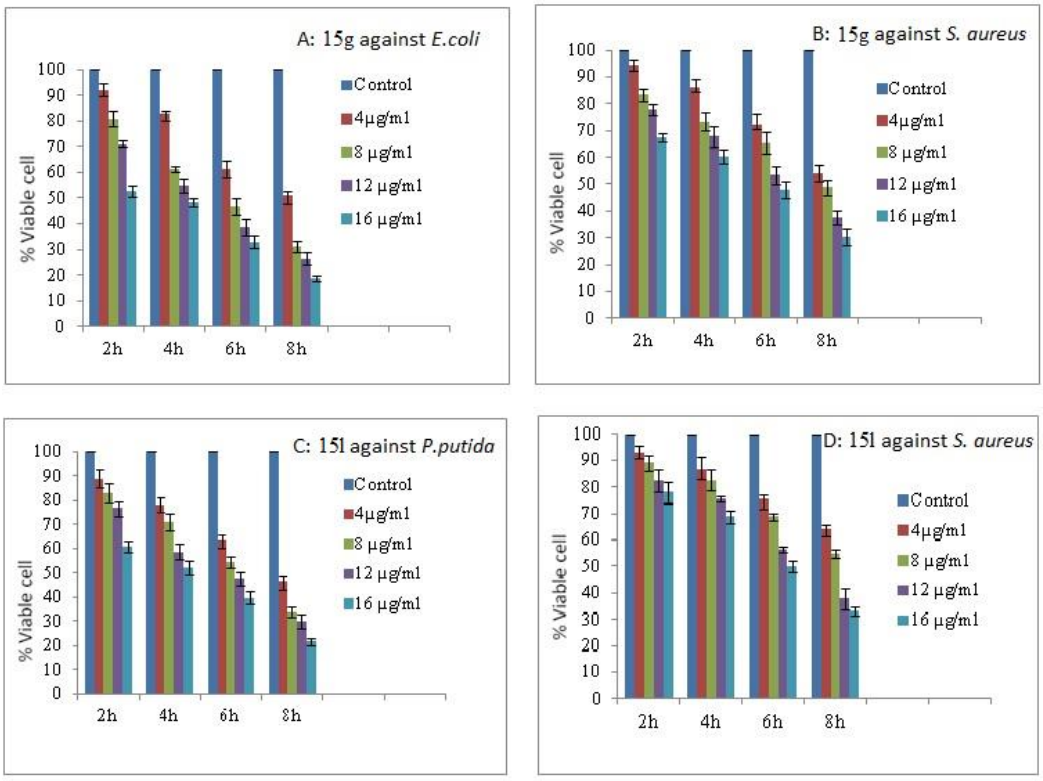


Figure 5.9 Cell viability assay of **15g** and **15l** against selected bacteria

5.3.4 Structure-activity relationship (SAR) studies

The structure-activity relationship (SAR) studies of carbazolyl glyoxamides imply that a combination of carbazole *N*-H and glyoxamide unit possessing *p*-fluorophenyl and methoxyphenyl substituents are beneficial for the activity (Figure 5.10).

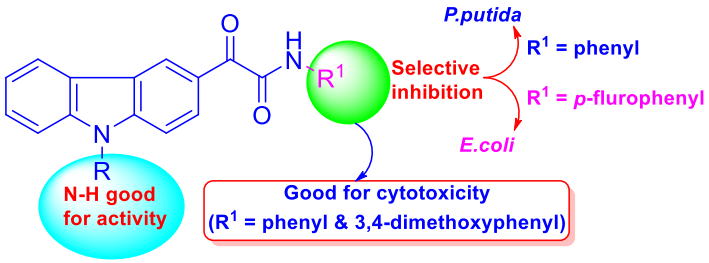


Figure 5.10 Structure-activity relationship of carbazolylglyoxamides **15a-x**

All the synthesized compounds demonstrated good to moderate cytotoxicity against a panel of cancer cell lines and excellent to moderate antibacterial activity towards tested bacterial strains. Notably, compound **15l** with carbazole *N*-H and phenyl moiety in glyoxamide part exhibited potent cytotoxicity and antibacterial activities. Exclusively, with carbazole *N*-H and dimethoxyphenyl moieties, compound **15q** was found to be the most active against the tested cancer cell lines and less potent towards tested bacterial strains. However, compound **15g** with *N*-chlorobenzylcarbazole and fluorophenyl units, and analogue **15n** having carbazole *N*-H and 3-methoxyphenyl moieties were the most active carbazolyl glyoxamides against the tested bacteria but exhibited low cytotoxicity towards tested cancer cell lines.

5.3.5 LDH assay

The toxicity of potent compounds **15i-m** and **15q** were evaluated using LDH (Lactate dehydrogenase) assay. The LDH activity shows that all the tested compounds **15i-m** and **15q** exhibited lower toxicity than the standard drug, doxorubicin which justifies the potential use of these compounds as anticancer as well as antibacterial agents (Figure 5.11).

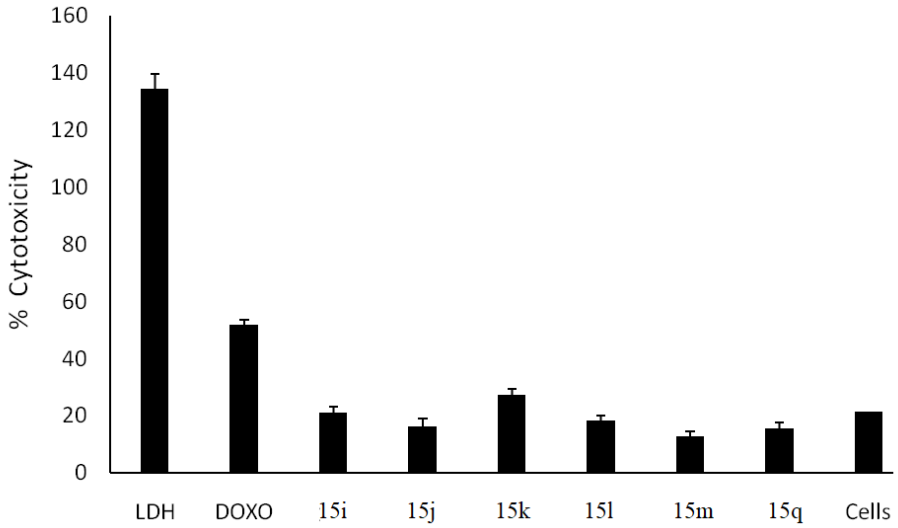


Figure 5.11 Cytotoxicity induced by carbazolyl glyoxamides in terms of LDH release

5.4 Conclusions

In summary, we have synthesized various carbazolyl glyoxamides from readily accessible glyoxalic acids **19** and arylamines by employing HATU as a coupling reagent. Synthesized glyoxamides were assessed for their cytotoxicity which enabled us to identify **15l** and **15q** as the most potent compounds against MCF-7 cells with IC_{50} values of 9.3 µM and 9.8 µM, respectively. Preliminary mechanism of action studies indicated that carbazolyl glyoxamides induced apoptosis in Jurkat cells *via* caspase-3 and-7 activation. In addition, antibacterial activity evaluation led us to compounds **15g, 15k-l** and **15n** with significant potency against Gram-positive and Gram-negative bacteria (MIC = 8-16 µg/mL and ZOI = 16-20 mm). Antibacterial activities of potent compounds **15g**, **15k-l** and **15n** were found to be comparable to the reference drug, chloramphenicol. Cell viability assay revealed that analogues **15g** and **15l** were capable of inhibiting the bacterial growth within few hours of initial interactions. Interesting activity results indicate that the identified potent carbazolyl glyoxamides **15g** and **15l** can be exploited further to develop either highly specific or potent antibacterial/anticancer agents, or if required both of these properties could be incorporated in the same molecule.

5.5 Experimental Section

5.5.1 General methods and materials

All the reagents were purchased from commercial sources (Sigma-Aldrich, Alfa Aesar, Merck, and Spectrochem). Solvents used for all reaction were dried prior to use by standard procedure. The synthesis of all the final compounds was carried out in a CEM Focused microwave. The progress of the reactions was followed by thin layer chromatography (TLC) analysis (Merck, silica gel 60 F254 in aluminium foil). Solvents were evaporated using Büchi rotary evaporator. Melting points were determined with electro thermal capillary melting point apparatus (*E*-*Z* melting) and were uncorrected. NMR spectra were measured on a Brucker Avance II 400 MHz (400 MHz for ^{1}H and 100 MHz for ^{13}C) instrument using solvents DMSO-d_6 and $CDCl_3$. Chemical shifts are reported in ppm and multiplicities are given as s (singlet), d (doublet), t (triplet), m (multiplet), dd (double doublet), and coupling constants (*J*) in Hertz (Hz). IR spectra were recorded on Shimadzu IR Prestige-21 FT-IR spectrophotometer using KBr pellets. The purity of all the synthesized compounds was > 97% as determined by WATERS 515 HPLC system with a Sunfire C-18 column (5 µm, 4.6 × 250 mm) and PDA detector using a flow rate of 1 mL/min. and a gradient of acetonitrile. HRMS analysis was performed on Bruker Compass Data Analysis 4.1 mass instrument.

5.5.2 General Experimental procedure

General Procedure for the synthesis of 9-(4-chlorobenzyl)-9*H*-carbazoles (17a-c)

A solution of potassium hydroxide (180 mmol) in 80 mL *N*,*N*-dimethylformamide (DMF) was stirred at room temperature for 20 min. Carbazole **16** (60 mmol) was added and the mixture was stirred for 40 min at room temperature. A solution of alkyl halide (72 mmol) in DMF (50 mL) was added drop wise with stirring. The resulting reaction mixture was then stirred at room temperature for 12 h. After completion of reaction as indicated by TLC, contents were poured into cold water (500 mL). White precipitate obtained was filtered, washed with cold water (100 mL) and recrystallized from ethanol to give pure **17a-c** in 90-95% yields.

9-(4-Chlorobenzyl)-9*H*-carbazole (17a): Off-white solid; Yield 92%; Mp 171-172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.0 Hz, 2H), 7.47 (t, J = 8.2 Hz, 2H), 7.37-7.24 (m, 6H), 7.09 (d, J = 8.0 Hz, 2H), 5.51 (s, 2H); IR (KBr, ν, cm^{-1}): 3047, 2938, 1597, 1450, 1327, 748, 725.

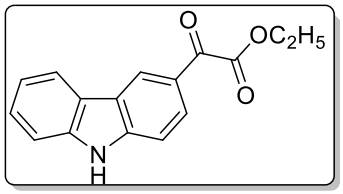
9-Methyl-9*H*-carbazole (17b): Off white solid; Yield 95%; Mp 95 °C (lit. 95-97 °C).[1]

9-Ethyl-9*H*-carbazole (17c): Off white solid; Yield 90%; Mp 70-72 °C (lit. 72-74 °C).[2]

General procedure for the synthesis of carbazolyl glyoxalic esters (18a-d)

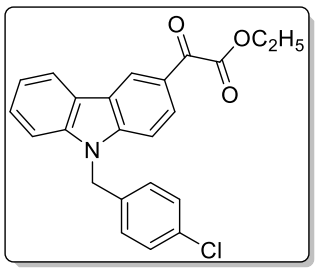
To a solution of dichloromethane (100 mL) at below 5 °C was added $AlCl_3$ (6.85 g, 51.4 mmol) portion wise, followed by ethyl chlorooxalate (9.36 g, 68.5 mmol) and slowly carbazole **17** (34.2 mmol) solution in 50 mL dichloromethane added over a period of 30 min. The reaction mixture was stirred at room temperature for 3 h. After complete disappearance of the starting material as indicated by TLC, contents were added slowly to a solution of ammonium acetate in water (200 mL). The aqueous layer was extracted with DCM (2 × 200 ml) and the combined organic layer was concentrated *in vaccuo*. The obtained crude product was purified by column chromatography using ethyl acetate and hexane as eluent to obtain pure compounds **18a-d** as yellow solids in good yields (50-70%).

Ethyl 2-(9*H*-carbazol-3-yl)-2-oxoacetate (18a)



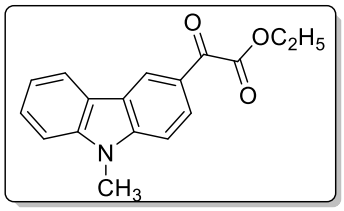
Off white solid; Yield 60%; Mp 121-122 °C; IR (KBr, *v*, cm^{-1}): 3302, 2901, 1728, 1659, 1628, 1582, 1335, 1250, 1119, 1057, 717, 671.

Ethyl 2-(9-(4-chlorobenzyl)-9*H*-carbazol-3-yl)-2-oxoacetate (18b)



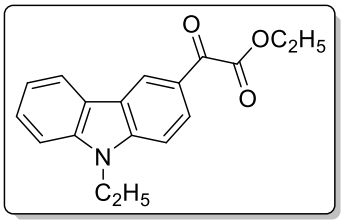
Off white solid; Yield 67%; Mp 129-131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.19 (d, J = 7.7 Hz, 1H), 8.13 (d, J = 8.7 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.39 (dd, J = 9.2, 4.6 Hz, 3H), 7.29–7.24 (m, 2H), 7.06 (d, J = 8.3 Hz, 2H), 5.52 (s, 2H), 4.54 (q, J = 7.1 Hz, 2H), 1.49 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.0, 164.9, 144.4, 141.4, 134.7, 133.9, 130.1, 129.1, 128.6, 128.2, 127.6, 127.1, 124.9, 124.6, 123.5, 121.9, 120.5, 108.5, 63.9, 62.4, 46.4; IR (KBr, *v*, cm^{-1}): 3063, 2978, 2932, 1728, 1666, 1589, 1474, 1188, 1026, 841, 802, 748.

Ethyl 2-(9-methyl-9*H*-carbazol-3-yl)-2-oxoacetate (18c)



Yellow oil; Yield 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, J = 2.1 Hz, 1H), 8.19–8.12 (m, 2H), 7.58–7.54 (m, 1H), 7.44 (t, J = 8.7 Hz, 2H), 7.38–7.32 (m, 1H), 4.54 (q, J = 7.2 Hz, 2H), 3.88 (s, 3H), 1.50 (t, J = 7.2 Hz, 3H).

Ethyl 2-(9-ethyl-9H-carbazol-3-yl)-2-oxoacetate (18d)

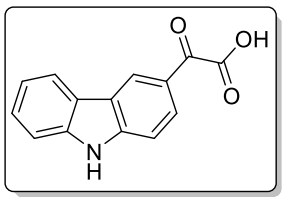


Yellow oil; Yield 57%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (d, J = 1.3 Hz, 1H), 8.17–8.13 (m, 2H), 7.57–7.53 (m, 1H), 7.44 (t, J = 8.7 Hz, 2H), 7.36–7.31 (m, 1H), 4.54 (q, J = 7.2 Hz, 2H), 4.37 (q, J = 7.2 Hz, 2H), 1.50 (t, J = 7.2 Hz, 3H), 1.46 (t, J = 7.3 Hz, 3H).

General procedure for the synthesis of carbazolyl glyoxalic acids (19a-d)

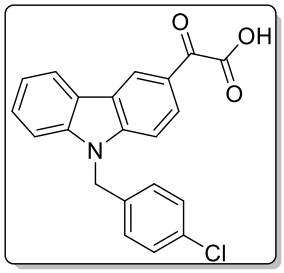
Carbazolyl glyoxalic esters **18** (12.8 mmol) was dissolved in 100 mL THF: H_2O (1:1). Lithium hydroxide monohydrate (2.7 g, 64 mmol) was added to the reaction mixture in portion wise. The resulting reaction mixture was stirred for 90 min at room temperature. Upon completion of the reaction as indicated by TLC, an excess of THF was evaporated under *vaccuo* to give a residue which was dissolved in water (50 mL) and acidified up to pH~2 using 2N HCl (50 mL). The obtained solid was filtered, washed with water and dried to afford compounds **19a-d** as yellow solids in excellent yields (90-97%).

2-(9*H*-Carbazol-3-yl)-2-oxoacetic acid (19a)



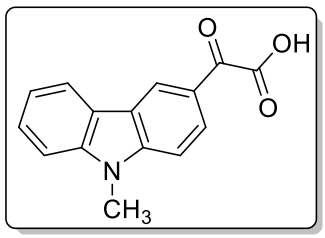
White solid; Yield 90%; Mp 215-217 °C; IR (KBr, v, cm^{-1}): 3358, 2953, 1713, 1659, 1589, 1327, 1180, 918, 795, 741.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-2-oxoacetic acid (19b)



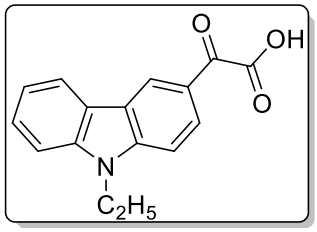
Yield 94%; off white solid; Mp 183-185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.35 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.34–7.30 (m, 3H), 7.18 (d, J = 7.9 Hz, 2H), 5.74 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 189.3, 167.9, 144.2, 141.4, 136.5, 132.6, 129.2, 129.1, 127.9, 127.6, 124.2, 123.8, 123.0, 122.9, 121.6, 121.2, 110.8, 110.5, 45.7; IR (KBr, v, cm^{-1}): 3232, 2946, 1759, 1660, 1582, 1497, 1342, 1142, 802, 741.

2-(9-Methyl-9*H*-carbazol-3-yl)-2-oxoacetic acid (19c)



Yellow solid; Yield 92%; Mp 162-163 °C. IR (KBr, v, cm^{-1}): 3318, 2953, 1739, 1663, 1580, 1323, 1243, 1180, 908, 795.

2-(9-Ethyl-9*H*-carbazol-3-yl)-2-oxoacetic acid (19d)

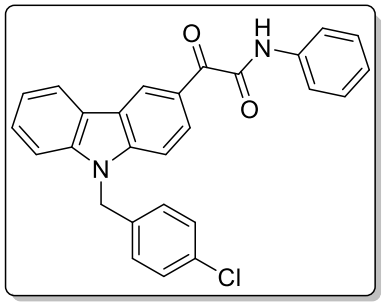


Yellow solid; Yield 97%; Mp 173 °C. IR (KBr, *v*, cm^{-1}): 3267, 2956, 1725, 1669, 1592, 1455, 1335, 1263, 1188, 803, 741.

General procedure for the synthesis of carbazolyl glyoxamides (15a-x)

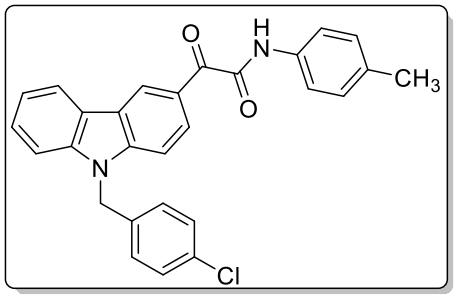
To a 10 mL microwave tube was added carbazole glyoxalic acid **19** (0.275 mmol), HATU (0.12 g, 0.317 mmol), *N*,*N*-diisopropylethylamine (0.09 g, 0.687 mmol) and an appropriate aryl/heteroarylamine (0.303 mmol) in DMF (2 mL). The tube was sealed with a pressure cap and placed in the microwave cavity. The sample was irradiated for 45 min at 70 °C and then allowed to cool at room temperature. The residue was poured into ice-cold water (30 mL) and stirred for 20 min at room temperature. The solid so obtained was filtered, dried and purified by column chromatography on silica gel using ethylacetate: hexane (3:7) as eluent to obtain pure (**15a-g**, **15j-o** and **15q**) in excellent yields. Some of the compounds (**15h-i** and **15p**) were crystallized from acetone to obtain pure yellow solid products in 70-91% yields.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-2-oxo-*N*-phenylacetamide (15a)



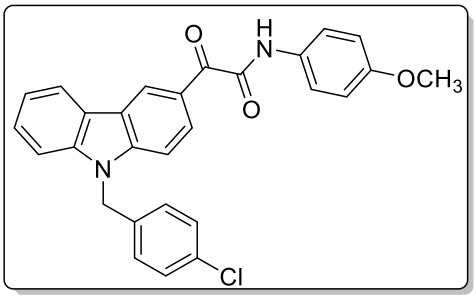
Yield 70%; Mp 191-193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (d, *J* = 1.3 Hz, 1H), 9.15 (s, 1H), 8.58 (dd, *J* = 8.8, 1.7 Hz, 1H), 8.25 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.78 (dd, *J* = 8.6, 1.0 Hz, 2H), 7.54–7.50 (m, 1H), 7.45 (dd, *J* = 8.3, 7.5 Hz, 3H), 7.41–7.36 (m, 3H), 7.26 (t, *J* = 2.6 Hz, 2H), 7.08 (d, *J* = 8.6 Hz, 2H), 5.55 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.9, 160.0, 144.2, 141.2, 136.9, 134.6, 133.7, 129.8, 129.3, 129.2, 127.7, 127.0, 126.2, 125.2, 125.0, 123.6, 123.1, 121.1, 121.1, 120.0, 109.5, 108.8, 46.24; IR (KBr, *v*, cm^{-1}): 3335, 3090, 3052, 2916, 1682, 1653, 1589, 1520, 1435, 1307, 1250, 1134, 1011, 825, 795; Anal. RP-HPLC t_R = 4.641 min, purity 98.55%; HRMS (ESI^+) Calculated for $C_{27}H_{20}ClN_2O_2$ $[M + H]^+$, 439.1213; Found 439.1208 and 461.1025 $[M + Na]^+$.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-2-oxo-*N*-(*p*-tolyl)acetamide (15b)



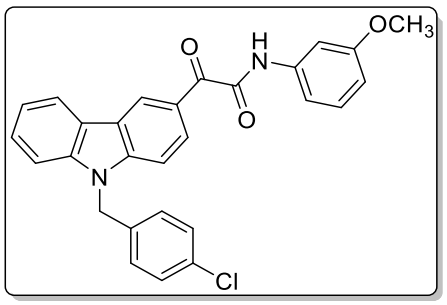
Yield 72%; Mp 172-173 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.48 (d, J = 1.6 Hz, 1H), 9.09 (s, 1H), 8.58 (dd, J = 8.8, 1.7 Hz, 1H), 8.27–8.23 (m, 1H), 7.67–7.64 (m, 2H), 7.53-7.49 (m, 1H), 7.44–7.35 (m, 3H), 7.26 (t, J = 2.2 Hz, 3H), 7.24 (s, 1H), 7.09 (d, J = 8.6 Hz, 2H), 5.55 (s, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.0, 159.9, 144.2, 141.1, 134.9, 134.6, 134.3, 133.7, 129.9, 129.7, 129.2, 127.7, 126.9, 126.1, 125.1, 123.6, 123.1, 121.1, 121.0, 119.9, 109.5, 108.7, 46.2, 21.0; IR (KBr, v, cm^{-1}): 3325, 3094, 3055, 2916, 1682, 1651, 1620, 1582, 1520, 1443, 1327, 1265, 1149; Anal. RP-HPLC t_R = 5.317 min, purity 98.10%; HRMS (ESI$^+$) Calculated for $C_{28}H_{21}ClN_2O_2$ $[M + H]^+$, 453.1369; Found 453.1365 and 475.1183 $[M + Na]^+$

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-*N*-(4-methoxyphenyl)-2-oxoacetamide (15c)



Yield 72%; Mp 168-170 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (d, J = 1.5 Hz, 1H), 9.04 (s, 1H), 8.56 (dd, J = 8.8, 1.7 Hz, 1H), 8.22 (d, J = 7.7 Hz, 1H), 7.69–7.65 (m, 2H), 7.51-7.47 (m, 1H), 7.42–7.32 (m, 4H), 7.25–7.23 (m, 1H), 7.06 (d, J = 8.5 Hz, 2H), 6.97–6.94 (m, 2H), 5.52 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.1, 159.8, 157.0, 144.2, 141.1, 134.6, 133.7, 130.1, 129.7, 129.2, 127.7, 126.9, 126.1, 125.1, 123.6, 123.1, 121.6, 121.1, 121.0, 114.4, 109.5, 108.7, 55.5, 46.2; IR (KBr, v, cm^{-1}): 3348, 3055, 2924, 1682, 1643, 1620, 1582, 1528, 1443, 1327, 1250, 1149; Anal. RP-HPLC t_R = 4.368 min, purity 98.67%; HRMS (ESI$^+$) Calculated for $C_{28}H_{22}ClN_2O_3$ $[M + H]^+$, 469.1319; Found 469.1310, 471.1271 $[M + H + 2]^+$ and 491.1129 $[M + Na]^+$.

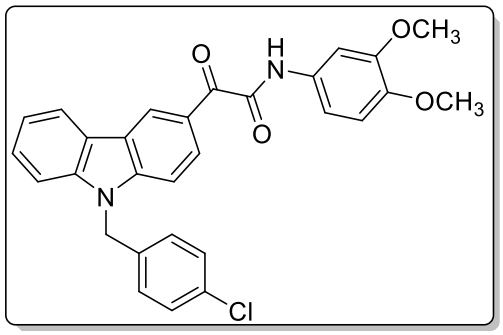
2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-*N*-(3-methoxyphenyl)-2-oxoacetamide (15d)



Yield 73%; Mp 106-107 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 9.11 (s, 1H), 8.57–8.54 (m, 1H), 8.23 (d, J = 7.7 Hz, 1H), 7.52–7.46 (m, 2H), 7.40–7.33 (m, 3H), 7.31–7.28 (m, 2H), 7.24–7.19 (m, 2H), 7.06 (d, J = 8.3 Hz, 2H), 6.77 (dd, J = 7.8, 1.6 Hz, 1H), 5.52 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.8, 160.3, 159.9, 144.2, 141.1, 138.1, 134.6, 133.7, 129.9, 129.7, 129.2, 127.7, 126.9, 126.1,

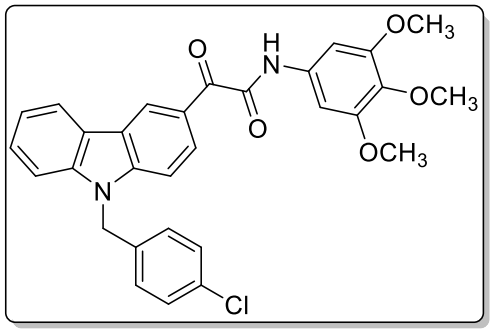
124.9, 123.5, 123.1, 121.1, 121.0, 112.2, 111.1, 109.5, 108.7, 105.5, 55.4, 46.2; IR (KBr, *v*, cm^{-1}): 3333, 3055, 2932, 2862, 1693, 1659, 1589, 1528, 1381, 1335, 1265, 1203, 1142, 1088, 1041,849, 795, 725, 687, 679; Anal. RP-HPLC t_R = 4.550 min, purity 98.36%; HRMS (ESI^+) Calculated for $C_{28}H_{22}ClN_2O_3$ $[M + H]^+$, 469.1319; Found 469.1313 and 491.1133 $[M + Na]^+$.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-*N*-(3,4-dimethoxyphenyl)-2-oxoacetamide (15e)



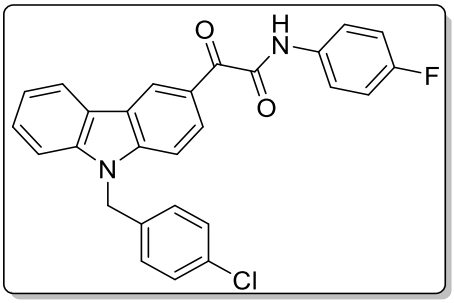
Yield 70%; Mp 136-137 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 9.08 (s, 1H), 8.56 (d, *J* = 8.7 Hz, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 2.0 Hz, 1H), 7.49 (t, *J* = 7.7 Hz, 1H), 7.40–7.33 (m, 3H), 7.24 (s, 2H), 7.16 (dd, J = 8.5, 2.1 Hz, 1H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 1H), 5.51 (s, 2H), 3.96 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.0, 159.7, 149.2, 146.5, 144.2, 141.1, 134.6, 133.7, 130.6, 129.7, 129.2, 127.7, 126.9, 126.1, 125.1, 123.6, 123.1, 121.1, 121.0, 112.1, 111.4, 109.5, 108.7, 104.5, 56.1, 56.0, 46.2; IR (KBr, *v*, cm^{-1}): 3317, 3055, 2924, 2847, 1690, 1651, 1589, 1520, 1458, 1412, 1381, 1335, 1242, 1203, 1134, 1026, 849, 795, 748, 663, 602, 540; Anal. RP-HPLC t_R = 4.031 min, purity 98.17%; HRMS (ESI^+) Calculated for $C_{29}H_{24}ClN_2O_4$ $[M + H]^+$, 499.1424; Found 499.1424, 501.1390 $[M + H + 2]^+$ and 521.1238 $[M + Na]^+$.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-2-oxo-*N*-(3,4,5-trimethoxyphenyl)acetamide (15f)



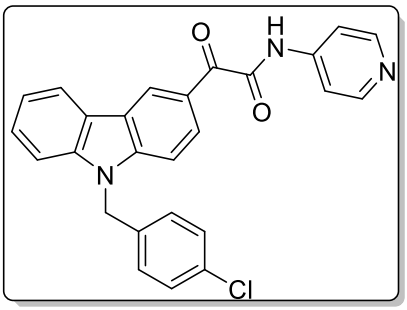
Yield 71%; Mp 183-184 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 9.15 (s, 1H), 8.57 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.25 (d, *J* = 7.6 Hz, 1H), 7.55–7.49 (m, 1H), 7.40 (t, *J* = 8.6 Hz, 3H), 7.29-7.25 (m, 3H), 7.08 (d, *J* = 8.6 Hz, 3H), 5.53 (s, 2H), 3.94 (s, 6H), 3.89 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.8, 159.9, 153.5, 144.2, 141.1, 135.3, 134.6, 133.7, 133.0, 129.7, 129.1, 127.7, 127.0, 126.0, 124.9, 123.5, 123.1, 121.1, 121.0, 109.5, 108.8, 97.5, 61.0, 56.2, 46.2; IR (KBr, *v*, cm^{-1}): 3356, 2932, 1690, 1651, 1597, 1528, 1504, 1450, 1412, 1373, 1335, 1296, 1250, 1126, 1011, 802; Anal. RP-HPLC t_R = 4.181 min, purity 98.17%; HRMS (ESI^+) Calculated for $C_{30}H_{26}ClN_2O_5$ $[M + H]^+$, 529.1530; Found 529.1531 and 551.1344 $[M + Na]^+$.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-*N*-(4-fluorophenyl)-2-oxoacetamide (15g)



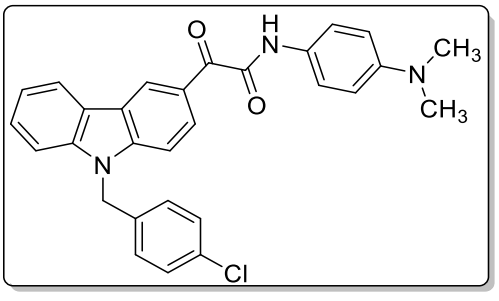
Yield 75%; Mp 202-203 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (d, 1.5 Hz, 1H), 9.13 (s, 1H), 8.55 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 7.74–7.71 (m, 2H), 7.53–7.46 (m, 1H), 7.40–7.33 (m, 3H), 7.24 (s, 2H), 7.14–7.03 (m, 4H), 5.51 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.7, 159.9, 144.2, 141.2, 134.6, 133.7, 133.0, 133.0, 129.7, 129.2, 127.7, 127.0, 126.2, 124.9, 123.6, 123.1, 121.8, 121.7, 121.1, 116.1, 115.9, 113.4, 109.5, 108.8, 46.2; IR (KBr, *v*, cm^{-1}): 3310, 3055, 2935, 1690, 1651, 1589, 1535, 1497, 1381, 1335, 1265, 1211, 1142, 833, 705.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-2-oxo-*N*-(pyridin-4-yl)acetamide (15h)



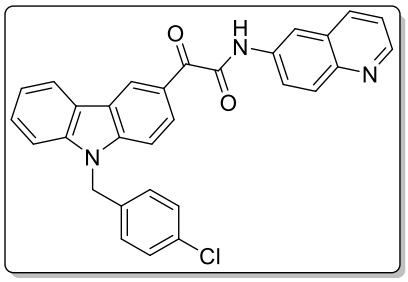
Yield 91%; Mp 116-118 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 1H), 9.32 (s, 1H), 8.61 (d, *J* = 5.0 Hz, 2H), 8.53 (dd, *J* = 8.8, 1.4 Hz, 1H), 8.22 (d, *J* = 7.5 Hz, 1H), 7.69 (d, J = 6.1 Hz, 2H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.31–7.35 (m, 3H), 7.24 (t, *J* = 4.8 Hz, 2H), 7.06 (d, *J* = 8.3 Hz, 2H), 5.52 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.6, 160.5, 151.0, 144.4, 143.8, 141.2, 134.5, 133.8, 129.8, 129.2, 127.7, 127.2, 126.2, 124.5, 123.5, 123.3, 121.2, 121.1, 113.8, 109.6, 108.9, 46.3; IR (KBr, *v*, cm^{-1}): 3340, 2924, 1696, 1659, 1489, 1327, 1265, 1142, 1021, 803; Anal. RP-HPLC t_R = 4.306 min, purity 98.02%; HRMS (ESI^+) Calculated for $C_{26}H_{19}ClN_3O_2$ $[M + H]^+$, 440.1166; Found 440.1171 and 442.1141 $[M+H+2]^+$.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-*N*-(4-(dimethylamino)phenyl)-2-oxoacetamide (15i)



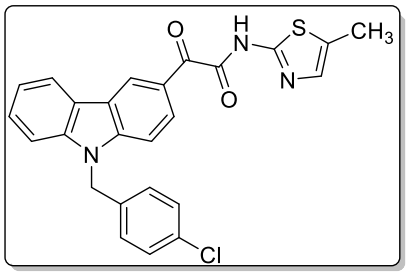
Yield 71%; Mp 211-213 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (d, *J* = 1.3 Hz, 1H), 8.98 (s, 1H), 8.56 (dd, *J* = 8.8, 1.7 Hz, 1H), 8.24–8.21 (m, 1H), 7.63–7.60 (m, 2H), 7.50-7.46 (m, 1H), 7.42–7.30 (m, 4H), 7.24 (s, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.79–6.76 (m, 2H), 5.52 (s, 2H), 2.97 (s, 6H); IR (KBr, *v*, cm^{-1}): 3333, 3047, 2924, 2854, 2800, 1674, 1643, 1582, 1520, 1443, 1350, 1265, 1136, 1011, 949, 895, 825, 795, 748, 694, 617; HRMS (ESI^+) Calculated for $C_{29}H_{25}ClN_3O_2$ $[M + H]^+$, 482.1635; Found 482.1621 and 484.1609 $[M + H + 2]^+$.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-2-oxo-*N*-(quinolin-6-yl)acetamide (15j)



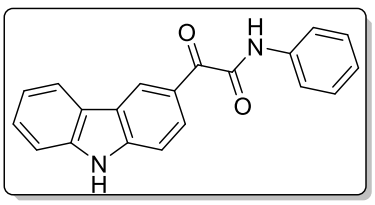
Yield 70%; Mp 129-131 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 (d, 1.3 Hz, 2H), 8.89 (dd, J = 4.3, 1.6 Hz, 1H), 8.61–8.55 (m, 2H), 8.25–8.18 (m, 2H), 8.16 (d, J = 9.0 Hz, 1H), 7.79 (dd, J = 9.0, 2.4 Hz, 1H), 7.52–7.41 (m, 2H), 7.41–7.30 (m, 3H), 7.23 (t, J = 2.1 Hz, 2H), 7.05 (d, J = 8.5 Hz, 2H), 5.49 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.4, 160.3, 149.5, 145.4, 144.3, 141.1, 136.4, 134.9, 134.5, 133.7, 130.2, 130.1, 129.8, 129.2, 128.8, 127.7, 127.1, 126.2, 124.8, 123.5, 123.4, 123.2, 121.9, 121.1, 116.5, 109.5, 108.8, 46.2; IR (KBr, v, cm^{-1}): 3356, 2932, 1690, 1651, 1597, 1528, 1504, 1450, 1412, 1373, 1335, 1296, 1250, 1126, 1011, 802; Anal. RP-HPLC t_R = 4.911 min, purity 97.89%; HRMS (ESI$^+$) Calculated for $C_{30}H_{21}ClN_3O_2$ [M + H]$^+$, 490.1322; Found 490.1327 and 492.1304 [M + H + 2]$^+$.

2-(9-(4-Chlorobenzyl)-9*H*-carbazol-3-yl)-*N*-(5-methylthiazol-2-yl)-2-oxoacetamide (15k)



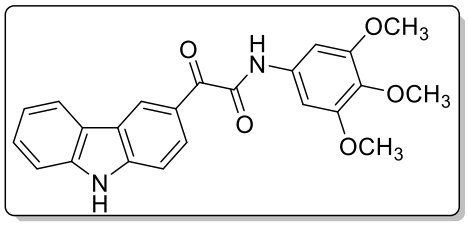
Yield 74%; Mp 178-179 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.63 (s, 1H), 9.48 (d, J = 1.3 Hz, 1H), 8.60 (dd, J = 8.8, 1.5 Hz, 1H), 8.25 (d, J = 8.2 Hz, 1H), 7.52 (t, J = 7.3 Hz, 1H), 7.46–7.32 (m, 4H), 7.26 (s, 1H), 7.08 (d, J = 8.3 Hz, 2H), 6.70 (s, 1H), 5.53 (s, 2H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9, 159.3, 156.1, 148.4, 144.4, 141.2, 134.5, 133.8, 129.7, 129.2, 128.7, 127.7, 127.1, 126.2, 124.6, 123.5, 123.3, 121.2, 109.6, 109.2, 108.9, 46.3, 17.1; IR (KBr, v, cm^{-1}): 3317, 3055, 2932, 1690, 1651, 1589, 1528, 1489, 1257, 1142, 1011, 802; Anal. RP-HPLC t_R = 4.562 min, purity 99.82%; HRMS (ESI$^+$) Calculated for $C_{25}H_{19}ClN_3O_2S$ [M + H]$^+$, 460.0886; Found 460.0888.

2-(9*H*-Carbazol-3-yl)-2-oxo-*N*-phenylacetamide (15l)



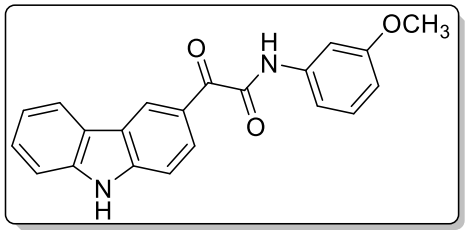
Yield 70%; Mp 221-222 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 9.12 (s, 1H), 8.55 (dd, J = 8.7, 1.5 Hz, 1H), 8.43 (s, 1H), 8.18 (d, J = 7.7 Hz, 1H), 7.76 (d, J = 7.7 Hz, 2H), 7.53–7.46 (m, 3H), 7.43 (t, J = 7.9 Hz, 2H), 7.36–7.30 (m, 1H), 7.21 (t, J = 7.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.4, 162.4, 143.9, 140.6, 137.5, 128.8, 128.3, 126.4, 125.1, 124.6, 124.0, 123.1, 122.8, 120.4, 120.2, 120.0, 111.4, 110.9; IR (KBr, v, cm^{-1}): 3348, 3055, 2932, 1674, 1651, 1528, 1443, 1335, 1296, 1257, 1134, 1011, 787, 748, 687, 602, 548; Anal. RP-HPLC t_R = 3.335 min, purity 98.25%; HRMS (ESI$^+$) Calculated for $C_{20}H_{15}N_2O_2$ [M + H]$^+$, 315.1133; Found 315.1129 and 337.0949 [M + Na]$^+$.

2-(9*H*-Carbazol-3-yl)-2-oxo-*N*-(3,4,5-trimethoxyphenyl)acetamide (15m)



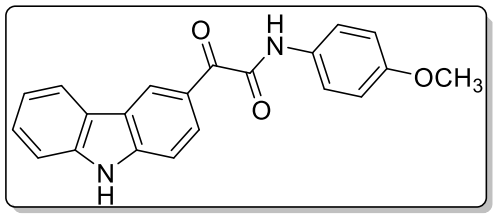
Yield 72%; Mp 207-208 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (s, 1H), 9.08 (s, 1H), 8.55 (dd, J = 8.7, 1.7 Hz, 1H), 8.47 (s, 1H), 8.19 (d, J = 7.8 Hz, 1H), 7.51–7.45 (m, 3H), 7.35–7.31 (m, 1H), 7.07 (s, 2H), 3.93 (s, 6H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.9, 160.0, 153.5, 143.5, 140.0, 135.3, 133.1, 129.5, 126.9, 126.1, 124.9, 123.6, 123.3, 120.9, 120.8, 111.1, 110.6, 97.6, 61.0, 56.2; IR (KBr, ν, cm^{-1}): 3340, 3301, 3055, 2932, 2839, 1690, 1651, 1597, 1528, 1458, 1412, 1373, 1335, 1234, 1203, 1126, 995, 802, 733, 617; Anal. RP-HPLC t_R = 3.113 min, purity 98.72%; HRMS (ESI$^+$) Calculated for $C_{23}H_{21}N_2O_5$ $[M + H]^+$, 405.1450; Found 405.1451 and 427.1273 $[M + Na]^+$.

2-(9*H*-Carbazol-3-yl)-*N*-(3-methoxyphenyl)-2-oxoacetamide (15n)



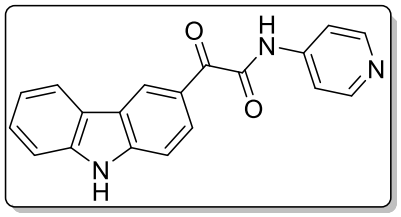
Yield 72%; Mp 171-173 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 1H), 9.10 (s, 1H), 8.55 (dd, J = 8.7, 1.4 Hz, 1H), 8.43 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.49 (dd, J = 8.5, 3.8 Hz, 3H), 7.35–7.29 (m, 2H), 7.22 (d, J = 8.0 Hz, 1H), 6.77 (dd, J = 7.9, 1.8 Hz, 1H), 6.38–6.21 (m, 1H), 3.87 (s, 3H); IR (KBr, ν, cm^{-1}): 3379, 3317, 3070, 2962, 2831, 1690, 1651, 1597, 1535, 1497, 1450, 1335, 1242, 1203, 1149, 1034, 795, 687; Anal. RP-HPLC t_R = 3.251 min, purity 98.37%; HRMS (ESI$^+$) Calculated for $C_{21}H_{17}N_2O_3$ $[M + H]^+$, 345.1239; Found 345.1237 and 367.1058 $[M + Na]^+$

2-(9*H*-Carbazol-3-yl)-*N*-(4-methoxyphenyl)-2-oxoacetamide (15o)



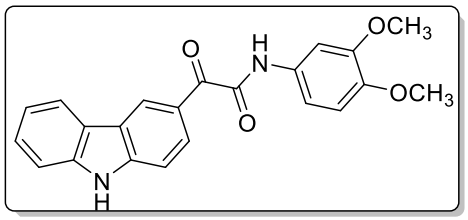
Yield 70%; Mp 199-201 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 9.04 (s, 1H), 8.54 (dd, J = 8.7, 1.7 Hz, 1H), 8.43 (s, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.71–7.67 (m, 1H), 7.67–7.65 (m, 1H), 7.48 (dd, J = 5.8, 2.1 Hz, 3H), 7.34–7.30 (m, 1H), 6.98–6.96 (m, 1H), 6.96–6.93 (m, 1H), 3.84 (s, 3H); IR (KBr, ν, cm^{-1}): 3294, 3256, 3094, 2962, 2831, 1692, 1659, 1597, 1504, 1450, 1412, 1335, 1281, 1234, 1134, 1103, 1011, 802, 795, 679; Anal. RP-HPLC t_R = 3.138 min, purity 99.62%; HRMS (ESI$^+$) Calculated for $C_{21}H_{17}N_2O_3$ $[M + H]^+$, 345.1239; found 345.1238 and 367.1059 $[M + Na]^+$

2-(9*H*-Carbazol-3-yl)-2-oxo-*N*-(pyridin-4-yl)acetamide (15p)



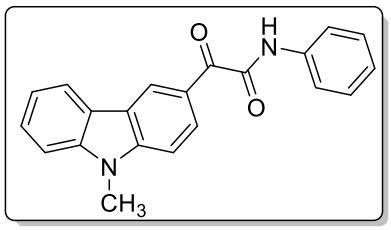
Yield 71%; Mp >300 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H), 11.34 (s, 1H), 8.91 (d, J = 1.4 Hz, 1H), 8.56 (d, J = 6.0 Hz, 2H), 8.31 (d, J = 7.8 Hz, 1H), 8.10 (dd, J = 8.6, 1.7 Hz, 1H), 7.78 (d, J = 6.3 Hz, 2H), 7.66 (d, J = 8.6 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.49 (t, J = 8.2 Hz, 1H), 7.27 (t, J = 7.9 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 188.6, 165.8, 151.1, 145.2, 144.2, 141.0, 128.1, 127.3, 124.7, 123.6, 123.0, 123.0, 121.5, 120.6, 114.6, 112.2, 112.0; IR (KBr, v, cm^{-1}): 3286, 3217, 3094, 2962, 2800, 1705, 1659, 1605, 1566, 1489, 1420, 1342, 1311, 1250, 1196, 1157, 1126, 1011, 802, 787, 748, 679; Anal. RP-HPLC t_R = 3.133 min, purity 98.12%; HRMS (ESI$^+$) Calculated for $C_{19}H_{14}N_3O_2$ [M + H]$^+$, 316.1086; found 316.1085.

2-(9*H*-Carbazol-3-yl)-*N*-(3,4-dimethoxyphenyl)-2-oxoacetamide (15q)



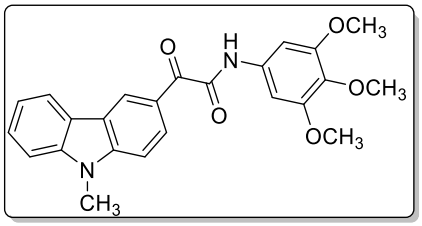
Yield 71%; Mp 202-203 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 9.07 (s, 1H), 8.55 (dd, J = 8.7, 1.7 Hz, 1H), 8.43 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.56 (d, J = 2.4 Hz, 1H), 7.51–7.45 (m, 3H), 7.35-7.31 (m, 1H), 7.16 (dd, J = 8.6, 2.4 Hz, 1H), 6.90 (d, J = 8.6 Hz, 1H), 3.96 (s, 3H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.0, 159.7, 149.2, 146.5, 143.4, 139.9, 130.6, 129.6, 126.9, 126.1, 125.1, 123.6, 123.3, 120.9, 120.9, 112.1, 111.4, 111.1, 110.5, 104.4, 56.1, 56.0; IR (KBr, v, cm^{-1}): 3472, 3286, 3086, 2924, 2839, 1692, 1659, 1589, 1450, 1335, 1219, 1134, 1018, 849, 795, 733, 671, 617; Anal. RP-HPLC t_R = 2.996 min, purity 98.66%; HRMS (ESI$^+$) Calculated for $C_{22}H_{19}N_2O_4$ [M + H]$^+$, 375.1344; Found 375.1348 and 397.1164 [M + Na]$^+$.

2-(9-Methyl-9*H*-carbazol-3-yl)-2-oxo-*N*-phenylacetamide (15r)



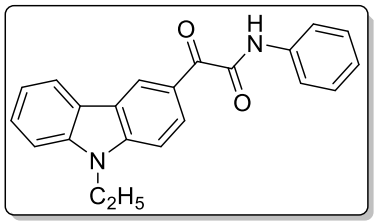
Yield 78%; Mp 165-166 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (d, J = 1.4 Hz, 1H), 9.18 (s, 1H), 8.60 (dd, J = 8.8, 1.7 Hz, 1H), 8.19 (d, J = 7.7 Hz, 1H), 7.79 (d, J = 7.6 Hz, 2H), 7.56 (t, J = 8.2 Hz, 1H), 7.47–7.41 (m, 4H), 7.35 (t, J = 7.9 Hz, 1H), 7.23 (t, J = 7.4 Hz, 1H), 3.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.8, 160.2, 144.6, 141.7, 136.9, 129.5, 129.2, 126.7, 126.0, 125.1, 124.4, 123.3, 122.7, 120.8, 120.6, 120.0, 109.1, 108.4, 29.4; IR (KBr, v, cm^{-1}): 3333, 2924, 2854, 1682, 1643, 1610, 1528, 1443, 1366, 1304, 1257, 1149, 1026, 895; Anal. RP-HPLC t_R = 3.461 min, purity 98.76%; MS (ESI$^+$) Calculated for $C_{21}H_{17}N_2O_2$ [M + H]$^+$, 329.12; Found 329.12.

2-(9-Methyl-9*H*-carbazol-3-yl)-2-oxo-*N*-(3,4,5-trimethoxyphenyl)acetamide (15s)



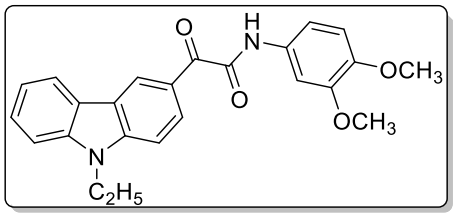
Yield 80%; Mp 187-188 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (d, J = 1.6 Hz, 1H), 9.17 (s, 1H), 8.57 (dd, J = 8.8, 1.6 Hz, 1H), 8.18 (d, J = 7.7 Hz, 1H), 7.54 (t, J = 8.2 Hz, 1H), 7.43–7.38(m, 2H), 7.34 (t, J = 7.2 Hz, 1H), 7.08 (s, 2H), 3.92 (s, 6H), 3.88 (s, 3H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.7, 160.1, 153.4, 144.5, 141.6, 135.2, 133.1, 129.4, 126.7, 125.9, 124.3, 123.2, 122.7, 120.8, 120.5, 109.1, 108.4, 97.5, 61.0, 56.2, 29.3; IR (KBr, v, cm^{-1}): 3310, 2924, 2854, 1683, 1659, 1607, 1589, 1504,1450, 1373, 1327, 1227, 1126, 895; Anal. RP-HPLC t_R = 2.837 min, purity 99.34%; MS (ESI^+) Calculated for $C_{24}H_{23}N_2O_5$ $[M + H]^+$, 419.16; Found 419.18.

2-(9-Ethyl-9*H*-carbazol-3-yl)-2-oxo-*N*-phenylacetamide (15t)



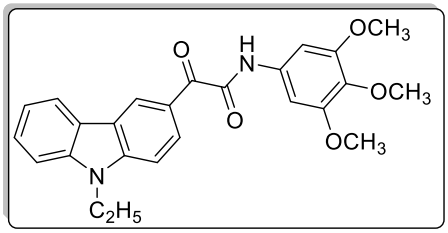
Yield 82%; Mp 159-160 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.44 (d, J = 1.3 Hz, 1H), 9.18 (s, 1H), 8.62 (dd, J = 8.8, 1.7 Hz, 1H), 8.22 (d, J = 7.7 Hz, 1H), 7.79 (d, J = 7.6 Hz, 2H), 7.59–7.53 (m, 1H), 7.49–7.43 (m, 4H), 7.35 (t, J = 7.4 Hz, 1H), 7.26–7.21 (m, 1H), 4.42 (q, J = 7.2 Hz, 2H), 1.50 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.8, 160.2, 143.7, 140.7, 136.9, 129.5, 129.2, 126.6, 126.2, 125.1, 124.3, 123.5, 122.9, 121.0, 120.5, 119.9, 109.1, 108.4, 37.9, 13.8; IR (KBr, v, cm^{-1}): 3310, 2924, 2854, 1690, 1643, 1589, 1535, 1443, 1381, 1335, 1257, 1149, 756; Anal. RP-HPLC t_R = 2.870 min, purity 98.74%; MS (ESI^+) Calcd for $C_{22}H_{19}N_2O_2$ $[M + H]^+$, 343.14; found 343.19.

***N*-(3,4-Dimethoxyphenyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-2-oxoacetamide (15u)**



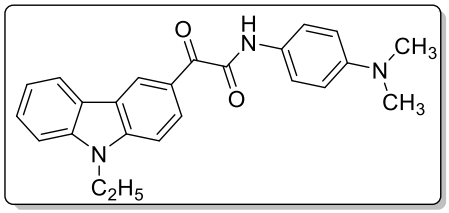
Yield 85%; Mp 110-111 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (d, J = 1.4 Hz, 1H), 9.15 (s, 1H), 8.60 (dd, J = 8.8, 1.6 Hz, 1H), 8.20 (d, J = 7.7 Hz, 1H), 7.58 (d, J = 2.4 Hz, 1H), 7.54 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 8.6 Hz, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.18 (dd, J = 8.6, 2.4 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 4.39 (q, J = 7.2 Hz, 2H), 3.98 (s, 3H), 3.92 (s, 3H), 1.47 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.9, 160.0, 149.2, 146.4, 143.6, 140.6, 130.6, 129.4, 126.6, 126.1, 124.4, 123.5, 122.9, 121.0, 120.4, 112.0, 111.3, 109.1, 108.3, 104.4, 56.0, 37.9, 13.8; IR (KBr, v, cm^{-1}): 3338, 2924, 2854, 1724, 1659, 1589, 1443, 1381, 1327, 1250, 1142, 1026, 768; Anal. RP-HPLC t_R = 2.849 min, purity 99.51%; MS (ESI^+) Calcd for $C_{24}H_{23}N_2O_4$ $[M + H]^+$, 403.16; found 403.21.

2-(9-Ethyl-9*H*-carbazol-3-yl)-2-oxo-*N*-(3,4,5-trimethoxyphenyl)acetamide (15v)



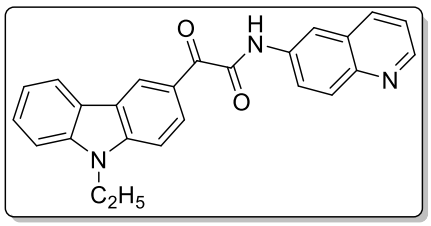
Yield 86%; Mp 175-176 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (d, J = 1.4 Hz, 1H), 9.18 (s, 1H), 8.59 (dd, J = 8.8, 1.6 Hz, 1H), 8.20 (d, J = 7.7 Hz, 1H), 7.54 (t, J = 7.7 Hz, 1H), 7.47–7.41 (m, 2H), 7.34 (t, J = 7.7 Hz, 1H), 7.10 (s, 2H), 4.38 (q, J = 7.2 Hz, 2H), 3.93 (s, 6H), 3.88 (s, 3H), 1.47 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.7, 160.1, 153.5, 143.6, 140.6, 135.2, 133.1, 129.4, 126.6, 126.0, 124.2, 123.4, 122.9, 121.0, 120.5, 109.1, 108.3, 97.6, 61.0, 56.2, 37.9, 13.8; IR (KBr, v, cm^{-1}): 3279, 2932, 2854, 1736, 1659, 1607, 1589, 1504,1450, 1389, 1342, 1234, 1126,1049, 995; Anal. RP-HPLC t_R = 2.866 min, purity 98.57%; MS (ESI$^+$) Calcd for $C_{25}H_{25}N_2O_5$ $[M + H]^+$, 433.17; found 433.18.

***N*-(4-(dimethylamino)phenyl)-2-(9-ethyl-9H-carbazol-3-yl)-2-oxoacetamide (15w)**



Yield 73%; Mp 173-174 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (d, J = 1.3 Hz, 1H), 9.04 (s, 1H), 8.63 (dd, J = 8.8, 1.6 Hz, 1H), 8.21 (d, J = 7.7 Hz, 1H), 7.65 (d, J = 9.0 Hz, 2H), 7.55 (t, J = 7.6 Hz, 1H), 7.49–7.43 (m, 2H), 7.34 (t, J = 7.0 Hz, 1H), 6.80 (d, J = 9.0 Hz, 2H), 4.41 (q, J = 7.2 Hz, 2H), 2.99 (s, 6H), 1.49 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.3, 159.8, 148.3, 143.6, 140.6, 129.5, 126.7, 126.6, 126.1, 124.7, 123.6, 122.8, 121.4, 121.0, 120.4, 112.9, 109.1, 108.3, 40.8, 37.9, 13.8; IR (KBr, v, cm^{-1}): 3290, 2924, 2854, 1723, 1666, 1582,1448, 1350, 1234, 1126, 1065, 895; Anal. RP-HPLC t_R = 2.862 min, purity 99.71%; MS (ESI$^+$) Calcd for $C_{24}H_{24}N_3O_2$ $[M + H]^+$, 386.18; found 386.24.

2-(9-Ethyl-9*H*-carbazol-3-yl)-2-oxo-*N*-(quinolin-6-yl)acetamide (15x)



Yield 78%; Mp 191-192 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.49 (s, 1H), 9.42 (d, J = 1.3 Hz, 1H), 8.89 (dd, J = 4.2, 1.7 Hz, 1H), 8.64–8.56 (m, 2H), 8.19 (t, J = 7.1 Hz, 2H), 8.14 (d, J = 8.0 Hz, 1H), 7.80 (dd, J = 9.0, 2.4 Hz, 1H), 7.57–7.52 (m, 1H), 7.47–7.40 (m, 3H), 7.34 (t, J = 7.5 Hz, 1H), 4.37 (q, J = 7.2 Hz, 2H), 1.47 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.3, 160.5, 149.8, 145.8, 143.7, 140.6, 136.0, 134.9, 130.5, 129.5, 128.8, 126.7, 126.2, 124.2, 123.4, 123.2, 122.9, 121.8, 121.0, 120.6, 116.5, 109.2, 108.4, 37.9, 13.8; IR (KBr, v, cm^{-1}): 3348, 2924, 2854, 1690, 1651, 1582,1466, 1327, 1234, 1126, 1065, 887; Anal. RP-HPLC t_R = 2.852 min, purity 98.11%; MS (ESI$^+$) Calculated for $C_{25}H_{20}N_3O_2$ $[M + H]^+$, 394.15; found 394.16.

Cytotoxicity assay

Cell culture. U937 (Histiocytic lymphoma), and breast (MCF-7 and MDA-MB-231) cancer cell lines were cultured in DMEM supplemented with 10% fetal bovine serum and 50 µg/mL of streptomycin and penicillin. Jurkat (Human T lymphocyte) cell was cultured in RPMI supplemented with 10% fetal bovine serum and 50 µg/mL of streptomycin and penicillin. All the cell lines were maintained in 5% CO_2 environment at 37 °C.

Cell viability assay. Cells were seeded in a 96-well plate at a density of 100,000/mL and grown for overnight. Cells were treated with various carbazolyl glyoxamides at a final concentration of 10 µM and incubated for 48 h. Cell viability assay was performed using an MTT cell proliferation kit from ATCC. In summary, 10 µL MTT reagent was added to each well, and cells were placed back in the incubator for 4 h. 100 µL of detergent (from a kit) was added and absorbance data were collected at 570 nm using Biotek synergy 2 spectrophotometers. Percentage of cell survival data was calculated using the following formula;

% cell survival = (100/At*As)

Where At and As are the absorbances of wells treated with test compounds and solvent control respectively.

Caspase assay. 1,00,000 cells were plated in a 24 well plate and treated with 1 µM compounds **15i-m** and **15q** for 24 h later, sample (100 µL) was taken and analyzed as per kit (Promega G7790). Fluorescence for the samples was measured at 0 min and 180 min. Camptothecin was used as a positive control for inducing apoptotic cell death.

LDH assay

50,000 Cells per 100 µL were plated per well in a 96-well format and were treated with 20 micro molar doxorubicin and test compound. After 40 h, samples were processed and analyzed for LDH activity using LDH cytotoxicity kit from Cayman chemicals (cat no 601170).

5.6 References

1. Chen, J.; Ahn, S.; Wang, J.; Lu, Y.; Dalton, J. T.; Miller, D. D.; Li, W., Discovery of novel 2-aryl-4-benzoyl-imidazole (ABI-III) analogues targeting tubulin polymerization as antiproliferative agents. *J. Med. Chem.,* **2012,** *55*, 7285-7289.
2. Patil, S. A.; Patil, R.; Miller, D. D., Indole molecules as inhibitors of tubulin polymerization: potential anticancer agents. *Future Med. Chem.,* **2012,** *4*, 2085-2115.
3. Roy, J.; Jana, A. K.; Mal, D., Recent trends in the synthesis of carbazoles: an update. *Tetrahedron,* **2012,** *68*, 6099-6121.
4. S Tsutsumi, L.; Gündisch, D.; Sun, D., Carbazole scaffold in medicinal chemistry and natural products: a review from 2010-2015. *Curr. Top. Med. Chem.,* **2016,** *16*, 1290-1313.
5. Nandy, B. C.; Gupta, A.; Mittal, A.; Vyas, V., Carbazole: It's biological activity. *J. Biomed. Pharm. Res.,* **2014,** *3*, 42-48.
6. Schmidt, A. W.; Reddy, K. R.; Knölker, H.-J., Occurrence, biogenesis, and synthesis of biologically active carbazole alkaloids. *Chem. Rev.,* **2012,** *112*, 3193-3328.
7. Kamal, A.; Shetti, R. V.; Ramaiah, M. J.; Swapna, P.; Reddy, K. S.; Mallareddy, A.; Rao, M. N.; Chourasia, M.; Sastry, G. N.; Juvekar, A., Carbazole–pyrrolo [2, 1-*c*][1, 4] benzodiazepine conjugates: design, synthesis, and biological evaluation. *MedChemComm,* **2011,** *2*, 780-788.
8. Bandgar, B. P.; Adsul, L. K.; Chavan, H. V.; Jalde, S. S.; Shringare, S. N.; Shaikh, R.; Meshram, R. J.; Gacche, R. N.; Masand, V., Synthesis, biological evaluation, and docking studies of 3-(substituted)-aryl-5-(9-methyl-3-carbazole)-1*H*-2-pyrazolines as potent anti-inflammatory and antioxidant agents. *Bioorganic Med. Chem. Lett.,* **2012,** *22*, 5839-5844.
9. Zhang, F.-F.; Gan, L.-L.; Zhou, C.-H., Synthesis, antibacterial and antifungal activities of some carbazole derivatives. *Bioorganic Med. Chem. Lett.,* **2010,** *20*, 1881-1884.
10. Caruso, A.; Iacopetta, D.; Puoci, F.; Rita Cappello, A.; Saturnino, C.; Stefania Sinicropi, M., Carbazole derivatives: A promising scenario for breast cancer treatment. *Mini-Rev. Med. Chem.,* **2016,** *16*, 630-643.
11. S Shaikh, M.; Karpoormath, R.; Thapliyal, N.; A Rane, R.; B Palkar, M.; Faya, A.; M Patel, H.; S Alwan, W.; Jain, K.; A Hampannavar, G., Current perspective of natural alkaloid carbazole and its derivatives as antitumor agents. *Anticancer Agents Med. Chem.,* **2015,** *15*, 1049-1065.

12. Asche, C.; Demeunynck, M., Antitumor carbazoles. *Anticancer Agents Med. Chem.,* **2007,** *7*, 247-267.
13. Ohira, M.; Iwasaki, Y.; Tanaka, C.; Kuroki, M.; Matsuo, N.; Kitamura, T.; Yukuhiro, M.; Morimoto, H.; Pang, N.; Liu, B.; Kiyono, T.; Amemiya, M.; Tanaka, K.; Yoshida, K.; Sugimoto, N.; Ohshima, T.; Fujita, M., A novel anti-microtubule agent with carbazole and benzohydrazide structures suppresses tumor cell growth *in vivo*. *Biochim. Biophys. ,* **2015,** *1850*, 1676-1684.
14. Miller, C. M.; McCarthy, F. O., Isolation, biological activity and synthesis of the natural product ellipticine and related pyridocarbazoles. *RSC Adv.,* **2012,** *2*, 8883-8918.
15. Vassal, G.; Merlin, J.-L.; Terrier-Lacombe, M.-J.; Grill, J.; Parker, F.; Sainte-Rose, C.; Aubert, G.; Morizet, J.; Sévenet, N.; Poullain, M.-G.; Lucas, C.; Kalifa, C., *In-vivo* antitumor activity of S16020, a topoisomerase II inhibitor, and doxorubicin against human brain tumor xenografts. *Cancer Chemother. Pharmacol.,* **2003,** *51*, 385-394.
16. Léonce, S.; Perez, V.; Casabianca-Pignede, M.-R.; Anstett, M.; Bisagni, E.; Pierré, A.; Atassi, G., *In vitro* cytotoxicity of S16020-2, a new olivacine derivative. *Invest. New Drugs,* **1996,** *14*, 169-180.
17. Guilbaud, N.; Kraus-Berthier, L.; Saint-Dizier, D.; Rouillon, M.; Jan, M.; Burbridge, M.; Pierré, A.; Atassi, G., Antitumor activity of S 16020-2 in two orthotopic models of lung cancer. *Anti-cancer drugs,* **1997,** *8*, 276-282.
18. Kraus-Berthier, L.; Guilbaud, N.; Jan, M.; Saint-Dizier, D.; Rouillon, M.; Burbridge, M.; Pierre, A.; Atassi, G., Experimental antitumour activity of S 16020-2 in a panel of human tumours. *Eur. J. Cancer,* **1997,** *33*, 1881-1887.
19. Conchon, E.; Anizon, F.; Aboab, B.; Golsteyn, R. M.; Léonce, S.; Pfeiffer, B.; Prudhomme, M., Synthesis, *in vitro* antiproliferative activities, and Chk1 inhibitory properties of pyrrolo[3,4-*a*]-carbazole-1,3-diones, pyrrolo[3,4-*c*]carbazole-1,3-diones, and 2-aminopyridazino[3,4-*a*]pyrrolo[3,4-*c*]carbazole-1,3,4,7-tetraone. *Eur. J. Med. Chem.,* **2008,** *43*, 282-292.
20. Caulfield, T.; Cherrier, M.; Combeau, C.; Mailliet, P. European Patent 1253141, 2002.
21. Liu, C.-H.; Lin, C.; Tsai, K.-J.; Chuang, Y.-C.; Huang, Y.-L.; Lee, T.-H.; Huang, L.-J.; Chan, H.-C., Biological evaluation of 9-[(6-chloropyridin-4-yl) methyl]-9*H*-carbazole-3-carbinol as an anticancer agent. *Oncol. Rep.,* **2013,** *29*, 1501-1509.
22. Nakamura, S.; Yamasaki, K.; Kaneda, M.; Watanabe, K.; Ueki, Y.; Ishimaru, K.; Nomi, R.; Yoshida, N.; Nakajima, T., New antibiotics, carbazomycins A and B. III. Taxonomy and biosynthesis. *J. Antibiot.,* **1983,** *36*, 552-558.

23. Knölker, H.-J., Occurrence, biological activity, and convergent organometallic synthesis of carbazole alkaloids. *Top. Curr. Chem.,* **2005,** *244*, 115-148.
24. Głuszyńska, A., Biological potential of carbazole derivatives. *Eur. J. Med. Chem.,* **2015,** *94*, 405-426.
25. Maneerat, W.; Ritthiwigrom, T.; Cheenpracha, S.; Promgool, T.; Yossathera, K.; Deachathai, S.; Phakhodee, W.; Laphookhieo, S., Bioactive carbazole alkaloids from Clausena wallichii roots. *J. Nat. Prod.,* **2012,** *75*, 741-746.
26. Maneerat, W.; Phakhodee, W.; Ritthiwigrom, T.; Cheenpracha, S.; Promgool, T.; Yossathera, K.; Deachathai, S.; Laphookhieo, S., Antibacterial carbazole alkaloids from *Clausena harmandiana* twigs. *Fitoterapia,* **2012,** *83*, 1110-1114.
27. Jiang, H. Y.; Wang, C. F.; Fan, L.; Yang, K.; Feng, J. B.; Geng, Z. F.; Xu, J.; Deng, Z. W.; Du, S. S.; Yin, H. B., Cytotoxic constituents from the stems of *Clausena lansium* (Lour.) Skeels. *Molecules,* **2013,** *18*, 10768-10775.
28. Francisco, C. S.; Rodrigues, L. R.; Cerqueira, N. M.; Oliveira-Campos, A. M.; Esteves, A. P., Novel benzopsoralen analogues: Synthesis, biological activity and molecular docking studies. *Eur. J. Med. Chem.,* **2014,** *87*, 298-305.
29. Hu, L.; Li, Z.-r.; Li, Y.; Qu, J.; Ling, Y.-H.; Jiang, J.-d.; Boykin, D. W., Synthesis and structure−activity relationships of carbazole sulfonamides as a novel class of antimitotic agents against solid tumors. *J. Med. Chem.,* **2006,** *49*, 6273-6282.
30. Auclair, C., Multimodal action of antitumor agents on DNA: The ellipticine series. *Arch. Biochem. Biophys.,* **1987,** *259*, 1-14.
31. Tao, Y.; Yang, C.; Qin, J., Organic host materials for phosphorescent organic light-emitting diodes. *Chem. Soc. Rev.,* **2011,** *40*, 2943-2970.
32. Álvarez, S.; Álvarez, R.; Khanwalkar, H.; Germain, P.; Lemaire, G.; Rodríguez-Barrios, F.; Gronemeyer, H.; de Lera, Á. R., Retinoid receptor subtype-selective modulators through synthetic modifications of RARγ agonists. *Bioorganic Med. Chem.,* **2009,** *17*, 4345-4359.
33. Gupta, L.; Talwar, A.; Nishi; Palne, S.; Gupta, S.; Chauhan, P. M. S., Synthesis of marine alkaloid: 8,9-Dihydrocoscinamide B and its analogues as novel class of antileishmanial agents. *Bioorganic Med. Chem. Lett.,* **2007,** *17*, 4075-4079.
34. Wienecke, A.; Bacher, G., Indibulin, a novel microtubule inhibitor, discriminates between mature neuronal and nonneuronal tubulin. *Cancer Res.,* **2009,** *69*, 171-177.

35. Ma, Y.; Yakushijin, K.; Miyake, F.; Horne, D., A concise synthesis of indolic enamides: coscinamide A, coscinamide B, and igzamide. *Tetrahedron Lett.,* **2009,** *50,* 4343-4345.

36. Bokesch, H. R.; Pannell, L. K.; McKee, T. C.; Boyd, M. R., Coscinamides A, B and C, three new bis indole alkaloids from the marine sponge Coscinoderma sp. *Tetrahedron Lett.,* **2000,** *41,* 6305-6308.

37. Singh, P.; Verma, P.; Yadav, B.; Komath, S. S., Synthesis and evaluation of indole-based new scaffolds for antimicrobial activities-identification of promising candidates. *Bioorganic Med. Chem. Lett.,* **2011,** *21,* 3367-3372.

38. Kumar, D.; Maruthi Kumar, N.; Tantak, M. P.; Ogura, M.; Kusaka, E.; Ito, T., Synthesis and identification of *α*-cyanobis(indolyl)chalcones as novel anticancer agents. *Bioorganic Med. Chem. Lett.,* **2014,** *24,* 5170-5174.

39. Tantak, M. P.; Kumar, A.; Noel, B.; Shah, K.; Kumar, D., Synthesis and biological evaluation of 2-arylamino-5-(3′-indolyl)-1,3,4-oxadiazoles as potent cytotoxic agents. *ChemMedChem,* **2013,** *8,* 1468-1474.

40. Kumar, D.; Maruthi Kumar, N.; Chang, K.-H.; Shah, K., Synthesis and anticancer activity of 5-(3-indolyl)-1,3,4-thiadiazoles. *Eur. J. Med. Chem.,* **2010,** *45,* 4664-4668.

41. Kumar, D.; Maruthi Kumar, N.; Noel, B.; Shah, K., A series of 2-arylamino-5-(indolyl)-1,3,4-thiadiazoles as potent cytotoxic agents. *Eur. J. Med. Chem.,* **2012,** *55,* 432-438.

42. Kumar, D.; Maruthi Kumar, N.; Chang, K. H.; Shah, K., Synthesis of novel indolyl-1,2,4-triazoles as potent and selective anticancer agents. *Chem. Biol. Drug Des.,* **2011,** *77,* 182-188.

43. Tantak, M. P.; Wang, J.; Singh, R. P.; Kumar, A.; Shah, K.; Kumar, D., 2-(3′-Indolyl)-*N*-arylthiazole-4-carboxamides: Synthesis and evaluation of antibacterial and anticancer activities. *Bioorganic Med. Chem. Lett.,* **2015,** *25,* 4225-4231.

44. Thornberry, N. A., The caspase family of cysteine proteases. *Br. Med. Bull,* **1997,** *53,* 478-490.

Chapter 6

Conclusions

6.1 General conclusions

A significant number of natural as well as synthetic indoles are currently in preclinical or clinical trials and many of these are anticancer agents. Among the indoles, carbazole and *β*-carboline have become leading pharmacophores which continues to receive increasing attention from medicinal chemists due to unique biological properties of its derived compounds, especially, anticancer activity. Chemotherapy is a very important and popular treatment among the available cancer therapies. Various types of chemotherapeutic medications such as antimetabolites (methotrexate, gemcitabine, cytarabine etc.), alkylating agents (chlorambucil, cisplatin, carmustine, procarbazine etc.), cytotoxic agents (bleomycin, mitomycin etc.), anti-microtubule agents (paclitaxel, vinblastine etc.), topoisomerase inhibitors (doxorubicin, etoposide etc.) have severe adverse effects like hair loss, nausea and vomiting, anemia etc. Therefore, the development of novel and effective anticancer agents with an improved pharmacological profile remains a major challenge.

The primary goal of this thesis is design, synthesis and investigation of indole related heterocycles like carbazoles and *β*-carbolines as potent cytotoxic agents against a panel of cancer cells. The research work in thesis deals with the design, synthesis and *in vitro* cytotoxicity studies of *β*-carboline chalcones, *β*-carbolinium bromides, carbazolyl glyoxamides and porphyrin-*β*-carboline conjugates. While synthesizing indole related heterocycles (carbazoles and *β*-carbolines), we developed mild, efficient, metal-free and high yielding synthetic protocols leading to *β*-carbolines, porphyrin-*β*-carboline conjugates and carbazolylglyoxamides. Using readily available indoles and tryptamine, we disclosed an efficient strategy to prepare pityriacitrin, alangiobussinine and related analogues. *In vitro* screening of the prepared library of diverse carbazole and *β*-carboline analogues enabled us to identify carbazolylglyoxamides, *β*-carboline chacones and *β*-carbolinium bromides as potent apoptosis inducing cytotoxic agents. Cationic porphyrin-*β*-carboline conjugates displayed remarkable photocytotoxicity (IC_{50} = 39-152 nM) against lung as well as colon cancer cell lines and triggered cell death in A549 cells.

6.2 Specific conclusions

The thesis deals with design, synthesis and *in vitro* anticancer activity studies of selected indole related heterocycles (carbazole and *β*-carboline derivatives). The research work undertaken in this thesis has been divided into six chapters.

The **first chapter** represented a brief overview about synthesis and antitumor properties of indole related heterocycles like carbazoles and *β*-carbolines. This chapter further elaborates about the rational design of carbazole and *β*-carboline containing chemical entities as potent anticancer agents. The chapter also discloses the scope for developing novel carbazole and *β*-carboline compounds by structural modifications of existing carbazole and *β*-carboline lead anticancer drug candidates.

The **second chapter** deals with the design, synthesis and cytotoxicity studies of two different series of novel *β*-carbolinium bromides. **Part A** of this chapter reports, synthesis of 2-phenacyl-*β*-carbolinium bromides **13a-t** involving MW-assisted rapid reaction of *β*-carboline with various 1-aryl-2-bromoethanones in ethanol as a solvent. Among the synthesized *β*-carbolinium bromide derivatives, 9-(4-chlorobenzyl)-2-(4-methoxyphenacyl)-*β*-carbolinium bromide **13l** displayed broad spectrum of anticancer activity against all the tested cancer cell lines (IC_{50} = 3.16-7.93 µM) and triggered apoptosis in C4-2 cells. In **part B** of this chapter various *β*-carboline-1-chalcones **23a-g** and their bromide salts **24a-i** were synthesized from the initial reaction of tryptamine with pyruvaldehyde under Pictet–Spengler reaction conditions and *in-situ* aromatization followed by *N*-protection with di-*tert*-butyl dicarbonate to afford *N*-boc-1-acetyl-*β*-carboline **27**, which was further condensed with appropriate arylaldehydes to give *β*-carboline-1-chalcones **23a-g.** Finally, the N^2-alkylation of three *β*-carboline chalcones **23c-e** with various alkylbromides led to the corresponding bromide salts **24a-i** in excellent yields. *In-vitro* cytotoxicity study of *β*-carboline-1-chalcones **23a-g** and their bromide salts **24a-i** resulted in compound **24g** with IC_{50} values lower than 22.5 µM against all the tested cancer cell lines. Further, preliminary mechanism of action studies indicated that *β*-carbolinium chalcone bromide **24g** induced apoptosis in MDA-MB-231cells.

The **third chapter** of thesis illustrates the development of an efficient, facile, high yielding molecular iodine and triphenylphosphine combination promoted protocol enabling to achieve a library of bioactive *β*-carbolines **14a-j** namely, Pityriacitrin **14a** and Hyrtiosulawesine **16** by employing cyclization of easily accessible bis(indolyl)ketoamides. This simple protocol offers advantages including shorter reaction times, milder reaction conditions, wider substrate scope and high product yield. Further, this methodology was utilized to prepare biologically interesting *β*-carboline Alangiobussinine **17** by the treatment of *in-situ* generated glyoxamide **30** with molecular iodine and triphenylphosphine.

Chapter four highlights the synthesis and photocytotoxicity studies of water-soluble cationic porphyrin-*β*-carboline conjugates **17a-c**. The synthesis of conjugates involve a facile and highly efficient MW-assisted coupling reaction of *β*-carboline acids **20a-c** with 5-(4-amino-phenyl)tripyridylporphyrin **31** followed by rapid and high yielding *N*-methylation of neutral porphyrin conjugates **32a-c** under MW irradiation. Prepared cationic porphyrin-*β*-carboline conjugates, **17a-c** displayed remarkable photocytotoxicity against lung (A549, IC_{50} = 39-152 nM) as well as colon (Colon-26, IC_{50} = 47-173 nM) cancer cell lines. Also, the conjugate **17b** (IC_{50} = 47 nM) was found to be 3.5 folds more potent than standard **H_2TMPyP 7** (IC_{50} = 164 nM) against Colon-26 cancer cell line.

Chapter five discloses the anticancer as well as antibacterial potential of carbazolyl glyoxamides. A library of twenty-four glyoxamides **15a–x** was achieved in good to excellent yields from easily accessible carbazolyl glyoxalic acids **19** and aryl/heteroarylamines by employing HATU as a coupling reagent. Prepared glyoxamides were assessed for their antitumor activity, which enabled us to identify **15l** (IC_{50} = 9.3 µM) and **15q** (IC_{50} = 9.8 µM) as the most potent compounds against MCF-7 cell lines. In a preliminary mechanistic study, carbazoles **15l** and **15q** induced caspase-dependent apoptotic cell death in Jurkat cells. In addition, the antibacterial activity studies of carbazolylglyoxamides resulted in compounds **15g, 15k-l** and **15n** endowed with prominent potency against the tested bacterial stains (MIC = 8-16 µg/mL and ZOI = 16-20 mm); comparable to the reference drug, chloramphenicol. Further, cell viability assay indicated that carbazoles **15g** and **15l** were capable to inhibit the growth of bacteria in few hours of initial interactions.

6.3 Future scope of the research work

The aim of thesis is to achieve diverse structural class of carbazoles and *β*-carbolines as novel anticancer agents. Anticancer activity potential of identified *β*-carboline and carbazole derivatives can be enhanced by extensive SAR study. Structural modifications of most potent compounds identified in different series is likely to produce drug-like candidates. Molecular targets and *in vivo* screening studies of the most potent compound in each series may also be investigated.

The relatively benign and high yielding microwave-assisted synthetic protocols developed for the preparation of carbazole and *β*-carboline derivatives and quaternization of azaheterocycle linked porphyrins have greater potential to produce a library of interesting bioactive scaffolds.

Appendices

List of publications [A-1]

1. **P. O. Venkataramana Reddy**, Mukund P. Tantak, Reyna Valdez, Rajnish Prakash Singh, Okram Mukherjee Singh, Rachna Sadana, and Dalip Kumar. "Synthesis and biological evaluation of novel carbazolyl glyoxamides as anticancer and antibacterial agents." ***RSC Adv.,* 2016, 12, 9379-9386.**
2. V. Arun, **P. O. Venkataramana Reddy**, Meenakshi Pilania, and Dalip Kumar. "Ligand-and base-free access to diverse biaryls by the reductive coupling of diaryliodonium salts." ***Eur. J. Org. Chem.,* 2016, 12, 2096-2100.**
3. Sridhar S. N. C, Ginson George, **P. O. Venkataramana Reddy**, Mukund P. Tantak, Dalip Kumar and Atish T. Paul. "Synthesis, evaluation and molecular modelling studies of 2-(carbazol-3-yl)-2-oxoacetamide analogues as a new class of potential pancreatic lipase inhibitors." ***Bioorganic Med. Chem.,* 2017, 25, 609–620.**
4. **P. O. Venkataramana Reddy**, Shriprada Mishra, Mukund P. Tantak, Kumar Nikhil, Rachna Sadana, Kavita Shah and Dalip Kumar. "Design, synthesis and *in-vitro* cytotoxicity studies of novel *β*-carbolinium bromides." ***Bioorganic Med. Chem. Lett.,* 2017, 27, 1379–1384.**
5. **P. O. Venkataramana Reddy**, Hridhay M, Kumar Nikhil, Shahid Khan, P N Jha, Kavita Shah and Dalip Kumar. "Synthesis and investigations into the anticancer and antibacterial activity studies of *β*-carboline chalcones and their bromide salts". ***Bioorganic Med. Chem. Lett.*, 2018, 28, 1278–1282.**
6. **P. O. Venkataramana Reddy,** Babita Malik, and Dalip Kumar. "Sequential one-pot synthesis of *β*-carboline analogs: An efficient strategy to prepare pityriacitrin and alangiobussinine natural products." ***Indian J. Heterocycl. Chem., 2018*, 28, 133-142.**
7. M. V. S. K. Chaitanya, **P. O. Venkataramana Reddy**, Nikhil Kumar, Anil Kumar, Kavita Shah, and Dalip Kumar. "Synthesis and anticancer activity studies of indolylisoxazoline analogues." ***Bioorganic Med. Chem. Lett.*, 2018, 28, 2842-2845**
8. **P. O. Venkataramana Reddy**, K. P. Chandra Shekar, Santosh B. Khandagale, Takeo Ito, Aoi Son, Daiki Hara, Kazuhito Tanabe, and Dalip Kumar. "Easy access to water-soluble cationic porphyrin-*β*-carboline conjugates as potent photocytotoxic and DNA cleaving agents" ***Asian J. Org. Chem.* 2019, 8, 269-274**

List of conference presentations [A-2]

1. **P. O. Venkataramana Reddy**, Mukund P. Tantak, and Dalip Kumar, "Design and Synthesis of 2-Arylamino-5-(indolyl)-1,3,4-oxadiazoles as Anticancer Agents" at National Conference on Recent Developments in Chemical Sciences, Guru Jambheshwar University of Science & Technology, Hisar, India, 25-26 February 2014 (Received best poster award).
2. **P. O. Venkataramana Reddy**, Mukund P. Tantak, and Dalip Kumar, "Synthesis and *in-vitro* Anticancer Activity of α-Cyano Bis(Indolyl)chalcones" at 21th ISCB International Conference on Current trends in drug discovery and developments, CDRI, Lucknow, India, 25-28 February 2015.
3. **P. O. Venkataramana Reddy**, Mukund P. Tantak, Rachana Sadana, and Dalip Kumar "Synthesis and Biological Evaluation of Novel Carbazolyl Glyoxamides as Anticancer and Antibacterial Agents" at International Conference on Nascent Developments in Chemical Sciences: Opportunities for Academia-Industry Collaboration, Department of Chemistry, BITS Pilani, Pilani Campus, Rajasthan, India, 16-18 October 2015.
4. **P. O. Venkataramana Reddy**, Mukund P. Tantak, Rachana Sadana, and Dalip Kumar "Synthesis and Biological Evaluation of Novel Carbazolyl Glyoxamides as Anticancer and Antibacterial Agents" at 22nd ISCB International Conference on Recent Trends in Affordable and Sustainable Drug Discovery and Developments, Uka Tarsadia University, Surat, India, 6-8 February 2016.
5. **P. O. Venkataramana Reddy,** Shriprada Mishra, Kavita Shah and Dalip Kumar "Synthesis and Identification of β-Carbolinium Salts as Novel Cytotoxic Agents "at National Conference on Organic Chemistry in Sustainable Development: Recent Advances and Future Challenges, Department of Chemistry, BITS Pilani, Pilani Campus, Rajasthan, India, 29-30 August 2016.
6. **P. O. Venkataramana Reddy,** Shriprada Mishra, Kavita Shah and Dalip Kumar "Synthesis and Identification of β-Carbolinium Salts as Novel Cytotoxic Agents" 23rd ISCB International Conference (ISCBC-2017) Interface of Chemical Biology in Drug Research Organized by Indian Society of Chemists and Biologists Venue: SRM University, Chennai, India; 8-10 February 2017

List of conference presentations [A-2]

7. **P. O. Venkataramana Reddy,** Shriprada Mishra, Kavita Shah and Dalip Kumar "Synthesis and Identification of *β*-Carbolinium Salts as Novel Cytotoxic Agents "International Conference on Challenges in Drug Discovery and Delivery" Department of Pharmacy, BITS Pilani, Pilani Campus, Rajasthan, India, 2-4 March 2017.
8. **P. O. Venkataramana Reddy**, K. P. Chandra Shekar, Kazuhito Tanabe and Dalip Kumar "Design and Synthesis of Water Soluble Cationic Porphyrin–β-Carboline Conjugates as Potent Photocytotoxic Agents" International Conference on Frontiers at the Chemistry – Allied Sciences Interface Organized by Centre of Advanced Study, Department of Chemistry, University of Rajasthan, Jaipur (INDIA); 22-23 July 2017
9. **P. O. Venkataramana Reddy** and Dalip Kumar, Molecular Iodine Mediated Efficient Synthesis of Pityriacitrin Analogues *via* Intramolecular Cyclization of Bis(indolyl)ketoamides, 24th ISCB International Conference (ISCBC-2018) Frontier Research in Chemistry & Biology Interface Organized by Indian Society of Chemists and Biologists Venue: Manipal University, Rajasthan, India; 11-13 January 2018

BRIEF BIOGRAPHY OF THE CANDIDATE [A-3]

P.O. Venkataramana Reddy born in Palamaner, Chittoor, Andhra Pradesh, India. He pursued his Bachelor's degree (Chemistry, Physics & Mathematics) from Railway Degree College, Hyderabad. He completed his Master's degree (Chemistry of Natural products) from S.K.U.P.G. Centre, Kurnool, Sri Krishnadevaraya University, Andhra Pradesh, India during 2006 to 2008. In June 2009, he qualified National Eligibility Test (NET) for Lectureship conducted by UGC-CSIR, Govt. of India. In July 2008, he joined Laurus Labs Pvt. Ltd, Hyderabad, India as a Trainee research chemist and continued till December 2012. During this period, he was involved in a variety of projects dealing with the synthesis of diverse drug intermediates and Active Pharma Ingredients (APIs). In January 2013, he joined in Department of Chemistry, BITS Pilani as project fellow in DBT Sponsored Research Project under the direction of Prof. Dalip Kumar. Subsequently, in January 2013, he registered for Ph.D program under the guidance of Prof. Dalip Kumar with the financial assistance from the DBT Sponsored Research Project. During the Ph.D tenure, he has presented (poster) his research work in various national and international conferences and received one time best poster presentation award. He has published research articles in well renowned international journals.

BRIEF BIOGRAPHY OF THE SUPERVISOR [A-4]

Dr. Dalip Kumar is a Professor of Chemistry at Department of Chemistry, Birla Institute of Technology and Science, Pilani. He completed his Ph.D degree from Kurukshetra University, Kurukshetra, Haryana in 1997 under the direction of Prof. Shiv P. Singh in the research area of heterocyclic chemistry. After his doctorate, he moved to Sam Houston State University, TX, USA for his post-doctoral research (1997-1999) with the guidance of Prof. Rajender S. Varma. He was also associated with Prof. Sean M. Kerwin as a post-doctoral fellow (1999-2000), College of Pharmacy, University of Texas at Austin, TX, USA. He joined BITS Pilani, Pilani campus, as a lecturer during 2000-2002. Later, in December 2002, he moved to University of Maryland, College Park, MD, USA as a Research Associate. In 2004, Prof. Kumar re-joined as an Assistant Professor at Department of Chemistry, BITS Pilani, Pilani campus, and since then he is continuing there. He was promoted to professor in year 2012. He has been involved in research for the last 20 years and in teaching for 13 years. As a result of his research accomplishment, he has greater than 120 international publications in peer reviewed journals. Prof. Kumar has guided nine Ph.D students and currently he is supervising six Ph.D. students. He has one US patent, one Indian patent and successfully completed several sponsored research projects from DST, DRDO, UGC, CSIR, DBT and DST-JSPS Indo-Japan project. Currently, he has two Government of India sponsored projects from CSIR and DST and a collaborative industrial project from Ranbaxy Research Laboratory Ltd.

Prof. Kumar is recipient of Honorary Diploma for Scientific Achievements and International Scientific Collaboration by Russian International Charitable Foundation "Scientific Partnership", Moscow, Russia (March 2013). He received the Prof. R. D. Desai 80th Birthday Commemoration Medal and Prize from Indian Chemical Society for year 2015. He is an Associate Editor of Chemistry & Biology Interface Journal published by Indian Society of Chemists and Biologists, Lucknow. Prof. Kumar is life member of Indian Chemical Society, Indian Society of Chemists and Biologists, and Indian Council of Chemists. His current research pursuit is focused on synthesis of indole and porphyrin derived potential anticancer agents by employing transition-metals and organoiodine reagents.