Synthesis, Study of Some Novel β-carboline Derivatives as Potential Agents Against HIV and Associated Infections

THESIS

Submitted in partial fulfillment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY

by

ASHOK P
ID. No. 2011PHXF042P

Under the Supervision of
Dr. S. MURUGESAN

BITS Pilani
Pilani | Dubai | Goa | Hyderabad

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI
2015

DEDICATED TO MY BELOVED FAMILY & ALMIGHTY

Table of Contents

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

CERTIFICATE

This is to certify that the thesis entitled **"Synthesis, Study of Some Novel β-carboline Derivatives as Potential Agents Against HIV and Associated Infections"** submitted by **ASHOK P,** ID No. **2011PHXF042P** for the award of Ph.D of the Institute embodies original work done by him under my supervision.

Signature of the Supervisor:

Name in capital letters: **Dr. S. MURUGESAN**

Designation: **Assistant Professor & Head,**

Department of Pharmacy,

Birla Institute of Technology & Science, Pilani

Pilani Campus

Date:

Acknowledgements

I would like to express my sincere gratitude to my supervisor **Dr. S. Murugesan**, Head, Department of Pharmacy, BITS Pilani, Pilani Campus who driven me to successful career and provided constant support for my pursuit of dissertation. I could not have it without him.

I am grateful to **Prof. B. N. Jain**, Vice-Chancellor, BITS Pilani, **Prof. A. K. Sarkar,** Director, BITS Pilani, Pilani Campus and **Prof. G. Raghurama**, Ex-Director, BITS Pilani, Pilani Campus for permitting me to pursue my research work in the Institute.

I express my earnest thanks to **Prof. R. N. Saha**, Director, BITS Pilani, Dubai Campus, **Prof. R. Mahesh,** Dean Faculty Affairs, BITS Pilani, Pilani Campus, for their valuable suggestions and cooperation during my research work. I am grateful to **Prof. S. K. Verma**, Dean, Academic Research Division, for his motivation, constant support and encouragement.

I would like to thank my Doctoral Advisory Committee (DAC) members **Dr. Hemant R. Jadhav**, Associate Dean, Academic Research Division and **Dr. Atish T. Paul**, Convener, Departmental Research Committee, Department of Pharmacy, Pilani Campus for helpful discussions and suggestions during the dissertation.

I am highly indebted to **Prof. Rafael Balana Fouce**, Department of Biomedical Sciences, University of Leon, Sweden and **Prof. Terry K. Smith,** The University of St Andrews, UK for their enormous help for anti-leishmanial screening of our compounds in their assay systems.

I would like to specially thank **Prof. Scott G. Franzblau** and **Prof. Baojie Wan,** Department of Medicinal Chemistry and Pharmacognosy, University of Illinois, USA, for anti-tubercular screening of our compounds. I would also like to thank **Dr. Prabhat Nath Jha** and **Mr. Rajinish Prakash Singh**, Department of Biological Sciences, BITS Pilani, Pilani Campus for their great help in anti-bacterial screening of our compounds in their assay system.

I am highly thankful to **Ranbaxy Laboratories Ltd**., INDIA, for their kind endowment of standard drug Efavirenz as gift sample on request.

I would also like to acknowledge **Dr. Shrikant Y. Charde**, Head, Department of Pharmacy, BITS Pilani, Hyderabad Campus, **Dr. Anil B. Gaikwad** and **Dr. Rajeev Taliyan,** for their assistance during the dissertation. I am highly indebted to **Dr. T. Devadoss, Dr. S. Sudharshan Rao, Dr. Kamesh Rao**, **Mr. Vadiraj Kurdekar, Mr. Arghya Kusum Dhar** and **Mr. Muthu S. Venkatesh** for the enthusiasm, support and timely guidance given by them during all the stages of my work.

My special thanks to **Dr. R. P. Pareek**, **Dr. Sushil Yadav**, **Dr. Sunil Dubey**, **Dr. Anil Jindal**, **Dr. Anupama Mittal**, **Dr. Deepak Chitkara**, **Mr. Mahaveer Singh**, **Mr. Shvetank Bhatt**, **Mr. Murali M. Pandey**, **Mr. Gautam Singhvi**, **Ms. Priti Jain**, and **Ms. Archana Kakkar** for their helpful advices.

I always had a hearty inspiration from **Mr. Jaipal A**, **Mr. Emil Joseph**, **Mr. Prashant Raut**, **Mr. Satish Reddi**, **Mr. Yeshwant Kurhe**, **Mr. S N C Sridhar, Mr. Subhash Chander, Mr. Almesh Kadakol, Mr. Pankaj Wadhwa and Mr. Santosh Kumar** for the wonderful moments we have shared and making my stay in Pilani, a pleasant and memorable one.

I express heartiest gratitude to all my dear friends and colleague research scholars of Department of Pharmacy, **Dr. Vasanthakumar Sekar, Ms. Garima Balwani**, **Ms. Vibhu Nagpal**, **Ms. Deepali Gupta**, **Mr. Saurabh Mundra**, **Mr. Sorabh Sharma**, **Ms. Anuradha Pandey**, **Ms. Sruthi, Mr. Vajir Malek** and **Dr. Pallavi Singh** for timely help and their friendship shall always be remembered.

I thank the non-teaching staff **Mr. Ram Suthar**, **Mr. Puran**, **Mr. Sajjan**, **Mr. Mahender**, **Mr. Naveen**, **Mr Tarachand**, **Mr. Laxman**, **Mr. Sitaram**, **Mr. Vishal**, **Mr. Shyam**, **Mr. Mukesh** and **Mr. Shiv** and all the students of Pharmacy department for their valuable help at each stage of the research work.

My special thanks to **SAIF**, **Panjab University**, Chandigarh, **SAIF, CSIR-CDRI**, Lucknow, **Central Instrumentation Laboratory**, **Guru Jambheshwar University of Science and Technology,** Hisar, for providing analytical facilities related to my research work.

I would like to thank **University Grants Commission** for Basic Science Research, **Council of Scientific and Industrial Research**, New Delhi for providing the fellowship.

Last but not least, I would also like to thank **Department of Pharmacy** and **BITS Pilani University** for giving me an opportunity to carry out my research successfully.

My sincere thanks and regrets to all friends and people I missed to acknowledge who had directly or indirectly helped me to accomplish this task.

Finally, I would like to express my heartfelt thanks to my parents **P. Rajaiah** and **P. Lakshmi**, Brother **P. Odelu**, Sister **Manjula**, Brother-in-law **Srinivas**, and other family friends, **Deepika P, G. Hussain, T. Tajesh, M. Tatarao, P. Raju, and P. Radhakrishna** for their untiring support.

Last but not the least; it is GOD, the 'Almighty' who graced me in finishing this task.

ASHOK P

List of Abbreviations and Symbols

^{1}H NMR	Proton Nuclear Magnetic Resonance
5-HT	5-Hydroxytryptamine
3TC	Lamivudine
Å	Angstrom
α-APA	Alpha-Anilinophenylacetamide
β	Beta
δ	Delta
ABC	Abacavir
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AChE	Acetylcholinesterase
ADMET	Absorption Distribution Metabolism Excretion Toxicity
ADP	Adenosine Diphosphate
AIDS	Acquired Immuno Deficiency Syndrome
AKR_1B_1	Aldo-keto reductase B1
ART	Anti-Retroviral Treatment
AZT	Zidovudine/ Azidothymidine
BACTEC	Bactenecin
BChE	Butyrylcholinesterase
BZDs	Benzodiazepines
brs	Broad singlet
CD4	Cluster of Differentiation 4
CDK	Cyclindependent Kinase
*c*DNA	Complementary DNA
CC_{50}	Cytotoxic Concentration
CCR5	Chemokine Receptor type 5
CFU	Colony Forming Unit
*c*GMP	Cyclic Guanosine Monophosphate
CL	Cutaneous Leishmaniasis
CNS	Central Nervous System
CXCR4	Chemokine Receptor type 4

d	Doublet
d4T	Stavudine
dd	Doublet of doublet
ddC	Zalcitabine
DDI	Didanosine
DA	Dopamine
DH β-Cs	Dihydro-β-carbolines
DIG	Digoxigenin
DLV	Delavirdine
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleoside Triphosphate
DMEM	Dulbecco Modified Eagle Medium
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
dUTP	Deoxyuridine Triphosphate
dTTP	Deoxythymidine Triphosphate
ds	Double stranded
EC_{50}	Effective Concentration 50%
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EFZ	Efavirenz
ELISA	Enzyme Linked Immunosorbent Assay
eqv	Equivalent
EtOAc	Ethylacetate
FDA	Food and Drug Administration
FTC	Emtricitabine
FT-IR	Fourier Transform-Infra Red
FU	Fluorescence Units
gp120	Glycoprotein 120
GSK-3	Glycogen Synthase Kinase-3
h	Hour (s)
HAART	Highly Active Anti Retroviral Therapy

HBSS	Hanks' Balanced Salt Solution
HIV	Human Immunedeficiency Virus
HOBt	1-Hydroxybenzotriazole
HSV	Herpes Simplex Virus
Hz	Hertz
IC_{50}	Half maximal Inhibitory Concentration
IκB	IkappaB
i.p.	Intra-peritoneal
i.v.	Intravenous
IBS	Irritable Bowel Syndrome
IR	Infra Red
µg/mL	Microgram per Milliliter
µL	Microliter
µM	Micromolar
m	Multiplet
MCL	Mucocutaneous Leishmaniasis
mmol	Millimole
mL	Milliliter
m.p.	Melting Point
m/z	Mass/charge
MAOIs	Monoamine Oxidase Inhibitors
mg	Milligram
MIC	Minimum Inhibitory Concentration
min	Minute(s)
mM	Millimolar
MS	Mass Spectra
MTP	Microtiter Plate
MTS-PMS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium and phenylmethasulfazone]
MW	Molecular Weight
NCE's	New Chemical Entity

nM	Nanomolar
NMDA	*N*-methyl-D-aspartate
NNIBP	Non-Nucleoside Inhibitory Binding Pocket
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NtRTIs	Nucleotide Reverse Transcriptase Inhibitors
NVP	Nevirapine
OPLS	Optimized Potential for Liquid Simulations
PAF	Platelet Activating Factor
PDB	Protein Data Bank
PDE5	Phosphodiesterase-5
PIC	Pre-Integration Complex
PKDL	Post-Kala-Azar Dermal Leishmaniasis
PMA-SiO_2	Phosphomolybdic Acid Supported on Silica
ppm	Parts per Million
q	Quartet
quin	Quintet
RCSB	Research Collaboratory for Structural Bioinformatics
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
rt	Room Temperature
s	Singlet
SBE	Smad Binding Element
ss	Single Stranded
t	Triplet
TB	Tuberculosis
TCAs	Tricyclic Antidepressants
TDF	Tenofovir
TEA	Triethylamine
THF	Tetrahydrofuran
TIBO	Tetrahydroimidazo[4,5,1-jkj][1,4]benzodiazepin-2(1*H*)-one

TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
TMV	Tobacco Mosaic Virus
TOPO-I	Topoisomerase-I
TOPO-II	Topoisomerase-II
*t*RNA	Transfer Ribonucleic Acid
UNAIDS	United Nations Programme on HIV and AIDS
VL	Visceral Leishmaniasis
WHO	World Health Organization

List of Tables

List of Figures

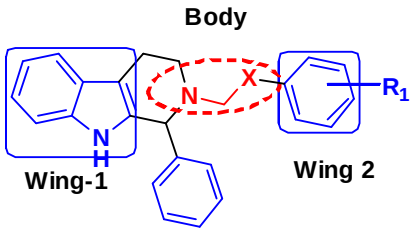
Abstract

Acquired Immuno Deficiency Syndrome (AIDS) is the advanced stage of the infection caused by Human Immunedeficiency Virus (HIV). According to *UNAIDS*-2014 report, it is estimated that, 35.3 millions are currently living with HIV infection, 2.3 million new infections and 1.5 million deaths are reported in the year 2013. AIDS mortality rate, decreased continuously in the last decade, due to the availability of Highly Active Anti Retroviral Therapy (HAART). HAART is the combination of three or more anti-HIV drugs, used to reduce the emergence of resistance and side effects. HAART turned fatal AIDS into chronic manageable disease. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are the key components of HAART due to their high potency, selectivity and less toxicity. Developed resistance to first generation NNRTIs and high mutation rate of the virus increases the need of novel anti-HIV agents.

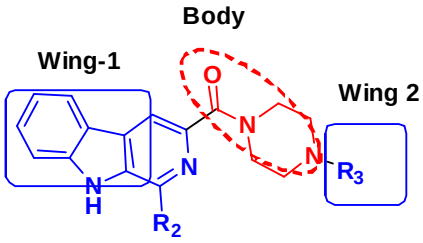
In the present, we have designed novel β-carboline derivatives based on butterfly shape pharmacophore as non-nucleoside inhibitors of HIV-1 reverse transcriptase. The designed β-carboline derivatives were synthesized from DL-tryptophan/tryptamine in series of reactions. Synthesized compounds were characterized by IR, ^{1}H NMR, Mass and elemental analysis. All the synthesized derivatives were evaluated for cytotoxicity against healthy vero cell line using MTT assay method. *In-vitro* HIV-1 reverse transcriptase inhibition activity of these compounds was evaluated using colorimetric assay method and first generation NNRTI efavirenz was used as reference drug. Molecular modeling studies were performed to study the molecular interactions and exact binding mode analysis in Non-nucleoside inhibitory binding pocket (NNIBP) using Schrödinger 2014. These anti-HIV β-carboline derivatives were also evaluated for their inhibitory potential against AIDS-opportunistic infections like leishmaniasis and tuberculosis. Standard drugs miltefosine (anti-leishmanial), isoniazid, rifampicin (anti-tubercular) were used as reference drugs for comparison purpose.

In these reported compounds, six 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives such as 6b, 10b, 10c, 13a, 13b, 13d (% RT inhibition 55, 61, 55, 72, 64 respectively) and eleven 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives 20aa, 20ab, 20ac, 20ad, 20ai, 20am, 20ao, 20bc, 20da, 20dm, 20do (% RT inhibition 66, 53, 56, 62, 60, 52, 56, 53, 63, 62, 53 respectively) exhibited significant inhibition of HIV-1 RT.

Molecular modeling studies suggested that, these β-carboline derivatives, displayed hydrogen bond interaction with Lys 101, Lys 103 and electrostatic interactions with aminoacids Leu100, Val106, Tyr181, Tyr188, Phe227, Trp229, Leu234, Tyr318 of HIV-1 RT.



X = CO, CONH, CONHNCH
R_1 = OCH_3, CH_3, Cl, Br,NO_2



R_2 = ph, 4-OCH_3-ph, 4-Cl-ph, thiophen-2-yl
R_3 = ph, OCH_3-ph, CH_3-ph, Cl-ph, F-ph, NO_2-ph, pyridyl

Structure Activity Relationship (SAR) studies of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives suggested that polar, 4-5 carbon length hydrophilic linker (carbohydrazide) and *para* substitution on wing-2 with *ortho-para* directing groups, favored HIV-1 RT inhibition activity. In 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives, un-substituted phenyl, hetero cyclic thiophene-2-yl ring on position-1, favored the activity and substitutions on position-1 phenyl ring resulted in drastic decline in activity. Although *para* substitutions on piperazine attached phenyl ring retained potency, *ortho* and *meta* substitutions resulted in decreased activity.

In addition to HIV-1 RT inhibition activity, these analogues displayed potent inhibitory activity against AIDS-opportunistic infections such as leishmaniasis and tuberculosis. In *in-vitro* anti-leishmanial screening, fourteen 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives 6d, 10a, 10e, 10h, 10i, 10k, 10l, 13a, 13b, 13e, 13g, 13m, 13n, 13o (EC_{50} 12.21, 12.37, 8.10, 7.63, 6.68, 1.99, 10.43, 3.50, 2.07, 5.98, 10.10, 9.83, 1.93, 11.90 respectively) and eighteen 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives 20aa, 20ab, 20ac, 20ag, 20ai, 20ba, 20bc, 20bk, 20bl, 20ci, 20cj, 20dc, 20df, 20dg, 20di, 20dj, 20dm, 20do (EC_{50} 12.50, 9.07, 9.63, 2.89, 3.35, 11.70, 6.38, 4.28, 11.90, 9.39, 10.90, 7.70, 7.00, 3.80, 7.10, 9.25, 3.10, 4.85 respectively) exhibited potent anti-leishmanial activity than reference drug miltefosine against promastigotes of *Leishmania infantum* with good selectivity index. SAR studies revealed that, among 1-phenyl-2,3,4,9-

tetrahydro-β-carboline derivatives (6, 10 and 13 series) irrespective to the substitutions on wing-2 phenyl ring, carbohydrazide (13 series) derivatives showed potent activity against *Leishmania* parasite followed by amide (10 series) and keto (6 series) derivatives respectively. In 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives, comparatively thiophene-2-yl (20d) derivatives exhibited potent anti-leishmanial activity followed un-substituted phenyl (20a), 4-methoxyphenyl (20b) and 4-chlorophenyl (20c) derivatives.

Among these reported compounds, two 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives 10m, 13j (MIC, 4.3, 6.24 µg/mL) and three 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives 20aa, 20ao, 20bo (MIC, 5.9, 5.5, 5.9 µg/mL, respectively) exhibited significant inhibition of *Mycobacterium tuberculosis* (H37Rv) with MIC values <6.25 µg/mL, is postulated as an upper threshold value of new anti-tubercular drugs for the evaluation of new *tuberculosis* therapy. In addition, ten 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives 6l, 10a, 10o, 13a, 13e, 13f, 13g, 3k, 13l, 13n (MIC 24.3, 19.4, 23.0, 17.57, 24.10, 7.04, 22.96, 22.71, 17.45, 20.14 µg/mL, respectively) and eight 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives 20ac, 20aj, 20am, 20bn, 20cm, 20cn, 20dm, 20do (MIC 11.5, 22.7, 23.4, 16.7, 23.4, 11.87, 12.08, 21.7 µg/mL, respectively) exhibited moderate anti-tubercular activity with MIC value below 25 µg/mL.

1. INTRODUCTION

Acquired Immuno Deficiency Syndrome (AIDS) is an infectious disease first identified in United States of America in 1981, then disseminated throughout the world, and became one of the leading causes of death. Human Immunedeficiency Virus (HIV) is the causative agent, first identified in 1983 from a French patient. HIV is a pathogenic lentivirus which belongs to the family *Retroviridae,* containing *ss*-RNA as genetic material [1]. HIV has two major categories, HIV-1 and HIV-2. Among these two, HIV-1 is the most common pathogenic virus distributed throughout the Globe. HIV-1 is further classified into major group M and three minor groups N, O and P [2]. Group M is again divided into sub-types such as A, B, C, D, E, F, G, H, I, J and K based on their genetic sequence [3, 4]. HIV-2 is less virulent, predominant in West Africa but also reported in some parts of Africa, Europe, India and United States. HIV-2 has lower transmission efficiency, a more lengthy asymptomatic phase and takes longer time to cause immune suppression when compared to HIV-1 [5]. HIV mainly attacks on cells like, helper T cells, macrophages and dendritic cells which plays vital role in cell mediated immunity [6]. AIDS is the advanced stage of HIV infection when T helper cell count is below 200 per µL, host becomes susceptible to opportunistic multiple infections like bacterial, fungal, protozoal and cancer which ultimately leads to death [7, 8]. According to *UNAIDS*-2014 report, it is estimated that, 35.3 millions are currently living with HIV infection, 2.3 million new infections and 1.5 million deaths have been reported in the year 2013 [9-11].

1.1. HIV life cycle

HIV life cycle comprises of various distinct phases such as infection, reverse transcription, integration, transcription, translation, assembly, budding and maturation [12] (fig. 1.1). HIV life cycle starts with the adsorption of HIV onto the CD4 receptors of the cells like, T-cell, macrophage and dendritic cells. HIV with its surface protein gp120 binds to CD4 receptors of the T-cell, interacts with CXCR4 or CCR5 co-receptors of the host cell [13], followed by fusion with cell membrane and injection of genetic material into cytoplasm of host cell by leaving the envelop behind plasma membrane [14]. Reverse transcriptase catalyzes the

conversion of *ss*RNA into *c*DNA and then *ds*DNA [15]. Due to lack of proof reading efficiency of reverse transcriptase enzyme, naturally large numbers of mutation are introduced in nascent DNA [16, 17]. Newly synthesized *ds*DNA transports in to host nucleus from cytoplasm as preintegration complex (PIC) [18], integrate with host DNA and integrated complex is called as provirus [1]. Enzyme integrase, catalyzes the whole process of transportation and integration of viral DNA into host genetic material [19]. HIV provirus remains in dormant stage until the activation of cell, once cell get activated, proviral DNA is transcribed in to messenger RNA with the help of host enzyme systems, which transports into cytosol and then translate into large number of viral proteins [20]. These newly produced proteins, enzymes and other components assemble together at the plasma membrane to produce complete virion and then release by cell disintegration [21]. The enzyme protease plays a vital role at this stage of the HIV life cycle by chopping up long strands of proteins into smaller pieces, which are used to construct mature viral cores [15, 20]. The newly released mature HIV particles are ready to infect another cell and begin replication process again.

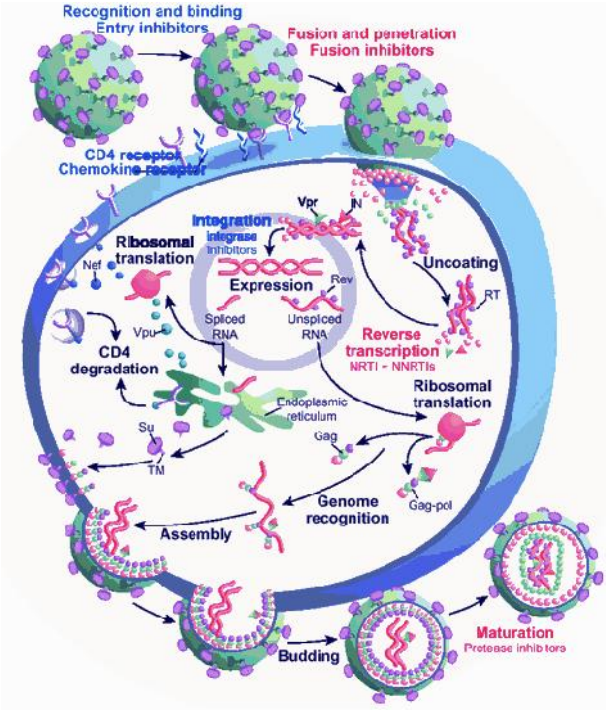


Fig. 1.1: HIV life cycle (http://img.medscape.com/fullsize/migrated/458/640/nm458640.gpf)

1.2. HIV Reverse Transcriptase

The Pol gene of HIV encodes three enzymes, namely Reverse Transcriptase (RT), protease and integrase which plays decisive role in HIV life cycle and are considered as vital targets to develop anti-HIV agents [22]. Among these enzymes, RT is a multi functional asymmetric heterodimer, comprising of two sub-units p66 (560 amino acids) and p51 (440 aminoacids) [23]. P66 sub-unit is an open and flexible structure consist of catalytic domain (active site) at amino terminal and RNAseH domain (120 amino acids) at carboxy terminal [15]. Catalytic domain is composed of three aspartic acid units (110, 185 and 186) and controls the polymerase activity [24]. RNAseH domain (427-560 aminoacids) linked to active site by the connection domain (amino acids 319-426) and catalyses the cleavage of RNA from *c*DNA. P66 sub-unit also contains non-nucleoside inhibitory binding pocket (NNIBP) which is located 10 Å away from catalytic site of the enzyme. This allosteric binding pocket is hydrophobic in nature and is surrounded by the hydrophobic (Pro59, Leu100, Val106, Val179, Leu234, Pro236), aromatic (Tyr181, Tyr188, Phe227, Trp229, Tyr232, Tyr318) and hydrophilic (Lys101, Lys103, Ser105, Asp132, Glu224) amino acids of the p66 sub-unit [15, 25] (fig. 1.2). P51 subunit contains same amino acid sequence as p66 but lack of RNAseH domain, is cleared by the protease enzyme during generation of mature viral particles. P51 subunit is compact, plays an important role in structural rigidity of the enzyme and it also provides a binding site for the lysine-*t*RNA primer to initiate DNA synthesis by reverse transcription [26].

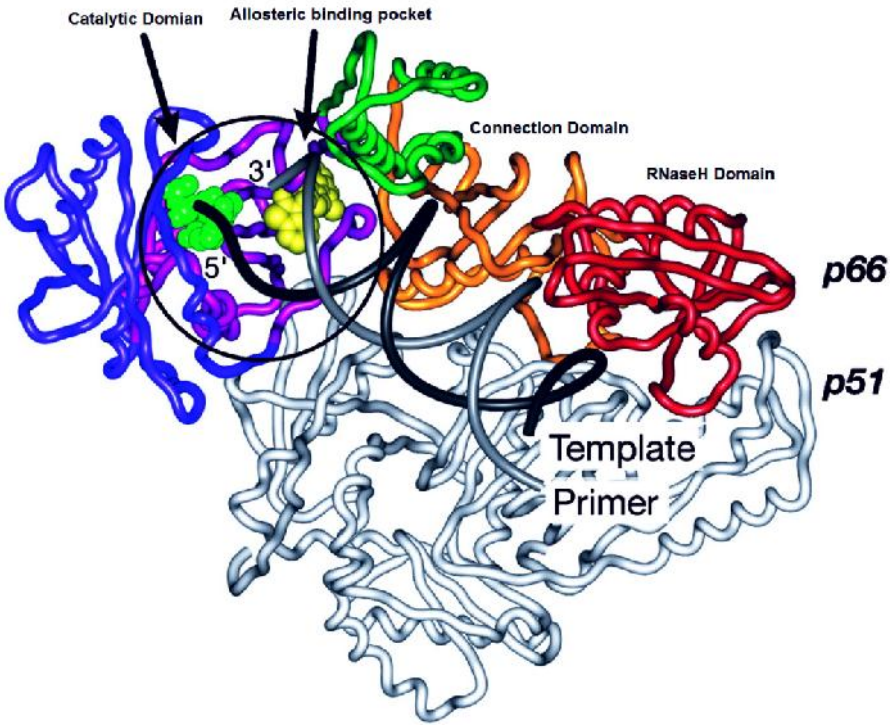


Fig. 1.2: 3D structure of HIV-1 Reverse Transcriptase [23]

1.3. Reverse Transcriptase Inhibitors

Reverse Transcriptase is one of the vital targets to develop anti-HIV agents, as it catalyzes the critical conversion of *ss*RNA to *ds*DNA. Reverse transcriptase inhibitors have great importance in anti-HIV treatment and are back bone to the currently available anti-retroviral combination regimen [27]. Drugs acting on reverse transcriptase are classified in to two groups, nucleotide or nucleoside reverse transcriptase inhibitors (NRTIs/NtRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) based on their enzyme binding domain [28].

1.3.1. Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs/NtRTIs)

NRTIs/NtRTIs are structural analogues of endogenous nucleosides/nucleotide with absence of hydroxyl group on position-3 of ribose sugar moiety. These NRTIs get converts into active form deoxyNucleoside Triphosphate (dNTP) by the successive phosphorylation in presence of host cellular enzymes [27]. These triphosphorylated active NRTIs are competitively incorporated in to nascent DNA by reverse transcriptase, inserted NRTIs are failed to form

phosphodiester bond with incoming nucleotides, ultimately results in termination of DNA synthesis [29, 30]. Currently, US-FDA have been approved nine NRTIs i.e. Zidovudine (AZT), Didanosine (DDI), Zalcitabine (HIVID, ddC), Stavudine (D4T), Lamivudine (3TC), Abacavir (ABC), Emtricitabine (FTC), Entecavir (ETV), Apricitabine (ATC) and two NtRTIs i.e. Adefovir, Tenofovir (TDF) for HIV treatment (fig. 1.3). Despite of their associated toxicity and resistance, still these are the major components of Highly Active Anti Retroviral Therapy **(**HAART) combination regimen [27, 31].

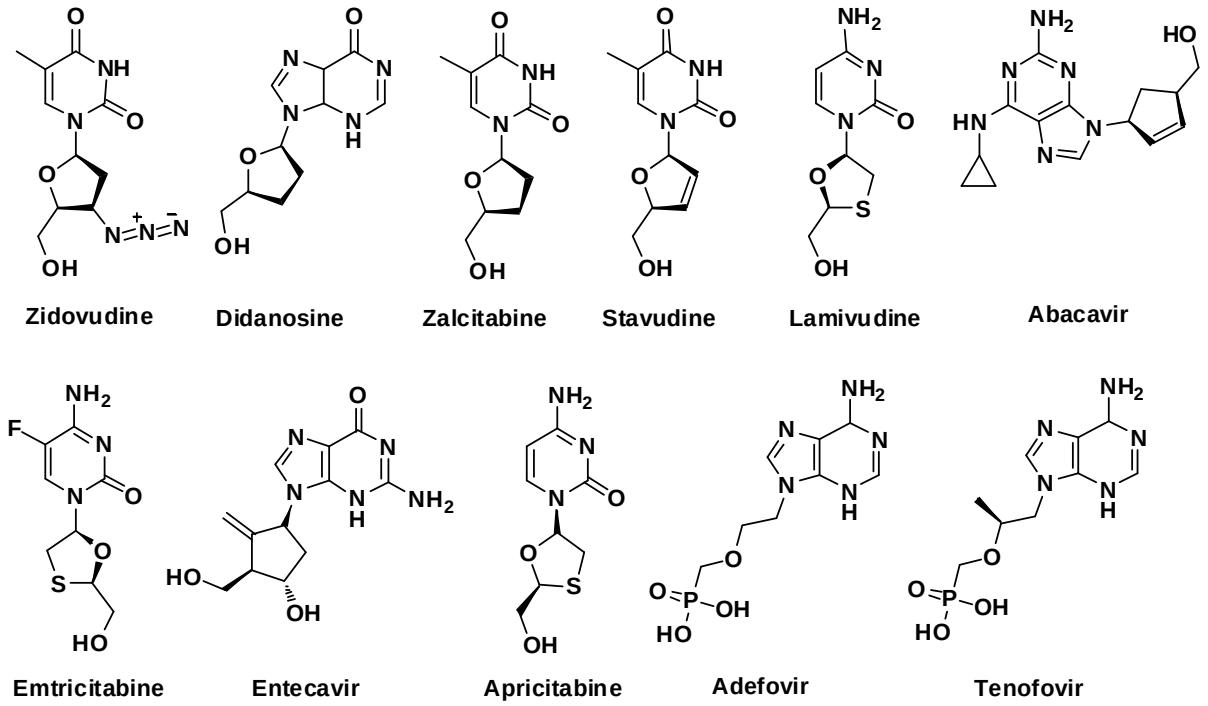


Fig. 1.3: Structure of FDA approved NRTI/NtRTI drugs

1.3.2. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

NNRTIs inhibit reverse transcriptase function by binding to allosteric hydrophobic binding pocket of the enzyme [32, 33]. This hydrophobic binding pocket does not appear in normal reverse transcriptase structure, but it is created when a non-nucleoside inhibitor binds to the enzyme due to some hydrophobic and hydrophilic interactions [25]. These interactions lead to movement of Tyr181 and Tyr188 amino acid side chains towards the active site and induce conformational changes in active site, which results in termination of reverse transcriptase activity [34]. Hydrophobic binding pocket amino acids such as, aromatic

(Tyr181, Tyr188, Phe227, Trp229, and Tyr232), hydrophobic (Pro59, Leu100, Val106, Val179, Leu234, and Pro236) and hydrophilic (Lys101, Lys103, Ser105, Asp132, and Glu224) plays an important role in receptor-ligand interaction there by inhibition of enzyme function [25].

Non-nucleoside reverse transcriptase inhibitors have gained privileged position in HIV treatment regimen HAART, due to their chemical diversity, high potency, specificity and low toxicity [35]. Currently five NNRTIs have been approved by FDA for the treatment of HIV infection. Among these, Nevirapine (NVP), Delavirdine (DLV), Efavirenz (EFZ) are the first generation, Etravirine and Rilpivirine are second generation NNRTIs [36] (fig. 1.4). These first generation NNRTIs, adopt butterfly shape confirmation with two hydrophobic wings and one hydrophilic body in NNIBP of HIV RT [28]. Hydrophobic wings are mainly responsible for hydrophobic/electrostatic interactions with aromatic aminoacids like Tyr181, Tyr188, Phe227, Trp229, Tyr318 and hydrophilic body forms hydrogen bond/hydrophilic interactions with aminoacids Leu100, Lys101, Lys103 and Val106 [37, 38]. Due to mutations of key aminoacids like L100I, K101E/Q, K103N/S, V106A/M, Y181C, Y188L, M230L, P236L, K238N/T and Y318F resistance was developed to these first generation NNRTIs and restricted their clinical usage [39]. Consequently, next generation NNRTIs are designed with flexible structures to orient easily in NNIBP and to form interactions with conserved aminoacids like Phe227, Trp229 and Tyr318 [40]. Etravirine and rilpivirine are second generation NNRTIs approved by FDA for the treatment in 2008 and 2010 respectively. These second generation NNRTIs adopt horseshoe or “U” shape configuration in NNIBP, represents the structural flexibility of these analogues which is essential to overcome the resistance problems. In this horseshoe shape pharmacophore model, core heterocycle ring is attached to two aromatic rings with hydrophilic polar linkers. Core heterocycle ring is mainly responsible for conformational adaptability, aromatic rings able to form hydrophobic interactions with aminoacids like Tyr181, Tyr188, Trp229, Phe227, Val106, Pro236, Leu100, Leu234, Tyr318 and hydrophilic polar linker acts as hydrogen bond donor/acceptor to interact with Lys101, Lys103 and Pro236 of allosteric binding pocket of HIV reverse transcriptase. On the other hand, etravirine displayed adverse effects like Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme and

hypersensitivity reactions in postmarketing surveillance reports [41]. In addition to this, resistance to etravirine has been reported due to multiple mutations like V90I, A98G, L100I, K101H/E/P, V106I, E138A, V179D/F/T, Y181C/I/V, G190A/S and M230L [42]. Structural similarity (2,4-diarylpyrimidine derivatives) of second generation NNRTIs, has increased the risk of cross resistance development to rilpivirine. It creates an emergency to develop novel NNRTIs with good pharmacokinetic profile [43].

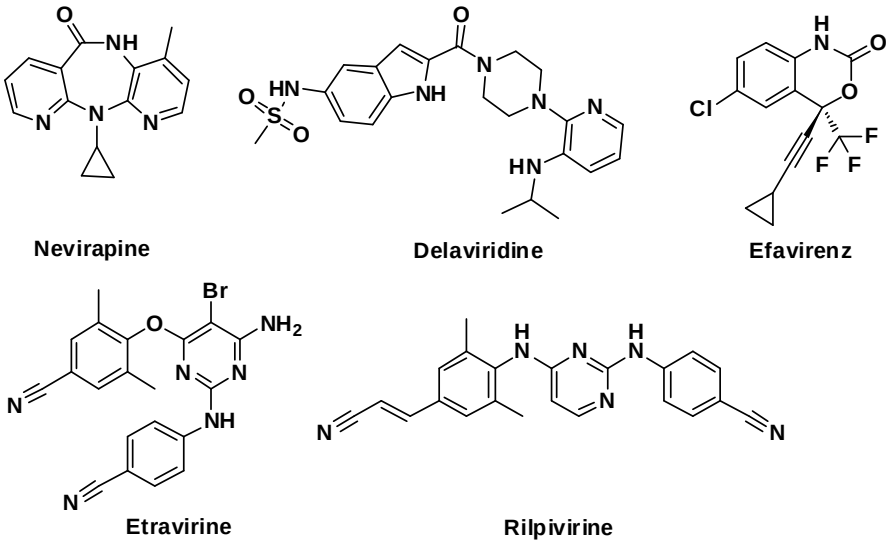


Fig. 1.4: Structure of FDA approved NNRTI drugs

1.4 Highly Active Anti Retroviral Therapy (HAART)

HAART is often called as drug-cocktail, is the combination of three or more different FDA approved anti-retroviral drugs. WHO recommends, the use of combination therapy for the treatment of HIV infection to decrease the emergence of drug resistance as well as to reduce side effects [44]. HAART includes the combination of two NRTIs with one Protease inhibitor or NNRTI or other drugs. Combination of NRTIs with NNRTIs is the preferred combination over other, because of their synergistic inhibitory effect on reverse transcriptase [45, 46]. With effectiveness of HAART regimen, lethal HIV infection is converted into chronic manageable disease, as number of deaths declined to 1.6 million in 2012, compared to 2.3 million in 2005 [5, 47]. HAART effectively reduces the viral replication rate and decreases plasma viral load below the detection level which resulted in significant delay in disease progression and reduced the risk of HIV transmission [48, 49].

1.5. Limitations of HAART

HAART effectively reduces viral load to below the detection level but unable to eradicate the virus completely from the patient [50]. Hence, permanent or lifelong treatment is required because of chronic nature of the infection which results in poor patient compliance [47]. Additionally, HAART is multi-drug treatment regimen which leads increased pill burden, toxic effects and interferes with pharmacokinetic profile of each other drugs [51, 52]. Moreover, due to high mutation rate of HIV, long-term clinical effectiveness of approved anti-HIV drugs has been hampered [50].

1.6. AIDS-Opportunistic Infections

As HIV infection progresses, patients became susceptible to bacterial, fungal, protozoal infections and cancers, are known as opportunistic infections. Opportunistic infections such as tuberculosis, leishmaniasis, kaposi carcinomas along other microbial infections are most frequently appears in immunocompromised patient and are the major cause of morbidity and mortality of HIV infected patients [11].

1.6.1. Tuberculosis

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis.* According to the recent statistics from WHO, 8.6 million new infections and 1.3 million TB deaths have been reported in the year 2012 [53]. It is estimated that, over 125 million people will get sick with more than one billion new infections and 30 million people will die with TB by 2020 [54]. Moreover, TB is the most common opportunistic infection in HIV patients and the risk of TB development is 20 times higher in immunocompromised patient than normal individuals. Tuberculosis remains as leading cause of death among the HIV infected patients, according to *UNAIDS* reports 2014, In 2013, 0.3 millions of HIV patients died with tuberculosis. Nearly 75% of the cases of HIV with active TB infection are living in only ten countries such as India, Ethiopia, Nigeria, Kenya, Mozambique, United Republic of Tanzania, South Africa, Uganda, Zambia and Zimbabwe [11, 55]. Treatment of HIV patients with active TB requires concomitant administration of antiretroviral and anti-TB drugs [56]. Co-administration of HAART and anti-TB drugs increases pill burden and lessens patient

compliance [57]. This parallel administration also suffered with severe adverse drug-drug interactions, as anti-retroviral and anti-TB drugs reduces (eg., efavirenz, saquinavir, Lopinavir, isoniazid,) and enhances (eg., efavirenz, Nevirapine, rifampicin) the metabolic activity of enzymes like cytochrome P450s, p-glycoprotein, which leads to variation in plasma concentration of drugs and ultimately resulted in therapy failure [58]. Additionally, majority of the antiretroviral drugs (including RTIs and PIs) are contraindicated with rifampicin [59]. Moreover, high probability to develop immune reconstitution inflammatory syndrome in HIV-TB co-infected patients with anti-retroviral treatment (ART) regimen which further worsen the TB symptoms [60].

1.6.2. Leishmaniasis

Leishmaniasis is a group of infective diseases caused by protozoan parasites of the genus *Leishmania*. It is considered as one of the most neglected diseases and is endemic in 90 countries throughout the world. Leishmaniasis is the most prevalent vector born infectious disease after malaria in terms of fatality and total number of patients. It is estimated that, currently 350 millions are living at risk places and 1.3 millions affected with annual mortality of 30,000 [37, 61, 62]. Traditionally, leishmaniasis has been classified in three different clinical forms (i. e) Cutaneous Leishmaniasis (CL), Mucocutaneous Leishmaniasis (MCL) and Visceral Leishmaniasis (VL) [41, 63]. CL is the most common form of the infection, 90% of cases occur in Afghanistan, Algeria, Brazil, Pakistan, Peru, Saudi Arabia, and Syria. It produces skin lesions on the exposed parts of the body, such as face, arms and legs [42]. Nearly about 20 species of *Leishmania* are responsible for CL which includes *Leishmania major, Leishmania braziliensis* (in Brazil)*, Leishmania mexicana, Leishmania infantum* (in southern France), *Leishmania donovani* and *Leishmania panamensi.* Although, CL is often self-healing, it can create serious permanent disfiguring scars [64]. Mucocutaneous Leishmaniasis (also called Espundia in South America,) produces lesion on mucous membranes of the nose, mouth, throat cavities and surrounding tissues. These lesions can lead to partial or total destruction of the affected organs [65]. Around 90% of the cases occur in Brazil, Bolivia and Peru and 20% of the infections caused by *Leishmania braziliensis* develop as MCL [66]. Pathogenesis of MCL is still not clear but genetic factor of infected

person plays an important role in progression of the disease [67]. Visceral Leishmaniasis also known as kala azar (Black Fever in Hindi) is the most severe form of Leishmaniasis, is caused by *Leishmania donovani* and *Leishmania infantum.* More than 90% of the cases occur in six countries: India, Bangladesh, Nepal, Sudan, Ethiopia and Brazil. Symptoms of VL are irregular fever, weight loss, mucosal ulcers, swelling of the liver, spleen and anaemia. Unlike, cutaneous forms of Leishmaniasis, VL affects the internal organs such as liver, spleen, bone marrow and is usually fatal if left untreated [68, 69]. After treatment and recovery of VL, generally patients develop chronic cutaneous Leishmaniasis, known as Post-kala-azar dermal leishmaniasis (PKDL). PKDL is prevalent after recovery of *L. donovani* infection but not from *L. infantum.* PKDL first appears as small, measle-like skin lesions on the face, which gradually increase in size and subsequently affect other parts of the body including conjunctival, nasal, oral and genital mucosa [70].

In 1985, first case of HIV-leishmaniasis co-infection was reported but number of co-infection cases has increased rapidly especially in leishmaniasis endemic countries like India, Bangladesh, Nepal, Sudan, Ethiopia and Brazil [71]. The prevalence of visceral leishmaniasis is about 100-2320 times higher in immunocompromised patients. It is estimated that, 50-60% of cases of visceral leishmaniasis in these countries are associated with HIV infection and 2-9% of HIV patients co-infected with leishmaniasis [72]. Despite of their toxicity, anti-leishmanial drugs such as, amphotericine-B, pentamidine and paromomycin are being used clinically but their usage against HIV-leishmania co-infection is restricted as probability of amplification of toxic effects is high in-combination with anti-retroviral drugs. Moreover, developed resistance to anti-leishmanial drugs antimonials and miltefosine have aggravated the HIV-leishmaniasis co-infection cases [73].

2. REVIEW OF LITERATURE

β-carboline represents a tricyclic pyrido[3,4-*b*] indole ring system, [74] present in a large number of natural products isolated from numerous sources like territorial plants [75], marine sponge [53], fast food [76] and mammals [77]. β-carbolines are categorized based on saturation level of the pyridine ring, compounds with fully unsaturated aromatic pyridine ring are named as β-carbolines (β-Cs), partially unsaturated compounds and completely saturated compounds are known as dihydro-β-carbolines (DH β-Cs), tetrahydro-β-carbolines (TH β-Cs) (fig. 2.1) respectively [78].

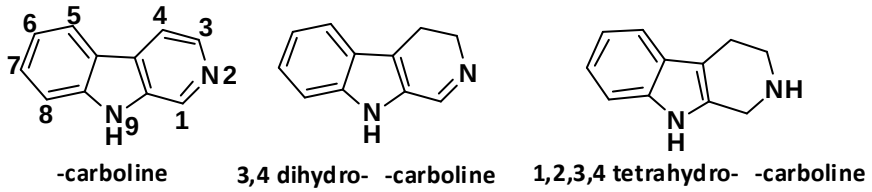
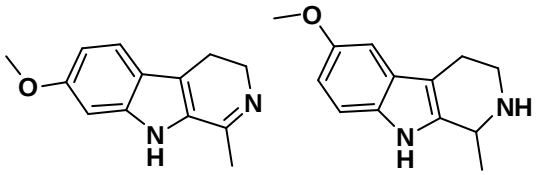


Fig. 2.1: Basic structure of β-carbolines

First β-carboline alkaloid harmaline (1) was isolated in 1841, from a wild flowering plant *Peganum harmala* (*Zygophillaceae*) found in Central Asia, Middle East and North Africa [79]. The seed extract of *P. harmala* has been used as abortifacient, emmenagogue, analgesic, anti-inflammatory, anti-malarial and to treat alimentary tract cancer for hundreds of years in Northwest China and North Africa [80, 81]. β-carboline alkaloids were also isolated from the South American vine *Banisteriopsis caapiwhich* (*Malpighiaceae*), a key plant ingredient in the sacramental hallucinogenic beverage Ayahuasca and some spiritual spirits (religious spirits) used in South America [82]. During last three decades, large number of β-carboline alkaloids were found in fruits, fruit derived products, fast foods, alcoholic and non-alcoholic beverages [78]. Consumption of beverages having β-carboline alkaloids contributed significantly for their endogenous presence in mammalian tissues along with endogenous bio-synthesis [83, 84]. Pinoline (2) was the first endogenous β-carboline alkaloid isolated from the extraction of pineal gland tissue in 1961 [85], followed by isolation of more number of β-carboline derivatives from various body fluids and tissues of mammals [78]. β-carboline alkaloids were produced from their bio-synthetic precursors tryptophan or tryptamine and aldehydes or α-keto acids under regular physiological conditions [86].



The bio-synthesis of β-carboline skeleton, takes place through well known pictet-spengler reaction (fig. 2.2) and it involves two steps, including initial Schiff base formation of tryptophan/tryptamine with aldehydes followed by cyclization to get 1,2,3,4-tetrahydro-β-carboline derivative [87]. Besides this, β-carboline moiety has privileged position in medicinal chemistry, as compounds with β-carboline skeleton displayed various biological activities such as anti-protozoal, anti-bacterial, anti-fungal, anti-viral, anti-cancer, anti-thrombotic, anti-filarial, phosphodiesterase-5 inhibitor, anti-inflammatory and other pharmacological actions [78].

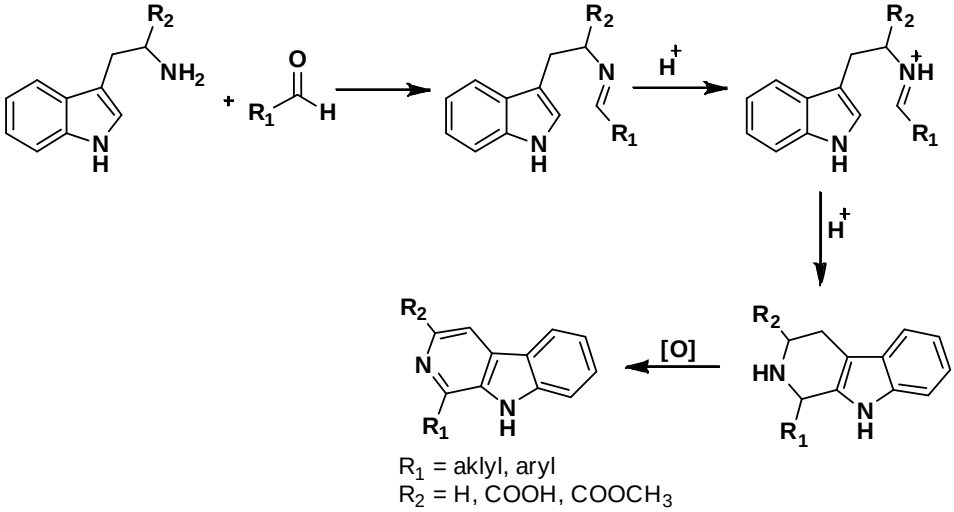


Fig. 2.2: Bio-synthesis of β-carboline skeleton by pictet-spengler reaction

2.1. Anti-protozoal activity

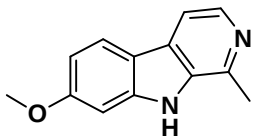
Natural alkaloids isolated from various sources are playing vital role in the treatment of protozoal infections from the ages. Large number of natural β-carboline alkaloids and their synthetic derivatives were well explored in the literature for their anti-protozoal activity such as anti-malarial, anti-leishmanial, anti-trypanosomal and anti-toxoplasmal.

2.1.1. Anti-malarial activity

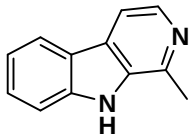
Large numbers of natural as well as synthetic β-carboline derivatives were reported for their anti-malarial activity. Hence for easy understanding, we have divided this section into two parts as anti-malarial activity of natural β-carbolines and synthetic β-carbolines.

2.1.1. a. Anti-malarial activity of natural β*-carboline alkaloids*

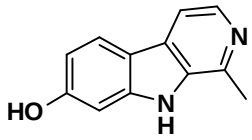
The first β-carboline alkaloid harmaline (1) was isolated from *Peganum harmala* (Syrian Rue), belongs to the family *Zygophillaceae* in 1841 [79]. Other β-carboline alkaloids, harmine (3), harmane (4), harmalol (5), tetrahydroharmine (6), norharmane (7) and harmalan (8) were also isolated from *P. harmala*, and were collectively known as harmala alkaloids. Among these harmala alkaloids, harmine (3) and harmaline (1) exhibited *in-vitro* anti-malarial activity against *Plasmodium falciparum* (IC_{50} 8.0 & 25.1 μg/mL) [41].



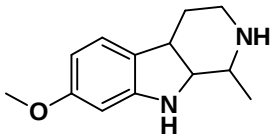
Harmine (3)



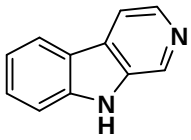
Harmane (4)



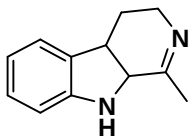
Harmalol (5)



Tetrahydroharmine (6)



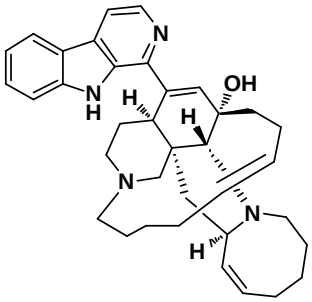
Norharmane (7)



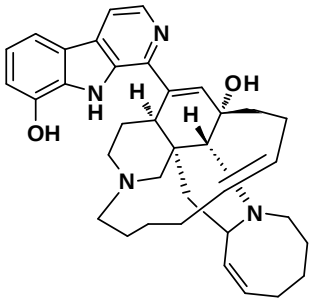
Harmalan (8)

Manzamines are a unique group of β-carboline alkaloids isolated from various species of marine sponge. Manzamines are complex natural alkaloids having β-carboline moiety attached to a pentacyclic diamine ring having both eight and thirteen member rings on a pyrrolo[2,3-*i*]isoquinoline framework [43]. Manzamine-A (9), is the first alkaloid from this group, was isolated from Okinawa sponge in 1986 [53]. Currently more than 100 manzamine natural alkaloids have been isolated from more than 16 species of marine sponges belongs to five families distributed from red sea to Indonesia [10, 88]. Manzamine group alkaloids are majorly explored for their inhibitory potential against infectious diseases like malaria, leishmaniasis, tuberculosis and HIV [89]. Among these group of

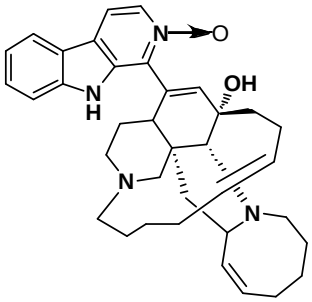
alkaloids, manzamine-A (9), 8-hydroxymanzamine-A (10) and Manzamine-A-*N*-oxide (11) exhibited potent anti-malarial activity against both wild D6 (IC_{50} 0.004, 0.006 and 0.011 µg/mL, respectively) and resistant W2 (IC_{50} 0.008, 0.008 and 0.013 µg/mL, respectively) strains of *P. falciparum* [90].



Manzamine-A (9)

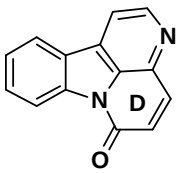


8-hydroxymanzamine-A (10)

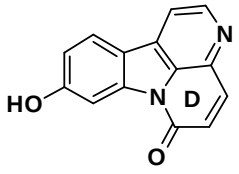


Manzamine-A-*N*-oxide (11)

Canthines are another group of β-carboline alkaloids with an additional D ring, isolated from several Asian and Australian plants known for anti-microbial and anti-tumor properties [91]. Canthin-6-one (12) is the first canthine alkaloid, isolated from Australian *Pentaceras australis* (Family: *Rutaceae*) [92]. Currently several dozens of alkaloids from this group have been reported for different biological activities. Among these canthine group alkaloids, canthin-6-one (12) and 9-hydroxycanthin-6-one (13) displayed anti-malarial activity against resistant W2 strain of *P. falciparum* (IC_{50} 2.2 and 2.3 µg/mL) [93].



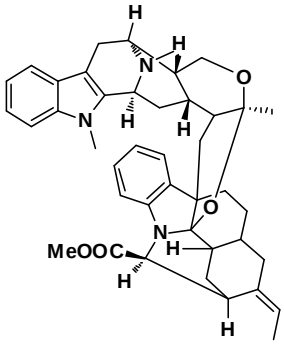
Canthin-6-one (12)



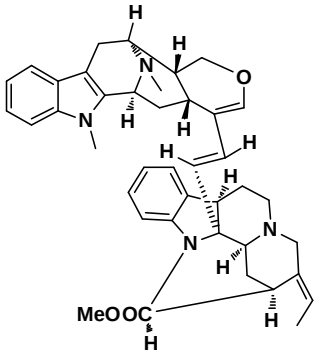
9-hydroxycanthin-6-one (13)

Fifteen novel indole alkaloids were isolated from the various parts of *Alstonia sp. (scholaris, macrophylla* and *glaucescens)* collected from Thailand in the year 1999. Among these reported alkaloids, two β-carboline alkaloids villalstonine (14) and macrocarpamine (15) showed moderate inhibition (IC_{50} 0.27 and 0.35 µg/mL) against resistant strain K1 of *P. falciparum* [94]. 10′-hydroxyusambarensine (16), a novel bisindole alkaloid isolated from the roots of *Strychnos usambarensis,* displayed moderate activity against both chloroquine

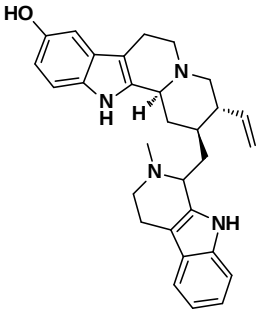
sensitive FCA 20 (IC_{50} 0.480 µg/mL) and resistant W2 (IC_{50} 0.160 µg/mL) strains of *P. falciparum* [95].



Villalstonine (14)



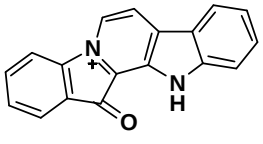
Macrocarpamine (15)



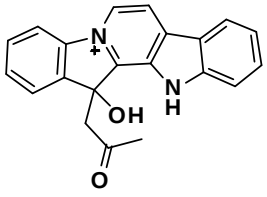
10′-hydroxyusambarensine (16)

β-carboline alklaoids, fascaplysin (17) [96] and homofascaplysin-A (18) [97] are isolated from the marine sponge *Hyrtios cf. erecta. Kirsch et al.,* 2000, reported the anti-malarial activity of these previously reported alkaloids. Fascaplysin (17) and homofascaplysin-A (18) displayed anti-malarial activity against chloroquine susceptible NF54 (IC_{50} 0.034 and 0.024 µg/mL) and chloroquine resistant K1 (IC_{50} 0.050 and 0.014 µg/mL) strains of *P. falciparum*. Homofascaplysin-A (18) exhibited superior potency against resistant K1 strain than chloroquine sensitive NF54 strain [98].

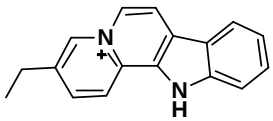
Novel β-carboline alkaloid, flavopereirine (19) along with three novel indole alkaloids, isolated from *Geissospermum sericeum* and evaluated for anti-malarial activity against the chloroquine sensitive (T9-96) and resistant (K1) strains by *Steele et al.,* 2002. Flavopereirine (19) showed significant inhibition of T9-96 (IC_{50} 0.1 µM) and K1 (IC_{50} 0.54 µM) strains of *P. falciparum* [99].



Fascaplysin (17)



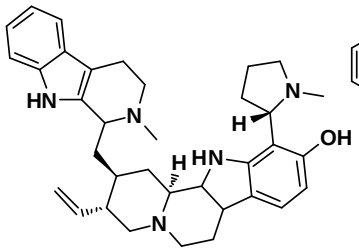
Homofascaplysin-A (18)



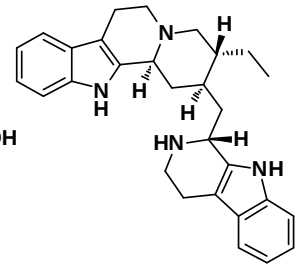
Flavopereirine (19)

*Frederich et al.*2002, reported anti-malarial activity of previously reported 69 alkaloids from various *Strychnos* species against different strains of *P. falciparum*. In these reported alkaloids, bisindole alkaloids isostrychnopentamine (20), ochrolifuanine-A (21) and 5,6-dihydrousambarensine (22) exhibited anti-malarial activity against chloroquine sensitive

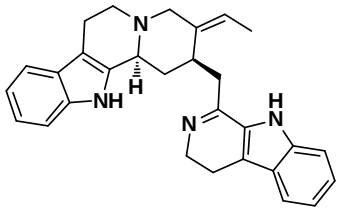
FCA 20 (IC_{50} 0.120, 0.118 and 0.857 µM, respectively) and resistant W2 (IC_{50} 0.152, 0.492 and 0.032 µM, respectively) strains of *P. falciparum* [100].



Isostrychnopentamine (20)

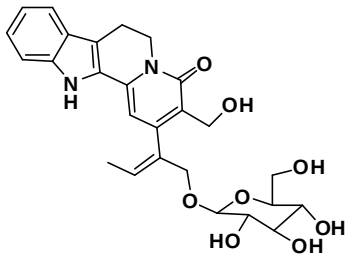


Ochrolifuanine-A (21)

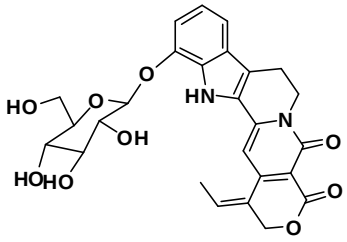


5,6-dihydrousambarensine (22)

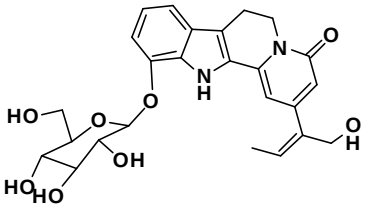
Sun et al., 2008, reported the isolation and anti-malarial activity of five β-carboline alkaloids, naucleofficine-A-E (23-27) from the stem bark of *Nauclea officinalis.* Among these reported alkaloids, naucleofficine-A (23) showed weak inhibitory activity (IC_{50} 9.7 µM) against *P. falciparum* FCC1-HN strain [101].



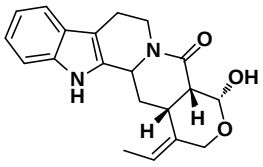
Naucleofficine-A (23)



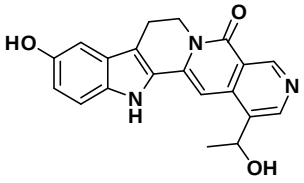
Naucleofficine-B (24)



Naucleofficine-C (25)



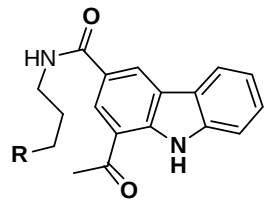
Naucleofficine-D (26)



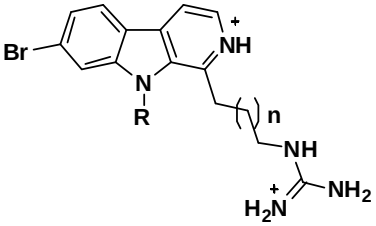
Naucleofficine-E (27)

Huang et al., 2011, isolated four new β-carboline alkaloids marinacarboline-A-D (28a-d), from the broth of *Marinactinospora thermotolerans* SCSIO 00652, a new actinomycete belonging to the family *Nocardiopsaceae*. From these reported alkaloids, marinacarboline-C (28c) and D (28d) exhibited weak anti-malarial activity against chloroquine sensitive 3D7 (IC_{50} 3.09 and 5.39 µM) and resistant 3D2 (IC_{50} 3.38 and 3.59 µM) strains of *P. falciparum* [102]. In the same year *Chan et al.,* reported the anti-malarial activity of three novel β-carboline alkaloids opacalines-A-C (29a-c) and one known tetrahydro-β-carboline alkaloid

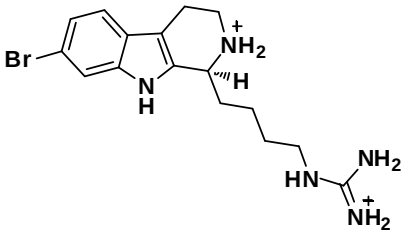
(-)-7-bromohomotrypargine (30) [103] from the New Zealand ascidian *Pseudodistoma opacum.* In these four alkaloids, two alkaloids opacaline-A (29a) and B (29b) displayed weak inhibition of *P. falciparum* K1 strain (IC_{50} 2.5 and 4.5 µM) [104].



R = 4-methoxyphenyl (28a)
R = 4-hydroxyphenyl (28b)
R = phenyl (28c)
R = 2-indolyl (28d)



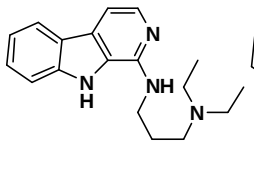
R= H, n= 2 (29a)
R= OH, n= 2(29b)
R= H, n= 1(29c)



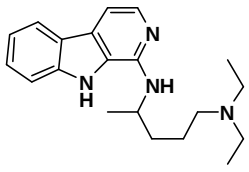
7-bromohomotrypargine (30)

2.1.1. b. Anti-malarial activity of synthetic β*-carboline alkaloids*

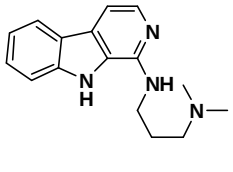
Boursereau et al., 2004, reported the synthesis and biological evaluation of 1-amino β-carboline derivatives against tropical infections. Eight derivatives (31a-h) from these simplified manzamine analogues displayed potent inhibitory activity (IC_{50} values range 0.35-0.64 µg/mL) against *P. falciparum.* These analogues suggested that, planar β-carboline scaffold plays an important role in anti-malarial activity of manzamines [105].



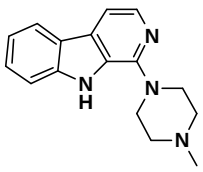
31a, IC_{50} = 0.64µM



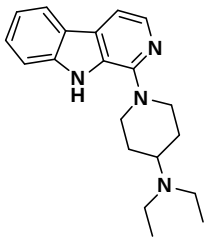
31b, IC_{50} = 0.42µM



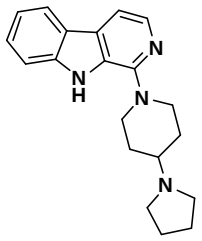
31c, IC_{50} = 0.45µM



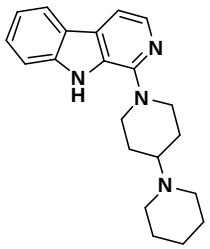
31d, IC_{50} = 0.62µM



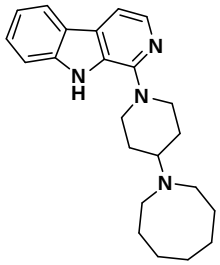
31e, IC_{50} = 0.41µM



31f, IC_{50} = 0.45µM



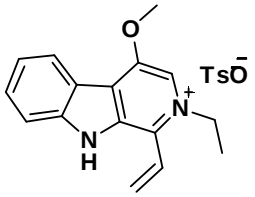
31g, IC_{50} = 0.39µM



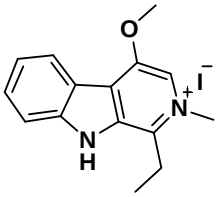
31h, IC_{50} = 0.35µM

In the same year, *Takasu et al.,* reported anti-malarial activity of simplified β-carboline derivatives and their quaternary salts. In these reported analogues, four quaternary salt derivatives (32a-d) displayed potent anti-malarial activity (IC_{50} 0.13, 0.95, 0.37 and 0.46

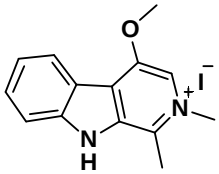
μg/mL, respectively) than their respective tertiary amine analogues and comparable potency with standard drug quinine (IC_{50} 0.11 μg/mL). This study concluded that, quaternization of pyridine nitrogen favors the anti-malarial activity [106].



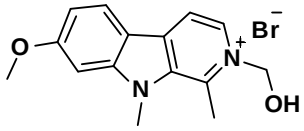
32a, IC_{50} = 0.13μM



32b, IC_{50} = 0.95μM

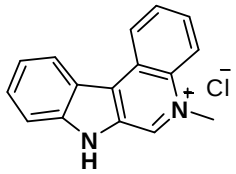


32c, IC_{50} = 0.37μM



32d, IC_{50} = 0.46μM

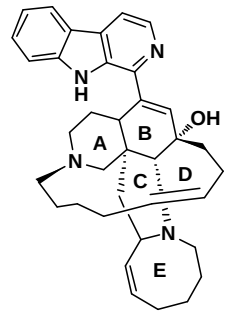
Miert et al., 2005, reported the anti-malarial activity of natural indoloquinoline alkaloids cryptolepine, neocryptolepine, isocryptolepine (δ-carboline derivatives) and their synthetic analogue 9-*N*-methyl-isocryptolepinium iodide and isoneocryptolepine (33). β-carboline alkaloid isoneocryptolepine (33) displayed potent inhibitory activity against *P. falciparum* (IC_{50} 0.23 μM). Further molecular mechanism studies suggested that, DNA intercalation and inhibition of β-hematin formation are plausible reasons for its anti-malarial activity [107].



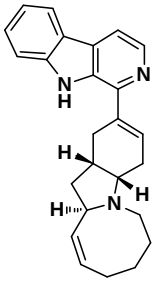
Isoneocryptolepine (33)

Manzamines are complex β-carboline alkaloids, displayed potent anti-malarial activity against drug sensitive and resistant strains, but associated toxicity is the major drawback with this class of alkaloids. Hence few attempts have made in the literature, to develop manzamine derivatives as potent anti-malarial agents with better therapeutic index. *Winkler et al.,* 2006 reported, some simplified analogues of manzamine-A (34-37) to study the effect of A, D ring presence, relative stereochemistry and orientation of β-carboline nucleus on anti-malarial potency. In these simplified synthetic analogues, compound 34 which has the both same relative stereochemistry and β-carboline ring orientation (C-10) as that of manzamine-A, displayed potent inhibition against D6 (IC_{50} 0.30 μg/mL) and W2 (IC_{50} 0.31 μg/mL) strains of *P. falciparum* respectively, although these analogues were produced significant decrease in anti-malarial potency. These results emphasized the importance of A and D rings and orientation of β-carboline ring on anti-malarial activity of manzamine-A

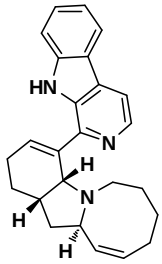
[108]. In a consequent study, anti-malarial activity of new synthetic manzamine analogues (38-42) consisting of monocyclic (B), bicyclic (AB and BC) and tetracyclic (ABCE) ring with β-carboline skeleton was discussed. The simplified manzamine analogues are ended with drastic reduction in anti-malarial potency [109]. In their subsequent studies, *Winkler et al., 2007,* reported the anti-malarial activity of new manzamine-A analogues (43-45) with lack of either or both of the D and E rings. In these new manzamine analogues, compounds 43 and 44 exhibited anti-malarial activity (IC_{50} 0.19 and 0.13 μM) against D6 strain but significantly less potent than manzamine-A. Results of these extensive studies advocated that, simplified structural analogues of manzamine-A could not serve as useful leads for the development of potent anti-malarial agents [110].



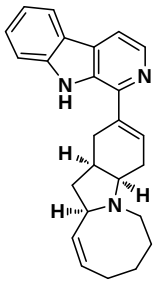
Manzamine-A (9)



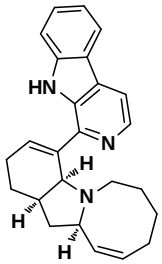
(34)



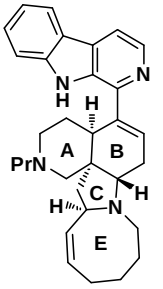
(35)



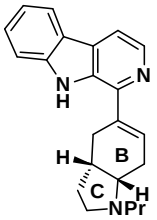
(36)



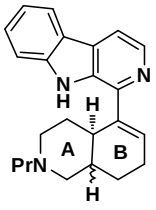
(37)



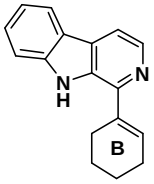
(38)



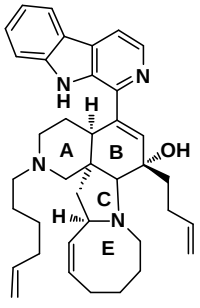
(39)



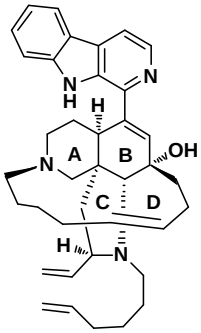
cisisomer: (40)
trans isomer: (41)



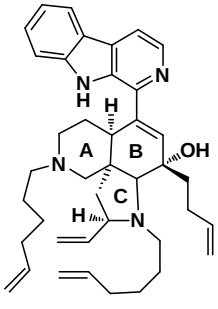
(42)



(43)

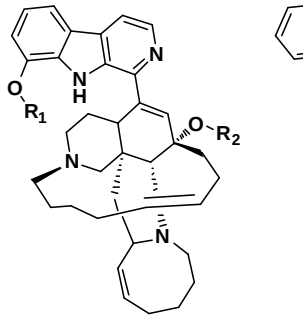


(44)

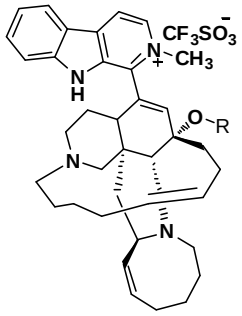


(45)

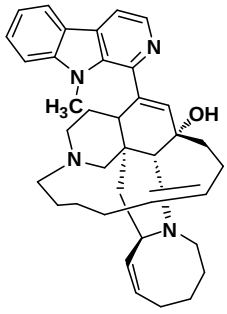
In their following studies, *Shilabin et al.* 2008 reported the anti-malarial activity of semi-synthetic derivatives of 8-hydroxymanzamine-A (10). In these *O*-substituted manzamine derivatives (46a-c), 8-acetyloxymanzamine-A (46a), 8-methoxymanzamine-A (46b) and 8, 12-di acetyloxymanzamine-A (46c) displayed potent inhibition against chloroquine sensitive D6 (IC_{50} 0.00930, 0.037 and 1.3 µg/mL, respectively) and resistant W2 (IC_{50} 0.030, 0.047 and 1.2 µg/mL, respectively) strains of *P. falciparum* respectively [111]. With their continuous interest to develop potent anti-malarial agents, *Ibrahim et al.,* reported biological evaluation of *N*-methyl manzamine-A derivatives (47a-b) such as, 2-*N*-methylmanzamine-A trifluoromethanesulfonate (47a), 2-*N*,12-*O*-dimethylmanzamine-A trifluoromethanesulfonate (47b) and 9-*N*-methylmanzamine-A (48) against *P. falciparum*. One derivative 2-*N*-methylmanzamine-A trifluoromethanesulfonate (47a) showed anti-malarial activity against D6 (IC_{50} 0.74 µg/mL) and W2 (IC_{50} 1.01 µg/mL) strains with comparable selectivity index to that of manzamine-A. Other 2-*N*,12-*O*-dimethylated (47b) and 9-*N*-methylated (48) derivatives of manzamine-A were devoid of anti-malarial activity [112].



$R_1 = COCH_3$, $R_2 = H$; (46a)
$R_1 = CH_3$, $R_2 = H$; (46b)
$R_1 = COCH_3$, $R_2 = COCH_3$; (46c)

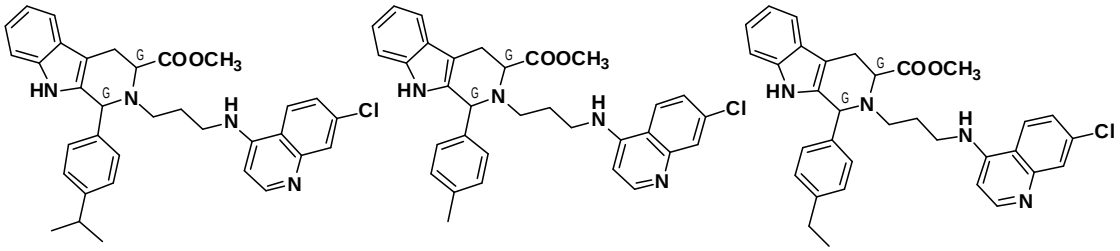


R = H; (47a)
$R = CH_3$; (47b)



(48)

Gupta et al., 2008 reported anti-malarial activity of synthetic β-carboline hybrid and 7-chloro-quinolin-4-ylamine hybrid molecules against chloroquine sensitive *P. falciparum* NF-54 strain. Seven analogues (49a-g) from these β-carboline hybrid molecules showed potent inhibition of *P. falciparum* (MIC ranges from 0.05 to 0.46 µM) and is comparable to that of chloroquine (MIC 0.39 µM) [113]. This study signifies the importance of linker length and stereochemistry at C-1 and C-3 centers of β-carboline derivatives on their anti-malarial activity as analogues with propyl linker have displayed potent activity.

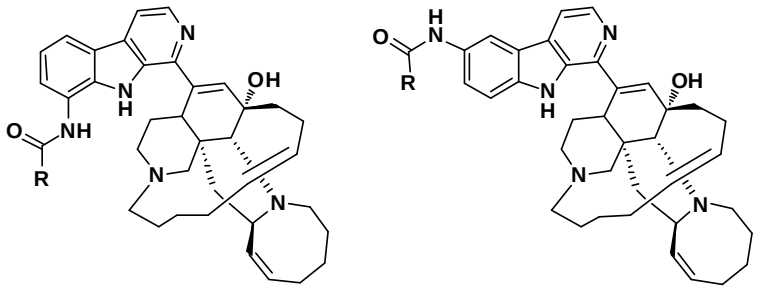


L/cis; (49a), IC_{50} = 0.22 μM
L/trans; (49b), IC_{50} = 0.22 μM
D/cis; (49c), IC_{50} = 0.22 μM
L/trans; (49d), IC_{50} = 0.05 μM

L/trans; (49e), IC_{50} = 0.46 μM
D/trans; (49f), IC_{50} = 0.06 μM

D/cis; (49g), IC_{50} = 0.11 μM

Wabha et al., 2009 reported anti-malarial activity of manzamine-A derivatives with amidation on position-6 and 8 of β-carboline moiety. Twenty, new manzamine-A derivatives were synthesized and evaluated for their anti-malarial activity. Among these reported manzamine-A derivatives, five analogues (50a-e) exhibited potent anti-malarial activity against D6 (0.035, 0.053, 0.032, 0.055, 0.034 μM, respectively) and W2 (0.121, 0.049, 0.065, 0.043, 0.053 μM, respectively) strains of *P. falciparum* and potency is comparable with manzamine-A with better selectivity. SAR analysis indicated that, bulky and cyclic groups adjacent to the amide carbonyl at position-6 favored the anti-malarial activity while, small and linear alkyl groups at position-8 provided the most active derivative [114].

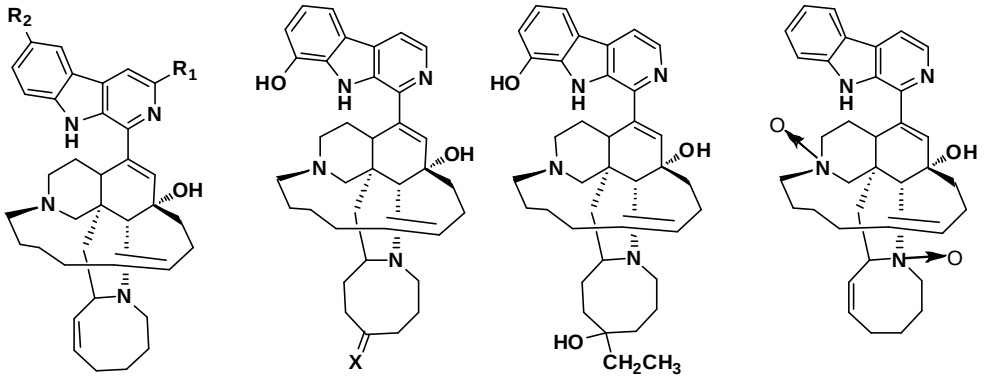


R = $CH_2(CH_2)_2CH_3$; (50a)
R = $CH_2(CH_2)_3CH_3$; (50b)
R = $CH_2(CH_2)_4CH_3$; (50c)
R = $CH_2(CH_2)_6CH_3$; (50d)

R = cyclohexyl; (50e)

Peng et al., 2010 reported anti-malarial activity of semi-synthetic manzamine-A derivatives, with substitutions like hydroxyl, nitro, alkyl and acetyl groups on β-carboline ring. Synthetic manzamine derivatives such as, manzamine-A-3-methyl ester (51), 6-nitromanzamine-A (52) and 6-methoxymanzamine-A (53) displayed potent anti-malarial activity against D6 (IC_{50} 0.011, 0.018 and 0.028 μg/mL, respectively) and W2 strains (IC_{50} 0.015, 0.028 and 0.058 μg/mL, respectively) of *P. falciparum,* but unfortunately these compounds also showed

elevated cytotoxicity (CC_{50} 0.2, 0.5 and 270 µg/mL, respectively). Synthetic manzamine-A derivatives having different substitutions on polycyclic ring system are also explored for anti-malarial activity. Manzamine-F (54) is a carbonyl derivative of potent anti-malarial alkaloid 8-hydroxymanzamine-A (10), exhibited weak anti-malarial activity. To understand the effect of carbonyl functional group on anti-malarial activity, manzamine-F derivatives were prepared mainly by modifying the carbonyl functionality into hydroxyl, hydrazone and alkyl groups. Manzamine-F-31-hydrazone (55), 31-Ethylmanzamine-F (56) and 21,27-*N*-oxamanzamine-A (57) showed potent inhibition against D6 (IC_{50} 0.029, 0.077 and 0.017 µg/mL, respectively) and W2 strains (IC_{50} 0.038, 0.086 and 0.067 µg/mL, respectively) of *P. falciparum*, indicated that carbonyl group on position-31 is not favorable for anti-malarial activity [115].



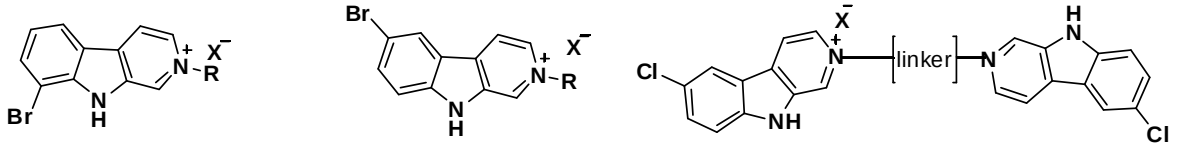
$R_1 = COOCH_3$, R_2 = H; (51)
R_1 = H, $R_2 = NO_2$; (52)
R_1 = H, $R_2 = OCH_3$; (53)

X = O; (54)
X = $NHNH_2$; (55)

(56)

(57)

Bonazzi et al., 2010, reported the synthesis and biological evaluation of nostocarboline derivatives (58), eudistomin derivatives (59) and eudistomin dimers (60) against tropical infections like malaria, leishmaniasis, trypanosomiasis and tuberculosis. Interestingly, bis-β-carboline derivatives showed significant increase in anti-malarial activity as compared to their respective monomers. These eudistomin dimer derivatives displayed significant to moderate inhibition against K1 strain of *P. falciparum* (IC_{50} ranges 0.018-6.738 µM) [116].

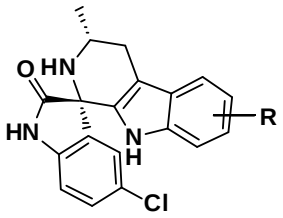


Nostocarboline derivatives (58)

Eudistomin derivatives (59)

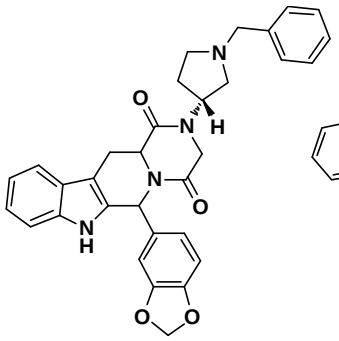
Eudistomin dimer (60)

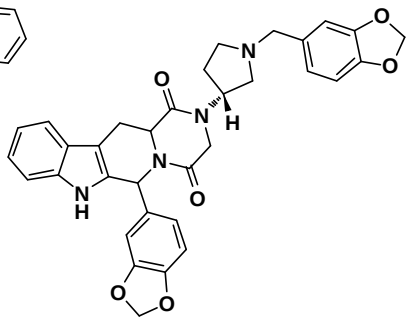
A new class of spirotetrahydro-β-carboline derivatives was developed with excellent anti-malarial activity by Novartis in 2010. Among the reported spirotetrahydro-β-carbolines, five compounds (61a-e) displayed potent anti-malarial activity (IC_{50} 9.0, 3.0, 4.0, 0.9 and 0.2 nM, respectively) against NF54 strain of *P. falciparum.* Interestingly, all the potent spiro-β-carboline derivatives have the same stereo orientation at C1 and C3 carbons, revealed that stereo chemistry at C1 and C3 (1*R*, 3*S*) has the significant effect on anti-malarial potency of these analogues [117]. Spirotetrahydro-β-carboline derivative (61d) has successfully completed phase-I clinical trial and presently it is undergoing phase-IIa clinical trials as anti-malarial agent [108, 118, 119].

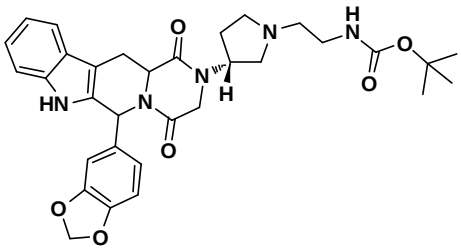


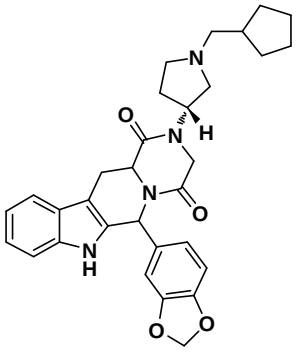
R = H, (61a)
R = 6-F, (61b)
R = 7-Cl, (61c)
R = 7-Cl, 6-F, (61d)
R = 6,7-di-F, (61e)

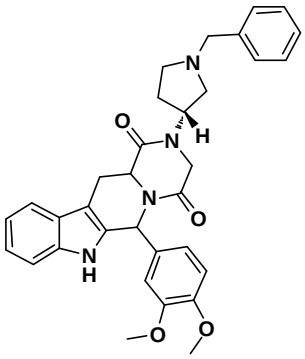
Beghyn et al., 2011, reported the anti-malarial activity of human PDE5 inhibitor tadalafil analogues based on drug to genome to drug approach. These novel tadalafil analogues displayed moderate to weak anti-malarial activity against chloroquine sensitive *P. falciparum* GHA strain. Compound 62a is the most active derivative and displayed potent inhibition (IC_{50} 0.5 µM) of the tested strain [120]. With their continuous interest to develop tadalafil analogues as anti-malarial agent with good selectivity index, they reported anti-malarial activity of tadalafil analogues by the replacement of piperonyl group with different bioisosteres. Five compounds (62b-f) of these new tadalafil analogues showed potent inhibition of plasmodium PDE5 (IC_{50} 0.53, 0.22, 0.25, 0.42 and 0.52 µM, respectively) with better selectivity. But few compounds showed good anti-malarial potency with poor selectivity between human and parasite PDE5 [121].

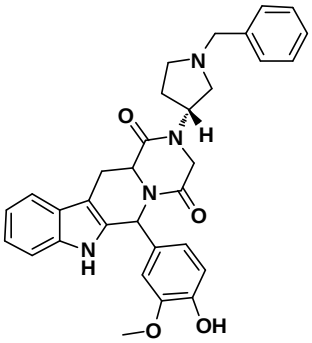

(62a)


(62b)

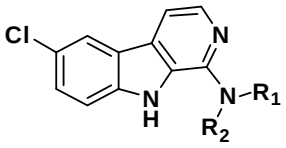

(62c)

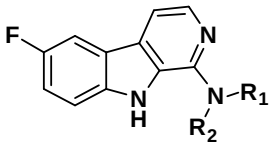

(62d)

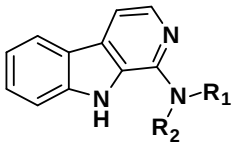

(62e)


(62f)

Thompson et al., 2012, reported biological evaluation of a novel series of 1-amino-6-halo-β-carboline derivatives as anti-malarial agents. These 1-amino-6-halo-β-carboline derivatives displayed moderate to weak inhibitory activity against 3D7 (IC_{50} ranges 0.15-10.2 µM) and D6 (IC_{50} ranges 0.24-4.95 µM) strains of *P. falciparum.* Among these reported β-carboline derivatives, 1-amino-6-chloro-β-carboline analogues (63a) displayed superior anti-malarial activity than respective 6-fluoro and un-substituted 1-amino-β-carboline analogues (63b, 63c), signifies the importance of chlorine substitution on β-carboline ring [61].

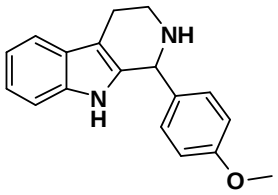

(63a)


(63b)

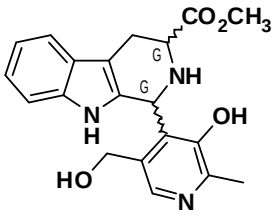

(63c)

In 2012, *Gellis et al.* reported the synthesis and anti-plasmodial activity of a novel series of 1-substituted-tetrahydro-β-carboline derivatives. Amongst reported analogues, *Para*-methoxy derivative (64) displayed significant inhibition of (IC_{50} of 0.7 µM) W2 strain of *P. falciparum* [122].

Brokamp et al., 2014, reported the synthesis of pyridoxal 1,2,3,4-tetrahydro-β-carboline derivatives and studied the effect of C1 and C3 stereo chemistry on their anti-malarial activity. Surprisingly, *trans*-isomers (65a-b) showed potent inhibition of *P. falciparum* (IC_{50} 8.0 and 22.0 µM) than respective *cis*-isomers (65c-d) (IC_{50} 108.0 and 91.0 µM), may be due to the better accommodation of axially located carboxylic group standing perpendicularly to the plane of the tetrahydro-β-carboline framework in the active site of the affected pyridoxal 5-phosphate (PLP) dependent enzymes of *P. falciparum* [66].



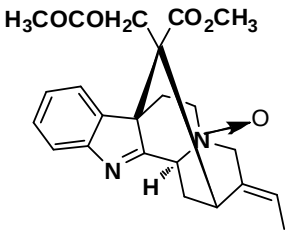
(64)



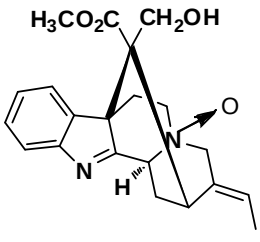
D/trans; (65a), L/trans; (65b)
D/cis; (65c), L/cis; (65d)

2.1.2. Anti-leishmanial activity

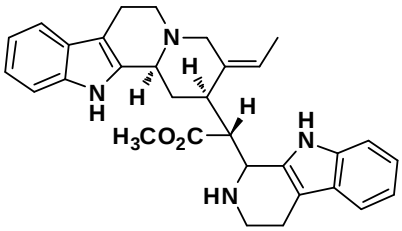
Kam et al., 1999, reported the isolation of two novel β-carboline alkaloids, akuammiline *N*(4)-oxide (66), 24,16-epideacetylakuammiline *N*(4)-oxide (67) from a plant *Kopsia grithii*, along with known β-carboline alkaloids harmane (4), buchtienine (68) [123] and their anti-leishmanial activity against *Leishmania donovani*. In these natural β-carboline alkaloids, buchtienine (68) displayed potent anti-leishmanial activity (IC_{50} 0.39 µg/mL) against the tested parasite [69]. Buchtienine (68) also isolated from bolivian plant *Peschiera buchtieni,* has been used for the treatment of leishmaniasis and identified as vital constituent for its anti-leishmanial activity [123].



Akuammiline *N*(4)-oxide (66)

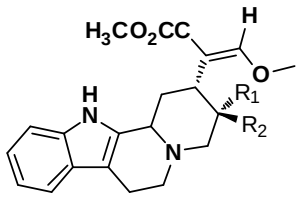


24,16-epideacetylakuammiline *N*(4)-oxide (67)



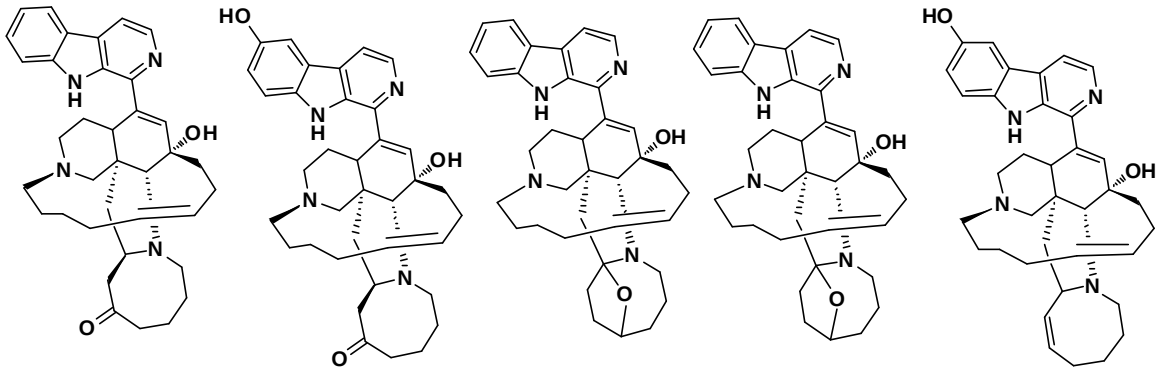
Buchtienine (68)

Staerk et al., 2000 reported the anti-leishmanial activity of five novel indole alkaloids, isolated from the bark of *Corynanthe pachyceras* (Rubiaceae). Three of these reported natural alkaloids, corynantheine (69a), dihydrocorynantheine (69b) and corynantheidine (69c) displayed significant inhibition of *L. donovani* promastigotes (IC_{50} 1.12, 1.65 and 2.81 μM, respectively) [62, 67]. Among the harmala group alkaloids, harmine (**3**) displayed potent anti-leishmanial activity (IC_{50} 3.7 μg/mL), and harmaline (**1**) showed weak activity (IC_{50} 116.8 μg/mL) against promastigotes of *L. donovani* [124].



R_1 = H, R_2 = C_2H_3; (69a)
R_1 = H, R_2 = C_2H_5; (69b)
R_1 = C_2H_5, R_2 = H; (69c)

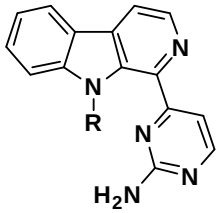
Natural manzamine alkaloids such as, Manzamine-A (9), 8-hydroxymanzamine-A (10), manzamine-A-*N*-oxide (11), manzamine-E (70), 6-hydroxymanzamine-E (71), manzamine-F (54), manzamine-X (72), 6-deoxymanzamine-X (73) and manzamine-Y (74) displayed significant to moderate anti-leishmanial activity (IC_{50} 0.9, 6.2, 1.1, 3.8, 2.5, 4.2, 5.7, 3.2 and 1.6 μg/mL, respectively) against promastigotes of *L. donovani* [90, 125].



Manzamine-E (70) 6-hydroxymanzamine-E (71) Manzamine-X (72) 6-deoxymanzamine-X (73) Manzamine-Y (74)

Novel pyrimidine-β-carboline alkaloid *N*-hydroxyannomontine (75) along with one previously reported annomontine (76) [126] was isolated from *Annona foetida* by *Costa et al.*, 2006 and evaluated for anti-leishmanial activity. These two pyrimidine-β-carboline

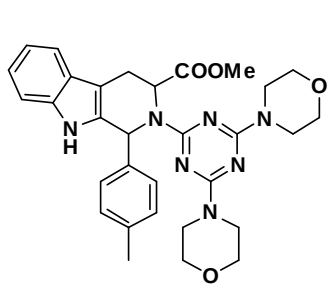
alkaloids, *N*-hydroxyannomontine (75) and annomontine (76) exhibited weak anti-leishmanial activity (IC_{50} 252.7 and 34.8 µM) against promastigotes of *L. braziliensis* [127].



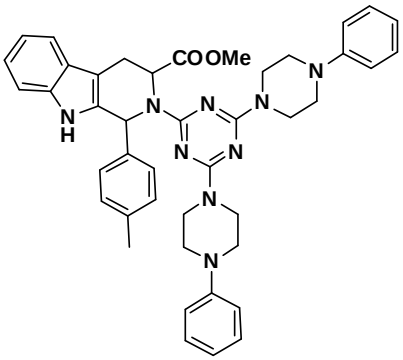
R = OH, *N*-hydroxyannomontine (75)
R = H, Annomontine (76)

Shilabin et al. 2008, reported the anti-leishmanial activity of 8-hydroxymanzamine-A (10) derivatives against promastigotes of *L. donovani*. In these analogues, 8-acetyloxymanzamine-A (46a) and 8-methoxymanzamine-A (46b) displayed moderate anti-leishmanial activity (IC_{50} 3.4 and 5 µg/mL), while 8, 12-diacetyloxy derivative (46c) exhibited weak anti-leishmanial activity against *L. donovani* (IC_{50} 16 µg/mL) [111].

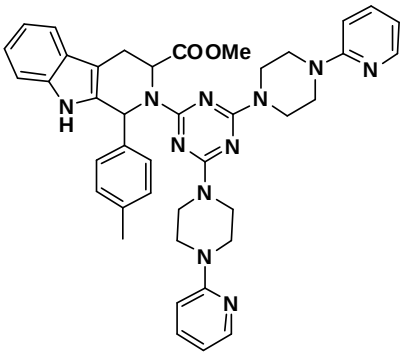
Kumar et al., 2006 reported the synthesis of a novel series of triazino-tetrahydro-β-carboline hybrid molecules and their *in-vivo* anti-leishmanial activity. Amongst the reported triazino-β-carboline hybrid molecules, compounds 77, 78 and 79 showed significant inhibition (78.6, 78.0 and 68.0%) of *L. donovani* at a dose of 50 mg/kg for 5 days [128].



(77)



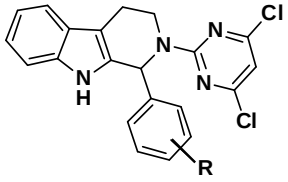
(78)



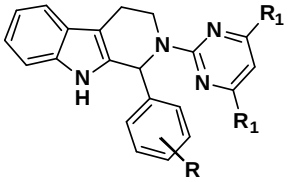
(79)

In their consequent studies, *Kumar et al.,* 2010 reported anti-leishmanial activity of a novel series of 2-(pyrimidin-2-yl)-tetrahydro-β-carboline derivatives against both promastigote and amastigote forms of *L. donovani*. In this pool of compounds, five compounds 80-84 exhibited potent inhibition (>90%) of promastigote forms at 10 µg/mL concentration. In

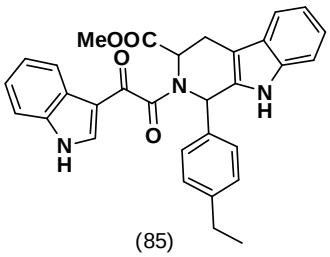
these five compounds, four compounds (80, 82-84) displayed significant anti-amastigote activity (IC_{50} 0.48, 0.49, 1.93 and 4.48 µg/mL, respectively) and one compound (81) remained inactive against amastigotes of *L. donovani* [129]. In their successive studies, *Chauhan et al.,* 2010 reported a novel series of indolyl-β-carboline hybrid molecules and their anti-leishmanial activity against amastigote forms of *L. donovani*. Among these reported indolyl glyoxylamide derivatives, compound 85 displayed potent inhibition of amastigotes (IC_{50} 5.17 µM) than standard drug pentamidine (IC_{50} 20.43 µM) with good selectivity index of 31.48 [130].



R = 3,4-dimethoxy (80)
R = 3,5-dimethoxy (81)

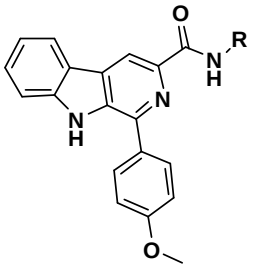


For
(82) R = 3,4-dioxymethylene, R_1= *N*-methylpiperazine
(83) R = 3,4-dioxymethylene, R_1= *N*-ethylpiperazine
(84) R = 3,4-dimethoxy, R_1= morpholine



(85)

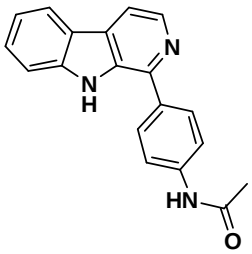
A novel series of 1-substituted-β-carboline-3-carboxamide derivatives were synthesized and evaluated for their anti-leishmanial activity. 1-(4-methoxy-phenyl) derivative with *N*-butylamido substitution (86a) produced the most active (IC_{50} 0.25 µM) analogue of this series of compounds against promastigotes of *L. amazonensis* [131]. In a subsequent study, *Pedroso et al.,* 2011 reported anti-leishmanial activity of β-carboline-3-carboxamide derivatives with different alkyl substitutions on amide nitrogen. In these reported analogues, three amido derivatives 86b-d exhibited potent anti-leishmanial activity against promastigote (IC_{50} 2.6, 3.6 and 5.3 µM, respectively) and amastigote (IC_{50} 1.0, 1.6 and 0.5 µM, respectively) forms of *L. amazonensis* [132]. In their subsequent studies, they reported exact mechanism of compound 86a for its potent anti-leishmanial activity. The exact mechanism was found to be, it impairs cytokinesis, thus preventing proliferation of the parasite [133].



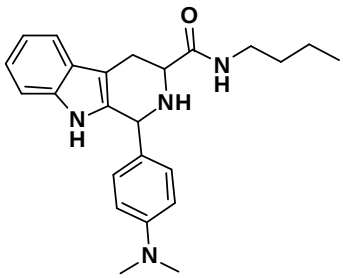
R = n-butyl; (86a)
R = benzyl; (86b)
R = cyclohexyl; (86c)
R = isopropyl; (86d)

Gohil et al., 2012 reported the synthesis of 1-(substituted-aryl)-β-carboline derivatives and their biological evaluation against promastigotes of *L. donovani*. Few analogues from this series of compounds displayed comparable activity with standard anti-leishmanial drug miltefosine (IC_{50} 12.07 μM) and moreover, most active compound 87 exhibited nearly six times (IC_{50} 2.16 μM) potent activity than standard drug [134].

Nakamura group, with their continuous interest to evaluate β-carbolines as anti-leishmanial agents, *Volpato et al.,* 2013 reported *in-vitro* inhibitory activity of *N*-butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide 88 against both promastigote and amastigote forms of *L. amazonensis.* This previously reported anti-trypanosomal agent exhibited moderate activity against promastigotes (IC_{50} 16.0 μM) and axenic amastigotes (IC_{50} 16.3 μM) of *L. amazonensis* and mechanistic studies suggested that, it might interrupted parasite mitochondrial functions [135].



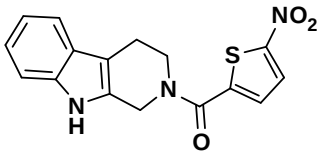
(87)



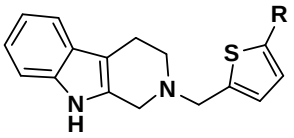
(88)

Recently *Manda et al.,* 2014 reported the anti-leishmanial activity of 2-substituted tetrahydro-β-carbolines against both promastigote and amastigote forms of *L. donovani*. Amongst the reported analogues, compounds 89, 90a, 90b exhibited moderate inhibition of

promastigote forms (IC_{50} 12.7, 9.1 and 22.1 µM, respectively) and one compound 89 showed weak activity (IC_{50} 87.6 µM) against amastigote forms [136].

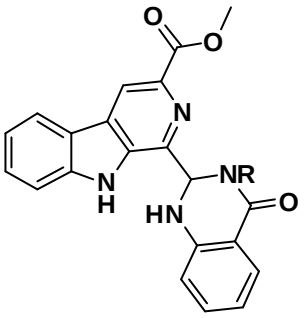


(89)



R = Cl; (90a)
R = $C_{10}H_7$; (90b)

Chauhan et al., 2015 reported synthesis and biological evaluation of novel β-carboline-quinazolinone hybrid molecules as *L.donovani* Trypanothione Reductase inhibitors. Among these hybrid molecules, three compounds 91a-c showed potent anti-leishmanial activity (IC_{50} 4.4, 6.0 and 4.3 µM respectively) against amastigotes of *L.donovani* and significant inhibition of tryptathione reductase inhibition (64, 81 and 77%, respectivley) at 50 µM concentration [137].



R = 4-Clph; (91a)
R = 4-OCH_3phCH_2; (91b)
R = 3,4-diClphCH_2; (91c)

2.1.3. Anti-trypanosomal activity

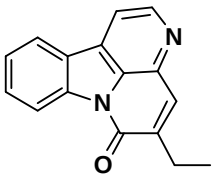
With their potent inhibitory activity against parallel protozoal parasites like *Plasmodium* and *Leishmania,* few numbers of natural as well as synthetic β-carboline alkaloids were evaluated against *Trypanosoma* parasite, responsible for infection known as trypanosomiasis. Among the harmala group alkaloids, harmine (3) showed moderate activity against both the nifurtimox-sensitive (Tulahuen) and resistant (LQ strain) strains (IC_{50} 19.69 and 17.99 µM), relatively nifurtimox-resistant LQ strain was more sensitive to harmine [138].

Boursereau et al., 2004, reported anti-trypanosomal activity of 1-amino β-carboline derivatives against *T. b. rhodesiense* and *T. cruzi* along with their anti-malarial activity. Among the reported analogues, seven compounds (31a-f, 31h) displayed potent inhibition (IC_{50} ranges 0.09-0.63 μg/mL) of *T. b. rhodesiense* and one compound, 36a showed moderate activity against *T. cruzi* (IC_{50} 1.62 μg/mL) [105].

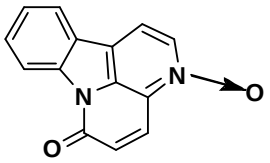
Ferreira et al., 2007, reported the isolation, *in-vivo* anti-trypanosomal activity of canthin-6-one (12), 5-methoxycanthin-6-one (92) and canthin-6-one-*N*-oxide (93) from the bark of *Zanthoxylum chiloperonestem*. In these natural canthine alkaloids, canthin-6-one has significantly (>90%) reduced the parasiteamia in both acute and chronic infections with dose of 5 mg/kg/day for 2 weeks [139].

In their successive studies, Nakamura group reported the anti-trypanosomal activity of cis and trans isomers of methyl 1-(*m*-nitro)phenyl (94a-b) and 1-(*p*-nitro)phenyl-1,2,3,4-tetrahydro-9*H*-β-carboline-3-carboxylates (95a-b). Amongst reported, four tertrahydro-β-carboline derivatives, trans-methyl 1-(*m*-nitro)phenyl-1,2,3,4-9*H*-tetrahydro-β-carboline-3-carboxylate (94b) displayed trypanocidal activity against epimastigotes of *T. cruzi* (IC_{50} 22.2 μM). Theoretical calculations such as molecular conformations and electronic properties suggested that, mulliken charges on C-1 and electronic potential on nitro group are playing important role in anti-trypanosomal potency of these reported analogues [140].

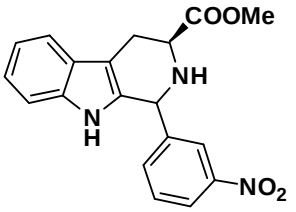
With their continuous efforts in biological evaluation of β-carboline derivatives by Nakamura group, *Valdez et al.,* 2009 reported anti-trypanosomal activity of 1,2,3,4-tetrahydro-β-carboline-3-carboxamide derivatives. From this group of 1,2,3,4-tetrahydro-β-carboline-3-carboxamide derivatives, compound 88 displayed inhibition of epimastigote, trypomastigote and amastigote forms of *T. cruzi* (IC_{50} 14.9, 45 and 33 μM, respectively) [141]. In the following study of *in-vivo* anti-trypanosomal activity evaluation, compound 88 showed weak *in-vivo* anti-trypanosomal activity at dose of 100mg/kg, but surprisingly in combination with benznidazole has shown synergistic inhibition of trypomastigote [142].



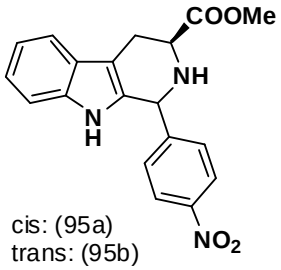
(92)



(93)



cis: (94a)
trans: (94b)

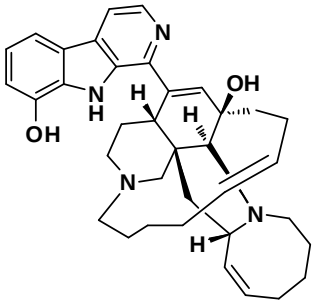


cis: (95a)
trans: (95b)

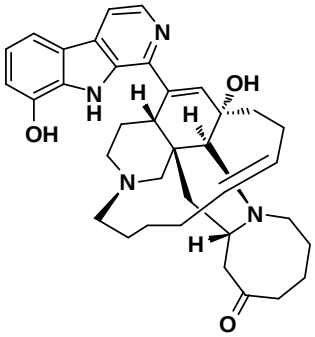
Costa et al., 2011 reported anti-trypanosomal activity of pyrimidine-β-carboline alkaloid annomontine (75) against epimastigote and trypomastigote forms of *T.cruzi*. Annomontine (75) displayed potent inhibition of trypomastigote (IC_{50} 4.2 µg/mL) than standard crystal violet (IC_{50} 12.8 µg/mL) but weak inhibiton against epimastigotes (IC_{50} 198.0 µg/mL) of *T.cruzi* [143].

2.1.4. Anti-toxoplasmal activity

Even though many of natural as well as synthetic β-carboline derivatives were exhibited potent protozoal activity, only few natural β-carboline alkaloids were evaluated against fellow protozoan *Toxoplasma.* In natural β-carboline alkaloids, manzamine-A (9) and *ent*-8-hydroxymanzamine-A (96) exhibited potent inhibition against *Toxoplasma gondii* with 70 and 71% parasite inhibition at 0.1 and 1 µM concentration respectively. *ent*-manzamine-F (97) showed 37% inhibition of parasite growth at 10 µM. Manzamine-A also showed *in-vivo* anti-toxoplasmal activity and increased the survival time of infected mice to 20 days (16 days of control) with i.p. dose of 8 mg/kg for 8 days [144].



ent-8-hydroxymanzamine-A (96)



ent-manzamine-F (97)

Alomar et al., 2013, reported *in-vitro* anti-toxoplasmal activity of harmala alkaloids, harmine (3), harmane (4) and norharmane (7). In the tested alkaloids, norharmane displayed highest

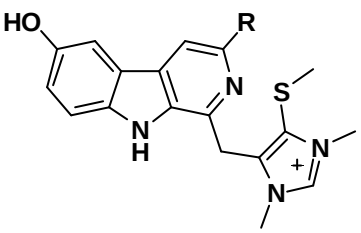
parasiticidal effect at 2.5 µM whereas harmane and harmine have shown maximum effect at 40 µM [145].

2.2. Anti-bacterial and anti-fungal activity

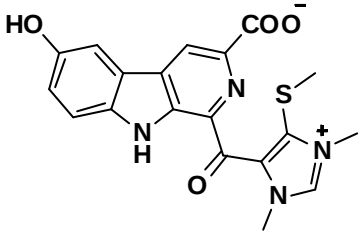
In 1991, *Prinsep et al.* reported anti-microbial activity of harmane (4) against gram (+)ve bacteria, *Bacillus subitilis* (MIC 7.5 µg/mL) and fungi *Candida albicans* (MIC 1.9 µg/mL) [146]. Aassila et al., 2003, evaluated harmane (4) against gram (-)ve bacteria *Vibrio anguillarum* and MIC was found to be 0.017 mM [147]. Other harmala alkaloids, harmine (3) and harmaline (1) exhibited inhibition activity against *Escherichia coli* (MIC 0.75 and 1.0 mg/mL)*, Proteus vulgaris* (MIC 0.83 and 0.75 mg/mL), *Staphyllococcus aureus* (MIC 1.0 and 0.75 mg/mL), *B. subitilis* (MIC 0.75 and 0.83 mg/mL), *Asperagillus niger* (MIC 0.66 and 1.0 mg/mL) and *Candida albicans* (MIC 0.50 and 0.66 mg/mL) [148].

Saify et al., 2005, evaluated the anti-bacterial activity of harmaline (1) and its synthetic derivative against both gram (+)ve and gram (-)ve bacteria. Natural β-carboline alkaloid harmaline (1) displayed inhibition against gram (+)ve bacteria, *B. subtilis* with zone of inhibition (20 mm) and gram (-)ve bacteria *Klebsiella pneumoniae* (17 mm). All these reported synthetic derivative of harmaline exhibited weak anti-bacterial activity [149]. β-carboline alkaloid norharmane (7) isolated from marine bacterium NJ6-3-1 and screened for inhibitory potency against gram (+)ve and gram (-)ve bacteria. Norharmane (7) showed inhibition of gram (+)ve bacteria, *B. subtilis* (zone of inhibition ≥5 mm) and gram (-)ve bacteria *K. pneumoniae* (zone of inhibition ≥5 mm) [150].

A novel imidazolyl-β-carboline alkaloid gesashidine-A (98a), isolated from an Okinawan marine sponge of the family *Thorectidae* along with two known alkaloids dragmacidonamines-A (98b) and B (99). Among these natural alkaloids, gesashidine-A showed anti-bacterial activity against *Micrococcus luteus* (MIC 16.6 µg/mL) without any cytotoxicity [151].

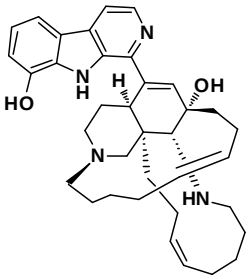


R = H; (98a)
R = COO⁻; (98b)

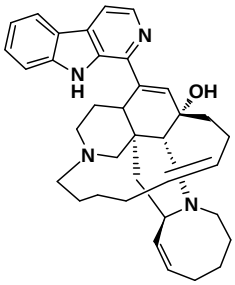


(99)

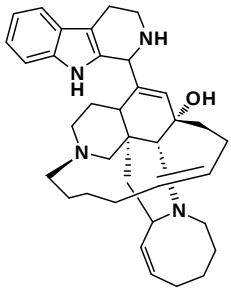
In manzamine alkaloids, manzamine-A (9), manzamine-A-*N*-oxide (11) and manzamine-X (72) exhibited potent inhibition of *S. aureus* (MIC 0.5, 0.5 and 1.0 µg/mL, respectively) and methicillin-resistant *S. aureus* (MIC 0.7, 0.4 and 0.75 µg/mL, respectively) while 8-hydroxymanzamine-A (10) displayed potent inhibition of *S. aureus* (MIC 0.9 µg/mL) and moderate activity against methicillin-resistant *S. aureus* (MIC 4.0 µg/mL). 8-hydroxymanzamine-J (100), and 6-deoxymanzamine-X (73) showed moderate inhibition of *S. aureus* (MIC 3.0 and 1.5 µg/mL) and methicillin-resistant *S. aureus* (MIC 5.0 and 1.5 µg/mL). These potent anti-bacterial manzamine alkaloids are also displayed moderate to weak anti-fungal activity [125]. *Wahba et al.,* 2009 reported anti-fungal activity of synthetic manzamine-A derivatives, amongst reported compounds, 8-*N*-octamidomanzamine-A (50d) exhibited potent inhibition of *Cryptococcus neoformans* (MIC 1.01 µg/mL) better than manzamine-A (9) (1.8µg/mL) [114]. Semi-synthetic manzamine derivatives, 32,33-dihydromanzamine-A (101), 1,2,3,4-tetrahydromanzamine-A (102) and manzamine-F-31-hydrazone (55) displayed potent anti-fungal activity against *C. neoformans* with MIC 0.9, 0.8 and 1.0 µg/mL respectively [115]. Maeganedin-A (103), a new manzamine-related tetrahydro-β-carboline alkaloid with a methylene carbon bridge between *N*-2 and *N*-27, was isolated from an Okinawan marine sponge *Amphimedon sp*. Maeganedin-A (103) displayed anti-bacterial activity against *Sarcina lutea*, *B. subtilis* and *Corynebacterium xerosis* (MIC 2.8, 2.8 and 5.7 µg/mL, respectively) [152].



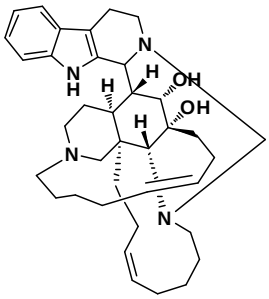
8-hydroxymanzamine-J (100)



32,33-dihydromanzamine-A (101)

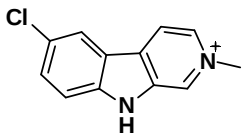


1,2,3,4-tetrahydro manzamine-A (102)

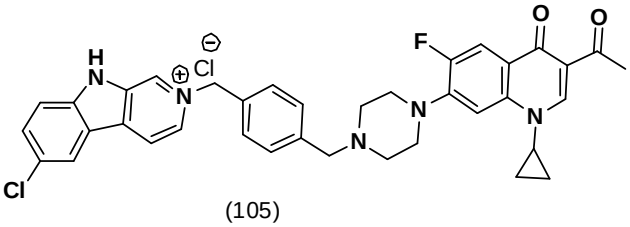


Maeganedin-A (103)

Natural β-carboline alkaloid nostocarboline and its synthetic derivatives were reported for inhibitory potential against bacterial strains *Microcystis aeruginosa*, *Synechococcus* and *Kirchneriella contorta*. Amongst the tested compounds, nostocarboline (104) exhibited equipotent activity against all the tested strains with IC_{50} 1 µM. Interestingly nostocarboline/ciprofloxacin hybrid (105) molecule displayed increased potency against *M. aeruginosa*, *Synechococcus, K. contorta* (IC_{50} 1, 1 and 10 µM, respectively) than ciprofloxacin (IC_{50} 1, 10 and >100 µM, respectively) [153].

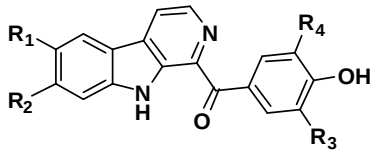


(104)



(105)

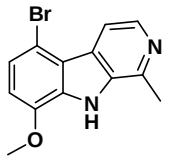
Eudistomins are another important group of β-carboline alkaloids, containing an oxathiazepino-tetrahydro-β-carboline ring system and initially isolated from the Caribbean tunicate of *Eudistoma olivaceum* [154, 155]. These eudistomin alkaloids were isolated from different tunicates and evaluated for anti-viral (discussed under the heading of anti-viral activity) and anti-bacterial activities. *Wang et al.,* 2004, isolated new β-carboline alkaloids, eudistomins Y_{1-7} (106a-g) from a Korean tunicate *Eudistoma* sp. and evaluated for anti-bacterial activity against gram (+)ve and gram (-)ve bacteria. In these natural alkaloids, eudistomins Y_6 (106f) showed inhibition of gram (+)ve bacteria *Staphylococcus epidermidis* (MIC 12.5 µg/mL) and *B. subtilis* (MIC 25µg/mL). Eudistomins Y_1 (106a), Y4 (106d) displayed weak antibacterial activity against *S. epidermidis* and *B. subtilis* (MIC 50 and 200µg/mL) and none of the compounds exhibited activity against gram (-)ve bacterial strains *E. coli* and *Salmonella typhimurium* [156].



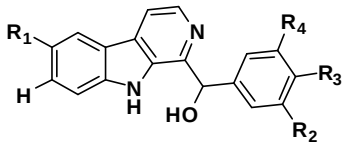
R_1 = H, R_2 = H, R_3 = H, R_4 = H; (106a)
R_1 = Br, R_2 = H, R_3 =H, R_4 = H; (106b)
R_1 = H, R_2 = H, R_3 = Br, R_4 = H; (106c)
R_1 = Br, R_2 = H, R_3 =Br, R_4 = H; (106d)
R_1 = H, R_2 = H, R_3 =Br, R_4 = Br; (106e)
R_1 = Br, R_2 = H, R_3 = Br, R_4 = Br; (106f)
R_1 = H, R_2 = Br, R_3 = Br, R_4 = Br; (106g)

A new β-carboline alkaloid, 5-bromo-8-methoxy-1-methyl-β-carboline (107) isolated from the New Zealand marine bryozoans *Pterocella vesiculosa* and evaluated for anti-microbial activity. This novel β-carboline alkaloid displayed moderate inhibition against *Bacillus subtilis,* fungi *Candida albicans* and *Trichophyton mentagrophytes* (MIC 3, 4.5 and 4.5 µg/mL, respectively) [157].

Won et al., 2012 reported anti-microbial activity of natural eudistomins Y_{2-7} (106b-g) and their semi-synthetic derivatives such as eudistomin Y_{8-13} (108a-f) against gram (+)ve *Staphylococcus aureus*, *B. subtilis*, *M. luteus*, and gram (-)ve *S. typhimurium*, *P. vulgaris*, *E. coli* bacteria. Natural alkaloids eudistomins Y_{4-7} (106d-g) displayed potent inhibition of gram (-)ve bacteria *S. typhimurium*, *P. vulgaris* (MIC ranges 0.39-1.56 µg/mL) and potent to moderate activity against gram (+)ve bacteria *S. aureus*, *B. subtilis*, *M. luteus* (MIC ranges 0.39-6.25 µg/mL). Comparatively, semi-synthetic derivatives have exhibited decreased anti-microbial activity against all the tested strains. In semi-synthetic analogues, eudistomin Y_{11} (108d) displayed moderate inhibition activity against *B. subtilis, M. luteus, S. typhimurium, P. vulgaris* with MIC 3.125 µg/mL and *S. aureus* (MIC 6.25 µg/mL). Eudistomin Y_{10} (108c) exhibited weak activity against *M. luteus, S. typhimurium, P. vulgaris* (MIC 12.5 µg/mL) and *S. aureus and B. subtilis* (MIC 25 µg/mL). Unfortunately, none of these eudistomins displayed anti-fungal activity against the tested *Aspergillus fumigatus*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Candida albicans* strains [158].



(107)

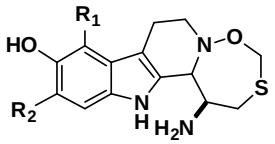


R_1 = H, R_2 = H, R_3 = OH, R_4 = H; (108a)
R_1 = Br, R_2 = Br, R_3 = OH, R_4 = H; (108b)
R_1 = H, R_2 = Br, R_3 = OH, R_4 = Br; (108c)
R_1 = Br, R_2 = Br, R_3 = OH, R_4 = Br; (108d)
R_1 = Br, R_2 = Br, R_3 =O-Tos, R_4 = Br; (108e)
R_1 = Br, R_2 = H, R_3 = H, R_4 = H; (108f)

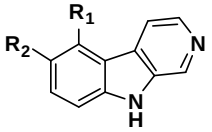
2.3. Anti-viral activity

As we discussed earlier, eudistomins are oxathiazepino-tetrahydro-β-carboline alkaloids, currently more than 50 alkaloids from this group have been reported from the genus *Eudistoma*. Amongst the eudistomin alkaloids, eudistomin C and E (109 & 110) with a

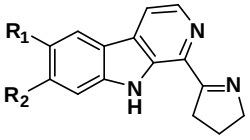
phenolic hydroxyl group displayed potent anti-viral activity at concentration 5-10 ng/disk. Eudistomins D, H, K, L, N and P (111-116) showed activity at L (100 ng), K (250 ng), D, H, N and P (500 ng) per disk against herpes simplex virus (HSV-1) [154, 155, 159]. Optimistic anti-viral potency of these natural eudistomins, encouraged the evaluation of semi-synthetic derivatives of eudistomin as anti-viral agents, but unfortunately semi-synthetic analogues exhibited decreased anti-viral activity than natural eudistomin alkaloids.



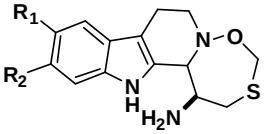
R_1 = H, R_2 = Br; (109)
R_1 = Br, R_2 = H; (110)



R_1 = Br, R_2 = OH; (111)
R_1 = H, R_2 = Br; (115)

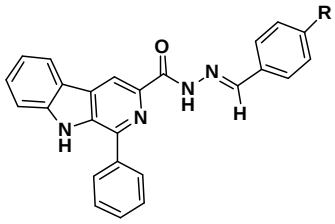


R_1 = Br, R_2 = H; (112)
R_1 = OH, R_2 = Br; (116)

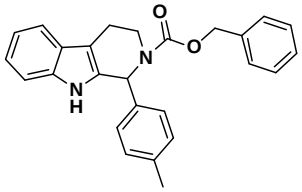


R_1 = H, R_2 = Br; (113)
R_1 = Br, R_2 = H; (114)

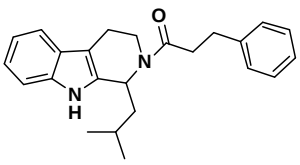
Ormagio et al., 2009 reported synthesis and anti-viral activity of 1,3-disubstituted-β-carboline derivatives against poliovirus and Herpes Simplex Virus-1(HSV). In these reported 3-hydrazino substituted β-carbolines, two compounds 117a-b displayed potent anti-viral activity against both poliovirus (IC_{50} 0.87 and 0.87 µM) and HSV-1 virus (IC_{50} 0.47 and 1.85 µM) [160]. *Miller et al.,* 2010 reported synthesis and biological evaluation of 1,2 disubstituted-tetrahydro-β-carboline derivatives against human papilloma virus. Amongst the reported tetrahydro-β-carboline derivatives, two compounds 118 and 119 exhibited potent inhibition of human papilloma virus (IC_{50} 0.9 and 0.33 µM) [161].



R = OCH_3; (117a)
R = $N(CH_3)_2$; (117b)



(118)



(119)

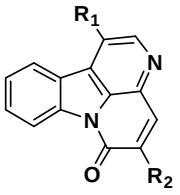
Anti-viral activity of natural harmala alkaloids and their semi-synthetic derivatives against tobacco mosaic virus (TMV) is reported by *song et al.,* 2014. Among these reported derivatives, natural harmala alkaloids, harmine (3), harmane (4), tetrahydroharmine (6) and harmalan (8) displayed significant inhibition of TMV (% inhibition 44.6, 54.5, 63.7 and 58.6,

respectively) than standard drug ribavirin (% inhibition 40) at 500 ng/mL concentration [162].

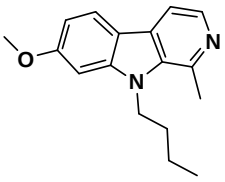
2.3.1. Anti-HIV activity

Cathin-6-one alkaloids, 1-methoxycanthinone (120a) and 5-methoxycanthinone (120b) are isolated from *Leitneria floridana* and evaluated for anti-HIV activity by *Xu et al.,* 2000. In these two previously reported β-carboline alkaloids, 1-methoxycanthinone (120a) displayed potent anti-HIV activity (IC_{50} 0.256 µg/mL) without any cytotoxicity at 100 µg/mL against H9 cell line [163]. *Ishida et al.,* 2001 evaluated the anti-HIV activity of harmine (3) and its semi-synthetic derivatives. Natural alkaloid harmine exhibited moderate anti-HIV activity (IC_{50} 10.7 µM) and among its synthetic derivatives, compound (121) exhibited potent anti-HIV activity (IC_{50} 0.037 µM) than standard drug zidovudine (IC_{50} 0.045 µM) [75].

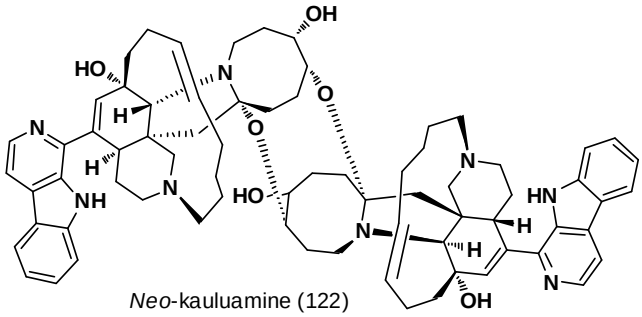
In manzamine group alkaloids, 8-hydroxymanzamine-A (10) exhibited potent anti-HIV activity (IC_{50} 0.59 µM), manzamine-A (9), manzamine-F (54), manzamine-X (72), 6-deoxymanzamine-X (73) and neokauluamine (122) have displayed moderate activity (IC_{50} 4.2, 7.3, 2.3, 1.6 and 2.3 µg/mL, respectively) [89, 125, 164].



$R_1 = OCH_3$, R_2 = H, (120a)
R_1 = H, $R_2 = OCH_3$, (120b)

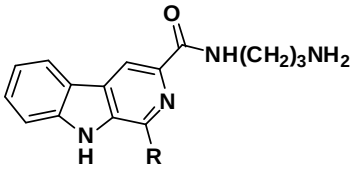


(121)

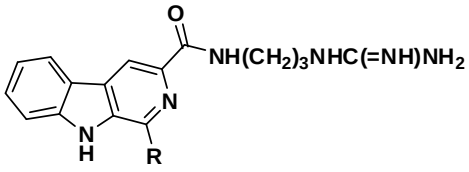


Neo-kauluamine (122)

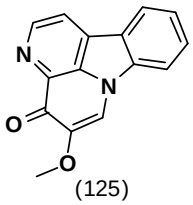
Yu et al., 2004 reported the synthesis, Tat–TAR inhibitory properties of 1,3-disubstituted-β-carboline derivatives as anti-HIV agents. Four compounds (123a-b and 124a-b) displayed potent inhibition of Tat-TAR interaction but none of the compounds showed *in-vitro* HIV cell line inhibition at 100 µM concentration [165]. In the same year, one novel β-carboline alkaloid drymaritin (125) isolated from *Drymaria diandra* and evaluated for anti-HIV activity. This new β-carboline alkaloid exhibited potent anti-HIV activity (IC_{50} 0.699 µg/mL) with selectivity index 20.6 [166].



R = H; (123a)
R = CH_3; (123b)



R = H; (124a)
R = CH_3; (124b)

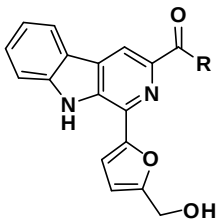


(125)

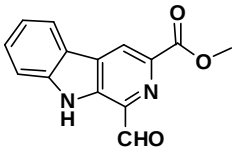
Wang et al., 2007 reported anti-HIV activity of natural β-carboline alkaloid flazin (126a) and its semi-synthetic amido derivative flazinamide (126b). Interestingly, natural alkaloid flazin exhibited moderate anti-HIV activity (IC_{50} 2.37 µM) while flazinamide showed potent activity against HIV-1_{IIIB}, HIV-2_{ROD} and HIV-2_{CBL-20} (IC_{50} 0.38, 0.57 and 0.89 µM, respectively). Flazinamide might interfere in the early stage of HIV life cycle, through preventing the entry of HIV into the host cell, and also showed weak inhibition of HIV-1 reverse transcriptase, protease or integrase [167]. In their subsequent studies, reported anti-HIV activity of semi-synthetic flazinamide analogues with different substitutions on various positions of β-carboline and furan ring. These synthetic analogues displayed significant to moderate activity but comparatively less potent than flazinamide. SAR studies concluded that, substitutions at position-3, 1' and 5' of flazin have considerable effect on their anti-HIV potency [168].

Brahmbhatt et al., 2010 reported the synthesis and biological evaluation of 1,3,9-trisubstituted-β-carboline derivatives as anti-HIV agents. Amongst the reported β-carboline derivatives, 1-formyl-β-carboline-3-carbxylic acid methyl ester (127) displayed moderate anti-HIV activity (IC_{50} 2.9 µM) [169].

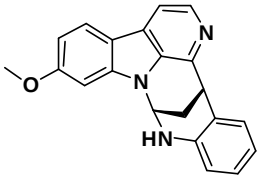
A novel β-carboline alkaloid trigonoine-A (128), isolated from the leaves of *Trigonostemon lii* along with one quinoline alkaloid by *Li et al.,* 2011 and structural elucidation has done using 1D and 2D NMR data. This novel trigonoine A, displayed weak anti-HIV activity (IC_{50} 13.1 µg/mL) with selectivity index 1.28 [170].



R = OH; (126a)
R = NH_2; (126b)



(127)

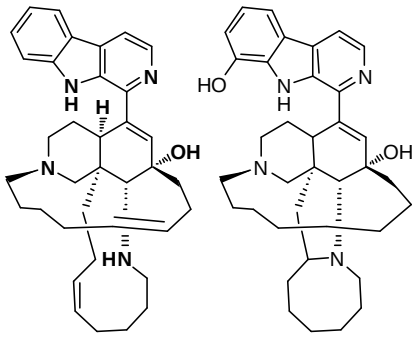


(128)

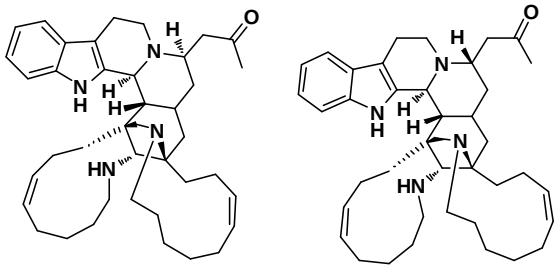
2.4. Anti-tubercular activity

Versatile manzamine alkaloids are evaluated for anti-tubercular activity and many of these alkaloids have displayed potent inhibition of *Mycobacterium tuberculosis* with MIC value below 6.25 µg/mL concentration. Among the natural manzamine alkaloids, manzamine-A (9), D (103), E (70), F (54), J (129), Y (74), 8-hydroxymanzamine-A (10), *ent*-8-hydroxymanzamine-A (96), manzamine-A-*N*-oxide (11), 6-hydroxymanzamine-E (71), 6-deoxymanzamine-X (73) and *neo*-kauluamine (122) exhibited potent inhibiton of *M. tuberculosis* (H37Rv) with MIC values 1.53, 0.99, 3.76, 2.56, 1.70, 5.20, 0.90, 3.10, 3.90, 0.40, 1.80 and 2.00 µg/mL, respectively and anti-tubercular potency of manzamine alkaloids is comparable with standard drug rifampicin 0.5 µg/mL [89, 125, 171]. Besides these natural manzamine alkaloids, semi-synthetic manzamine analogues such as, 15,16,32,33-tetrahydro-8-hydroxymanzamine-A (130), 6-nitromanzamine-A (52), manzamine-F-31-hydrazone (55) and 31-Ethylmanzamine-F (56) also displayed anti-tubercular activity with MIC values 1.84, 1.60, 1.90 and 1.90 µg/mL, respectively [115, 172].

Manadomanzamine are another group of β-carboline alkaloids and are structurally related to manzamines. Two manadomanzamine alkaloids namely manadomanzamine-A & B (131-132), isolated from an Indonesian sponge *Acanthostrongylophora* sp. (*Haplosclerida: Petrosiidae*) by *Peng et al.,* 2003. These manadomanzamine alkaloids showed potent inhibition of *M. tuberculosis* (MIC 1.9 and 1.5 *µ*g/mL) [173].



(129) (130)

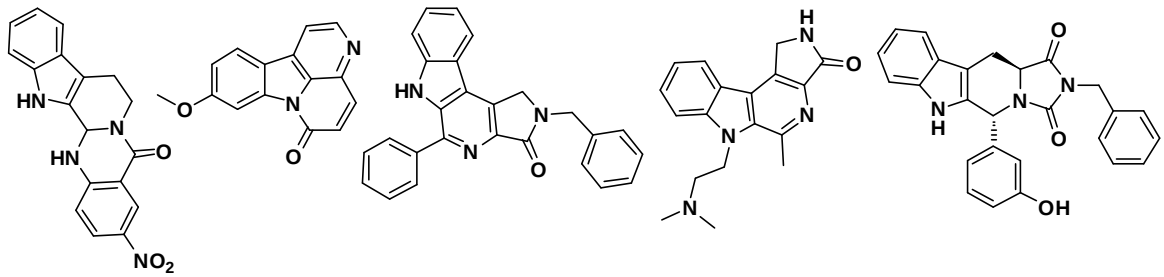


(131) (132)

2.5. Anti-cancer activity

In recent times, β-carboline derivatives gained interest as anti-cancer agents and have been extensively explored in the literature. Large number of β-carboline derivatives displayed potent anti-cancer activity through multiple mechanisms such as, interfering with enzymes like TOPO-I and II, CDKs (cyclindependent kinases), IκB kinases and intercalating DNA.

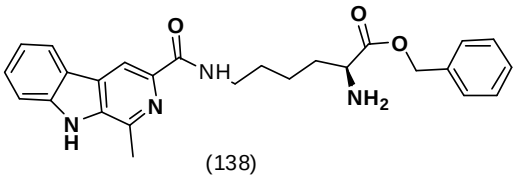
Boursereau et al., 2004, reported the synthesis and biological activity of 1-amino-β-carboline derivatives. Anti-parasitic activity of these analogues was discussed in the earlier part of this review. Among these simplified manzamine analogues, compound 31a displayed potent anti-cancer activity at micromolar concentration against the tested tumor cell-lines leukaemia K-562 (IC_{50} 3.2), non-small cell lung cancer HOP-92 (IC_{50} 0.38), colon HT29 (IC_{50} 2.7), melanoma M14 (IC_{50} 2.0), melanoma UACC-62 (IC_{50} 2.3), ovarian OVCAR-3 (IC_{50} 1.6) and breast MDA-MB-231/ATCC (IC_{50} 5.2). This study revealed that, manzamine analogues can be prepared by replacing the complex polycyclic ring system with simple substitutions as potent anti-cancer agents [105]. Baruah *et al.,* 2004, reported anti-cancer activity of quinazolino-β-carboline-5-one derivatives against eight different cancer cell lines. In these reported analogues, compound (133), displayed potent activity against seven tested cancer cell lines such as, breast MCF7/ADR (IC_{50} 2.0), CNS U251 (IC_{50} 3.0), colon SW620 (IC_{50} 2.0), lung H522 (IC_{50} 3.0), melanoma UACC62 (IC_{50} 2.0), prostate DU145 (IC_{50} 3.0) and renal ACHN (IC_{50} 3.0) at micromolar concentration [174]. In the same year, Kuo et al., 2004, reported the anti-cancer and anti-malarial properties of natural products isolated from *Eurycoma longifolia.* Canthin-6-one (12), 9-methoxycanthin-6-one (134) have exhibited cytotoxicity against breast cancer cell line MCF-7 (IC_{50} 7.3 and 4.5 µg/mL) and canthin-6-one (12) has also displayed inhibition of lung cancer cell line A-549 (IC_{50} 3.6 µg/mL) [175].



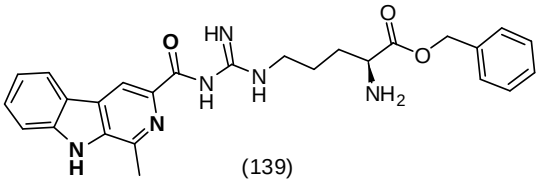
(133) (134) (135) (136) (137)

Cytotoxicity of synthetic 1,3 pyrrolidino-fused β-carboline derivatives was reported against lymphocytic cancer cell lines L1210, P338 and P338CPT5 by *Laronze et al.,* 2005. In these reported pyrrolo-β-carboline derivatives, compounds (135-136) exhibited anti-cancer activity against L1210 (IC_{50} 6.4 and 2.7 µM) and P338 (IC_{50} 5.3 and 2.6 µM) cell lines [176]. Plassmann *et al.,* 2005, evaluated a novel series of tetrahydro-β-carboline derivatives as mitotic kinesin Eg5 (KSP) inhibitors. Fifteen compounds from this small library of compounds displayed potent activity than standard monastrol and compound 137 is the most active compound with IC_{50} 0.65 µM [177].

Zhao et al., 2006 reported *in-vitro* cytotoxicity properties of β-carboline aminoacid ester conjugates against the liver (HepG-2), cervical (HeLa) and breast (MCF-7) cancer cell lines. Amongst reported analogues, Lysine and Arginine (138-139) conjugated analogues displayed activity against HepG-2 (IC_{50} 4.0 and 1.0 µM), HeLa (IC_{50} 2.0 and 5.0 µM) and MCF-7 cell lines (IC_{50} 5.0 and 7.0 µM) [178].



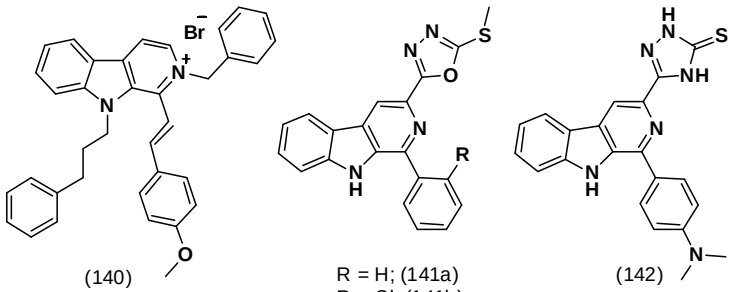
(138)



(139)

Cao et al., 2008, reported the synthesis and anti-cancer properties of 1-benzylidine substituted β-carboline derivatives against 10 human cancer cell lines such as cervical carcinoma (HeLa), liver carcinoma (Bel-7402 and HepG2), gastric carcinoma (BGC-823), non-small cell lung carcinoma (A549), malignant melanoma (A375), renal carcinoma (786-0 and 769-P), colon carcinoma (HT-29) and oral squamous carcinoma (KB). Many of these reported analogues exhibited potent anti-cancer activity against all the tested cell lines and N^2-benzylated quaternary-β-carbolinium salts have shown significantly more active than their corresponding tertiary-β-carboline derivative indicating that quaterinzation of pyridine nitrogen favored cytotoxic activity of these analogues. Compound 140 is the most active analogues with IC_{50} <5 µM against the tested cell lines [179]. Formagio et al., 2008 reported anti-cancer activity of novel hybrid β-carboline derivatives in conjugation with 1,3,4-oxadiazole and 1,2,4-triazole against Melanoma UACC-62, Breast MCF-7, Lung NCI-460,

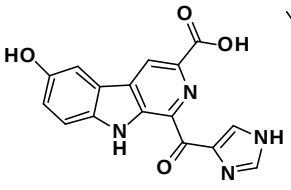
Leukemia K-562, Ovarian OVCAR, Prostate PCO-3, Colon HT-29 and Renal 786-0 cell lines. In β-carboline-1,3,4-oxadiazole hybrid molecules, compounds 141a-b displayed selective potent anti-cancer activity against ovarian cell line (IC_{50} 0.01, 0.01 μM) and leukemia K-562 cell line (IC_{50} 0.43, 0.17 μM). In β-carboline-1,2,4-triazole derivatives, compound 142 exhibited anti-cancer activity against lung (IC_{50} 0.06μM) and renal (IC_{50} 0.04 μM) cancer cell lines [180].



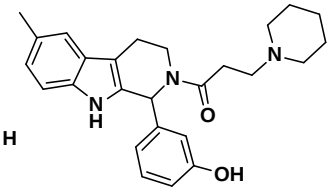
(140) (142)

n 2010, a novel 1-imidazoyl-3-carboxy-6-hydroxy-β-carboline alkaloid, hyrtiocarboline (143) isolated from the marine sponge *Hyrtios reticulates* and evaluated for cytotoxicity against thirteen different cancer cell lines. This novel β-carboline alkaloid displayed selective anti-proliferative activity against non-small cell lung H522-T1, lymphoma cancer U937 and melanoma MDA-MB-435 cell lines (IC_{50} values of 1.2, 1.5 and 3.0 μg/mL, respectively) [181]. *Barsanti et al.,* 2010 evaluated tetrahydro-β-carboline derivatives as kinesin Eg5 inhibitors and their cytotoxicity potency against human colon cell line HCT-116. Many of these reported β-carboline derivatives have displayed potent activity against kinesin Eg5 at nano molar concentration and compound 144 is the most active derivative with inhibition against HCT-116 cell line (IC_{50} 0.07 μM) and kinesin Eg5 (IC_{50} 0.10 μM) [182].

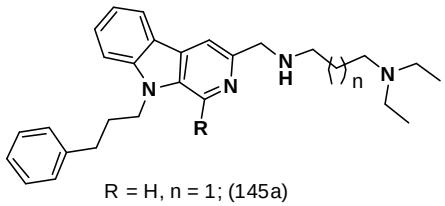
Chen *et al.,* 2010, reported the anti-cancer activity of 9-*N*-substituted-β-carbolines and their DNA binding capacity. Among the reported derivatives, compound 145a-c displayed potent cytotoxicity against ten human tumor cell lines (KB, BGC-823, 769-P, 786-0, OS-RC-2, HepG2, A375, HT-29, MCF-7 and prostate carcinoma 22RV1) with IC_{50} values <10 μM. SAR studies revealed that, 9-*N* substitution has suggested for anti-cancer activity of these analogues [183-187].



(143)

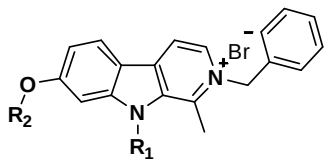


(144)

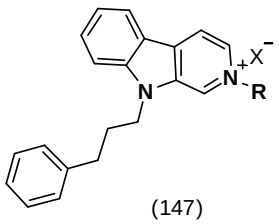


R = H, n = 1; (145a)
R = 3,4,5-trimethoxyphenyl, n = 1; (145b)
R = 3,4,5-trimethoxyphenyl, n = 2; (145c)

With their continuous interest to evaluate cytotoxic potential of quaternary β-carbolinium derivatives, they have reported library of quaternary-β-carboline derivatives and their anti-cancer activity against different cancer cells. These reported quaternary β-carboline derivatives have displayed potent to moderate anti-proliferative activity against the tested cell line and are significantly more active than respective tertiary-β-carboline derivative. In this library of compounds, derivatives 146a-d displayed potent anti-cancer activity against four cancer cell lines such as, liver carcinoma HepG2 (IC_{50} 1.6, 1.8, 1.9 and 3.9 µM, respectively), Bel-7402 (IC_{50} 4.0, 5.3, 5.0 and 2.0 µM, respectively), gastric carcinoma BGC-823 (IC_{50} 4.6, 6.8, 5.2 and 3.9 µM, respectively) and breast adenocarcinoma MCF-7 (IC_{50} 5.8, 5.7, 5.7 and 5.4 µM, respectively) [188, 189]. In their successive studies, Zhang *et al.,* 2013 described anti-cancer activity of new β-carbolinium derivatives (147) against ten different cancer cell lines such as, breast carcinoma (MCF-7), liver carcinoma (HepG2), prostate carcinoma (22RV1), colon carcinoma (HT-29), renal carcinoma (769-P), malignant melanoma (A375), ovarian carcinoma (SK-OV-3), esophageal carcinoma (Eca-109), gastric carcinoma (BGC-823) and Lewis lung carcinoma (LLC). Many of these reported analogues displayed potent anti-cancer activity against the tested cancer cell lines with IC_{50} values at micromolar level and compound 147a displayed most potent activity among the reported analogues against the tested cell lines (IC_{50} 3.9, 4.3, 2.2, 2.3, 2.5, 2.9, <2.0, 2.5, 2.3 and <2.0 µg/mL, respectivley) [190].



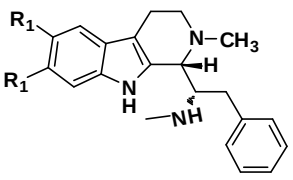
$R_1 = C_2H_5$, $R_2 = CH_2C_6F_5$; (146a)
$R_1 = i\text{-}C_4H_9$, $R_2 = n\text{-}C_4H_9$; (146b)
$R_1 = i\text{-}C_4H_9$, $R_2 = CH_2C_6H_5$; (146c)
$R_1 = (CH_2)_3C_6H_5$, $R_2 = n\text{-}C_8H_{17}$; (146d)



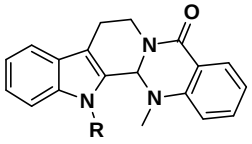
(147)

R = $n\text{-}C_6H_{13}$; (147a)

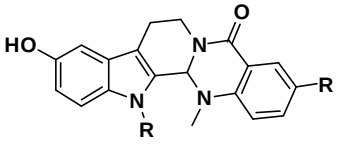
Eudistomidin G (148a), a tetrahydro-β-carboline alkaloid, isolated from the Okinawan marine tunicate *Eudistoma glaucus* in 2010. Eudistomidin G and along previously reported eudistomidin B (148b)[191] are evaluated for *in-vitro* cytotoxic potenial and exhibited anti-cancer activity (IC_{50} 4.8 and 4.7 µg/mL) against L1210 murine leukemia cells [192]. In structure based virtual screening study of SPECS database, β-carboline alkaloid, evodiamine (149a) is identified as topoisomerase-I inhibitor (Jiang and Hu 2009). Evodiamine has displayed *in-vitro* cytotoxicity against breast cancer MDA-MB-435 cell line (IC_{50} 29 µM). A pool of semi-synthetic evodiamine analogues were prepared with different substitutions on indole nitrogen and evaluated for their cytotoxicity. Among these analogues, 4-Cl benzoyl derivative (149b) exhibited potent anti-cancer activity against lung A549, breast MDA-MB-435 and colon HCT116 cancer cell lines (IC_{50} 0.86, 0.49 and 2.6 µM, respectively) and much more potent than evodiamine [193]. In a subsequent study, library of evodiamine derivatives has prepared with various substitutions on different positions and evaluated for anti-cancer activity. Many of synthetic evodiamine analogues displayed exceptional anti-proliferative activity against A549, MDA-MB-435 and HTC116 cell lines at nano Molar concentration. Further mechanistic studies suggested that, these analogues displayed potent inhibition of both topoisomerase-I and II. Moreover, compounds 10-hydroxy-evodiamine (149c) and 3-amino-10-hydroxy evodiamine (149d) exhibited good *in-vivo* anti-cancer activity at 2 mg/kg dose with good selectivity. With this excellent *in-vitro* and *in-vivo* potency, these compounds can be considered as promising molecules to develop new anti-proliferative agents [194].



R_1 = Br, R_2 = H; (148a)
R_1 = H, R_2 = Br; (148b)



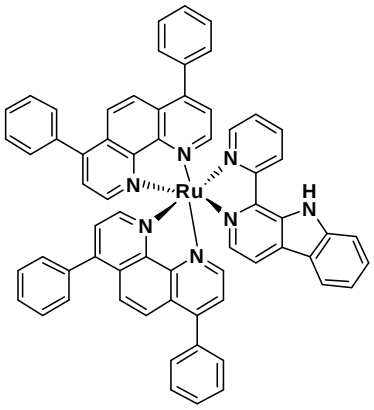
R = H; (149a)
R = 4-Cl-COPh; (149b)



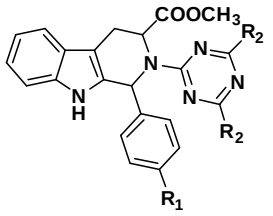
R = H; (149c)
R = NH_2; (149d)

Anti-cancer potency of ruthenium (II) complexes containing β-carboline was reported against four cancer cell lines by Tan et al. 2010. Among these β-carboline-ruthenium (II) complex derivatives, compound 150 exhibited potent inhibition of liver HepG2, breast MCF-

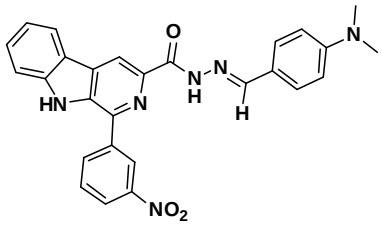
7 and MCF-10 cancer cell lines (IC_{50} 3.5, 5.9 and 40.6 µM, respectively) [195]. *Kumar et al.,* 2010 reported the synthesis and anti-cancer activity of β-carboline and triazine hybrids against breast (MCF7), colon (SW620), prostate (DU145), oral (KB), ovary (PA1), leukemia (K562), pancreas (Miapaca2) and lung (A549) cancer cell lines. In these reported β-carboline hybrid molecules, compounds 151a-c displayed selective anti-cancer activity towards oral cancer KB cell line (IC_{50} 105.8, 664.7 and 122.2 nM, respectively) [196].



(150)



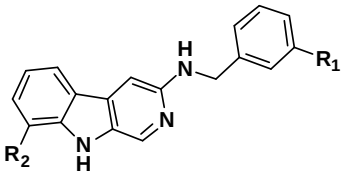
R_1 = CH_3, R_2 = *N*-methylpiperazine; (151a)
R_1 = CH_3, R_2 = n-propylamine; (151b)
R_1 = OCH_3, R_2 = n-propylamine; (151c)



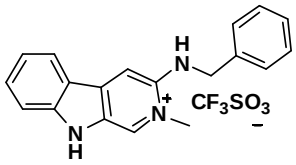
(152)

A series of β-carboline-3-carbohydrazide derivatives were evaluated for cytotoxicity potential against a panel of cancer cell lines (Melanoma UACC-62, Breast MCF7, Lung NCI-460, Leukemia K-562, Ovarian OVCAR, Prostate PCO-3, Colon HT29 and Renal 786-0). Large number of β-carboline-3-carbohydrazide derivatives displayed potent inhibition (IC_{50} <10 µM) of different cancer cell lines. Compound 152 is the most active derivative and exhibited potent selective inhibition of renal cancer cell line 786-0 (IC_{50} 40 nM) [197].

Ikeda et al., 2011 reported a novel series of 3-substituted-β-carboline derivatives and evaluated for anti-cancer activity. From this series of compounds, 3-benzylamino derivatives (153a-c) exhibited potent anti-proliferative activity (IC_{50} 0.12, 0.54 and 0.92 µM, respectively) against HeLa S-3 cell line [198]. In their subsequent study, reported anti-cancer activity of a library of 3-benzylamino-β-carboline derivatives with different substituents on position-6 and 8 of β-carboline ring. In these analogues, compounds 153d-e exhibited significant anti-cancer activity against HeLa S-3 (IC_{50} 0.074 and 0.046 µM), Sarcoma 180 (IC_{50} 0.03 and 0.024 µM) cell lines than parent compound. Compound 153f showed selective inhibition of HeLa S-3 (IC_{50} 0.032 µM) cell line [199].



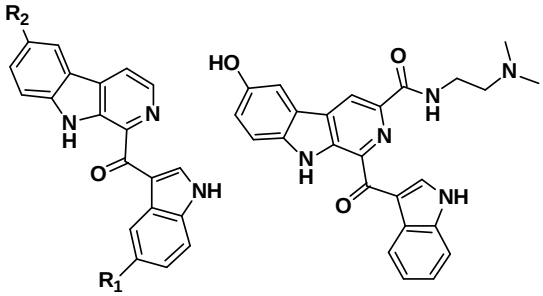
R_1 = H, R_2 = H; (153a)
R_1 = OH, R_2 = H; (153c)
R_1 = OC_6H_5, R_2 = H; (153d)
R_1 = H, R_2 = $NHCH_3$; (153e)
R_1 = OC_6H_5, R_2 = $NHCH_3$; (153f)



(153b)

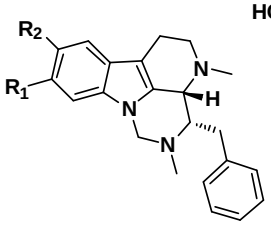
A novel 1-indoloxyl-β-carboline alkaloid, pityriacitrin (154a), isolated from the yeast *Malassezia furfur* in the year 2002 [200]. Total synthesis of pityriacitrin and anti-cancer activity of pityriacitrin derivatives were reported in 2011. Natural alkaloid pityriacitrin displayed weak anti-cancer activity (IC_{50} 55.1 µM) against prostate carcinoma cell line PC3. Liew et al., 2015, reported anti-malarial and anti-cancer activities of semi-synthetic pityriacitrin derivatives, among these reported analogues, compound 154b showed modest anti-leukemic activity against HL-60 (TB) cell line (IC_{50} 4.2 µM) and two compounds 154c, 154d showed weak anti-malarial activity (IC_{50} 1.3 and 1.0 µM) against *Plasmodium falciparum*[201]. Zhang et al., 2011, reported biological evaluation of semi-synthetic pityriacitrin derivatives against breast carcinoma cell line MDA-231. In these semi-synthetic derivatives, compound (155) showed potent inhibition of MDA-231 cell line (IC_{50} 1.33 µM) [202].

Four eudistomidin alkaloids, eudistomidin-H-K (156a-d) are isolated from an Okinawan marine tunicate *Eudistoma glaucus* in 2011. In these eudistomidin alkaloids, eudistomidin-J (156c) has shown *in-vitro* potent inhibition of murine leukemia cells P388 (IC_{50} 0.043 µg/mL), L1210 (IC_{50} 0.047 µg/mL) and human epidermoid carcinoma cells KB (IC_{50} 0.063 µg/mL) [203].

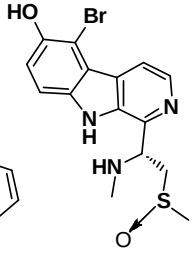


R_1 = H; R_2 = H; (154a)
R_1 = OH; R_2 = H; (154b)
R_1 = OCH_3; R_2 = H; (154c)
R_1 = OCH_3; R_2 = OCH_3; (154d)

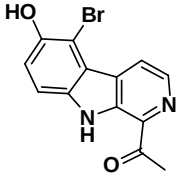
(155)



R_1 = Br; R_2 = H; (156a)
R_1 = H; R_2 = Br; (156b)



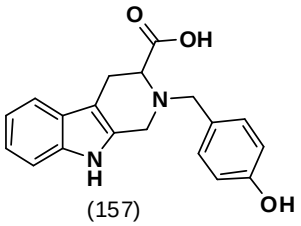
(156c)



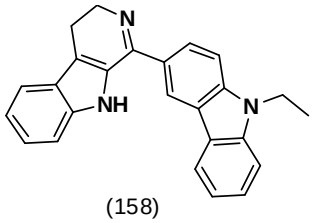
(156d)

A novel tetrahydro-β-carboline alkaloid, Callophycin-A (157) isolated from the red alga *Callophycus oppositifolius* and evaluated for cytotoxicity activity against a panel of human carcinoma cell lines. Callophycin-A has displayed potent anti-cancer activity against central nervous system-glioblastoma (SF-268), MCF-7, lung (H460), colon (HT-29) and ovary (CHO-K1) cancer cell lines (IC_{50} 1.3, 4.2, 2.0, 1.7 and 0.59 µM, respectively) [204].

Shen et al., 2011 reported the anti-cancer activity of 1-carbazolyl-β-carboline derivatives against five cell lines such as human mouth epidermoid carcinoma (KB), human colon adenocarcinoma (DLD), human lung large cell carcinoma (NCI-H661) and human hepatoma (Hepa and HepG2/A2). In these hybrid molecules, carbazolyl-dihydro-β-carboline derivatives showed potent anti-cancer activity than the respective tetrahydro derivatives. Compound 158 is the most active compound and displayed potent inhibition of KB, DLD, NCI-H661and HepG2/A2 cell lines (IC_{50} 0.71, 1.09, 0.84 and 0.60 µg/mL, respectively) [205].



(157)

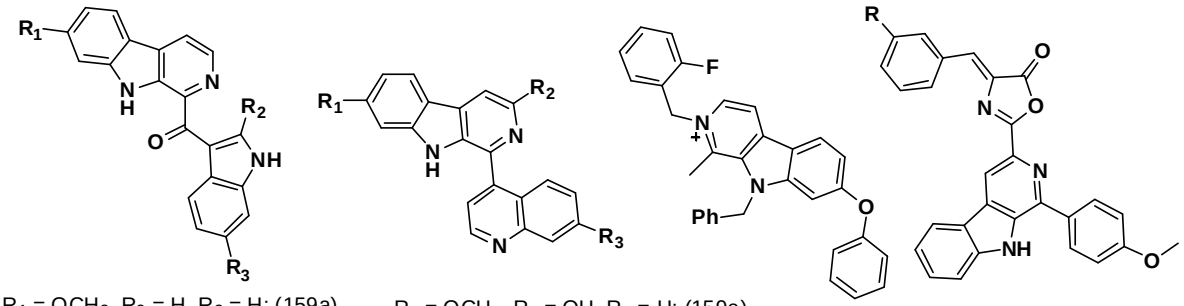


(158)

Li *et al.,* 2012 reported the isolation and biological evaluation of six novel β-carboline alkaloids trigonostemines A-F (159a-f) from *Trigonostemon lii*. Natural alkaloid trigonostemine-B (159b) displayed significant anti-cancer activity against cell lines SMMC-7721, A-549, MCF-7 and SW480 (IC_{50} 4.78, 8.67, 2.98 and 3.55 µM, respectively) than standard drug cisplatin (IC_{50} 9.25, 23.52, 10.64 and 12.32 µM, respectively) [206].

Frederick et al., 2012, reported cytotoxicity activity of tri-substituted harmine derivatives. In this library of harmine derivatives, large number of compounds exhibited potent anti-cancer activity against tested cancer cell lines. Compound 160 displayed potent inhibition of glioma U373, T98G, Hs68317 (IC_{50} 0.5, 0.37 and 0.44 µM, respectively) and esophageal OE21, OE33 cancer cell lines (IC_{50} 0.38, 0.70, respectively) [207]. *Savariz et al.,* 2012, reported the synthesis and anti-cancer activity of β-carboline-3-oxazol-5-one derivatives. Among these

reported analogues, compounds 161a-b displayed selective inhibition of glioma (U251) (IC_{50} 0.35 and 0.48 µM) and prostate (PC-3) (IC_{50} 2.18 and 1.07 µM) cancer cell lines [208].



$R_1 = OCH_3$, $R_2 = H$, $R_3 = H$; (159a)
$R_1 = OCH_3$, $R_2 = H$, $R_3 = OCH_3$; (159b)
$R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$; (159c)
$R_1 = H$, $R_2 = OH$, $R_3 = OCH_3$; (159d)

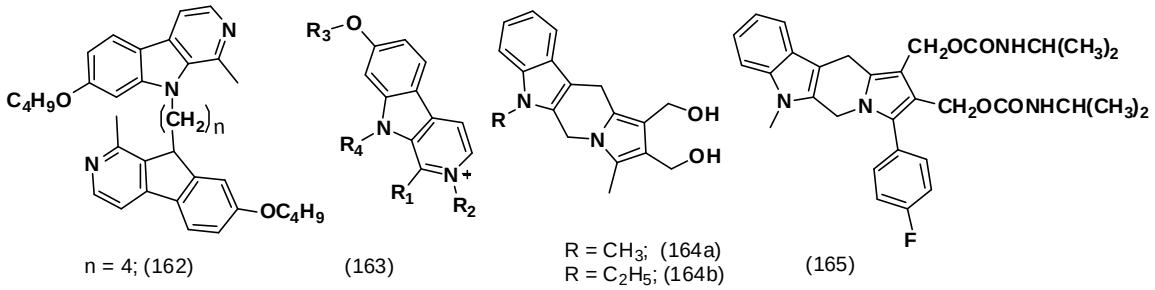
$R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$; (159e)
$R_1 = H$, $R_2 = OH$, $R_3 = OCH_3$; (159f)

(160)

$R = NO_2$; (161a)
$R = H$; (161b)

Shi et al., 2013 reported cytotoxicity evaluation of a library of β-carboline dimers against gastric carcinoma (BGC-823), malignant melanoma (A375), renal carcinoma (769-P, OS-RC-2) and epidermoid carcinoma of the nasopharynx (KB). In these β-carboline dimers, compound 162 exhibited significant inhibition of BGC, A375 and KB cell line (IC_{50} 8.5, 3.8 and 6.8 µM, respectively). SAR studies revealed that, connecting linker between two carboline ring has significant effect on cytotoxicity potency and linkers with four to six methylene units favored anti-cancer activity [209]. In their subsequent studies, anti-cancer activity of harmine derivatives (163) was reported against a panel of fifteen human cancer cell lines. Many of these reported compounds displayed potent anti-cancer activity against the tested cell lines and SAR studies suggested that, substitutions on position-2, 7 and 9 of β-carboline has significant impact on their anti-cancer potency [210].

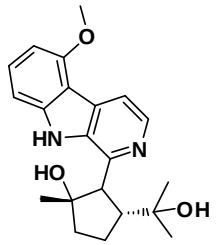
Synthesis and biological evaluation of a novel series of tetrahydro-β-carboline derivatives against a panel of cancer cells (CCRF/CEM, PC3, OECM1, MX-1, HCT-116, CL141T, A549, H460, HT-29 and HEL299) was reported by *Chaniyara et al.,* 2013. Among these reported β-carboline hybrid molecules, most of the compounds have shown potent *in-vitro* inhibition of the tested human cancer cell line. Most active compounds 164a-b and 165 are also exhibited potent *in-vivo* anti-cancer activity with 99% tumor remission in a mice model. Exact mechanism of action for this cytotoxicity was not clear as these compounds showed inhibition of topoisomerase-I and II, DNA cross-linking and cell-cycle arrest at the *S*-phase [211].



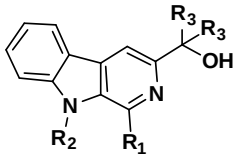
n = 4; (162) (163) R = CH_3; (164a) R = C_2H_5; (164b) (165)

Figueiredo et al., 2014 reported isolation and cytotoxicity evaluation of novel β-carboline alkaloid, 1-methyl-3-(2-hydroxypropan-2-yl)-2-(5-methoxy-9*H*-β-carbolin-1-yl)-cyclopentanol (166) from the roots of *Galianthe thalictroides.* This novel β-carboline alkaloid 166, showed inhibition of both topoisomerase-1 and 2α at 1 μM concentration and also displayed anti-cancer activity against kidney carcinoma (786-0), breast (MCF-7), human melanoma (UACC-62) and murine melanoma (B16-F10) cell lines (IC_{50} 3.0, 3.0, 2.5, and 19.5 μg/mL, respectively) [212].

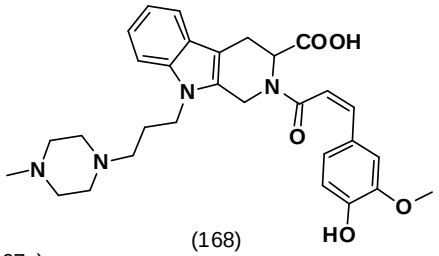
In 2014, *Bai et al.,* reported the anti-cancer activity of 1,3-di-substituted-β-carboline derivatives against a panel of five cancer cell lines. In this series of compounds, four analogues 167a-d exhibited potent inhibition of cell lines leukemia HL-60 (IC_{50} 0.75, 0.85, 1.0, and 0.9 μg/mL, respectively), liver SMMC-7721 (IC_{50} 0.91, 3.82, 4.55 and 3.1 μg/mL, respectively), human alveolar basal epithelial cells A-549 (IC_{50} 1.0, 9.4, 3.1 and 1.3 μg/mL, respectively), MCF-7 (IC_{50} 1.13, 3.8, 1.3 and 1.2 μg/mL, respectively) and colon SW480 (IC_{50} 2.5, 5.3, 11.5 and 6.3 μg/mL, respectively). SAR studies suggested that, alcohol group substitution on position-3 of β-carboline favors the anti-cancer activity [213]. *Lin et al.,* evaluated the cytotoxicity potentials of β-carboline and cinnamic acid hybrid molecules against liver SMMC-7721, human gastric SGC-7901, HCT116, liver Hep G2, breast MCF-7 and ovary SKOV-3. Many of these hybrid molecules exhibited superior anti-cancer activity than 5-fluorouracil against all cell lines. Compound 168 is the most active among the reported derivatives with potent inhibition of SMMC-7721 (IC_{50} 6.5), SGC-7901 (IC_{50} 6.6), HCT116 (IC_{50} 9.3), Hep G2 (IC_{50} 5.9) MCF-7 (IC_{50} 7.7) and SKOV-3 (IC_{50} 9.1) cell lines at micromolar concentration [214].



(166)



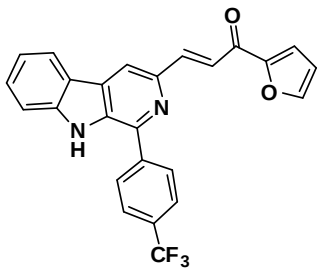
R_1 = phenanthren-9-yl, R_2 = H,R_3 = H; (167a)
R_1 = naphthalen-2-yl, R_2 = CH_3, R_3 = CH_3; (167b)
R_1 = 6-methoxynaphthalen-2-yl, R_2 = CH_3, R_3 = H; (167c)
R_1 = phenanthren-9-yl, R_2 = CH_3, R_3 = H; (167d)



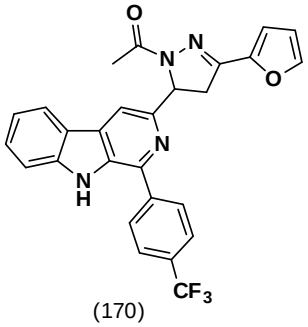
(168)

Kamal et al., reported anti-proliferative evaluation of β-carboline-pyrazole/chalcone hybrid molecules against lung cancer (A549), prostate cancer (DU-145), breast cancer (MCF-7), cervical cancer (HeLa), renal cancer (ACHN), normal human embryonic kidney (HEK-293) cell lines. Nearly half of the reported compounds showed potent activity against five cell lines (A549, DU-145, MCF-7, HeLa, ACHN) and weak inhibition of HEK-293. Potent compounds were also evaluated for DNA cleavage and topoisomerase-I inhibition activity to find out the exact molecular mechanism of action. Compounds 169-170 effectively cleaved pBR322 plasmid DNA upon irradiation with UV light and compound 170 also showed significant inhibition of topoisomerase-I at 100 µM concentration [215].

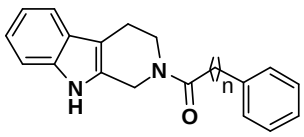
Recently, in a virtual screening study of ChemDiv database against transforming growth factor beta (TGFβ) kinase, 2-benzoyl-1,3,4,9-tetrahydro-β-carboline (171a) was identified as initial hit molecule. To optimize the identified lead molecule, library of molecules were designed with different substituents on various positions of β-carboline and phenyl ring and evaluated anti-proliferative activity. In these reported synthetic analogues, compound 171b exhibited 72.4% SBE luciferase inhibition rate at 1.0 µM and 59% anti-proliferation activity at 10 µM against PC-9 cell line. Compound 171b also displayed significant *in-vivo* activity against lung cancer and breast cancer with the dose of 5.0 mg/kg/day (i.p.) [216].



(169)



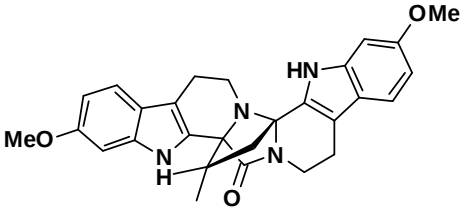
(170)



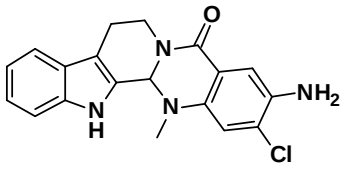
n = 0; (171a)
n = 1; (171b)

Peganumine A (172), is a novel β-carboline dimer alkaloid isolated from *Peganum harmala* and evaluated for cytotoxic activity against MCF-7, PC-3, HepG2, HL-60 cell lines by wang et al., 2014. This novel alkaloid, Peganumine A exhibited selective activity against HL-60 cell line (IC_{50} 5.8 µM) [217].

Fang et al., 2014 reported, synthesis and anti-tumor activity of eodiamine synthetic derivatives against MDA-MB-435, A-549, HCT-116 cell lines. Among these eodiamine synthetic derivatives, compound 173 showed selective inhibition of MDA-MB-435 cell line (IC_{50} 1.35 µM) [218].



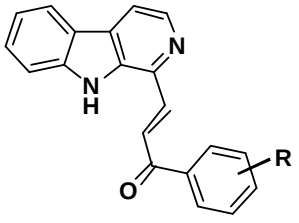
Peganumine A (172)



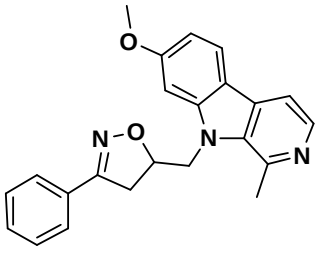
(173)

Chauhan et al., 2014, reported synthesis and anti-caner activity of β-carboline based chalcone derivatives against a panel of human cancer cell lines (MiaPaca-2, PLC/PRF-5, SKOV-3, DU-145, A-172, A-549, MCF-7 and DLD-1). In these β-carboline derivatives, two compounds (174a-b) exhibited siginificant activity against breast cancer (MCF-7) cell line (IC_{50} 2.25, 3.29 µM, respectively) [219].

Filali et al., 2014, reported, synthesis and biological evaluation of novel isoxazoline derivatives of harmine alkaloid. Among these reported harmine derivatives, one compound (175) displayed significant inhibition of HCT-116 and MCF-7 cell lines (IC_{50} 9.7 and 0.2 µM) and these analogues showed weak inhibition of acetylcholinesterase [220].



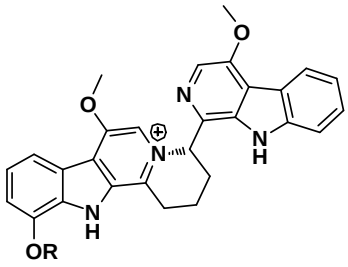
R = 3,4-dimethoxy; (174a)
R = 3,4,5-trimethoxy; (174b)



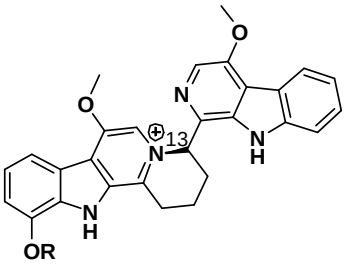
(175)

Jiao et al., 2014 reported the isolation, structural elucidation and anti-cancer activity of two novel bis-β-carboline alkaloids Quassidines I (176a-b) and J (177a-b) from *Picrasma quassioides*. These isolated racemic mixtures of two alkaloids were resolved in to two pairs of enantiomer using HPLC. In these natural alkaloids, 176a and 177a showed more potent cytotoxicity against HeLa (IC_{50} 5.75, 4.03 µM, respectively) and MKN28 (6.30, 4.91 µM, respectively) cancer cell lines [221].

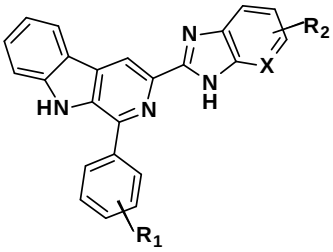
Kamal et al., 2014, reported synthesis and biological evaluation of novel β-carboline-benzimidazole conjugates (178) against 60 different human cancer cell lines. Most of these β-carboline hybrid molecules exhibited potent anti-cancer activity againt the tested cell line with IC_{50} value below 10 µM [222].



R = CH_3; (176a)
R = H; (177a)



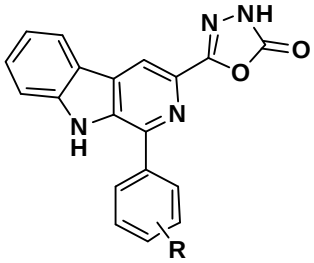
R = CH_3; (176b)
R = H; (177b)



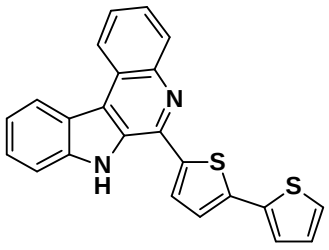
(178) X = C, N
R_1 = CH_3, OCH_3, Cl, F
R_2 = CH_3, OCH_3, Cl, F

Savariz et al., 2014, reported, synthesis and anti-anti-cancer activity of 1-substituted phenyl 3-(2-oxo-1,3,4-oxadiazol-5-yl)-β-carboline derivatives against a panel of human cancer cell line (MCF-7, NCI-ADR//RES, NCI-H460, OVCAR-03, HT-29, PC-3 and UACC-62). Among these reported library of β-carboline derivatives, three compounds (179a-c) displayed significant anti-cancer activity with mean IC_{50} value 8.41, 1.68 and 5.74 µM, respectively [223].

Srihari et al., 2015, reported PMA-SiO_2 catalyzed synthesis of 1-substituted β-carboline derivatives and their cyctotoxic properties against DU-145, A-549, MCF-7, HeLa and NIH-3T3 cell line. Among this series of β-carboline derivatives, compound 180 showed siginificant anti-cancer activity against DU-145, A-549, MCF-7, HeLa cell lines with IC_{50} 0.52, 3.03, 10.72 and 6.59 µM, respectively [224].

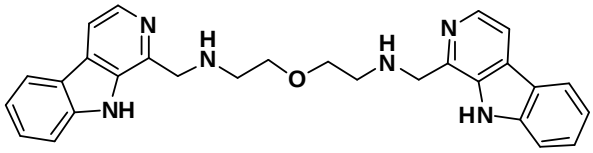


R = H; (179a)
R = 4-N(CH$_3$)$_2$; (179b)
R = 2-Cl; (179c)

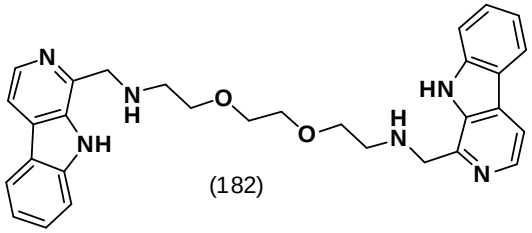


(180)

Chatwichien et al., 2015 reported synthesis and biological evaluation of β-carboline dimers based on neokauluamine structure. In these reported dimer molecules, two compounds 181-182 showed selective inhibition of H-1299 (IC_{50} 1.6, 1.8 µM, respectively) and A-375 (IC_{50} 3.0, 2.2 µM, respectively) cell lines. These β-carboline dimer derivatives also exhibited moderate anti-bacterial activity [225].



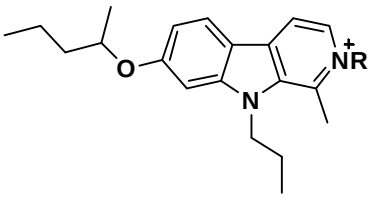
(181)



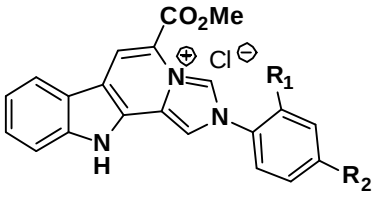
(182)

Meinguet et al., 2015 reported the design, synthesis, anti-proliferative activity and *in-silico* molecular properties of trisubstituted harmine derivatives. Among this reported library of harmine derivatives, two compounds 183a-b showed potent activity against tested cell lines A-549, SKMEL-28, U-373, T98G, HS-683 with mean IC50 value 0.6 and 0.6 µM [226].

Dighe et al., 2015, studied the anti-cancer properties of β-carboline-based *N*-heterocyclic carbene derivative against human breast cancer cell line. In these reported novel β-carboline derivatives, three compounds displayed potent anti-cancer activity 184a (IC_{50} 7.80, 5.96, 8.02 and 6.46 µM, respectively), 184b (IC_{50} 4.49, 6.80, 5.94 and 5.13 µM, respectively), 184c (IC_{50} 7.28, 6.90, 5.78 and 7.70 µM, respectively) against MDA-MB-231, MCF-7, MDA-MB-468, H-1299 cell lines [227].



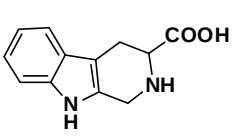
R = C_2H_5; (183a)
R = C_3H_7; (183b)



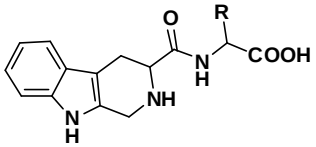
R_1 = I, R_2 = H; (184a)
R_1 = Cl, R_2 = F; (184b)
R_1 = Br, R_2 = CH_3; (184c)

2.6. Anti-thrombotic agents

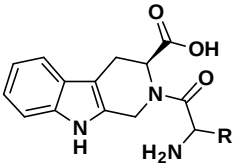
Simple β-carboline derivative, 3-(*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (185), isolated from *Allium chinense G. Don*, exhibited *in-vitro* and *in-vivo* anti-thrombotic activity but compound has poor aqueous solubility and bio-availability [228]. *Zhao et al.,* 2006, designed peptide derivatives of 1,2,3,4-tetrahydro-β-carboline to increase the bio-availability and biologically evaluated for anti-aggregation property. Among the synthesized peptide derivatives, compounds 186a-h showed significant *in-vitro* activity than parent compound 185 and aspirin in both ADP and PAF-induced platelet aggregation assay with increased bioavailability. Compounds 186c-e and 186g also displayed better *in-vivo* anti-thrombotic activity than aspirin at equal dose of 5 μmol/kg [229]. In a subsequent study, anti-thrombotic activity of 2*N*-substituted-tetrahydro-β-carboline-3-carboxylic acid derivatives (187) was reported. This novel series of compounds displayed many fold increase in anti-platelet potency (IC_{50} ranges 6.5-33.4 nM) than parent compound 185 (IC_{50} 701 nM) [230]. In their successive studies, same group reported, anti-platelet activity of 3-acylhydrazine derivatives of tetrahydro-β-carboline (188) and β-carboline derivatives (189). These tetrahydro-β-carboline and β-carboline-3-acylhydrazine derivatives exhibited significant anti-thrombotic activity at micro molar concentrations. Interestingly, oxidized β-carboline derivatives (189) displayed relatively higher potency than their respective tetrahydro-β-carboline derivative (188) [231, 232].



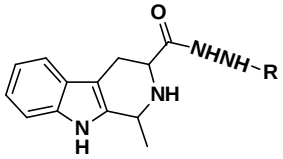
(185)



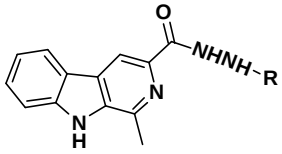
R = $CH_2C_6H_5$; (186a)
R = $CH(CH_3)_2$; (186b)
R = CH_2SH; (186c)
R = $(CH_2)_2SCH_3$; (186d)
R = $CH_2(CH_2)_2NHC(NH_2)$=NH; (186e)
R = H; (186f)
R = $CH_2(CH_2)_3NH_2$; (186g)
R = $CH_2CH_2CONH_2$; (186h)



(187)



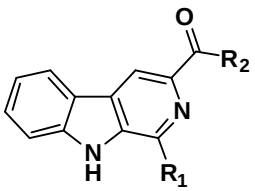
(188)



(189)

2.7. Anti-filarial agents

In the late 90's Chauhan group reported *in-vivo* anti-filarial activity of 1 and 3 substituted β-carboline derivatives (190). Interestingly, few of these derivatives have shown potent inhibition of microfilariae and macrofilariae forms of *Litomosoides carinii*. SAR studies suggested that, methyl ester group on position-3 and *para*-substituted-phenyl ring on position-1 of carboline has favored anti-filarial activity [233, 234]



(190)

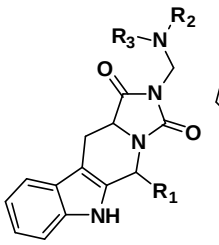
2.8. Phosphodiesterase-5 (PDE5) inhibitors

PDE5 belongs to the family of cyclic nucleotide hydrolyzing enzymes, which catalyzes the conversion of cyclic guanosine monophosphate (cGMP) in to guanosine monophosphate. PDE5 is presented throughout vascular smooth muscle tissue, lesser extent in kidney, lung and platelets. PDE5 is considered as useful target to treat cancer, erectile dysfunction and cardiovascular diseases such as, hypertension and angina. Tetrahydro-β-carboline derivatives (191) have explored extensively in the literature as PDE5 inhibitors. Tetrahydro-

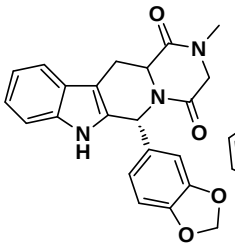
β-carboline derivative, tadalafil (192) is a PDE5 inhibitor approved in 2003 by US-FDA for the treatment of erectile dysfunction and approved for the treatment of pulmonary arterial hypertension in the year 2009. With the invention of tadalafil, huge numbers of tetrahydro-β-carboline derivatives were evaluated for PDE5 inhibitory activity. Most of these β-carboline derivatives displayed significant inhibition of PDE5 [235-237].

In the recent times, these tadalafil analogues were evaluated for other biological activities like anti-cancer, anti-malarial and anti-trypanosomal activity. Abadi group reported inhibition of PDE5 as well as anti-cancer activity of tetrahydro-β-carboline-hydantoin (193) and tetrahydro-β-carboline-piperazinedione derivatives (194). In these reported analogues, most of the compounds exhibited potent PDE5 inhibition and moderate anti-cancer activity, but there is no apparent correlation observed among these two activities. From these studies, it was concluded that, these compounds have shown anti-cancer activity by acting on different targets not by inhibition of PDE5 enzyme [238, 239].

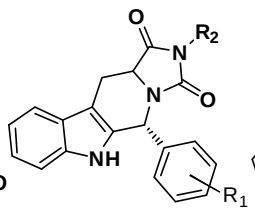
Ochiana et al., 2012 reported the biological evaluation of tadalafil analogues (195) as trypanosomal PDE (TbrPDEB1) inhibitor and these compounds exhibited weak inhibition of TbrPDEB1 at tested 100 µM concentration. This study concluded that, tadalafil analogues are selective human PDE5 inhibitors and not useful structural scaffolds to develop TbrPDEB1 inhibitors [240]. Anti-malarial activity of these tadalafil analogues was also discussed in the earlier parts of this review [108, 118].



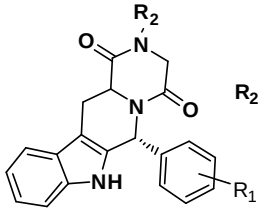
(191)



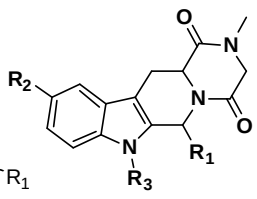
(192)



(193)



(194)



(195)

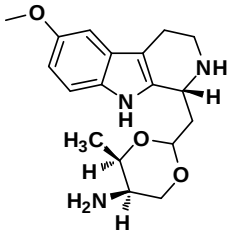
2.9. Anti-inflammatory agents

In the recent literature, few reports are available on anti-inflammatory activity of β-carboline derivatives. Bi *et al.,* 2010, designed some β-carboline hybrid molecules in conjugation with anti-inflammatory pharmacophore 1,3-dioxane moiety and evaluated for

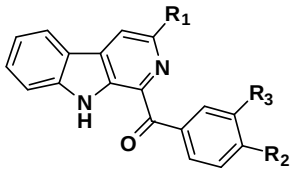
anti-inflammatory activity. In these reported hybrid molecules, compound 196 is the most active and exhibited potent *in-vivo* anti-inflammatory, anti-oxidant effect in xylene induced ear edema as well as carrageenan-induced rat paw inflammation models. Anti-inflammatory potency of the compound 196 is comparable with standard drug indomethacin [241].

In 2011 *Yang et al.,* studied the *in-vitro* anti-inflammatory activity of 1,3 di-substituted-β-carboline derivatives (197a-d) by measuring the inhibition of prostaglandin E_2, nitric oxide production (NO) and superoxide (O_2^-) formation. Most of these reported analogues have shown potent inhibition of prostaglandin E_2, nitric oxide production (NO) and superoxide (O_2^-) formation. Compounds 197a-b displayed 100% inhibition of prostaglandin E_2 at 1 µM concentration where as compound 197c exhibited potent inhibition of NO production (EC_{50} 0.20 µM). Compound 197d showed comparable activity against superoxide anion generation and elastase release (IC_{50} 2.28 and 1.99 µM) with standards LY294002 and indomethacin [242].

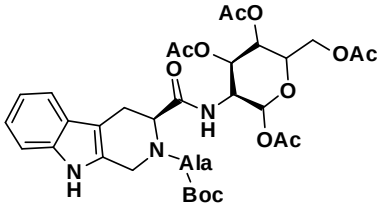
Zeng et al., 2012 reported the biological evaluation of 2,3-disubstituted-tetrahydro-β-carboline derivatives as anti-inflammatory agents using xylene induced rat ear edema model. Among these reported β-carbolines, compound 198 exhibited significant anti-inflammatory activity with 82% ear edema reduction and comparable with aspirin 89% reduction [243].



(196)



R_1 = COOH, R_2= CH_3, R_3 = H; (197a)
R_1 = COOH, R_2= Br, R_3 = H; (197b)
R_1 = H, R_2 = H, R_3 = CH_3; (197c)
R_1 = COOH, R_2= Br, R_3 = H; (197d)



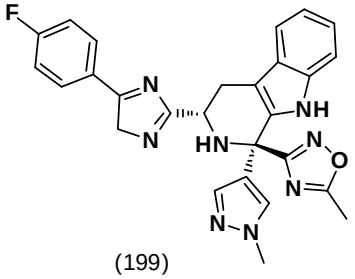
(198)

2.10. Other Pharmacological Uses

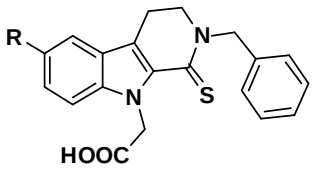
2.10.1. Anti-diabetic agents

In the recent times, β-carboline derivatives gained interest as anti-diabetic agents. In 2010, Merck company has been granted a patent on 3-imidazolyl-β-carboline derivatives (199) as

potent somatostatin subtype receptor 3 (SSTR3) antagonists in the treatment of Type 2 diabetes mellitus [244, 245].



(199)



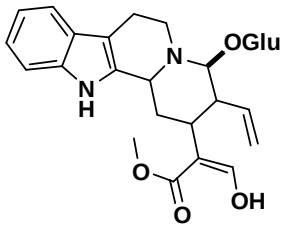
R = OCH_3, (200a)
R = COOH, (200b)

Minehira et al., 2012, evaluated the novel 1-(thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acid derivatives as selective aldo-keto reductase B1 (AKR1B1) inhibitor and their anti-diabetic activity. Among these designed analogues, compounds 200a-b exhibited potent inhibition of AKR1B1 (IC_{50} 0.15 and 0.17 µM) and potency is comparable with standard drug epalrestat (IC_{50} 0.10 µM). Interestingly, compound 200b displayed selective inhibition against AKR1B1 than AKR1B10 with selectivity index of 312 [246].

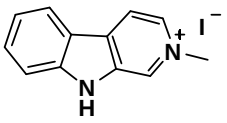
2.10.2. Anti-alzheimer agents

Few natural as well as synthetic β-carboline derivatives were evaluated against Glycogen Synthase Kinase-3β (GSK-3β), acetyl and butyryl cholinesterase (AChE and BChE) as anti-alzheimer's agenst. Natural β-carboline alkaloids, turbinatine (201) showed moderate acetyl cholinesterase inhibitory activity (IC_{50} 1.86 µM) in an *in-vitro* rat brain assay method [247]. Nostocarboline (104), and 2-methylnorharmane iodide (202) displayed butyryl cholinesterase inhibitiory (IC_{50} 19.4 and 11.2 µM) activity [248]. *Schott et al. 2006* reported biological evaluation of 6-hydroxy and 6-methoxy (203a-b) harmine derivatives as acetyl and butyrylcholinesterase inhibitors and these analogues showed significant to moderate inhibition of both enzymes [249]. Manzamine alkaloids, manzamine-A (9), 8-hydroxymanzamine-A (10) and manzamine-Y (74) have exhibited 73.2, 86.7 and 74.3% inhibition of GSK-3 respectively at 25 µM concentration. Among these alkaloids, manzamine-A and 8-hydroxymanzamine-A displayed strong inhibition of cell line Tau phosphorylation at 5 µM without any cytotoxicity. Further studies revealed that, these

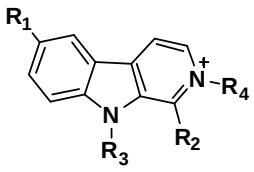
manzamine-A analogues exhibited selective inhibition of GSK-3β isomer and considered as promising structural targets to develop novel anti-alzheimer agents [10, 89].



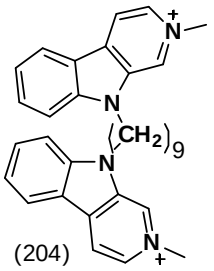
(201)



(202)



R_1 = OH; (203a)
R_1 = OCH_3, (203b)

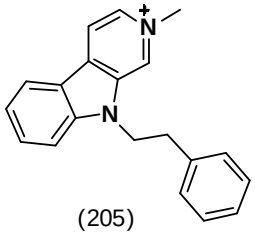


(204)

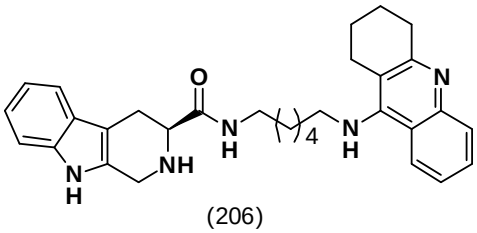
Rook et al., 2010, reported the anti-alzheimer activity of synthetic bivalent β-carboline derivatives with *in-vitro* evaluation against enzymes such as, acetylcholinesterase and butrylcholinestrase enzymes. Among the reported derivatives, compound (204) exhibited potent inhibition of AChE, BChE (IC_{50} 0.0005, 0.0057 μM) respectively [250].

Otto et al., 2014 studied the anti-alzheimer activity of β- and γ-carboline derivatives. Among these reported β-carboline derivatives, compound 205 showed significant inhibition of AChE (IC_{50} 0.6 μM) and BChE (IC_{50} 0.6 μM) [251].

Lan et al., 2014, reported the biological evaluation of β-carboline-tacrine hybrid molecules against acetylcholinesterase and butrylcholinestrase enzymes. In these reported hybrid molecules, compound 206 exhibited potent inhibition of both AChE (IC_{50} 0.021 μM) and BChE (IC_{50} 0.039 μM) enzymes [252].



(205)



(206)

2.10.3. Effects on Central Nervous System

β-carboline alkaloids are well known for their effects on Central Nervous System. They alter the functions of multiple receptors (benzodiazepine, 5-HT, dopamine and imidazoline) and enzymes involved in the CNS functions. Several literature reports have been published in late 90's on benzodiazepine receptor affinity of β-carboline derivatives. They act as agonist

[253], partial agonist [254], antagonist [255], partial inverse agonist [256] and inverse agonist [257, 258] to produce either anxiolytic, anxiogenic, sedative, tremorgenic, pro-convulsant or convulsant effect. SAR studies revealed that, ester, amide substitutions on position-3 and fully aromatic β-carboline ring favors the benzodiazepine receptor binding whereas reduced pyridine ring derivatives tetrahydro-β-carbolines and dihydro-β-carbolines displayed decreased binding affinity towards benzodiazepine receptor [78].

β-carboline derivatives are also reported in the literature for their agonist and antagonist effects on 5-hydroxytryptamine receptors like $5HT_{1A}$, $5HT_{2A,}$ $5HT_{2B}$ and $5HT_{2C}$. Unlike the benzodiazepine receptor affinity, tetrahydro and dihydro-β-carboline derivatives displayed increased affinity towards 5-hydroxytryptamine receptors. SAR studies suggested that, bromo substitution on position-6 of carboline ring enhances the binding affinity, while 6-methoxy substitution does not have significant effect on binding affinity [259, 260]. Affinity of β-carboline derivatives towards the imidazoline receptors such as I_1, I_2, I_3 [261], dopamine receptors D_1 and D_2 [262] was well studied in the literature. β-carbolines were also reported for inhibitory activity against the enzymes like monoamino oxidase-A and B [263, 264]. This broad range of affinity towards these CNS receptors, enzymes and lack of inherent selectivity of β-carboline derivatives, ultimately limited their applications as CNS agents.

3. OBJECTIVES AND PLAN OF WORK

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) are one of the key components in the HAART treatment regimen because of their high potency, specificity and low toxicity. Unfortunately, clinical effectiveness of first generation NNRTIs (Nevirapine, Delavirdine and Efavirenz) has been decreased due to developed resistance. Moreover, signs of cross resistance and treatment failures of second generation NNRTI etravirine in clinical studies have amplified the need of novel NNRTIs. As we discussed in introduction section, the prevelance of opportunistic infection is high and are majorly responsible for the mortality of HIV infected patients. Hence, development of new anti-HIV agents with potent activity against assosciated infections is highly desirable. With the highest consideration of all the above facts, in the present study, we have designed novel β-carboline derivatives as anti-HIV agents and biologically evaluated against HIV-1 RT, assosciated infections like leishmaniasis and tuberculosis. Diverse biological activities of β-carboline scaffold (especially anti-HIV and other anti-infective activities) fascinated us to develop novel non-nucleoside inhibitors of HIV-1 RT using ligand based drug design approach.

3.1. Objectives

Objectives of present dissertation work are, design and synthesis of novel β-carboline derivatives as non-nucleoside inhibitors of HIV-1 reverse transcriptase. *In-vitro* biological evaluation of these synthesized compounds against HIV-1 RT, assosciated infections such as, leishmaniasis and tuberculosis.

3.2. Plan of work

The plan of work is as follows,

3.2.1. Design of novel NNRTIs

Novel β-carboline derivatives were designed as non-nucleoside inhibitors of HIV-1 reverse transcriptase based on butterfly shape pharmacophoric requirements. In the present study, we have chosen, β-carboline ring, substituted aromatic rings as hydrophobic fragments (wing-1 and wing-2), those are connected with, NCH_2CO, NCH_2CONH and $NCH_2CONHNCH$

hydrophilic body. Molecular properties like, mol. wt., number of hydrogen bond acceptors, number of hydrogen bond donors, logP, number of rotatable bonds, % oral absorption, drug likeness, drug score were predicted using online servers like molinspiration (http://www.molinspiration.com), Osiris property explorer (http://www.organic-chemistry.org/prog/peo) and Qikprop module of Schrodinger.

3.2.2. Synthesis of designed novel β-carboline derivatives

Synthesis of designed β-carboline derivatives was done using tryptamine/L-tryptophan as starting materials. β-carboline scaffold was synthesized from tryptamine/L-tryptophan using pictet spengler reaction and followed by sequence of reactions to obtain the desired β-carboline derivatives as final products.

3.2.3. Characterization

Synthesized novel β-carboline derivatives were characterized by using physical and spectral data such as IR, ^{1}H NMR, Mass and elemental analysis.

3.2.4. Biological evaluation

Synthesized β-carboline derivatives were evaluated for the following activities:

1. Synthesized β-carboline derivatives were evaluated for cytotoxicity against vero cell line using MTT assay method.
2. HIV-1 RT inhibition potency of the synthesized compounds was evaluated using calorimetric ELISA assay method.
3. Anti-leishmanial activity of the synthesized analogues was determined against the promastigotes of *Leishmania infantum* using MTT assay method.
4. Synthesized compounds were evaluated for anti-tubercular activity against *Mycobacterium tuberculosis* H37Rv using Microplate Alamar Blue Assay (MABA).

3.2.5. Molecular docking

Molecular docking study was performed to assess their exact binding mode and binding interactions of the reported compounds in the NNIBP of HIV-1 RT using molecular modeling suite Schrodinger 2014.

4. EXPERIMENTAL METHODS

4.1. Molecular properties

Molecular properties like CLogP, molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, aqueous solubility, topological polar surface area, number of rotatable bonds, drug likeness and drug score were predicted using online servers molinspiration chemoinformatics (http://www.molinspiration.com), osiris property explorer (http://www.organic-chemistry.org/prog/peo). Percentage of oral absorption of ligands was predicted using QikProp module of Schrodinger suite 2014 [265].

4.2. Chemistry

All the reagents and solvents purchased from Sigma, Merck, Spectrochem, Avra chemicals, and Roche companies are used as received without further purification. Solvent system used throughout the experimental work for running Thin Layer Chromatography (TLC) was Ethyl acetate and Hexane Mixture in order to monitor the reaction. Column chromatography was performed using silica gel (100-200 mesh, SRL, India) as stationary phase, Ethyl acetate and Hexane Mixture as mobile phase for purification of the synthesized compounds. Melting points are uncorrected and were determined in open capillary tubes on a Precision Buchi B530 (Flawil, Switzerland) melting point apparatus containing silicone oil. IR spectra of the compounds were recorded using FT-IR spectrophotometer (Shimadzu IR Prestige 21, India). ^{1}H NMR spectra were recorded on a Bruker DPX-400 spectrometer (Bruker India Scientific Pvt. Ltd., Mumbai) using TMS as an internal standard (chemical shifts in δ). Elemental analysis was performed on Vario EL III M/s Elementar C, H, N and S analyzer (Elementar Analysensysteme GmbH, Germany). The ESMS were recorded on MICROMASS Quattro-II LCMS/MS system (Waters Corporation, Milford, USA).

4.3. Synthesis

Synthesis of the present work is discussed under the following heads.

4.3.1. Synthesis of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

4.3.1.1. Synthesis of 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives (6a-l)

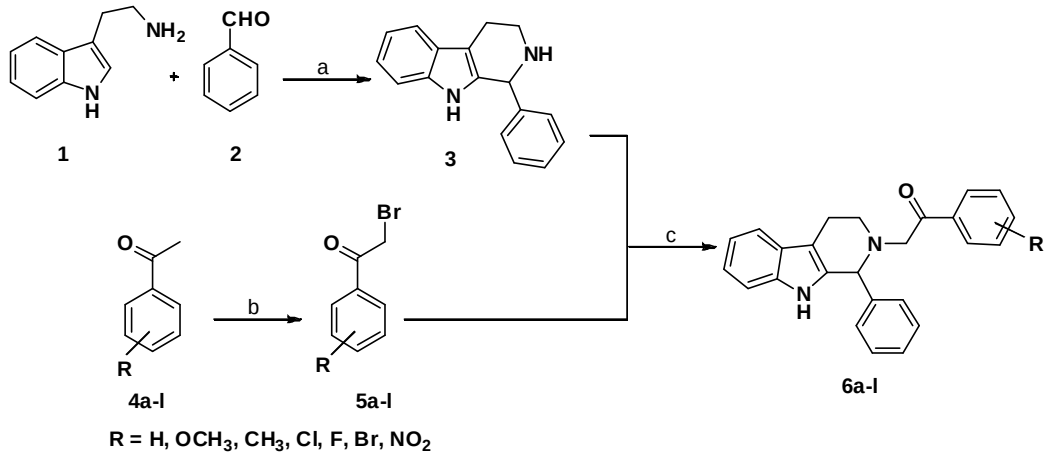
4.3.1.2. Synthesis of *N*-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetamide derivatives (10a-p)

4.3.1.3. Synthesis of *N*'-benzylidene-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetohydrazide derivatives (13a-o)

4.3.2. Synthesis of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives [20(a-d)(a-o)]

4.3.1. Synthesis of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

4.3.1.1. Synthesis of 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives (6a-l)



Scheme 1: Reagents and conditions: (a) trifluoroacetic acid, DCM, rt, 24 h; (b) *p*-toluenesulfonic acid, *N*-bromosuccinimide, acetonitrile, reflux, 3 h; (c) K_2CO_3, DMF, rt, 3 h.

Synthesis of 2,3,4,9-tetrahydro-1-phenyl-1H-pyrido[3,4-b]indole (3)

To the mixture of tryptamine (**1**) (4 g, 0.025 mol) and benzaldehyde (**2**) (2.6 mL, 0.022 mol) in 100 mL of dichloromethane, 2.5 mL of trifluoroacetic acid was added drop wise at 0 °C for 10 min. Then, the reaction mixture was stirred at room temperature for 24 h. After completion of reaction as monitored by TLC, the reaction mixture was poured into saturated $NaHCO_3$ solution. Organic layer was separated and washed twice with brine solution. Organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **3** as white solid, yield 78% [266-269].

General procedure for the synthesis of 2-bromo-1-phenylethanones (exemplified as 5a)

To the solution of acetophenone (**4a**) (1 g, 0.008 mol) in acetonitrile, *p*-toluenesulfonic acid monohydrate (1.58 g, 0.008 mol) and *N*-bromosuccinimide (1.48 g, 0.008 mol) were added. Then, the reaction mixture was refluxed for 3 h. After completion of reaction as monitored by TLC, solvent was evaporated *in-vacuo* and obtained residue was dissolved in 30 mL of ethyl acetate. Organic layer was washed twice with (2 x 30 mL) distilled water, collected organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **5a** as white solid, yield 72% [270].

General procedure for synthesis of 1-phenyl-2-(1-phenyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)ethanone derivatives (exemplified as 6a)

To a stirred solution of 2,3,4,9-tetrahydro-1-phenyl-1*H*-pyrido[3,4-*b*]indole (**3**) (0.47 g, 2 mmol) in dry dimethylformamide (2 mL), K_2CO_3 (0.83 g, 6 mmol) was added and stirred the reaction mixture at room temperature for 30 min. Thereafter, 2-bromo-1-phenylethanone (**5a**) (0.40g, 2 mmol) was added and continued stirring for additional 3 h at room temperature. On completion of reaction as monitored by TLC, the contents were poured into ice water (50 mL) whilst stirring with glass rod. Then aqueous layer was extracted with EtOAc (3 x 20 mL), organic layers were combined and washed with brine (40 mL). Separated organic layer was dried over Na_2SO_4, concentrated under vacuum and passed through short silica gel (100-200 mesh) column chromatography (ethylacetate:hexane = 2:8, R_f 0.40) to get **6a** as white solid [266, 271].

% Yield 80; mp 154-156 °C; IR νmax/cm (KBr): 3326, 2914, 1697, 1456; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89-7.85 (m, 2H), 7.55-7.51 (m, 2H), 7.42-7.33 (m, 8H), 7.21-7.19 (m, 1H), 7.15-7.08 (m, 2H), 4.99 (s, 1H), 4.14 (d, J = 16.6 Hz, 1H), 3.82 (d, J = 16.6 Hz, 1H), 3.30-3.25 (m, 1H), 3.10-3.04 (m, 1H), 3.01-2.94 (m, 1H), 2.90-2.84 (m, 1H); MS (ESI) m/z 367.2 $[M + 1]^+$; Anal. Calcd. for $C_{25}H_{22}N_2O$: C, 81.94; H, 6.05; N, 7.64, Found: C, 81.92; H, 6.08; N, 7.66.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-1-(4-methoxyphenyl)ethanone (6b)

White solid; % Yield 86; mp 138-140 °C; IR νmax/cm (KBr): 3342, 2946, 1698, 1487, 1249; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93-7.89 (m, 2H), 7.57-7.55 (m, 1H), 7.44-7.41 (m, 3H), 7.39-7.34 (m, 3H), 7.23-7.21 (m, 1H), 7.16-7.12 (m, 2H), 6.89-6.87 (m, 2H), 4.97 (s, 1H), 4.10 (d, J = 16.1 Hz, 1H), 3.87 (s, 3H), 3.75 (d, J = 17.9 Hz, 1H), 3.31-3.26 (m, 1H), 3.09-3.01 (m, 1H), 2.99- 2.95 (m, 1H), 2.91-2.87 (m, 1H); MS (ESI) m/z 397.0 $[M + 1]^+$; Anal. Calcd. for $C_{26}H_{24}N_2O_2$: C, 78.76; H, 6.10; N, 7.07, Found: C, 78.79; H, 6.14; N, 7.11.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-1-p-tolylethanone (6c)

White solid; % Yield 76; mp 132-134 °C; IR νmax/cm (KBr): 3352, 2941, 1686, 1486; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 8.2 Hz, 2H), 7.57-7.55 (m, 1H), 7.44-7.42 (m, 2H), 7.39-7.35 (m, 4H), 7.24-7.20 (m, 3H), 7.15-7.12 (m, 2H), 5.00 (s, 1H), 4.14 (d, J = 16.4 Hz, 1H), 3.80 (d, J = 16.4 Hz, 1H), 3.32-3.26 (m, 1H), 3.11-3.05 (m, 1H), 3.03-2.95 (m, 1H), 2.92-2.86 (m, 1H), 2.41 (s, 3H); MS (ESI) m/z 381.2 $[M + 1]^+$; Anal. Calcd. for $C_{26}H_{24}N_2O$: C, 82.07; H, 6.36; N, 7.36, Found: C, 82.04; H, 6.39; N, 7.34.

1-(4-chlorophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)ethanone (6d)

White solid; % Yield 70; mp 132-134 °C; IR νmax/cm (KBr): 3296, 1684, 1481, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85-7.83 (m, 2H), 7.57-7.54 (m, 1H), 7.42-7.38 (m, 8H), 7.23-7.21 (m, 1H), 7.16-7.12 (m, 2H), 4.91 (s, 1H), 4.10 (d, J = 16.1 Hz, 1H), 3.73 (d, J = 16.1 Hz, 1H), 3.28-3.23 (m, 1H), 3.06-2.94 (m, 2H), 2.90-2.86 (m, 1H); MS (ESI) m/z 401.9 $[M + 1]^+$, 403.9 $[M + 3]^+$; Anal. Calcd. for $C_{25}H_{21}ClN_2O$: C, 74.90; H, 5.28; N, 6.99, Found: C, 74.88; H, 5.31; N, 6.95.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-1-(4-nitrophenyl)ethanone (6e)

Yellow solid; % Yield 64; mp 92-94 °C; IR νmax/cm (KBr): 3282, 1714, 1519, 1456, 748; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, J = 8.6 Hz, 2H), 7.97 (d, J = 8.6 Hz, 2H), 7.57-7.54 (m, 3H), 7.40-7.34 (m, 4H), 7.25-7.10 (m, 3H), 4.83 (s, 1H), 3.53-3.37 (m, 2H), 3.27 (d, J = 16.6 Hz, 1H), 3.20-3.09 (m, 1H), 3.03-2.89 (m, 2H).

1-(4-fluorophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)ethanone (6f)

White solid; % Yield 74; mp 154-156 °C; IR νmax/cm (KBr): 3306, 1702, 1481, 755; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96-7.93 (m, 2H), 7.57-7.54 (m, 2H), 7.43-7.36 (m, 5H), 7.23-7.21 (m, 1H), 7.17-7.12 (m, 2H), 7.09-7.05 (m, 2H), 4.92 (s, 1H), 4.10 (d, J = 16.1 Hz, 1H), 3.74 (d, J = 16.1 Hz, 1H), 3.29-3.24 (m, 1H), 3.07-3.01 (m, 1H), 2.98-2.94 (m, 1H), 2.90-2.86 (m, 1H); MS (ESI) m/z 385.2 $[M + 1]^+$; Anal. Calcd. for $C_{25}H_{21}FN_2O$: C, 78.10; H, 5.51; N, 7.29, Found: C, 78.09; H, 5.48; N, 7.33.

1-(4-bromophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)ethanone (6g)

White solid; % Yield 68; mp 134-136 °C; IR νmax/cm (KBr): 3286, 1684, 1436, 822; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 8.5 Hz, 2H), 7.56-7.53 (m, 3H), 7.41-7.35 (m, 6H), 7.23-7.21 (m, 1H), 7.17-7.11 (m, 2H), 4.90 (s, 1H), 4.09 (d, J = 16.1 Hz, 1H), 3.72 (d, J = 16.1 Hz, 1H), 3.27-3.23 (m, 1H), 3.06-3.00 (m, 1H), 2.97-2.94 (m, 1H), 2.89-2.85 (m, 1H); GCMS (ESI) m/z 445.4 $[M]^+$, 447.3 $[M + 2]^+$; Anal. Calcd. for $C_{25}H_{21}BrN_2O$: C, 67.42; H, 4.75; N, 6.29, Found: C, 67.45; H, 4.73; N, 6.34.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-1-m-tolylethanone (6h)

White solid; % Yield 72; mp 144-146 °C; IR νmax/cm (KBr): 3394, 2926, 1698, 1474, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57-7.53 (m, 2H), 7.44-7.32 (m, 8H), 7.26-7.20 (m, 2H), 7.19-7.11 (m, 2H), 5.00 (s, 1H), 4.03 (d, J = 17.1 Hz, 1H), 3.71 (d, J = 17.1 Hz, 1H), 3.34-3.25 (m, 1H), 3.15-3.05 (m, 1H), 2.99-2.88 (m, 2H), 2.48 (s, 3H).

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-1-(2-methoxyphenyl)ethanone (6i)

White solid; % Yield 64; mp 150-152 °C; IR νmax/cm (KBr): 3371, 2918, 1697, 1487, 1248; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69-7.67 (m, 1H), 7.58-7.56 (m, 1H), 7.47-7.43 (m, 1H), 7.37-7.32 (m, 6H), 7.22-7.21 (m, 1H), 7.16-7.10 (m, 2H), 7.01 (t, J = 7.5 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 5.14 (s, 1H), 4.14 (d, J = 18.2 Hz, 1H), 3.92 (d, J = 18.2 Hz, 1H), 3.76 (s, 3H), 3.34-3.29 (m, 1H), 3.23-3.17 (m, 1H), 3.03-2.96 (m, 1H), 2.92-2.88 (m, 1H).

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-1-o-tolylethanone (6j)

White solid; % Yield 68; mp 132-134 °C; IR νmax/cm (KBr): 3287, 2934, 1692, 1468, 762; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58-7.53 (m, 1H), 7.43-7.32 (m, 8H), 7.26-7.11 (m, 5H), 5.01 (s, 1H), 4.03 (d, J = 17.1 Hz, 1H), 3.71 (d, J = 17.1 Hz, 1H), 3.34-3.24 (m, 1H), 3.15-3.05 (m, 1H), 3.02-2.84 (m, 2H), 2.48 (s, 3H).

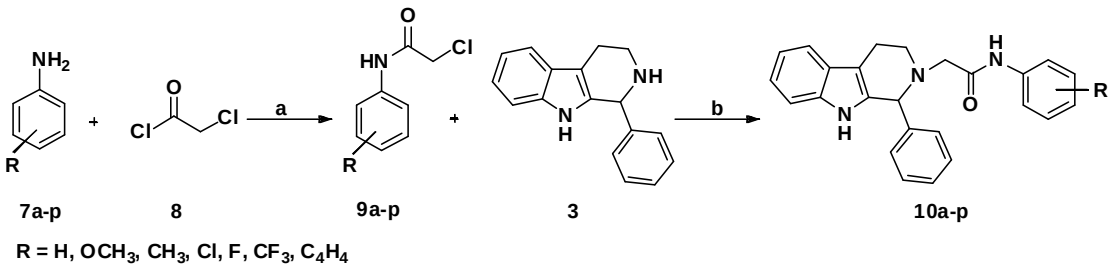
1-(2-chlorophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)ethanone (6k)

White solid; % Yield 60; mp 136-138 °C; IR νmax/cm (KBr): 3315, 1704, 1496, 766; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56-7.54 (m, 1H), 7.39-7.31 (m, 8H), 7.28-7.26 (m, 2H), 7.21-7.19 (m, 1H), 7.16-7.10 (m, 2H), 4.98 (s, 1H), 4.05 (d, J = 17.4 Hz, 1H), 3.73 (d, J = 17.4 Hz, 1H), 3.35-3.29 (m, 1H), 3.15-3.08 (m, 1H), 3.00-2.93 (m, 1H), 2.89-2.83 (m, 1H).

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-1-(3,4-dimethoxyphenyl)ethanone (6l)

White solid; % Yield 78; mp 110-112 °C; IR νmax/cm (KBr): 3342, 2932, 1682, 1468, 1246; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, J = 8.1 Hz, 3H), 7.45-7.42 (m, 2H), 7.40-7.36 (m, 4H), 7.24-7.22 (m, 1H), 7.17-7.13 (m, 2H), 6.81 (d, J = 8.1 Hz, 1H), 4.97 (s, 1H), 4.12 (d, J = 16.1 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 3.77 (d, J = 16.1 Hz, 1H), 3.32-3.26 (m, 1H), 3.10-2.96 (m, 2H), 2.92-2.87 (m, 1H).

4.3.1.2. Synthesis of *N*-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetamide derivatives (10a-p)



Scheme 2: Reagents and conditions: (a) triethylamine, DCM, 0°C-rt, 30 min; (b) K_2CO_3, DMF, rt, 3 h.

General procedure for the synthesis of 2-chloro-N-phenylacetamides (exemplified as 9a)

To the mixture of aniline (**7a**) (1 g, 0.011 mol) and triethylamine (4.5 mL, 0.033 mol) in dichloromethane, chloroacetylchloride (**8**) (0.84 mL 0.011 mol) was added slowly under ice cold condition. The reaction mixture was allowed to stir at room temperature for 30 min. On completion of reaction as monitored by TLC, the reaction mixture was diluted with dichloromethane and poured into $NaHCO_3$ solution (25 mL) whilst stirring with glass rod. Organic layer was washed twice with $NaHCO_3$ solution (2 x 25 mL), collected organic layer was dried over Na_2SO_4 and concentrated under vacuum to yield the intermediate **9a** as white solid, yield 92% [120, 272].

General procedure for the synthesis of N-phenyl-2-(1-phenyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)acetamide derivatives *(exemplified as 10a)*

To a stirred solution of 2,3,4,9-tetrahydro-1-phenyl-1*H*-pyrido[3,4-*b*]indole (**3**) (0.47 g, 2 mmol) in dry dimethylformamide (3 mL), K_2CO_3 (0.83 g, 6 mmol) was added and stirred the reaction mixture at room temperature for 30 min, 2-chloro-*N*-phenylacetamide (**9a**) (0.34 g, 2 mmol) was added and continued stirring for additional 3 h at room temperature. On completion of reaction as monitored by TLC, the contents were poured into ice water (50 mL). Thereafter, the aqueous layer was extracted with EtOAc (3 x 20 mL). The organic layers were combined and washed with brine (40 mL), dried over Na_2SO_4 and concentrated to yield the product. For analytical grade, the compound was passed through short silica gel

(100-200 mesh) column chromatography (ethylacetate:hexane = 6:4, R_f 0.40) to obtain **10a** as a white solid [120, 266, 271].

% Yield 78; mp 110-112 °C; IR vmax/cm (KBr): 3302, 1672, 1519, 1448; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.26 (brs, 1H), 7.60-7.58 (m, 1H), 7.54 (d, J = 7.6 Hz, 2H), 7.40-7.33 (m, 8H), 7.25-7.23 (m, 1H), 7.20-7.11 (m, 3H), 4.80 (s, 1H), 3.44 (d, J = 16.6 Hz, 1H), 3.36-3.32 (m, 1H), 3.21 (d, J = 16.6 Hz, 1H), 3.14-3.08 (m, 1H), 3.01-2.96 (m, 2H); MS (ESI) m/z 382.2 $[M + 1]^+$; Anal. Calcd. for $C_{25}H_{23}N_3O$: C, 78.71; H, 6.08; N, 11.02, Found: C, 78.72; H, 6.05; N, 11.04.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-(4-methoxyphenyl)acetamide (10b)

White solid; % Yield 90; mp 98-100 °C; IR vmax/cm (KBr): 3298, 1668, 1517, 1452, 1246; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.12 (brs, 1H), 7.58-7.56 (m, 1H), 7.43-7.34 (m, 8H), 7.19-7.12 (m, 3H), 6.86-6.84 (m, 2H), 4.75 (s, 1H), 3.78 (s, 3H), 3.41-3.29 (m, 2H), 3.18-3.05 (m, 2H), 2.96-2.92 (m, 2H); MS (ESI) m/z 412.4 $[M + 1]^+$; Anal. Calcd. for $C_{26}H_{25}N_3O_2$: C, 75.89; H, 6.12; N, 10.21, Found: C, 75.92; H, 6.13; N, 10.20.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-p-tolylacetamide (10c)

White solid; % Yield 86; mp 102-104 °C; IR vmax/cm (KBr): 3307, 1668, 1523, 1486; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.17 (brs, 1H), 7.57-7.55 (m, 1H), 7.41-7.35 (m, 8H), 7.22-7.20 (m, 1H), 7.15-7.11 (m, 4H), 4.77 (s, 1H), 3.42-3.38 (m, 1H), 3.32-3.30 (m, 1H), 3.19-3.06 (m, 2H), 2.97-2.94 (m, 2H), 2.31 (s, 3H); MS (ESI) m/z 396.4 $[M + 1]^+$; Anal. Calcd. for $C_{26}H_{25}N_3O$: C, 78.96; H, 6.37; N, 10.62, Found: C, 78.94; H, 6.39; N, 10.60.

N-(4-chlorophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetamide (10d)

White solid; % Yield 76; mp 130-132 °C; IR vmax/cm (KBr): 3323, 1678, 1519, 1490, 738; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.28 (brs, 1H), 7.60-7.58 (m, 1H), 7.51-7.47 (m, 2H), 7.39-7.35 (m, 6H), 7.32-7.29 (m, 2H), 7.25-7.22 (m, 1H), 7.19-7.16 (m, 2H), 4.79 (s, 1H), 3.43 (d, J = 16.7 Hz, 1H), 3.35-3.31 (m, 1H), 3.22 (d, J = 16.7 Hz, 1H), 3.16-3.08 (m, 1H), 3.02-2.97 (m, 2H); MS (ESI) m/z 416.9 $[M + 1]^+$, 418.8 $[M + 3]^+$; Anal. Calcd. for $C_{25}H_{22}ClN_3O$: C, 72.19; H, 5.33; N, 10.10, Found: C, 72.20; H, 5.32; N, 10.12.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-(4-nitrophenyl)acetamide (10e)

Yellow solid; % Yield 70; mp 210-212 °C; IR νmax/cm (KBr): 3288, 1681, 1537, 1506, 1342, 1300; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.62 (brs, 1H), 8.25-8.19 (m, 2H), 7.72-7.66 (m, 2H), 7.56-7.58 (m, 1H), 7.39-7.36 (m, 6H), 7.26-7.24 (m, 1H), 7.22-7.15 (m, 2H), 4.82 (s, 1H), 3.48 (d, J = 16.9 Hz, 1H), 3.36-3.27 (m, 2H), 3.16-3.11 (m, 1H), 3.09-2.98 (m, 2H); Anal. Calcd. for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.20; N, 13.14, Found: C, 70.38; H, 5.24; N, 13.12.

N-(4-fluorophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetamide (10f)

White solid; % Yield 72; mp 102-104 °C; IR νmax/cm (KBr): 3290, 1693, 1514; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.23 (brs, 1H), 7.60-7.58 (m, 1H), 7.51-7.47 (m, 2H), 7.39-7.36 (m, 6H), 7.25-7.22 (m, 1H), 7.21-7.14 (m, 2H), 7.07-7.01 (m, 2H), 4.79 (s, 1H), 3.43 (d, J = 16.7 Hz, 1H), 3.36-3.31 (m, 1H), 3.21 (d, J = 16.7 Hz, 1H), 3.16-3.08 (m, 1H), 3.02-2.95 (m, 2H); MS (ESI) m/z 400.5 $[M + 1]^+$; Anal. Calcd. for $C_{25}H_{22}FN_3O$: C, 75.17; H, 5.55; N, 10.52, Found: C, 75.18; H, 5.53; N, 10.54.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-(3-methoxyphenyl)acetamide (10g)

White solid; % Yield 80; mp 138-140 °C; IR νmax/cm (KBr): 3284, 1666, 1537, 1248; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 7.65-7.54 (m, 1H), 7.41-7.34 (m, 6H), 7.32 (t, *J* = 2.0 Hz, 1H), 7.27-7.21 (m, 2H), 7.21-7.14 (m, 2H), 7.00 (d, *J* = 7.9 Hz, 1H), 6.70-6.68 (m, 1H), 4.79 (s, 1H), 3.83 (s, 3H), 3.43 (d, *J* = 16.7 Hz, 1H), 3.38-3.29 (m, 1H), 3.24-3.06 (m, 2H), 3.04-2.93 (m, 2H); Anal. Calcd. for $C_{26}H_{25}N_3O_2$: C, 75.89; H, 6.12; N, 10.21, Found: C, 75.87; H, 6.15; N, 10.18.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-m-tolylacetamide (10h)

White solid; % Yield 72; mp 124-126 °C; IR νmax/cm (KBr): 3267, 1666, 1537, 1282; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1H), 7.60-7.58 (m, 1H), 7.45-7.30 (m, 8H), 7.27-7.12 (m, 4H), 6.95 (d, J = 7.5 Hz, 1H), 4.79 (s, 1H), 3.43 (d, J = 16.6 Hz, 1H), 3.34-3.32 (m, 1H), 3.24-3.08 (m, 2H), 3.04-2.93 (m, 2H), 2.37 (s, 3H); MS (ESI) m/z 396.4 $[M + 1]^+$; Anal. Calcd. for $C_{26}H_{25}N_3O$: C, 78.96; H, 6.37; N, 10.62, Found: C, 78.97; H, 6.36; N, 10.64.

N-(3-chlorophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetamide (10i)

White solid; % Yield 70; mp 120-122 °C; IR νmax/cm (KBr): 3251, 1672, 1523, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.30 (brs, 1H), 7.64 (t, J = 1.9 Hz, 1H), 7.60-7.58 (m, 1H), 7.41-7.352 (m, 7H), 7.26-7.23 (m, 2H), 7.19-7.16 (m, 2H), 7.10 (dd, J = 8.0, 1.0 Hz, 1H), 4.79 (s, 1H), 3.43 (d, J = 16.7 Hz, 1H), 3.33-3.30 (m, 1H), 3.22 (d, J = 16.7 Hz, 1H), 3.17-3.08 (m, 1H), 3.02-2.96 (m, 2H); Anal. Calcd. for $C_{25}H_{22}ClN_3O$: C, 72.19; H, 5.33; N, 10.10, Found: C, 72.17; H, 5.35; N, 10.14.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-(3-nitrophenyl)acetamide (10j)

Yellow solid; % Yield 68; mp 156-158 °C; IR νmax/cm (KBr): 3288, 1672, 1535, 1454, 1350; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.49 (brs, 1H), 8.34 (t, J = 2.1 Hz, 1H), 7.98-7.92 (m, 2H), 7.60-7.59 (m, 1H), 7.50 (t, J = 8.2 Hz, 1H), 7.41-7.37 (m, 6H), 7.26-7.24 (m, 1H), 7.20-7.17 (m, 2H), 4.82 (s, 1H), 3.48 (d, J = 16.8 Hz, 1H), 3.37-3.27 (m, 2H), 3.17-3.12 (m, 1H), 3.06-2.91 (m, 2H); Anal. Calcd. for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.20; N, 13.14, Found: C, 70.44; H, 5.18; N, 13.10.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-(2-methoxyphenyl)acetamide (10k)

White solid; % Yield 68; mp 106-108 °C; IR νmax/cm (KBr): 3305, 1666, 1537, 1282; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.91 (s, 1H), 8.37 (dd, J = 8.0, 1.6 Hz, 1H), 7.61-7.59 (m, 1H), 7.44 (dd, J = 7.5, 2.0 Hz, 2H), 7.37-7.34 (m, 4H), 7.25-7.23 (m, 1H), 7.20-7.14 (m, 2H), 7.08 (td, J = 7.8, 1.6 Hz, 1H), 6.98 (td, J = 7.8, 1.1 Hz, 1H), 6.92 (dd, J = 8.1, 1.1 Hz, 1H), 4.78 (s, 1H), 3.91 (s, 3H), 3.46 (d, J = 16.6 Hz, 1H), 3.37-3.33 (m, 1H), 3.19-3.12 (m, 2H), 2.97-2.92 (m, 2H); Anal. Calcd. for $C_{26}H_{25}N_3O_2$: C, 75.89; H, 6.12; N, 10.21, Found: C, 75.88; H, 6.10; N, 10.22.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-o-tolylacetamide (10l)

White solid; % Yield 74; mp 170-172 °C; IR νmax/cm (KBr): 3286, 1664, 1531, 1456; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.23 (brs, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.60-7.58 (m, 1H), 7.42-7.38 (m, 5H), 7.30 (brs, 1H), 7.25-7.15 (m, 5H), 7.05 (td, J = 7.5, 1.1 Hz, 1H), 4.77 (s, 1H), 3.52 (d, J = 16.5 Hz, 1H), 3.42-3.37 (m, 1H), 3.21-3.10 (m, 2H), 3.01-2.94 (m, 2H), 2.29 (s, 3H); Anal. Calcd. for $C_{26}H_{25}N_3O$: C, 78.96; H, 6.37; N, 10.62, Found: C, 78.92; H, 6.40; N, 10.60.

N-(2-chlorophenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetamide (10m)

White solid; % Yield 70; mp 156-158 °C; IR νmax/cm (KBr): 3234, 1672, 1519, 756; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.99 (brs, 1H), 8.48 (dd, J = 8.3, 1.5 Hz, 1H), 7.60-7.58 (m, 1H), 7.48-7.46 (m, 2H), 7.41-7.37 (m, 4H), 7.30-7.28 (m, 2H), 7.24-7.22 (m, 1H), 7.18-7.15 (m, 2H), 7.07-7.03 (m, 1H), 4.78 (s, 1H), 3.51 (d, J = 16.6 Hz, 1H), 3.89-3.34 (m, 1H), 3.30-3.16 (m, 2H), 3.01-2.93 (m, 2H); Anal. Calcd. for $C_{25}H_{22}ClN_3O$: C, 72.19; H, 5.33; N, 10.10, Found: C, 72.23; H, 5.29; N, 10.14.

N-(3-(trifluoromethyl)phenyl)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl) acetamide (10n)

White solid; % Yield 72; mp 160-162 °C; IR νmax/cm (KBr): 3342, 1658, 1519, 1454, 972; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56-7.53 (m, 1H), 7.35-7.30 (m, 5H), 7.27-7.19 (m, 7H), 7.16-7.13 (m, 2H), 4.61 (s, 1H), 3.24 (d, J = 16.5 Hz, 1H), 3.09-3.06 (m, 1H), 2.99 (d, J = 16.5 Hz, 1H), 2.86-2.82 (m, 1H), 2.79-2.73 (m, 2H); Anal. Calcd. for $C_{26}H_{22}F_3N_3O$: C, 69.48; H, 4.93; N, 9.35, Found: C, 69.50; H, 4.90; N, 9.37.

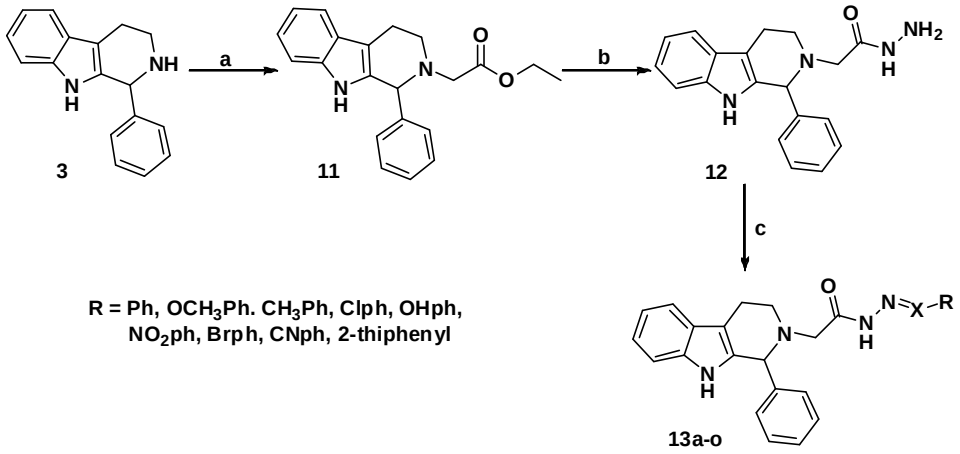
N-(2,4-dimethylphenyl)-2-(1-phenyl-3,4-dihydro-1H-pyrido[3,4-b]indol-2(9H)-yl)acetamide (10o)

White solid; % Yield 70; mp 88-90 °C; IR νmax/cm (KBr): 3302, 1687, 1519, 1454, 742; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (brs, 1H), 7.96 (d, J = 8.1 Hz, 1H), 7.59-7.57 (m, 1H), 7.41-7.37 (m, 5H), 7.31 (brs, 1H), 7.24-7.22 (m, 1H), 7.18-7.14 (m, 2H), 7.04-7.00 (m, 2H), 4.77 (s, 1H), 3.50 (d, J = 16.5 Hz, 1H), 3.40-3.36 (m, 1H), 3.19-3.10 (m, 2H), 3.00-2.91 (m, 2H), 2.30 (s, 3H), 2.25 (s, 3H); Anal. Calcd. for $C_{27}H_{27}N_3O$: C, 79.19; H, 6.65; N, 10.26, Found: C, 79.22; H, 6.63; N, 10.29.

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N-(naphthalen-1-yl)acetamide (10p)

White solid; % Yield 80; mp 160-162 °C; IR vmax/cm (KBr): 3291, 1672, 1531, 1494; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.91 (brs, 1H), 8.26-8.24 (m, 1H), 7.91-7.89 (m, 1H), 7.83-7.80 (m, 1H), 7.68 (d, J = 8.2 Hz, 1H), 7.63-7.61 (m, 1H), 7.55-7.52 (m, 2H), 7.50-7.47 (m, 3H), 7.42-7.39 (m, 4H), 7.27-7.25 (m, 1H), 7.22-7.18 (m, 2H), 4.85 (s, 1H), 3.63 (d, J = 16.6 Hz, 1H), 3.52-3.48 (m, 1H), 3.32 (d, J = 16.6 Hz, 1H), 3.26-3.21 (m, 1H), 3.09-3.00 (m, 2H); Anal. Calcd. for $C_{29}H_{25}N_3O$: C, 80.72; H, 5.84; N, 9.74, Found: C, 80.70; H, 5.82; N, 9.76.

4.3.1.3. Synthesis of *N*'-benzylidene-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetohydrazide derivatives (13a-o)



Scheme 3: Reagents and conditions: (a) ethyl 2-chloroacetate, K_2CO_3, acetonitrile, 8 h, 75%; (b) $NH_2NH_2.H_2O$, ethanol, reflux, 12 h, 62%; (c) substituted aromatic aldehydes, glacial acetic acid, ethanol, reflux, 3 h, 62-76%.

Synthesis of ethyl 2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetate (11)

To the solution of 2,3,4,9-tetrahydro-1-phenyl-1*H*-pyrido[3,4-*b*]indole (**3**) (2.35 g, 1 mol) in acetonitrile, K_2CO_3 (4.14 g, 3 mol) was added and stirred the reaction mixture at room temperature for 30 min. Thereafter, ethyl 2-chloroacetate (2.12 ml, 2 mol) was added and refluxed the reaction mixture for 8 h. After completion of reaction as monitored by TLC, solvent was evaporated, obtained residue was dissolved in 50 mL of ethyl acetate and organic layer was washed twice with (2 x 30 mL) distilled water. Organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum to get intermediate **11** as white solid, yield 75% [120].

Synthesis of 2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (12)

To a stirred solution of ethyl 2-(3,4-dihydro-1-phenyl-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetate (**11**) (1.67 g, 0.5 mol) in 30 mL of absolute ethanol, hydrazine hydrate (0.75 mL, 1.5 mol) and two drops of glacial aceticacid were added and refluxed the reaction mixture for 12 h. After completion of reaction as monitored by TLC, precipitated solid was filtered using vacuum,

residue was washed with cold ethanol (2 x 10 mL) and dried in oven at 50 °C to get intermediate **12** as white solid, yield 62% [160, 273, 274].

General procedure for synthesis of N'-benzylidene-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide derivatives (exemplified as 13a)

To the solution of 2-(3,4-dihydro-1-phenyl-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetohydrazide (**12**) (0.32 g, 1 mmol) in absolute ethanol, two drops of glacial aceticacid and benzaldehyde (0.1 mL, 1 mmol) were added and refluxed the reaction mixture for 3 h. After completion of reaction as monitored by TLC, solvent was evaporated, obtained residue was dissolved in 30 mL of ethyl acetate and organic layer was washed twice with (2 x 30 mL) distilled water. Organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum to get product **13a** as white solid [160].

% yield 70; mp 144-146 °C; IR vmax/cm (KBr): 3362, 3228, 1686, 1531, 1456; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.13 (s, 1H), 8.13 (s, 1H), 7.77-7.75 (m, 2H), 7.59 (d, J = 6.7 Hz, 1H), 7.42-7.37 (m, 9H), 7.25-7.23 (m, 1H), 7.18-7.13 (m, 2H), 4.75 (s, 1H), 3.48 (d, J = 16.6 Hz, 1H), 3.36-3.33 (m, 1H), 3.27 (d, J = 16.7 Hz, 1H), 3.12-3.09 (m, 1H), 2.99-2.96 (m, 2H); MS (ESI) m/z 409.6 $[M + 1]^+$; Anal. Calcd. for $C_{26}H_{24}N_4O$: C, 76.45; H, 5.92; N, 13.72, Found: C, 76.49; H, 5.90; N, 13.74.

N'-(4-methoxybenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydraz-ide (13b)

White solid; % Yield 64; mp 174-176 °C; IR vmax/cm (KBr): 3321, 3296, 1676, 1542, 1456, 1262; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.04 (s, 1H), 8.04 (s, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.59-7.57 (m, 1H), 7.41-7.34 (m, 6H), 7.25-7.23 (m, 1H), 7.20-7.14 (m, 2H), 6.93 (d, J = 8.8 Hz, 2H), 4.75 (s, 1H), 3.86 (s, 3H), 3.48 (d, J = 16.6 Hz, 1H), 3.36-3.32 (m, 1H), 3.26 (d, J = 16.6 Hz, 1H), 3.12-3.08 (m, 1H), 2.99-2.94 (m, 2H); MS (ESI) m/z 439.4 $[M + 1]^+$; Anal. Calcd. for $C_{27}H_{26}N_4O_2$: C, 73.95; H, 5.98; N, 12.78, Found: C, 73.93; H, 5.95; N, 12.81.

N'-(4-methylbenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydraz-ide (13c)

White solid; % Yield 64; mp 102-104 °C; IR vmax/cm (KBr): 3278, 3216, 1684, 1522, 1463; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.09 (s, 1H), 8.06 (s, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.62-7.55 (m, 1H), 7.44-7.32 (m, 6H), 7.26-7.14 (m, 5H), 4.75 (s, 1H), 3.48 (d, J = 16.7 Hz, 1H), 3.38-3.30 (m, 1H), 3.26 (d, J = 16.6 Hz, 1H), 3.18-3.06 (m, 1H), 3.00-2.93 (m, 2H), 2.40 (s, 3H); GCMS (ESI) m/z 423.1 $[M + 1]^+$; Anal. Calcd. for $C_{27}H_{26}N_4O$: C, 76.75; H, 6.20; N, 13.26, Found: C, 76.78; H, 6.17; N, 13.22.

N'-(4-chlorobenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (13d)

White solid; % Yield 72; mp 172-174 °C; IR vmax/cm (KBr): 3298, 3262, 1692, 1522, 1454, 748; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.25 (s, 1H), 8.47 (s, 1H), 8.16-8.14 (m, 1H), 7.60-7.58 (m, 1H), 7.43-7.34 (m, 8H), 7.32-7.30 (m, 1H), 7.25-7.23 (m, 1H), 7.19-7.16 (m, 2H), 4.76 (s, 1H), 3.50 (d, J = 16.8 Hz, 1H), 3.35-3.28 (m, 2H), 3.13-3.09 (m, 1H), 3.01-2.95 (m, 2H); MS (ESI) m/z 443.9 $[M + 1]^+$, 445.9 $[M + 3]^+$; Anal. Calcd. for $C_{26}H_{23}ClN_4O$: C, 70.50; H, 5.23; N, 12.65, Found: C, 70.48; H, 5.28; N, 12.62.

N'-(4-nitrobenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (13e)

Yellow solid; % Yield 74; mp 118-120 °C; IR vmax/cm (KBr): 3408, 3271, 1687, 1508, 1456, 1388; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.32 (s, 1H), 8.34 (s, 1H), 8.27 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 6.7 Hz, 1H), 7.42-7.35 (m, 6H), 7.25-7.16 (m, 3H), 4.77 (s, 1H), 3.52 (d, J = 16.8 Hz, 1H), 3.36-3.28 (m, 2H), 3.14-3.12 (m, 1H), 3.01-2.97 (m, 2H).

N'-(4-bromobenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (13f)

White solid; % Yield 70; mp 158-160 °C; IR vmax/cm (KBr): 3328, 1681, 1545, 1456; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.15 (s, 1H), 8.11 (s, 1H), 7.63-7.53 (m, 5H), 7.41-7.34 (m, 6H), 7.25-7.23 (m, 1H), 7.20-7.14 (m, 2H), 4.75 (s, 1H), 3.48 (d, J = 16.7 Hz, 1H), 3.35-3.31 (m, 1H), 3.27 (d, J = 16.7 Hz, 1H), 3.16-3.08 (m, 1H), 3.00-2.94 (m, 2H), MS (ESI) m/z 488.1 $[M + 1]^+$, 490.1 $[M + 3]^+$; Anal. Calcd. for $C_{26}H_{23}BrN_4O$: C, 64.07; H, 4.76; N, 11.50, Found: C, 64.11; H, 4.74; N, 11.53.

N'-(4-hydroxybenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydraz-ide (13g)

White solid; % Yield 62; mp 220-222 °C; IR vmax/cm (KBr): 3321, 3268, 1692, 1512, 1462; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (s, 1H), 8.00 (s, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.59-7.57 (m, 1H), 7.39-7.32 (m, 7H), 7.23-7.15 (m, 3H), 6.89 (d, J = 8.6 Hz, 2H), 4.76 (s, 1H), 4.15 (d, J = 7.1 Hz, 1H), 3.48 (d, J = 16.7 Hz, 1H), 3.36-3.25 (m, 2H), 3.16-3.07 (m, 1H), 2.99-2.96 (m, 1H).

N'-(4-cyanobenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (13h)

White solid; % Yield 76; mp 96-98 °C; IR vmax/cm (KBr): 3298, 3082, 2220, 1697, 1493, 1276; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.27 (s, 1H), 8.27 (s, 1H), 7.85 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 8.3 Hz, 2H), 7.58 (d, J = 6.8 Hz, 1H), 7.39-7.35 (m, 6H), 7.25-7.23 (m, 1H), 7.19-7.16 (m, 2H), 4.76 (s, 1H), 3.50 (d, J = 16.7 Hz, 1H), 3.35-3.27 (m, 2H), 3.16-3.09 (m, 1H), 3.00-2.96 (m, 2H).

N'-(3-methoxybenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydraz-ide (13i)

White solid; % Yield 66; mp 108-110 °C; IR vmax/cm (KBr): 3292, 3266, 1697, 1514, 1454, 1265; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.14 (s, 1H), 8.10 (s, 1H), 7.58 (d, J = 7.0 Hz, 1H), 7.39-7.17 (m, 12H), 6.98 (d, J = 7.6 Hz, 1H), 4.75 (s, 1H), 3.85 (s, 3H), 3.48 (d, J = 16.6 Hz, 1H), 3.32-3.25 (m, 2H), 3.12-3.10 (m, 1H), 2.99-2.96 (m, 2H).

N'-(3-nitrobenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (13j)

Yellow solid; % Yield 64; mp 98-100 °C; IR vmax/cm (KBr): 3386, 3275, 1696, 1526, 1423, 1372; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.29 (s, 1H), 8.51-8.50 (m, 1H), 8.38 (s, 1H), 8.26-8.23 (m, 1H), 8.14 (d, J = 7.8 Hz, 1H), 7.60-7.56 (m, 2H), 7.42-7.35 (m, 6H), 7.25-7.23 (m, 1H), 7.20-7.15 (m, 2H), 4.76 (s, 1H), 3.50 (d, J = 16.7 Hz, 1H), 3.36-3.27 (m, 2H), 3.15-3.09 (m, 1H), 3.01-2.97 (m, 2H).

N'-(2-bromobenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (13k)

White solid; % Yield 74; mp 126-128 °C; IR vmax/cm (KBr): 3329, 3256, 1686, 1526, 1456; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.19 (s, 1H), 8.10 (s, 1H), 7.93 (s, 1H), 7.63-7.58 (m, 3H), 7.46-7.33 (m, 6H), 7.28-7.16 (m, 4H), 4.73 (s, 1H), 3.46 (d, J = 16.7 Hz, 1H), 3.32-3.22 (m, 2H), 3.11-3.07 (m, 1H), 2.98-2.92 (m, 2H).

N'-(3-hydroxybenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydraz-ide (13l)

White solid; % Yield 68; mp 114-116 °C; IR vmax/cm (KBr): 3326, 3255, 1699, 1506, 1454; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.22 (s, 1H), 7.94 (s, 1H), 7.58-7.53 (m, 2H), 7.35-7.13 (m, 11H), 7.03 (d, J = 7.4 Hz, 1H), 6.91 (d, J = 8.0 Hz, 1H), 4.73 (s, 1H), 3.45 (d, J = 16.8 Hz, 1H), 3.28-3.24 (m, 2H), 3.07- 3.04 (m, 1H), 2.96-2.89 (m, 2H).

N'-(2-chlorobenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohydrazide (13m)

White solid; % Yield 62; mp 160-162 °C; IR vmax/cm (KBr): 3284, 3226, 1697, 1539, 1458; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.15 (s, 1H), 8.12 (s, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.59-7.57 (m, 1H), 7.40-7.34 (m, 9H), 7.25-7.23 (m, 1H), 7.19-7.14 (m, 2H), 4.75 (s, 1H), 3.48 (d, J = 16.7 Hz, 1H), 3.36-3.30 (m, 1H), 3.27 (d, J = 16.7 Hz, 1H), 3.12-3.08 (m, 1H), 2.99-2.93 (m, 2H).

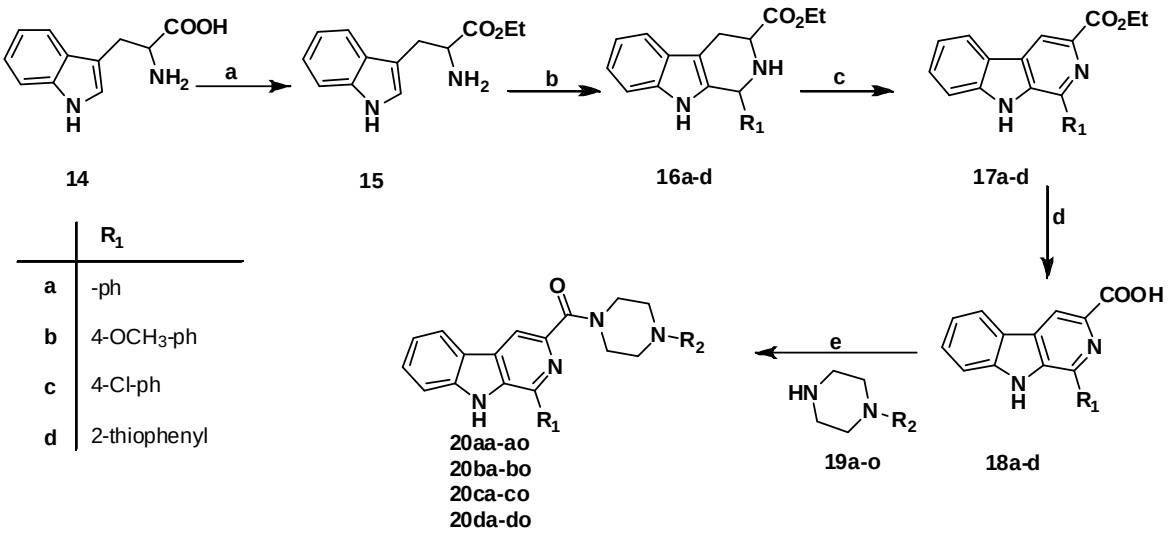
N'-(3,4-dimethoxybenzylidene)-2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)acetohy-drazide (13n)

White solid; % Yield 68; mp 226-228 °C; IR νmax/cm (KBr): 3371, 3255, 1693, 1504, 1267, 1238; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.07 (s, 1H), 8.03 (s, 1H), 7.59-7.57 (m, 1H), 7.46 (d, J = 1.8 Hz, 1H), 7.43 (brs, 1H), 7.38-7.34 (m, 5H), 7.25-7.23 (m, 1H), 7.20-7.11 (m, 3H), 6.88 (d, J = 8.3 Hz, 1H), 4.75 (s, 1H), 3.93 (s, 6H), 3.47 (d, J = 16.7 Hz, 1H), 3.35-3.31 (m, 1H), 3.25 (d, J = 16.6 Hz, 1H), 3.12-3.07 (m, 1H), 2.99-2.93 (m, 2H).

2-(3,4-dihydro-1-phenyl-1H-pyrido[3,4-b]indol-2(9H)-yl)-N'-((thiophen-2-yl)methylene)acetohy-drazide (13o)

White solid; % Yield 76; mp 160-162 °C; IR νmax/cm (KBr): 3318, 3196, 1682, 1513, 1446; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.03 (s, 1H), 8.68 (s, 1H), 7.59-7.57 (m, 1H), 7.43-7.31 (m, 8H), 7.25-7.14 (m, 3H), 7.09-7.06 (m, 1H), 4.73 (s, 1H), 3.47 (d, J = 16.6 Hz, 1H), 3.35-3.31 (m, 1H), 3.23 (d, J = 16.6 Hz, 1H), 3.12-3.08 (m, 1H), 2.98-2.92 (m, 2H).

4.3.2. Synthesis of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives [20a-d(a-o)]



Scheme 4: Reagents and conditions: (a) $SOCl_2$, ethanol, 0 °C, reflux, 1 h, 76%; (b) aromatic aldehydes, trifluoroacetic acid, DCM, rt, 3 h, 80-86%; (c) $KMnO_4$, THF, rt, 24 h, 64-74%, (d) NaoH, ethanol: water (1:1), rt, 30 min, 70-78%; (e) THF, EDCI. HCl, HOBt, rt, 6 h, 60-92%.

Synthesis of ethyl 2-amino-3-(1H-indol-3-yl)propanoate (15)

To a solution of DL-tryptophan (**14**) (5 g, 0.0245 mol) in 50 mL of ethanol, $SOCl_2$ (5.32 ml, 0.073 mol) was added drop wise at 0 °C. Subsequently, the reaction mixture was refluxed for 1 h. After completion of reaction as monitored by TLC, solvent was evaporated *in-vacuo* and the residue obtained was dissolved in ethyl acetate. Organic layer was washed twice with $NaHCO_3$ solution. The separated organic layer was dried over Na_2SO_4 and concentrated under vacuum to get intermediate **15** as white solid, yield 76% [266].

Synthesis of ethyl 2,3,4,9-tetrahydro-1-substituted-1H-pyrido[3,4-b]indole-3-carboxylate (exemplified as 16a)

To the mixture of ethyl 2-amino-3-(1*H*-indol-3-yl)propanoate (**15**) (5 g, 0.022 mol) and benzaldehyde (**2**) (2.2 mL, 0.022 mol) in 100 mL of dichloromethane, 2.5 mL of trifluoroacetic acid was added drop wise at 0 °C for 10 min. Then, reaction mixture was stirred at room temperature for 3 h. After completion of reaction as monitored by TLC, the

reaction mixture was poured into saturated $NaHCO_3$ solution. Organic layer was separated and washed twice with brine solution, dried over Na_2SO_4 and concentrated under vacuum to get **16a** as white solid, yield 82% [266-268, 271].

Synthesis of ethyl 1-substituted-9H-pyrido[3,4-b]indole-3-carboxylate (exemplified as 17a)

To a solution of ethyl 2,3,4,9-tetrahydro-1-phenyl-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**16a**) (4 g, 0.013 mol) in 200 mL of dry THF, $KMnO_4$ (6.15 g, 0.039 mol) was added and the reaction mixture was stirred at room temperature for 24 h. After completion of reaction as monitored by TLC, the reaction mixture was passed through celite bed to filter off the un-reacted solid $KMnO_4$. Then, solvent was evaporated *in-vacuo*, the residue was dissolved in ethyl acetate and washed twice with 50 mL of distilled water. The separated organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **17a** as yellowish white solid, yield 68% [266, 267, 271].

Synthesis of 1-substituted-9H-pyrido[3,4-b]indole-3-carboxylic acid (exemplified as 18a)

To a solution of ethyl 1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**17a**) (3 g, 0.01 mol) in ethanol water (1:1) mixture, NaOH (1.2 g, 0.03 mol) was added and refluxed for 30 min. After completion of reaction as monitored by TLC, ethanol from the reaction mixture was removed under vacuum and neutralized with dil. HCl. Then, the reaction mixture was extracted with ethyl acetate (2 x 30 mL), collected organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **18a** as white solid, yield 78%.

*General procedure for the synthesis of (1-phenyl-9H-pyrido[3,4-b]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives (exemplified as 20aa***)**

To a stirred solution of 1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (**18a**) (0.29 g, 1 mmol) in dry THF, HOBt (0.16 g, 1.2 mmol) and EDCI. HCl (0.23 g, 1.2 mmol) were added and continued stirring for 30 min. To the reaction mixture, 1-phenylpiperazine (**19a**) was added under ice cold temperature and the reaction mixture was further stirred at room temperature for 6 h. After completion of reaction as monitored by TLC, solvent was evaporated under vacuum. Reaction mixture was extracted with ethyl acetate (2 x 20 mL), collected organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **20aa**.

The obtained crude product was passed through short bed of silica gel (100-200) by using mobile phase ethyl acetate and *n*-hexane mixture (4:6) to obtain analytically pure compound as white solid [275, 276].

% Yield 74; mp 158-160 °C; IR vmax/cm (KBr): 3157, 1614, 1556, 1489, 738; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.53 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 7.64-7.58 (m, 3H), 7.54-7.52 (m, 3H), 7.48-7.46 (m, 1H), 7.37-7.32 (m, 1H), 7.31-7.26 (m, 2H), 6.95-6.89 (m, 3H), 4.10-4.01 (m, 4H), 3.23-3.21 (m, 4H); MS m/z 433.4 $[M + 1]^+$; Anal. Calcd. for $C_{28}H_{24}N_4O$: C, 77.75; H, 5.59; N, 12.95, Found: C, 77.78; H, 5.63; N, 12.93.

(4-(4-methoxyphenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ab)

White solid; % Yield 80; mp 182-184 °C; IR vmax/cm (KBr): 3234, 1625, 1546, 1510, 1255, 746; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.49 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.95 (d, J = 7.2 Hz, 2H), 7.62-7.48 (m, 7H), 7.37-7.32 (m, 1H), 6.88 (d, J = 8.9 Hz, 2H), 4.25-4.10 (m, 4H), 3.79 (s, 3H), 3.32-3.26 (m, 4H); MS m/z 463.4 $[M + 1]^+$; Anal. Calcd. for $C_{29}H_{26}N_4O_2$: C, 75.30; H, 5.67; N, 12.11, Found: C, 75.28; H, 5.64; N, 12.14.

(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)(4-p-tolylpiperazin-1-yl)methanone (20ac)

White solid; % Yield 74; mp 208-210 °C; IR vmax/cm (KBr): 3228, 1620, 1587, 1490, 1433, 746; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.51 (s, 1H), 8.19 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 7.2 Hz, 2H), 7.65-7.52 (m, 7H), 7.37 (t, J = 7.3 Hz, 1H), 7.15 (d, J = 7.1 Hz, 2H), 4.15-4.03 (m, 4H), 3.35-3.26 (m, 4H), 2.32 (s, 3H); MS m/z 447.4 $[M + 1]^+$; Anal. Calcd. for $C_{29}H_{26}N_4O$: C, 78.00; H, 5.87; N, 12.55, Found: C, 78.02; H, 5.83; N, 12.58.

(4-(4-chlorophenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ad)

White solid; % Yield 76; mp 186-188 °C; IR vmax/cm (KBr): 3203, 1610, 1588, 1481, 738; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.67 (s, 1H), 8.44 (s, 1H), 8.24 (d, J = 7.9 Hz, 1H), 8.10-8.06 (m, 2H), 7.67-7.60 (m, 5H), 7.30-7.26 (m, 1H), 7.19 (dd, J = 9.8, 6.4 Hz, 2H), 6.94 (d, J = 9.1 Hz, 2H), 3.99-3.95 (m, 4H), 3.30-3.25 (m, 4H); MS m/z 468.2 $[M + 1]^+$, 470.2 $[M + 3]^+$; Anal. Calcd. for $C_{28}H_{23}ClN_4O$: C, 72.02; H, 4.96; N, 12.00, Found: C, 72.05; H, 4.93; N, 12.03.

(4-(4-nitrophenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ae)

Yellow solid; % Yield 62; mp 134-136 °C; IR vmax/cm (KBr): 3184, 1624, 1595, 1489, 1319, 752; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.55 (s, 1H), 8.19-8.14 (m, 3H), 7.97 (d, J = 7.1 Hz, 2H), 7.64-7.52 (m, 5H), 7.36 (t, J = 7.3 Hz, 1H), 6.84 (d, J = 9.4 Hz, 2H), 4.20-4.06 (m, 4H), 3.64-3.56 (m, 4H).

(4-(4-fluorophenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20af)

White solid; % Yield 72; mp 178-180 °C; IR vmax/cm (KBr): 3209, 1614, 1550, 1508, 744; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.49 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 7.98-7.96 (m, 2H), 7.63-7.40 (m, 5H), 7.37-7.33 (m, 1H), 7.01-6.89 (m, 4H), 4.09-4.06 (m, 4H), 3.26-3.18 (m, 4H); MS m/z 451.3 $[M + 1]^+$; Anal. Calcd. for $C_{28}H_{23}FN_4O$: C, 74.65; H, 5.15; N, 12.44, Found: C, 74.64; H, 5.18; N, 12.49.

(4-(3-methoxyphenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ag)

White solid; % Yield 74; mp 118-120 °C; IR vmax/cm (KBr): 3221, 1606, 1536, 1492, 1251, 741; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.51 (s, 1H), 8.19 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 7.2 Hz, 2H), 7.65-7.52 (m, 5H), 7.37 (t, J = 7.3 Hz, 1H), 7.22 (t, J = 8.2 Hz, 1H), 6.60-6.58 (m, 1H), 6.51-6.48 (m, 2H), 4.10-4.06 (m, 4H), 3.82 (s, 3H), 3.37-3.30 (m, 4H).

(4-(3-chlorophenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ah)

White solid; % Yield 72; mp 138-140 °C; IR vmax/cm (KBr): 3201, 1610, 1558, 1481, 738; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 1H), 8.52 (s, 1H), 8.19 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 7.0 Hz, 2H), 7.66-7.53 (m, 5H), 7.39-7.35 (m, 1H), 7.21 (t, J = 8.1 Hz, 1H), 6.93 (t, J = 2.1 Hz, 1H), 6.88-6.82 (m, 2H), 4.12-4.06 (m, 4H), 3.38-3.31 (m, 4H).

(4-(2-methoxyphenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ai)

White solid; % Yield 64; mp 110-112 °C; IR vmax/cm (KBr): 3244, 1624, 1558, 1498, 1242, 740; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.47 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 8.01-7.98 (m, 2H), 7.64-7.51 (m, 5H), 7.38-7.34 (m, 1H), 7.08-7.04 (m, 1H), 7.01-6.90 (m, 3H), 4.20-4.10 (m, 4H), 3.91 (s, 3H), 3.25-3.16 (m, 4H).

(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)(4-o-tolylpiperazin-1-yl)methanone (20aj)

White solid; % Yield 68; mp 122-124 °C; IR vmax/cm (KBr): 3201, 1622, 1556, 1490, 1431, 740; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 8.46 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 8.05-7.96 (m, 2H), 7.67-7.55 (m, 4H), 7.52 (t, J = 7.4 Hz, 1H), 7.38-7.34 (m, 1H), 7.27-7.18 (m, 2H), 7.12-7.00 (m, 2H), 4.10-4.00 (m, 4H), 3.10-3.01 (m, 4H), 2.38 (s, 3H).

(4-(2-chlorophenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ak)

White solid; % Yield 64; mp 108-110 °C; IR vmax/cm (KBr): 3203, 1610, 1558, 1481, 738; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.50 (s, 1H), 8.19 (d, J = 7.9 Hz, 1H), 8.01-7.99 (m, 2H), 7.65-7.54 (m, 5H), 7.42-7.35 (m, 2H), 7.27-7.24 (m, 1H), 7.09-7.01 (m, 2H), 4.12-4.02 (m, 4H), 3.24-3.16 (m, 4H).

(4-(2-fluorophenyl)piperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone(20al)

White solid; % Yield 68; mp 104-106 °C; IR vmax/cm (KBr): 3182, 1610, 1556, 1498, 750; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.50 (s, 1H), 8.18 (d, J = 7.9 Hz, 1H), 8.02-7.98 (m, 2H), 7.65-7.51 (m, 5H), 7.39-7.35 (m, 1H), 7.12-7.05 (m, 2H), 7.02-6.97 (m, 2H), 4.11-4.10 (m, 4H), 3.28-3.20 (m, 4H).

(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)(4-(pyridin-4-yl)piperazin-1-yl)methanone (20am)

Pale yellow solid; % Yield 66; mp 130-132 °C; IR vmax/cm (KBr): 3162, 1641, 1541, 1419; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.70 (s, 1H), 8.47 (s, 1H), 8.26-8.19 (m, 2H), 8.09-8.06 (m, 2H), 7.69-7.53 (m, 6H), 7.30-7.27 (m, 1H), 6.84 (t, J = 11.7 Hz, 2H), 4.03-3.93 (m, 4H), 3.57-3.51 (m, 4H).

(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (20an)

Pale yellow solid; % Yield 62; mp 110-112 °C; IR vmax/cm (KBr): 3275, 1635, 1581, 1435, 734; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.49 (s, 1H), 8.23 (d, J = 3.6 Hz, 1H), 8.18 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 7.1 Hz, 2H), 7.62-7.50 (m, 6H), 7.36 (t, J = 7.3 Hz, 1H), 6.71-6.67 (m, 2H), 4.07-4.03 (m, 4H), 3.80-3.65 (m, 4H).

(4-benzylpiperazin-1-yl)(1-phenyl-9H-pyrido[3,4-b]indol-3-yl)methanone (20ao)

White solid; % Yield 72; mp 160-162 °C; IR vmax/cm (KBr): 3174, 1626, 1556, 1487, 1431, 738; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.43 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 7.2 Hz, 2H), 7.66-7.47 (m, 5H), 7.35-7.28 (m, 6H), 4.00-3.92 (m, 4H), 3.59 (s, 2H), 2.62-5.20 (m, 4H); MS m/z 447.6 ($M + 1^{+}$); Anal. Calcd. for $C_{29}H_{26}N_4O$: C, 78.00; H, 5.87; N, 12.55, Found: C, 78.04; H, 5.84; N, 12.58.

(1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives (20ba-bo)

General procedure for the synthesis of (1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives *(exemplified as 20ba***)**

To a stirred solution of 1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (**18b**) (0.32 g, 1 mmol) in dry THF, HOBt (0.16 g, 1.2 mmol) and EDCI. HCl (0.23 g, 1.2 mmol) were added and continued stirring for 30 min. To the reaction mixture, 1-phenylpiperazine (**19a**) was added under ice cold temperature and the reaction mixture was further stirred at room temperature for 6 h. After completion of reaction as monitored by TLC, solvent was evaporated under vacuum. Reaction mixture was extracted with ethyl acetate (2 x 20 mL), collected organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **20ba**. The obtained crude product was passed through short bed of silica gel (100-200) by using mobile phase ethyl acetate and *n*-hexane mixture (5:5) to obtain analytically pure compound as white solid [275, 276].

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-phenylpiperazin-1-yl)methanone (20ba)

White solid; % Yield 78; mp 240-242 °C; IR vmax/cm (KBr): 3360, 1649, 1477, 1234, 796; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.47 (s, 1H), 8.19 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 8.7 Hz, 2H), 7.61-7.56 (m, 2H), 7.42-7.25 (m, 3H), 7.16 (d, J = 8.7 Hz, 2H), 7.05-6.88 (m, 3H), 4.15-4.05 (m, 4H), 3.94 (s, 3H), 3.42-3.25 (m, 4H); MS (ESI) m/z 463.4 $[M + 1]^+$; Anal. Calcd. for $C_{29}H_{26}N_4O_2$: C, 75.30; H, 5.67; N, 12.11, Found: C, 75.28; H, 5.64; N, 12.15.

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(4-methoxyphenyl)piperazin-1-yl)methan-one (20bb)

White solid; % Yield 92; mp 122-124 °C; IR vmax/cm (KBr): 3156, 1654, 1592, 1492, 1252, 1236; ^{1}H NMR (400 MHz, $CDCl_3$): δ8.67 (s, 1H), 8.46 (s, 1H), 8.19 (d, J = 7.9 Hz, 1H), 7.99-7.91 (m, 2H), 7.62-7.55 (m, 2H), 7.38-7.34 (m, 1H), 7.18-7.11 (m, 2H), 6.99-6.92 (m, 2H), 6.92-6.84 (m, 2H), 4.12-4.05 (m, 4H), 3.94 (s, 3H), 3.80 (s, 3H), 3.28-3.16 (m, 4H); MS (ESI) m/z 493.3 $[M + 1]^+$; Anal. Calcd. for $C_{30}H_{28}N_4O_3$: C, 73.15; H, 5.73; N, 11.37, Found: C, 73.13; H, 5.76; N, 11.34.

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-p-tolylpiperazin-1-yl)methanone (20bc)

White solid; % Yield 86; mp 142-144 °C; IR νmax/cm (KBr): 3381, 1634, 1512, 1481, 1257; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.44 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 7.98-7.91 (m, 2H), 7.64-7.53 (m, 2H), 7.37-7.33 (m, 1H), 7.19-7.08 (m, 4H), 6.90 (d, J = 8.6 Hz, 2H), 4.10-4.02 (m, 4H), 3.92 (s, 3H), 3.31-3.23 (m, 4H), 2.31 (s, 3H); MS (ESI) m/z 477.5 $[M + 1]^+$; Anal. Calcd. for $C_{30}H_{28}N_4O_2$: C, 75.61; H, 5.92; N, 11.76, Found: C, 75.65; H, 5.96; N, 11.79.

(4-(4-chlorophenyl)piperazin-1-yl)(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20bd)

White solid; % Yield 80; mp 170-172 °C; IR νmax/cm (KBr): 3157, 1629, 1550, 1492, 1234, 740; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.46 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 7.98-7.89 (m, 2H), 7.64-7.54 (m, 2H), 7.38-7.34 (m, 1H), 7.27-7.23 (m, 2H), 7.17-7.12 (m, 2H), 6.92-6.87 (m, 2H), 4.12-4.02 (m, 4H), 3.93 (s, 3H), 3.33-3.26 (m, 4H); MS (ESI) m/z 498.2 $[M + 1]^+$, 500.1 $[M + 3]^+$; Anal. Calcd. for $C_{29}H_{25}ClN_4O_2$: C, 70.08; H, 5.07; N, 11.27, Found: C, 70.12; H, 5.05; N, 11.30.

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(4-nitrophenyl)piperazin-1-yl)methanone (20be)

White solid; % Yield 70; mp 214-216 °C; IR νmax/cm (KBr): 3162, 1616, 1504, 1487, 1238; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H), 8.52 (s, 1H), 8.23-8.13 (m, 3H), 7.97-7.90 (m, 2H), 7.65-7.55 (m, 2H), 7.39-7.35 (m, 1H), 7.19-7.12 (m, 2H), 6.90-6.83 (m, 2H), 4.25-4.05 (m, 4H), 3.93 (s, 3H), 3.65-3.58 (m, 4H).

(4-(4-fluorophenyl)piperazin-1-yl)(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20bf)

White solid; % Yield 84; mp 162-164 °C; IR vmax/cm (KBr): 3223, 1622, 1508, 1431, 1234, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H), 8.44 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 7.8 Hz, 1H), 7.62-7.56 (m, 2H), 7.38-7.34 (m, 2H), 7.15-7.08 (m, 2H), 7.03-6.97 (m, 2H), 4.19-4.00 (m, 4H), 3.92 (s, 3H), 3.26-3.17 (m, 4H); MS (ESI) m/z 481.3 $[M + 1]^+$; Anal. Calcd. for $C_{29}H_{25}FN_4O_2$: C, 72.48; H, 5.24; N, 11.66, Found: C, 72.51; H, 5.22; N, 11.70.

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(3-methoxyphenyl)piperazin-1-yl)methano-ne (20bg)

White solid; % Yield 78; mp 132-134 °C; IR vmax/cm (KBr): 3143, 1610, 1492, 1431, 1255, 1240; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 (s, 1H), 8.45 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 8.8 Hz, 2H), 7.60-7.56 (m, 2H), 7.37-7.33 (m, 1H), 7.22 (t, J = 8.2 Hz, 1H), 7.14 (d, J = 8.8 Hz, 2H), 6.60 (d, J = 8.0 Hz, 1H), 6.56-6.45 (m, 2H), 4.13-4.03 (m, 4H), 3.92 (s, 3H), 3.82 (s, 3H), 3.37-3.30 (m, 4H).

(4-(3-chlorophenyl)piperazin-1-yl)(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20bh)

White solid; % Yield 74; mp 104-106 °C; IR vmax/cm (KBr): 3153, 1614, 1487, 1433, 1234, 740; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (s, 1H), 8.45 (s, 1H), 8.16 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 8.6 Hz, 2H), 7.59 (d, J = 5.6 Hz, 2H), 7.35 (t, J = 6.0 Hz, 1H), 7.21 (t, J = 8.1 Hz, 1H), 7.14 (d, J = 8.5 Hz, 2H), 6.93 (s, 1H), 6.90-6.80 (m, 2H), 4.07 (d, J = 21.0 Hz, 4H), 3.92 (s, 3H), 3.36-3.30 (m, 4H).

*(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(2-methoxyphenyl)piperazin-1-yl)methan-one (20bi***)**

White solid; % Yield 68; mp 88-90 °C; IR vmax/cm (KBr): 3166, 1629, 1500, 1440, 1255, 1240; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.37 (s, 1H), 8.18-8.12 (m, 1H), 7.93-7.89 (m, 2H), 7.59-7.54 (m, 2H), 7.37-7.33 (m, 5H), 7.14-7.09 (m, 2H), 3.95-3.85 (m, 7H), 3.59 (s, 3H), 2.63-2.53 (m, 4H).

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-o-tolylpiperazin-1-yl)methanone (20bj)

White solid; % Yield 78; mp 110-112 °C; IR vmax/cm (KBr): 3184, 1625, 1594, 1460, 1255; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.02 (s, 1H), 8.41 (s, 1H), 8.16 (d, J = 7.4 Hz, 1H), 7.94 (d, J = 8.2 Hz, 2H), 7.63-7.55 (m, 3H), 7.37-7.32 (m, 1H), 7.25-7.15 (m, 2H), 7.08-6.99 (m, 3H), 4.10-3.90 (m, 7H), 3.10-2.95 (m, 4H), 2.37 (s, 3H).

(4-(2-chlorophenyl)piperazin-1-yl)(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20bk)

White solid; % Yield 64; mp 116-118 °C; IR vmax/cm (KBr): 3242, 1622, 15580, 1479, 1253, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.45 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.95 (d, J = 8.7 Hz, 2H), 7.60-7.56 (m, 2H), 7.42-7.39 (m, 1H), 7.39-7.33 (m, 1H), 7.27-7.25 (m, 1H), 7.15 (d, J = 8.8 Hz, 2H), 7.11-7.00 (m, 2H), 4.15-4.05 (m, 4H), 3.93 (s, 3H), 3.23-3.15 (m, 4H).

(4-(2-fluorophenyl)piperazin-1-yl)(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20bl)

White solid; % Yield 76; mp 82-84 °C; IR vmax/cm (KBr): 3147, 1643, 1502, 1433, 1236, 761; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76 (s, 1H), 8.46 (s, 1H), 8.18 (d, J = 7.9 Hz, 1H), 7.98-7.91 (m, 2H), 7.64-7.54 (m, 2H), 7.38-7.34 (m, 1H), 7.19-7.11 (m, 2H), 7.05-6.93 (m, 4H), 4.12-4.05 (m, 4H), 3.93 (s, 3H), 3.32-3.18 (m, 4H).

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(pyridin-4-yl)piperazin-1-yl)methanone (20bm)

White solid; % Yield 76; mp 94-96 °C; IR νmax/cm (KBr): 3165, 1618, 1485, 1433, 1242, 742; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.00 (s, 1H), 8.44 (s, 1H), 8.23 (d, J = 3.6 Hz, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 8.6 Hz, 2H), 7.66-7.49 (m, 3H), 7.40-7.31 (m, 1H), 7.10 (d, J = 8.6 Hz, 2H), 6.71-6.67 (m, 2H), 4.1-4.00 (m, 4H), 3.90 (s, 3H), 3.75-3.65 (m, 4H).

(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (20bn)

White solid; % Yield 60; mp 82-84 °C; IR νmax/cm (KBr): 3192, 1627, 1510, 1419, 1246, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19 (s, 1H), 8.50 (s, 1H), 8.28 (d, J = 5.2 Hz, 2H), 8.17 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.61-7.57 (m, 2H), 7.38-7.34 (m, 1H), 7.11 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 5.5 Hz, 2H), 4.16-4.04 (m, 4H), 3.90 (s, 3H), 3.58-3.51 (m, 4H).

(4-benzylpiperazin-1-yl)(1-(4-methoxyphenyl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20bo)

White solid; % Yield 76; mp 106-108 °C; IR νmax/cm (KBr): 3259, 1612, 1554, 1427, 1260; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.44 (s, 1H), 8.19 (d, J = 7.9 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.61-7.57 (m, 2H), 7.38-7.34 (m, 1H), 7.14 (d, J = 8.8 Hz, 2H), 7.10-7.02 (m, 1H), 7.00-6.90 (m, 4H), 4.15-4.05 (m, 4H), 3.93 (s, 3H), 3.91 (s, 2H), 3.25-3.15 (m, 4H); MS (ESI) m/z 477.4 $[M + 1]^+$; Anal. Calcd. for $C_{30}H_{28}N_4O_2$: C, 75.61; H, 5.92; N, 11.76, Found: C, 75.64; H, 5.96; N, 11.73.

(1-(4-chlorophenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives (20ca-co)

General procedure for the synthesis of (1-(4-chlorophenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives *(exemplified as 20ca***)**

To a stirred solution of 1-(4-chlorophenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (**18c**) (0.32 g, 1 mmol) in dry THF, HOBt (0.16 g, 1.2 mmol) and EDCI. HCl (0.23 g, 1.2 mmol) were added and continued stirring for 30 min. To the reaction mixture, 1-phenylpiperazine (**19a**) was added under ice cold temperature and the reaction mixture was further stirred at room temperature for 6 h. After completion of reaction as monitored by TLC, solvent was evaporated under vacuum. Reaction mixture was extracted with ethyl acetate (2 x 20 mL), collected organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **20ca**. The obtained crude product was passed through short bed of silica gel (100-200) by using mobile phase ethyl acetate and *n*-hexane mixture (4:6) to obtain analytically pure compound as white solid [275, 276].

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-phenylpiperazin-1-yl)methanone (20ca)

White solid; % Yield 68; mp 126-128 °C; IR vmax/cm (KBr): 3196, 1622, 1508, 1490, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.03 (s, 1H), 8.43 (s, 1H), 8.12 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 8.5 Hz, 2H), 7.62-7.52 (m, 4H), 7.37-7.29 (m, 3H), 6.99 (d, J = 8.1 Hz, 2H), 6.94 (t, J = 7.3 Hz, 1H), 4.11-4.02 (m, 4H), 3.36-3.27 (m, 4H); MS (ESI) m/z 468.2 $[M + 1]^+$, 469.5 $[M + 3]^+$; Anal. Calcd. for $C_{28}H_{23}ClN_4O$: C, 72.02; H, 4.96; N, 12.00, Found: C, 72.00; H, 4.98; N, 12.04.

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(4-methoxyphenyl)piperazin-1-yl)methanone (20cb)

White solid; % Yield 84; mp 198-200 °C; IR vmax/cm (KBr): 3256, 1624, 15221, 1442, 752; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.06 (s, 1H), 8.41 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 7.95-7.88 (m, 2H), 7.63-7.51 (m, 4H), 7.37-7.33 (m, 1H), 6.97 (d, J = 9.0 Hz, 2H), 6.91-6.86 (m, 2H), 4.08-4.02 (m, 4H), 3.80 (s, 3H), 3.24-3.15 (m, 4H); MS (ESI) m/z 498.2 $[M + 1]^+$, 500.2 $[M + 3]^+$; Anal. Calcd. for $C_{29}H_{25}ClN_4O_2$: C, 70.08; H, 5.07; N, 11.27, Found: C, 70.12; H, 5.04; N, 11.31.

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-p-tolylpiperazin-1-yl)methanone (20cc)

White solid; % Yield 76; mp 192-194 °C; IR vmax/cm (KBr): 3157, 1624, 1542, 1489, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.36 (s, 1H), 8.35 (s, 1H), 8.09 (d, J = 20.3 Hz, 1H), 7.94-7.84 (m, 2H), 7.68-7.44 (m, 4H), 7.34-7.28 (m, 1H), 7.13 (d, J = 8.0 Hz, 2H), 6.90 (d, J = 7.9 Hz, 2H), 4.10-4.00 (m, 4H), 3.30-3.21 (m, 4H), 2.31 (s, 3H); GCMS (ESI) m/z 482.3 $[M + 1]^+$, 484.2 $[M + 3]^+$; Anal. Calcd. for $C_{29}H_{25}ClN_4O$: C, 72.42; H, 5.24; N, 11.65, Found: C, 72.46; H, 5.22; N, 11.69.

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(4-chlorophenyl)piperazin-1-yl)methanone (20cd)

White solid; % Yield 78; mp 208-210 °C; IR vmax/cm (KBr): 3325, 1624, 1580, 1490, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.15 (s, 1H), 8.41 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 8.2 Hz, 2H), 7.58-7.54 (m, 4H), 7.34 (dt, J = 7.6, 3.8 Hz, 1H), 7.29-7.22 (m, 2H), 6.88 (d, J = 8.8 Hz, 2H), 4.04 (s, 4H), 3.31-3.23 (m, 4H); GCMS (ESI) m/z 501.3 $[M]^+$, 503.1 $[M + 2]^+$, 505.0 $[M + 4]^+$; Anal. Calcd. for $C_{28}H_{22}Cl_2N_4O$: C, 67.07; H, 4.42; N, 11.17, Found: C, 67.10; H, 4.46; N, 11.21.

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(4-nitrophenyl)piperazin-1-yl)methanone (20ce)

Yellow solid; % Yield 64; mp 170-172 °C; IR vmax/cm (KBr): 3332, 1616, 1508, 1487, 1317, 752; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.56 (s, 1H), 8.24-8.14 (m, 3H), 7.94 (d, J = 8.5 Hz, 2H), 7.67-7.58 (m, 4H), 7.43-7.35 (m, 1H), 6.90-6.82 (m, 2H), 4.23-4.06 (m, 4H), 3.66-3.57 (m, 4H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(4-fluorophenyl)piperazin-1-yl)methanone (20cf)

White solid; % Yield 70; mp 204-206 °C; IR vmax/cm (KBr): 3180, 1608, 1508, 1458, 754; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (s, 1H), 8.46 (s, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.63-7.57 (m, 4H), 7.38-7.35 (m, 1H), 7.04-6.97 (m, 2H), 6.97-6.90 (m, 2H), 4.11-4.01 (m, 4H), 3.28-3.18 (m, 4H); MS (ESI) m/z 486.4 $[M + 1]^+$, 488.2 $[M + 3]^+$; Anal. Calcd. for $C_{28}H_{22}ClFN_4O$: C, 69.35; H, 4.57; N, 11.55, Found: C, 69.31; H, 4.62; N, 11.52.

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(3-methoxyphenyl)piperazin-1-yl)methanone (20cg)

White solid; % Yield 80; mp 140-142 °C; IR vmax/cm (KBr): 3186, 1614, 1523, 1490, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86 (s, 1H), 8.46 (s, 1H), 8.15 (d, J = 7.9 Hz, 1H), 7.98-7.88 (m, 2H), 7.62-7.58 (m, 4H), 7.38-7.34 (m, 1H), 7.22 (t, J = 8.1 Hz, 1H), 6.59 (dd, J = 8.2, 1.9 Hz, 1H), 6.54-6.47 (m, 2H), 4.10-4.01 (m, 4H), 3.82 (s, 3H), 3.37-3.27 (m, 4H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(3-chlorophenyl)piperazin-1-yl)methanone (20ch)

White solid; % Yield 64; mp 152-154 °C; IR vmax/cm (KBr): 3167, 1653, 1516, 1435, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (s, 1H), 8.45 (s, 1H), 8.13 (d, J = 7.9 Hz, 1H), 7.95-7.89 (m, 2H), 7.62-7.55 (m, 4H), 7.38-7.34 (m, 1H), 7.22 (t, J = 8.1 Hz, 1H), 6.93 (t, J = 2.0 Hz, 1H), 6.88-6.83 (m, 2H), 4.10-4.00 (m, 4H), 3.37-3.28 (m, 4H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(2-methoxyphenyl)piperazin-1-yl)methanone (20ci)

White solid; % Yield 72; mp 116-118 °C; IR vmax/cm (KBr): 3246, 1618, 1508, 1436, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.39 (s, 1H), 8.35 (s, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 8.5 Hz, 2H), 7.61-7.54 (m, 2H), 7.49 (d, J = 8.5 Hz, 2H), 7.35-7.29 (m, 1H), 7.10-7.06 (m, 1H), 6.99-6.91 (m, 3H), 4.07-4.03 (m, 4H), 3.91 (s, 3H), 3.22-3.14 (m, 4H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-o-tolylpiperazin-1-yl)methanone (20cj)

White solid; % Yield 68; mp 186-188 °C; IR vmax/cm (KBr): 3262, 1632, 1522, 1464, 760; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.64 (s, 1H), 8.36 (s, 1H), 8.15-8.02 (m, 1H), 7.94-7.91 (m, 2H), 7.57-7.44 (m, 4H), 7.28-7.21 (m, 1H), 7.11-7.08 (m, 2H), 6.96-6.92 (m, 2H), 4.00-3.90 (m, 4H), 2.99-2.88 (m, 4H), 2.27 (s, 3H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(2-chlorophenyl)piperazin-1-yl)methanone (20ck)

White solid; % Yield 68; mp 138-140 °C; IR vmax/cm (KBr): 3255, 1620, 1514, 1435, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.17 (s, 1H), 8.38 (s, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 3.7 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.43-7.41 (m, 1H), 7.37-7.31 (m, 1H), 7.29-7.23 (m, 1H), 7.10-7.00 (m, 2H), 4.07-4.03 (m, 4H), 3.22-3.13 (m, 4H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(2-fluorophenyl)piperazin-1-yl)methanone (20cl)

White solid; % Yield 68; mp 180-182 °C; IR vmax/cm (KBr): 3251, 1618, 1504, 1435, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.86 (s, 1H), 8.47 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.96-7.91 (m, 2H), 7.61-7.57 (m, 4H), 7.39-7.35 (m, 1H), 7.15-7.04 (m, 2H), 7.02-6.97 (m, 2H), 4.12-4.02 (m, 4H), 3.26-3.18 (m, 4H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(pyridin-4-yl)piperazin-1-yl)methanone (20cm)

White solid; % Yield 74; mp 196-198 °C; IR vmax/cm (KBr): 3168, 1651, 1506, 1456, 736; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.03 (s, 1H), 8.54 (s, 1H), 8.32 (d, J = 6.5 Hz, 2H), 8.19 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.67-7.55 (m, 4H), 7.43-7.35 (m, 1H), 6.71 (d, J = 6.5 Hz, 2H), 4.13-4.06 (m, 4H), 3.61-3.43 (m, 4H).

(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)(4-(pyridin-2-yl)piperazin-1-yl)methanone (20cn)

White solid; % Yield 70; mp 154-156 °C; IR vmax/cm (KBr): 3207, 1626, 1508, 1485, 746; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (s, 1H), 8.47 (s, 1H), 8.26-8.20 (m, 1H), 8.15 (d, J = 7.9 Hz, 1H), 7.96-7.88 (m, 2H), 7.65-7.50 (m, 5H), 7.38-7.34 (m, 1H), 6.74-6.65 (m, 2H), 4.07-3.97 (m, 4H), 3.77-3.65 (m, 4H).

(4-benzylpiperazin-1-yl)(1-(4-chlorophenyl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20co)

White solid; % Yield 64; mp 168-170 °C; IR vmax/cm (KBr): 3192, 1622, 1511, 1486, 782; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.18 (s, 1H), 8.36 (s, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.92-7.85 (m, 2H), 7.59-7.56 (m, 2H), 7.54-7.50 (m, 2H), 7.38-7.29 (m, 6H), 3.96-3.82 (m, 4H), 3.59 (s, 2H), 2.67-2.47 (m, 4H); MS (ESI) m/z 482.9 $[M + 1]^+$, 484.7 $[M + 3]^+$; Anal. Calcd. for $C_{29}H_{25}ClN_4O$: C, 72.42; H, 5.24; N, 11.65, Found: C, 72.44; H, 5.28; N, 11.61.

(4-phenylpiperazin-1-yl)(1-(thiophen-2-yl)-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone derivatives (20da-do)

General procedure for the synthesis of (4-phenylpiperazin-1-yl)(1-(thiophen-2-yl)-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone derivatives *(exemplified as 20da***)**

To a stirred solution of 1-(thiophen-2-yl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (**18d**) (0.29 g, 1 mmol) in dry THF, HOBt (0.16 g, 1.2 mmol) and EDCI. HCl (0.23 g, 1.2 mmol) were added and continued stirring for 30 min. To the reaction mixture, 1-phenylpiperazine (**19a**) was added under ice cold temperature and the reaction mixture was further stirred at room temperature for 6 h. After completion of reaction as monitored by TLC, solvent was evaporated under vacuum. Reaction mixture was extracted with ethyl acetate (2 x 20 mL), collected organic layer was dried over Na_2SO_4 and concentrated under vacuum to get **20da**. The obtained crude product was passed through short bed of silica gel (100-200) by using mobile phase ethyl acetate and *n*-hexane mixture (4:6) to obtain analytically pure compound as white solid [275, 276].

(4-phenylpiperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20da)

White solid; % Yield 84; mp 152-154 °C; IR vmax/cm (KBr): 3250, 1622, 1556, 1494, 1433, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.71 (s, 1H), 8.40 (s, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 3.4 Hz, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.42 (d, J = 5.0 Hz, 1H), 7.24-7.15 (m, 2H), 6.91 (d, J = 8.1 Hz, 2H), 6.87-6.74 (m, 3H), 4.09-3.96 (m, 4H), 3.35-3.25 (m, 4H); MS (ESI) m/z 439.4 $[M + 1]^+$; Anal. Calcd. for $C_{26}H_{22}N_4OS$: C, 71.21; H, 5.06; N, 12.78; S, 7.31, Found: C, 71.18; H, 5.10; N, 12.82; S, 7.29.

(4-(4-methoxyphenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20db)

White solid; % Yield 86; mp 182-184 °C; IR vmax/cm (KBr): 3232, 1626, 1554, 1506, 1431, 1238, 723; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.98 (s, 1H), 8.22 (s, 1H), 7.93-7.77 (m, 2H), 7.50 (d, J = 8.0 Hz, 1H), 7.36-7.26 (m, 2H), 7.12-6.95 (m, 2H), 6.73 (d, J = 9.0 Hz, 2H), 6.63 (d, J = 9.0 Hz, 2H), 3.91-3.79 (m, 4H), 3.54 (s, 3H), 3.01-2.89 (m, 4H); MS (ESI) m/z 469.9 $[M + 1]^+$; Anal. Calcd. for $C_{27}H_{24}N_4O_2S$: C, 69.21; H, 5.16; N, 11.96; S, 6.84, Found: C, 69.24; H, 5.18; N, 11.92; S, 6.82.

(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)(4-p-tolylpiperazin-1-yl)methanone (20dc)

White solid; % Yield 80; mp 232-234 °C; IR vmax/cm (KBr): 3296, 1616, 1556, 1433, 709; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76 (s, 1H), 8.51 (s, 1H), 8.17 (d, J = 7.5 Hz, 1H), 7.79 (d, J = 2.0 Hz, 1H), 7.65-7.53 (m, 3H), 7.41-7.35 (m, 1H), 7.29-7.28 (m, 1H), 7.14 (d, J = 7.4 Hz, 2H), 6.94 (d, J = 7.4 Hz, 2H), 4.25-4.15 (m, 4H), 3.40-3.25 (m, 4H), 2.32 (s, 3H); MS (ESI) m/z 453.4 $[M + 1]^+$; Anal. Calcd. for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38; S, 7.09, Found: C, 71.67; H, 5.38; N, 12.35; S, 7.05.

(4-(4-chlorophenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20dd)

White solid; % Yield 78; mp >250 °C; IR vmax/cm (KBr): 3192, 1642, 1556, 1492, 1433, 748; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.98 (s, 1H), 8.48 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 3.1 Hz, 1H), 7.66-7.58 (m, 2H), 7.54 (d, J = 5.0 Hz, 1H), 7.38-7.63 (m, 1H), 7.28-7.26 (m, 1H), 7.23 (t, J = 8.1 Hz, 1H), 6.96 (t, J = 2.1 Hz, 1H), 6.91-6.83 (m, 2H), 4.21-4.01 (m, 4H), 3.45-3.35 (m, 4H); MS (ESI) m/z 474.9 $[M + 1]^+$, 476.8 $[M + 3]^+$; Anal. Calcd. for $C_{26}H_{21}ClN_4OS$: C, 66.02; H, 4.48; N, 11.85; S, 6.78, Found: C, 66.00; H, 4.49; N, 11.88; S, 6.81.

(4-(4-nitrophenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20de)

White solid; % Yield 76; mp >250 °C; IR vmax/cm (KBr): 3226, 1648, 1502, 1492, 1330, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 1H), 8.52 (s, 1H), 8.25-8.07 (m, 3H), 7.71-7.47 (m, 4H), 7.42-7.25 (m, 1H), 7.14-7.02 (m, 1H), 6.90-6.78 (m, 2H), 4.35-3.82 (m, 4H), 3.65-3.55 (m, 4H); Anal. Calcd. for $C_{26}H_{21}N_5O_3S$: C, 64.58; H, 4.38; N, 14.48; S, 6.63, Found: C, 64.59; H, 4.35; N, 14.51; S, 6.67.

(4-(4-fluorophenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20df)

White solid; % Yield 78; mp >250 °C; IR vmax/cm (KBr): 3207, 1624, 1554, 1433, 748; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 1H), 8.52 (s, 1H), 8.18 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 2.8 Hz, 1H), 7.63 (d, J = 3.6 Hz, 2H), 7.58-7.53 (m, 1H), 7.42-7.36 (m, 1H), 7.32-7.29 (m, 1H), 7.06-6.94(m, 4H), 4.22-4.04 (m, 4H), 3.34-3.28 (m, 4H); Anal. Calcd. for $C_{26}H_{21}FN_4OS$: C, 68.40; H, 4.64; N, 12.27; S, 7.02, Found: C, 68.43; H, 4.62; N, 12.30; S, 7.06.

(4-(3-methoxyphenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20dg)

White solid; % Yield 82; mp 170-172 °C; IR vmax/cm (KBr): 3387, 1602, 1554, 1485, 1433, 1249, 742; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H), 8.52 (d, J = 0.5 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.80-7.78 (m, 1H), 7.64-7.62 (m, 2H), 7.57-7.55 (m, 1H), 7.41-7.35 (m, 1H), 7.32-7.29 (m, 1H), 7.24 (t, J = 8.2 Hz, 1H), 6.63 (dd, J = 8.2, 1.8 Hz, 1H), 6.55 (t, J = 2.3 Hz, 1H), 6.49 (dd, J = 7.9, 2.1 Hz, 1H), 4.22-4.14 (m, 2H), 4.13-4.02 (m, 2H), 3.84 (s, 3H), 3.44-3.33 (m, 4H); Anal. Calcd. for $C_{27}H_{24}N_4O_2S$: C, 69.21; H, 5.16; N, 11.96; S, 6.84, Found: C, 69.19; H, 5.12; N, 11.93; S, 6.80.

(4-(3-chlorophenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20dh)

White solid; % Yield 76; mp 160-162 °C; IR vmax/cm (KBr): 3326, 1634, 1556, 1487, 744; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.98 (s, 1H), 8.48 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 3.1 Hz, 1H), 7.65-7.58 (m, 2H), 7.54 (d, J = 5.0 Hz, 1H), 7.38-7.34 (m, 1H), 7.28-7.26 (m, 1H), 7.23 (t, J = 8.1 Hz, 1H), 6.96 (t, J = 2.1 Hz, 1H), 6.90-6.84 (m, 2H), 4.20-4.02 (m, 4H), 3.43-3.37 (m, 4H); Anal. Calcd. for $C_{26}H_{21}ClN_4OS$: C, 66.02; H, 4.48; N, 11.85; S, 6.78, Found: C, 66.05; H, 4.52; N, 11.82; S, 6.82.

(4-(2-methoxyphenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20di)

White solid; % Yield 70; mp 148-150 °C; IR vmax/cm (KBr): 3356, 1638, 1526, 1427, 1246, 740; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.88 (s, 1H), 8.47 (s, 1H), 8.14 (d, J = 7.9 Hz, 1H), 7.82-7.80 (m, 1H), 7.65-7.59 (m, 2H), 7.56-7.54 (m, 1H), 7.38-7.34 (m, 1H), 7.28-7.26 (m, 1H), 7.10-6.97 (m, 3H), 6.95-6.93 (m, 1H), 4.22-4.08 (m, 4H), 3.92 (s, 3H), 3.32-3.22 (m, 4H); GCMS (ESI) m/z 468.9 $[M]^+$; Anal. Calcd. for $C_{27}H_{24}N_4O_2S$: C, 69.21; H, 5.16; N, 11.96; S, 6.84, Found: C, 69.22; H, 5.20; N, 11.98; S, 6.79.

(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)(4-o-tolylpiperazin-1-yl)methanone (20dj)

White solid; % Yield 78; mp 186-188 °C; IR vmax/cm (KBr): 3398, 1612, 1556, 1427, 740; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (s, 1H), 8.49 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.80 (d, J = 3.5 Hz, 1H), 7.62 (d, J = 3.7 Hz, 2H), 7.54 (d, J = 4.4 Hz, 1H), 7.37 (dt, J = 8.0, 4.1 Hz, 1H), 7.30 (s, 1H), 7.23 (t, J = 7.6 Hz, 2H), 7.11 (d, J = 7.7 Hz, 1H), 7.05 (t, J = 7.4 Hz, 1H), 4.17-4.08 (m, 4H), 3.14-3.10 (m, 4H), 2.39 (s, 3H); Anal. Calcd. for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38; S, 7.09, Found: C, 71.63; H, 5.32; N, 12.39; S, 7.12.

(4-(2-chlorophenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20dk)

White solid; % Yield 72; mp 148-150 °C; IR vmax/cm (KBr): 3306, 1624, 1562, 1446, 752; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.50 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.81-7.79 (m, 1H), 7.63 (d, J = 3.7 Hz, 2H), 7.56-7.54 (m, 1H), 7.45-7.34 (m, 2H), 7.31-7.26 (m, 2H), 7.14-7.12 (m, 1H), 7.05-7.03 (m, 1H), 4.22-4.06 (m, 4H), 3.32-3.21 (m, 4H); Anal. Calcd. for $C_{26}H_{21}ClN_4OS$: C, 66.02; H, 4.48; N, 11.85; S, 6.78, Found: C, 66.06; H, 4.52; N, 11.82; S, 6.76.

(4-(2-fluorophenyl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20dl)

White solid; % Yield 68; mp 154-156 °C; IR vmax/cm (KBr): 3271, 1634, 1502, 1433, 707; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.16 (s, 1H), 8.45 (s, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.94-7.88 (m, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.58-7.53 (m, 1H), 7.51-7.45 (m, 1H), 7.32-7.28 (m, 1H), 7.24-7.22 (m, 1H), 7.13-6.93 (m, 4H), 4.16-4.05 (m, 4H), 3.30-3.22 (m, 4H); Anal. Calcd. for $C_{26}H_{21}FN_4OS$: C, 68.40; H, 4.64; N, 12.27; S, 7.02, Found: C, 68.42; H, 4.68; N, 12.25; S, 7.01.

(4-(pyridin-4-yl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20dm)

White solid; % Yield 68; mp 140-142 °C; IR vmax/cm (KBr): 3207, 1641, 1554, 1415, 740; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.12 (s, 1H), 8.55 (s, 1H), 8.32 (d, J = 6.5 Hz, 2H), 8.17 (d, J = 7.9 Hz, 1H), 7.83-7.81 (m, 1H), 7.64-7.62 (m, 2H), 7.56-7.54 (m, 1H), 7.40-7.36 (m, 1H), 7.28-7.26 (m, 1H), 6.74 (d, J = 6.6 Hz, 2H), 4.23-4.07 (m, 4H), 3.65-3.55 (m, 4H); Anal. Calcd. for $C_{25}H_{21}N_5OS$: C, 68.32; H, 4.82; N, 15.93; S, 7.30, Found: C, 68.35; H, 4.83; N, 15.89; S, 7.32.

(4-(pyridin-2-yl)piperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20dn)

White solid; % Yield 64; mp 128-130 °C; IR vmax/cm (KBr): 3383, 1624, 1577, 1485, 1436, 738; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 1H), 8.54 (s, 1H), 8.25 (d, J = 3.8 Hz, 1H), 8.19 (d, J = 7.8 Hz, 1H), 7.80 (d, J = 3.6 Hz, 1H), 7.63 (d, J = 3.8 Hz, 2H), 7.58-7.51 (m, 2H), 7.43-7.34 (m, 1H), 7.32-7.29 (m, 1H), 6.74-6.66 (m, 2H), 4.18-4.02 (m, 4H), 3.86-3.75 (m, 4H); Anal. Calcd. for $C_{25}H_{21}N_5OS$: C, 68.32; H, 4.82; N, 15.93; S, 7.30, Found: C, 68.29; H, 4.79; N, 15.96; S, 7.26.

(4-benzylpiperazin-1-yl)(1-(thiophen-2-yl)-9H-pyrido[3,4-b]indol-3-yl)methanone (20do)

White solid; % Yield 76; mp 160-162 °C; IR vmax/cm (KBr): 3388, 1602, 1554, 1485, 1425, 700; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90 (s, 1H), 8.42 (s, 1H), 8.11 (d, J = 7.9 Hz, 1H), 7.82-7.75 (m, 1H), 7.70-7.46 (m, 3H), 7.43-7.18 (m, 7H), 3.98-3.94 (m, 4H), 3.62 (s, 2H), 2.68-2.30 (m, 4H); MS (ESI) m/z 453.2 $[M + 1]^+$; Anal. Calcd. for $C_{27}H_{24}N_4OS$: C, 71.65; H, 5.35; N, 12.38; S, 7.09, Found: C, 71.69; H, 5.32; N, 12.41; S, 7.12.

4.4. Biological evaluation

4.4.1. Cytotoxicity

Vero cells (ATCC CRL-1586) were cultured in 10% FBS in Dulbecco Modified Eagle Medium (DMEM). The cells were incubated at 37 °C under 5% CO_2 until confluent and then diluted with phosphate-buffered saline to 10^6 cells/mL. In a transparent 96-well plate (Falcon Microtest 96), threefold serial dilutions of the macrolide stock solutions resulted in final concentrations of 102.4 to 0.42 µM in a final volume of 200 µL. After incubation at 37 °C for 72 h, medium was removed and monolayers were washed twice with 100 µL of warm Hanks' Balanced Salt Solution (HBSS). One hundred microliters of warm medium and 20 µl of freshly made MTS-PMS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium and phenylmethasulfazone] (100:20) (Promega) were added to each well, plates were incubated for 3 h, and absorbance was determined at 490 nm. The CC50 were obtained from nonlinear regression analysis of concentration-effect curves by the GraphPad Prism 5 program and represent the mean ± standard deviation of three independent experiments [277, 278].

4.4.2. HIV-1 Reverse Transcriptase inhibition assay

In-vitro HIV-RT inhibitory potential of the synthesized β-carboline derivatives was evaluated using colorimetric assay kit procured from Roche diagnostics. All the reagents required to perform RT inhibitory assay were supplied in the kit and the ELISA procedure for RT inhibition assay was performed as described in the kit protocol. Standard drug efavirenz was (kindly donated by Ranbaxy Laboratories Ltd., INDIA) used for comparison purpose [279].

Briefly, the reaction mixture containing HIV-1 reverse transcriptase (RT) enzyme, viral nucleotides (digoxigenin (DIG)-dUTP, biotin-dUTP and dTTP) and reconstituted template in the incubation buffer with or without inhibitors (100 µM concentration) was incubated at 37 °C for 1 h in the reaction tubes. The reaction mixture was transferred to streptavidine coated microtitre plate (MTP) and incubated for another 1 h at 37 °C. The biotin-labeled viral nucleotides incorporated in nascent cDNA synthesized was catalyzed by RT were bound to streptavidine. The un-bound and un-reacted reaction mixture was removed by

gentle washing with washing buffer. Then anti-DIG-POD working solution was added into the MTPs and incubated for 1 h at 37 °C. The DIG-labeled dNTPs incorporated in cDNA were bound to the anti-DIG-POD antibody. The unbound anti-DIG-PODs were removed by washing and working peroxide substrate (ABST) solution was added to the MTPs. Finally, the reaction mixture was incubated at 25 °C for 15-20 min, until the sufficient green color was developed for detection. The absorbance of the sample was determined at O.D. 405 nm using microtiter plate ELISA reader (Stat Fax 2100) (Chen et al. 2013). The percentage of RT inhibitory activity was calculated by formula as given below:

% inhibition = 100-[OD value with inhibitor-Blank/OD value without inhibitor-Blank] x 100

4.4.3. Anti-leishmanial activity

The anti-leishmanial activity assay has been carried out using promastigotes of *Leishmania infantum,* which were cultured in a 96-well plate in the presence of graded concentrations (1-50 µg/mL) of the test compounds in duplicates, 200 µL of a promastigote culture ($1x10^6$ cells/mL) and 2 µL of the drugs per well. Plates were incubated for 48 h at 26 °C, and then growth of promastigotes was monitored by previously reported Alamar blue-based assay method [280]. Standard drug miltefosine was used for comparison of results.

4.4.4. Anti-tubercular activity

Anti-microbial susceptibility testing was performed in black, clear-bottomed, 96-well microplates (black view plates; Packard Instrument Company, Meriden, Conn.) in order to minimize background fluorescence. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial drug dilutions were prepared in either dimethyl sulfoxide or distilled deionized water and subsequent two fold dilutions were performed in 0.1 ml of 7H9GC (no Tween 80) in the microplates. BACTEC 12B-passaged inocula were initially diluted 1:2 in 7H9GC and 0.1 ml was added to wells. Subsequent determination of bacterial titers yielded 1×10^6, 2.5×10^6 and 3.25×10^5 CFU/ml in plate wells for H37Rv strain. Frozen inocula were initially diluted 1:20 in BACTEC 12B medium followed by 1:50 dilution in 7H9GC. Addition of 0.1 mL to wells resulted in final bacterial

titers of 2.0 × 10^5 and 5 × 10^4 CFU/mL for H37Rv strain. Wells containing drug only were used to detect autofluorescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M) and the plates were incubated at 37 °C. Starting at day 4 of incubation, 20 µl of 10 × alamar Blue solution (Alamar Biosciences/Accumed, Westlake, Ohio) and 12.5 µl of 20% Tween 80 were added to one B well and one M well and plates were re-incubated at 37 °C. Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ≥50,000 fluorescence units (FU). Fluorescence was measured in a cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, Mass.) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. If the B wells became pink by 24 h, reagent was added to the entire plate. If the well remained blue or ≤ 50,000 FU was measured, additional M and B wells were tested daily until a color change occurred, at which time reagents were added to all remaining wells. Plates were then incubated at 37 °C and results were recorded at 24 h post-reagent addition. Visual MICs were defined as the lowest concentration of drug that prevented a color change. For fluorometric MICs, a background subtraction was performed on all wells with a mean of triplicate M wells. Percent inhibition was defined as,

$$\% \text{ inhibition} = 1-[\text{test well FU}/\text{mean FU of triplicate B wells}] \times 100$$

The lowest drug concentration effecting an inhibition of ≥90% was considered the MIC [281].

4.5. Molecular docking

4.5.1. Protein preparation and grid generation

Molecular docking study was performed using Glide 5.9 docking module of maestro 9.4 version installed on Intel Xenon W 3565 processor and Cent OS Linux enterprise version 6.3 as the operating system. HIV-1 reverse transcriptase [PDB code: 3MEE] was retrieved from the RCSB protein data bank [282]. Target protein's PDB structure was further refined using protein preparation wizard, by selecting default options such as, assign bond order, add hydrogen, create zero-order bond to metals, create di-sulfide bonds and delete water

beyond 5 Å from ligand, followed by hydrogen bond optimization and energy minimization of the protein using force field Optimized Potentials for Liquid Simulations-2005 (OPLS). This energy minimized receptor protein was further used to generate grid, grid box was generated around co-crystallized ligand by using default setting pick to identify ligand and by allowing the rotation of receptor hydroxyl and thiol groups [283].

4.5.2. Ligand preparation

Ligands were drawn using maestro interface and further prepared for docking using LigPrep module by selecting default settings such as generate possible states at target pH 7.0±2.0, desalt, generate tautomers, retain specified chiralities and generate low energy ring conformations one per ligand [284].

4.5.3. Docking

Ligand flexible molecular docking was performed using Glide module of maestro interface in extra precision mode, by selecting the write XP descriptor information and add Epik state penalties to docking score [285].

5. RESULTS AND DISCUSSIONS

5.1. Design of novel NNRTIs

Many NNRTIs, including Tetrahydroimidazo[4,5,1-jkj][1,4]benzodiazepin-2(1*H*)-one (TIBO) and α -anilinophenylacetamide (α-APA) derivatives, adopt typical butterfly-like conformations in NNIBP, with hydrophilic body and hydrophobic wings (wing-I and wing-II). Hydrophilic body contain mainly functional groups like -NH, -C=O, -C=S and -OH which are able to form hydrogen bonding interactions with Lys101, Lys103 and Pro236 of main chain aminoacid residues [28, 286]. Hydrophobic wings are π electron containing aromatic ring system, which can form hydrophobic interactions with side chain residues of amino acids Tyr181, Tyr188, Trp229, Phe227, Val106, Po236, Leu100, Leu234 and Tyr318 [287, 288]. Recent literature suggested that, extension of the wing interacting with Tyr181 and Tyr188 and orienting towards the Trp229 could greatly enhance the activity of the ligand against the wild-type virus and its resilience to mutation [289]. Based on the above mentioned facts, we purposely incorporated bulkier β-carboline scaffold as one of the hydrophobic wings in butterfly shaped pharmacophore model, to establish interactions with conserved amnioacids. Diverse biological activity of β-carboline skeleton is an additional advantage feature of these designed anti-HIV compounds to exhibit inhibition activity against assosciated infections. We presupposed that, with the presence of previlaged β-carboline ring, our designed compounds were highly satisfied our intention to develop novel anti-HIV agents with inhibition potential against assosciated infectios like leishmaniasis and tuberculosis. In present designed anti-HIV compounds, besides 1-substituted β-carboline scaffold (wing-1), substituted aromatic rings acts as hydrophobic wing (wing-2) and were connected with hydrophilic body such as, NCH_2CO, NCH_2CONH, $NCH_2CONHNCH$ and $CON(CH_2)_4N$. The general structure of the designed β-carboline derivatives were showed in Fig: 5.1.

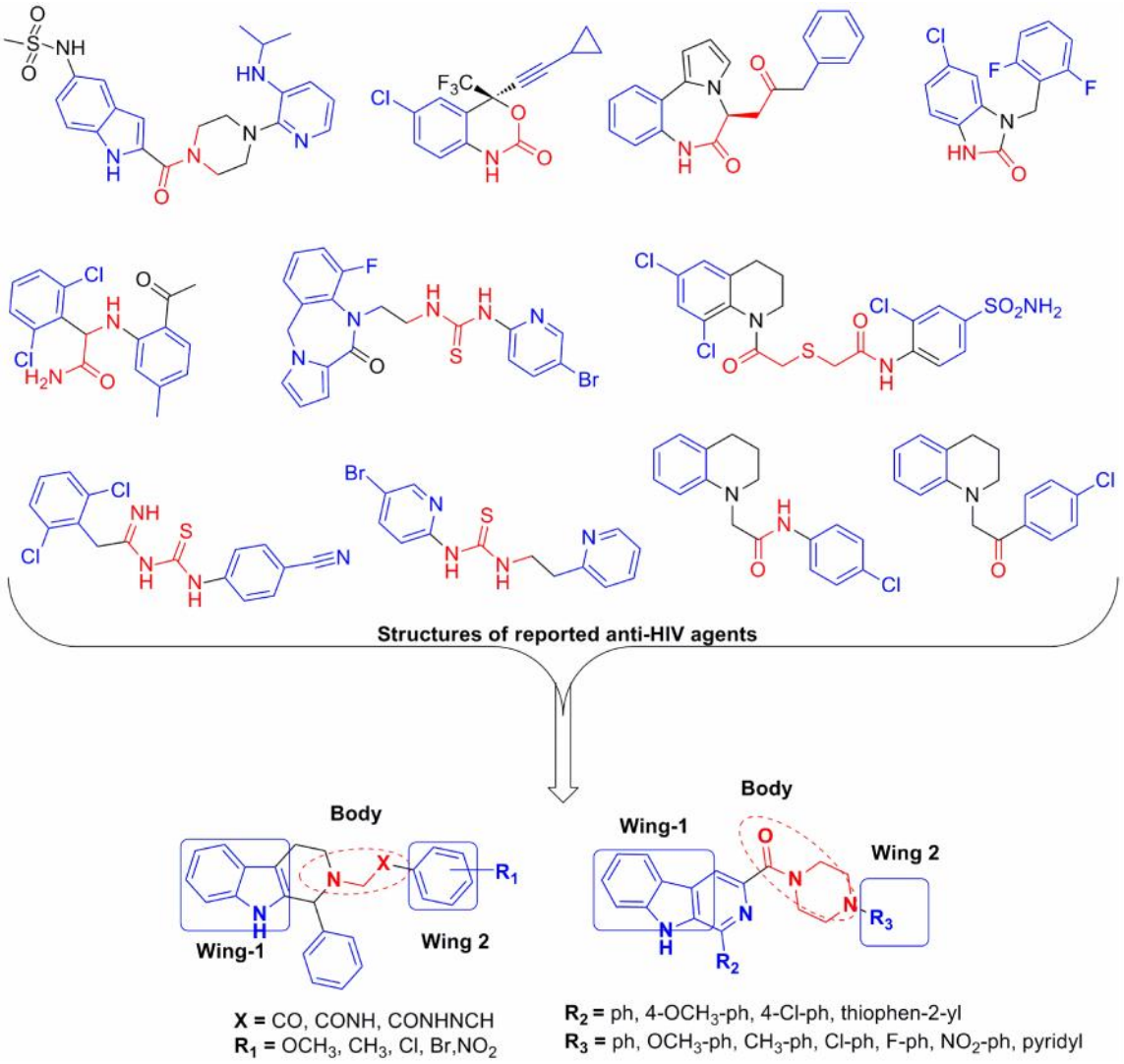


Fig. 5.1: Basic structure of the designed β-carboline derivatives

5.2. *In-silico* molecular descriptor prediction

Pharmacokinetic (ADMET) properties of new chemical entities (NCE's) plays significant role in drug discovery process and to become a successful drug. It has been anticipated that, nearly 40% of NCE's fail in drug discovery process due to their poor pharmacokinetic properties [290]. Molecular properties of NCE's plays major role in their pharmacokinetic properties, recently *in-silico* study of molecular descriptors is getting more attention in medicinal chemistry [290]. Molecular properties such as, CLogP (≤5), molecular weight (≤500), number of hydrogen bond acceptors (≤10), number of hydrogen bond donors (≤5), aqueous solubility, topological polar surface area (≤140 $Å^2$), number of rotatable bonds (≤10), percentage of oral absorption, drug likeness and drug score were studied using online servers molinspiration chemoinformatics (http://www.molinspiration.com) [291], Osiris property explorer (http://www.organic-chemistry.Org /prog/peo) [292] and QikProp module of Schrodinger suite 2014 [276]. In these molecular descriptors, CLogP, molecular weight, number of hydrogen bond acceptors and donors are known as Lipinski parameters [293, 294]. CLogP is the calculated partition co-efficient in octanol/water system and is useful to predict the lipophilicity of the molecule, which plays an important role in membrane permeability. Higher the LogP (>5) value signifies the high lipophilicity and poor membrane permeability of the molecule. Molecular weight is another molecular descriptor useful to predict the membrane diffusion and permeability. The most common acceptable cut off for molecular weight is ≤500, but it doesn't significantly separate the molecules of good and poor oral bioavailability. Since molecular weight of the molecule is directly proportional to its molecular size but the presence of number of hetero atoms and rotatable bonds significantly affect its lipophilicity, aqueous solubility and bioavailability. Hydrogen bond donor and acceptor forms hydrogen bond interaction with water molecules and facilitate the aqueous solubility of the molecules. Optimum number of hydrogen bond donor (≤10) and acceptors (≤5) indicates good aqueous solubility, disintegration and membrane permeability [294]. Topological polar surface area is defined as sum of surface area of polar atoms (oxygen, nitrogen and attached hydrogens). Although, molecular flexibility or rigidity prediction of a molecule is much more complex issue, number of

rotatable bonds present in the molecule gives an idea about it. Rotatable bond is defined as any non-ring single bond attached to non hydrogen atoms [295]. TPSA and number of rotatable bonds are useful to predict aqueous solubility, lipophilicity, intestinal absorption, bioavailability, blood-brain barrier penetration and the recommended values are ≤140 $Å^2$ and ≤10 respectively [295-297]. Percentage oral absorption gives an idea about permeability of the molecule through intestine layer and is depends on molecule LogP, aqueous solubility and TPSA. Compounds showed calculated percentage oral absorption value >80 are predicted to have good oral absorption [19]. Druglikeness is a qualitative value frequently used in medicinal chemistry; is useful to assess the drug like properties of a substance based on molecular descriptors like TPSA, Mol. Wt., LogP which affects oral bioavailability and around 80% of the marketed drugs have positive druglikeness value. Drug score is a calculated value and is useful to judge the overall potential of a substance to become a successful drug and is depends on druglikeness and toxicity risk alerts of the molecule [292]. With this immense importance of molecular properties of NCEs in the drug discovery process, in the present study, we studied *in-silico* molecular properties of these novel β-carboline derivatives.

5.2.1. Molecular descriptors of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

In the present study, we have designed three different series (6, 10 and 13, fig. 5.1) of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives with different hydrophilic linkers. *In-silico* molecular descriptors of these tetrahydro-β-carboline derivatives were showed in table 5.1, 5.2 and 5.3 respectively.

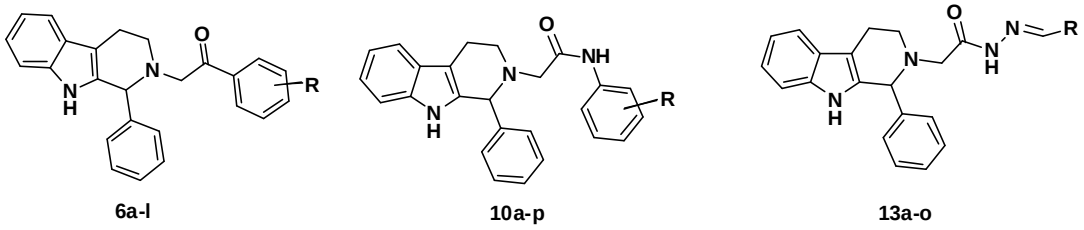


Fig. 5.2: General Structure of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

Table 5.1: Molecular descriptors of 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives

Comp. Code	R	M.wt.	HBA[a]	HBD[b]	ClogP	TPSA[c]	Rot[d]	% oral absorption	Drug Likeness	Drug Score
6a	H	366.5	3	1	4.56	36.1	3	96.6	4.6	0.56
6b	4-OCH_3	396.5	4	1	4.49	45.33	4	100.0	5.3	0.55
6c	4-CH_3	380.5	3	1	4.90	36.1	3	100.0	4.27	0.49
6d	4-Cl	400.9	3	1	5.17	36.1	3	100.0	6.87	0.43
6e	4-NO_2	411.5	6	1	3.64	81.92	4	89.0	-6.79	0.28
6f	4-F	384.4	3	1	4.66	36.1	3	100.0	5.37	0.51
6g	4-Br	445.4	3	1	5.28	36.1	3	100.0	2.15	0.37
6h	3-CH_3	380.5	3	1	4.90	36.1	3	100.0	3.36	0.49
6i	2-OCH_3	396.5	4	1	4.49	45.33	4	100.0	5.1	0.55
6j	2-CH_3	380.5	3	1	4.90	36.1	3	100.0	4.63	0.49
6k	2-Cl	400.9	3	1	5.17	36.1	3	100.0	5.2	0.43
6l	3,4-di-OCH_3	426.5	5	1	4.42	54.56	5	100.0	6.7	0.53

[a] No. of Hydrogen Bond Acceptors, [b] No. of Hydrogen Bond Donors, [c] Topological Polar Surface Area, [d] No. of Rotatable Bonds

Table 5.2: Molecular descriptors of *N*-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetamide derivatives

Comp. Code	R	M.wt.	HBA[a]	HBD[b]	ClogP	TPSA[c]	Rot[d]	% oral absorption	Drug Likeness	Drug Score
10a	H	381.5	4	2	4.39	48.13	3	100.0	3.97	0.3.6
10b	4-OCH_3	411.5	5	2	4.32	57.36	4	100.0	4.31	0.35
10c	4-CH_3	395.5	4	2	4.74	48.13	3	96.4	4.17	0.32
10d	4-Cl	415.9	4	2	5.00	48.13	3	100.0	6.33	0.28
10e	4-NO_2	426.5	7	2	3.47	93.95	4	86.9	-9.79	0.17
10f	4-F	399.5	4	2	4.49	48.13	3	96.0	4.34	0.33
10g	3-OCH_3	411.5	5	2	4.32	57.38	4	100.0	5.29	0.35
10h	3-CH_3	395.5	4	2	4.74	48.13	3	96.3	5.2	0.32
10i	3-Cl	415.9	4	2	5.00	48.13	3	100.0	5.57	0.28
10j	3-NO_2	426.5	7	2	3.47	93.95	4	86.9	0.31	0.27
10k	2-OCH_3	411.5	5	2	4.32	57.36	4	100.0	5.35	0.35
10l	2-CH_3	395.5	4	2	4.74	48.13	3	96.2	5.36	0.32
10m	2-Cl	415.9	4	2	5.00	48.13	3	100.0	5.47	0.28
10n	3-CF_3	449.5	4	2	5.24	48.13	3	100.0	-1.2	0.16
10o	2,4-di-CH_3	409.5	4	2	5.08	48.13	3	100.0	2.4	0.21
10p	2,3-C_4H_4	431.5	4	2	5.59	48.13	3	100.0	5.12	0.08

[a] No. of Hydrogen Bond Acceptors, [b] No. of Hydrogen Bond Donors, [c] Topological Polar Surface Area, [d] No. of Rotatable Bonds

Table 5.3: Molecular descriptors of *N*'-benzylidene-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)-acetohydrazide derivatives

Comp. Code	R	M.wt.	HBA[a]	HBD[b]	ClogP	TPSA[c]	Rot[d]	% oral absorption	Drug Likeness	Drug Score
13a	-ph	408.5	5	2	4.79	60.49	5	94.1	7.78	0.5
13b	-4-OCH_3-ph	438.5	6	2	4.72	69.72	6	94.7	7.94	0.48
13c	4-CH_3-ph	422.5	5	2	5.13	6.49	5	96.0	6.43	0.43
13d	4-Cl-ph	442.9	5	2	5.39	60.49	5	100.0	8.49	0.38
13e	4-NO_2-ph	453.5	8	2	3.87	106.3	6	86.6	-2.37	0.26
13f	4-Br-ph	487.4	5	2	4.89	6.49	5	100.0	6.86	0.46
13g	4-OH-ph	424.5	6	3	4.44	80.72	6	93.2	7.98	0.54
13h	4-CN-ph	433.5	5	2	4.62	84.28	6	90.5	0.64	0.29
13i	3-OCH_3-ph	438.5	6	2	4.72	69.72	6	94.7	7.64	0.48
13j	3-NO_2-ph	453.5	8	2	3.87	106.3	6	86.5	-2.7	0.47
13k	3-Br-ph	487.4	5	2	5.51	60.49	5	100.0	4.6	0.34
13l	3-OH-ph	424.5	6	3	4.44	80.72	6	93.2	7.92	0.54
13m	2-Cl-ph	442.9	5	2	5.39	60.49	5	100.0	8.21	0.38
13n	3,4-di-OCH_3-ph	468.5	7	2	4.65	78.95	7	95.7	9.38	0.46
13o	2-thiophenyl	414.5	5	2	4.65	88.73	5	93.3	9.04	0.50

[a] No. of Hydrogen Bond Acceptors, [b] No. of Hydrogen Bond Donors, [c] Topological Polar Surface Area, [d] No. of Rotatable Bonds

Predicted *in-silico* molecular descriptors study of these tetrahydro-β-carboline derivatives, suggested that, all these designed molecules followed Lipinski rule of five. Most of these derivatives showed all Lipinski parameters (Mol. Wt., ClogP, HBA, HBD) within the satisfactory limit and few compounds such as 6d, 6g, 6k, 10n, 10o, 10p, 13c, 13d, 13k and 13m displayed ClogP value >5, indicating their hydrophobic nature. As Lipinski rule states that, compounds with single violation from any of these lipinski parameters will not have

any oral bio-availability and pharmacokinetic problems. Hence, these β-carboline derivatives were predicted to have good oral bioavailability and pharmacokinetic profile [298]. Molecular descriptors like TPSA, number of rotatable bonds of these derivatives were within the acceptable limits and predicted % oral absorption values ranges from 86.5 to 100% has revealed their good membrane permeability as well as bio-availability. Additionally, these designed tetrahydro-β-carboline derivatives showed good druglikeness, drug score values except nitro (6e, 10e, 13e, 13j) and trifluoromethyl (10n) derivatives. From this *in-silico* molecular descriptor prediction study, we summarized, these designed tetrahydro-β-carboline derivatives might have good oral absorption and pharmacokinetic profile as the studied molecular descriptors of these derivatives are within the acceptable limits.

5.2.2. Molecular descriptors of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

In the present work, four series (20a-d) of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives were designed and studied for biological evaluation. General structure of these 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives were showed in fig. 5.3 and molecular descriptors in table 5.4 to 5.7 respectively.

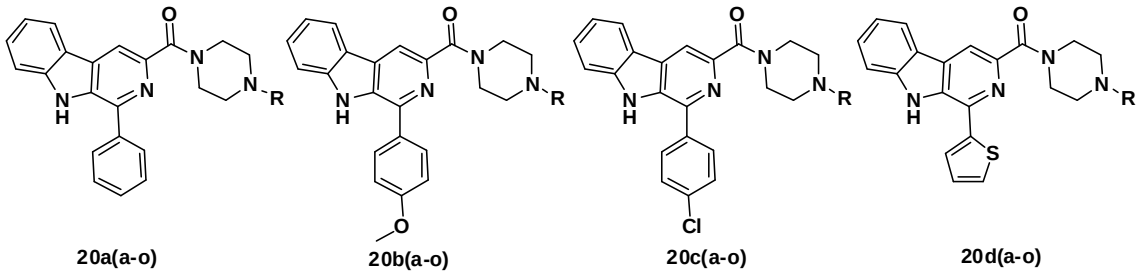


Fig. 5.3: General structure of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

Table 5.4: Molecular descriptors of (1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives

Comp. Code	R	M.wt.	HBA[a]	HBD[b]	ClogP	TPSA[c]	Rot[d]	% oral absorption	Drug Likeness	Drug Score
20aa	-ph	432.5	5	1	5.22	52.23	2	100.0	8.43	0.24
20ab	-4-OCH_3-ph	462.5	6	1	5.12	61.46	3	100.0	5.23	0.19
20ac	-4-CH_3-ph	446.5	5	1	5.50	52.23	2	100.0	5.37	0.21
20ad	-4-Cl-ph	467.0	5	1	5.84	52.23	2	100.0	7.27	0.19
20ae	-4-NO_2-ph	477.5	8	1	5.09	98.05	3	90.0	-4.55	0.11
20af	-4-F-ph	450.5	5	1	5.28	52.23	2	100.0	5.44	0.22
20ag	-3-OCH_3-ph	462.5	6	1	5.12	61.46	3	100.0	6.52	0.14
20ah	-3-Cl-ph	467.0	5	1	5.84	52.23	2	100.0	7.58	0.19
20ai	-2-OCH_3-ph	462.5	6	1	5.12	61.46	3	100.0	7.94	0.24
20aj	-2-CH_3-ph	446.5	5	1	5.50	52.23	2	100.0	7.64	0.21
20ak	-2-Cl-ph	467.0	5	1	5.84	52.23	2	100.0	7.75	0.15
20al	-2-F-ph	450.5	5	1	5.28	52.23	2	100.0	6.25	0.22
20am	-4-pyridyl	433.5	6	1	4.15	65.12	2	100.0	6.64	0.33
20an	-2-pyridyl	433.5	6	1	4.63	65.12	2	100.0	8.39	0.28
20ao	-CH_2ph	446.5	5	1	5.34	52.23	4	100.0	7.96	0.26

[a] No. of Hydrogen Bond Acceptors, [b] No. of Hydrogen Bond Donors, [c] Topological Polar Surface Area, [d] No. of Rotatable Bonds

Table 5.5: Molecular descriptors of (1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives

Comp. Code	R	M.wt.	HBA[a]	HBD[b]	ClogP	TPSA[c]	Rot[d]	% oral absorption	Drug Likeness	Drug Score
20ba	-ph	462.5	6	1	5.12	61.46	3	100.0	8.37	0.24
20bb	-4-OCH_3-ph	492.6	7	1	5.01	70.69	4	100.0	5.35	0.18
20bc	-4-CH_3-ph	476.6	6	1	5.43	61.46	3	100.0	5.44	0.21
20bd	-4-Cl-ph	497.0	6	1	5.73	61.46	3	100.0	7.30	0.18
20be	-4-NO_2-ph	507.5	9	1	4.99	107.2	4	80.0	-4.48	0.1
20bf	-4-F-ph	480.5	6	1	5.18	61.46	3	100.0	5.47	0.21
20bg	-3-OCH_3-ph	492.6	7	1	5.01	70.69	4	100.0	6.67	0.14
20bh	-3-Cl-ph	497.0	6	1	5.73	61.46	3	100.0	7.63	0.18
20bi	-2-OCH_3-ph	492.6	7	1	5.01	70.69	4	100.0	8.04	0.23
20bj	-2-CH_3-ph	476.6	6	1	5.43	61.46	3	100.0	7.66	0.21
20bk	-2-Cl-ph	497.0	6	1	5.73	61.46	3	100.0	7.76	0.14
20bl	-2-F-ph	480.5	6	1	5.18	61.46	3	100.0	6.24	0.21
20bm	-4-pyridyl	463.5	7	1	4.04	74.35	3	100.0	6.67	0.32
20bn	-2-pyridyl	463.5	7	1	4.52	74.35	3	100.0	8.37	0.27
20bo	-CH_2ph	476.6	6	1	5.23	61.46	5	100.0	7.94	0.25

[a] No. of Hydrogen Bond Acceptors, [b] No. of Hydrogen Bond Donors, [c] Topological Polar Surface Area, [d] No. of Rotatable Bonds

Table 5.6: Molecular descriptors of (1-(4-chlorophenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives

Comp. Code	R	M.wt.	HBA[a]	HBD[b]	ClogP	TPSA[c]	Rot[d]	% oral absorption	Drug Likeness	Drug Score
20ca	-ph	467.0	5	1	5.84	52.23	2	100.0	9.05	0.19
20cb	-4-OCH_3-ph	497.0	6	1	5.73	61.46	3	100.0	5.97	0.14
20cc	-4-CH_3-ph	481.0	5	1	6.15	52.23	2	100.0	6.12	0.17
20cd	-4-Cl-ph	501.0	5	1	6.45	52.23	2	100.0	7.71	0.15
20ce	-4-NO_2-ph	512.0	8	1	5.71	98.05	3	81.1	-3.18	0.08
20cf	-4-F-ph	485.0	5	1	5.90	52.23	2	100.0	6.20	0.17
20cg	-3-OCH_3-ph	497.0	6	1	5.73	61.46	3	100.0	7.29	0.11
20ch	-3-Cl-ph	501.0	5	1	6.45	52.23	2	100.0	8.30	0.15
20ci	-2-OCH_3-ph	497.0	6	1	5.73	61.46	3	100.0	8.64	0.18
20cj	-2-CH_3-ph	481.0	5	1	6.15	52.23	2	100.0	8.36	0.17
20ck	-2-Cl-ph	501.0	5	1	6.45	52.23	2	100.0	8.46	0.12
20cl	-2-F-ph	485.0	5	1	5.90	52.23	2	100.0	6.98	0.17
20cm	-4-pyridyl	467.9	6	1	4.76	65.12	2	100.0	7.40	0.25
20cn	-2-pyridyl	467.9	6	1	5.24	65.12	2	100.0	9.08	0.21
20co	-CH_2ph	481.0	5	1	5.95	52.23	4	100.0	8.61	0.19

[a] No. of Hydrogen Bond Acceptors, [b] No. of Hydrogen Bond Donors, [c] Topological Polar Surface Area, [d] No. of Rotatable Bonds

Table 5.7: Molecular descriptors of (4-phenylpiperazin-1-yl)(1-(thiophen-2-yl)-9*H*-pyrido[3,4-*b*]indol-3-yl)methanone derivatives

Comp. Code	R	M.wt.	HBA[a]	HBD[b]	ClogP	TPSA[c]	Rot[d]	% oral absorption	Drug Likeness	Drug Score
20da	-ph	438.5	5	1	5.35	80.47	1	100.0	8.72	0.24
20db	-4-OCH_3-ph	468.6	6	1	5.24	89.7	2	100.0	5.62	0.18
20dc	-4-CH_3-ph	452.6	5	1	5.66	80.47	1	100.0	5.77	0.21
20dd	-4-Cl-ph	473.0	5	1	5.96	80.47	1	100.0	7.66	0.18
20de	-4-NO_2-ph	483.5	8	1	5.22	126.2	2	100.0	-4.15	0.11
20df	-4-F-ph	456.5	5	1	5.41	80.47	1	100.0	5.84	0.22
20dg	-3-OCH_3-ph	468.6	6	1	5.24	89.7	2	100.0	6.91	0.14
20dh	-3-Cl-ph	473.0	5	1	5.96	80.47	1	100.0	7.96	0.18
20di	-2-OCH_3-ph	468.6	6	1	5.24	89.7	1	100.0	8.30	0.23
20dj	-2-CH_3-ph	452.6	5	1	5.66	80.47	1	100.0	8.01	0.21
20dk	-2-Cl-ph	473.0	5	1	5.96	80.47	1	100.0	8.13	0.15
20dl	-2-F-ph	456.5	5	1	5.41	80.47	1	100.0	6.64	0.22
20dm	-4-pyridyl	439.5	6	1	4.27	93.36	1	100.0	7.04	0.32
20dn	-2-pyridyl	439.5	6	1	4.75	93.36	1	100.0	8.75	0.28
20do	-CH_2ph	452.6	5	1	5.46	80.47	3	100.0	8.27	0.25

[a] No. of Hydrogen Bond Acceptors, [b] No. of Hydrogen Bond Donors, [c] Topological Polar Surface Area, [d] No. of Rotatable Bonds

Predicted *in-silico* molecular descriptors study indicates, these designed 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives are hydrophobic in nature than tetrahydro-β-carboline derivatives, as most of these derivatives displayed ClogP value >5. In these reported derivatives, few compounds 20cd, 20ce, 20ch and 20ck were showed two violations from Lipinski parameters such as ClogP and mol. wt. with small margin. As literature suggests that, pharmacokinetic properties of ligand depends, not only on number

of violations but also an extent of individual violation from lipinski parameters [298]. The extent of violation from one lipinski parameter (mol. wt.) of these derivatives was very marginally (~2%), hence it might not have any significant effect on their pharmacokinetic profile. Molecular descriptors like TPSA, number of rotatable bonds were in desirable limits and % oral absorption values ranges from 80.0 to 100% signifies their good oral absorption as well as membrane permeability. Moreover, these derivatsives displayed good druglikeness and drug score values (except nitro derivatives such as 20ae, 20be, 20ce, 20de) illustrated their ability to overcome the pharmacokinetic problems. From this *in-silico* prediction study, we concluded that, these designed 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives might have good oral bio-availability and pharmacokinetic profile.

5.3. Chemistry

5.3.1. Synthesis of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

5.3.1.1. Synthesis of 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives (6a-l)

Synthetic protocol for the preparation of 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives (**6a-l**) is illustrated in scheme 1. Key intermediate 1-phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**3**) was synthesized from tryptamine (**1**) and benzaldehyde (**2**) in presence of trifluoroacetic acid via pictet-spengler reaction with good yield [265]. Other intermediates such as, substituted phenacyl bromides (**5a-l**) were prepared from the bromination of substituted acetophenones (**4a-l**), using *N*-bromosuccinimide and phase transfer catalyst *p*-toluenesulfonic acid in moderate to good yields [299]. Key intermediate (**3**) and substituted phenacyl bromides (**5a-l**) were subjected to nucleophilic substitution reaction in presence of base K_2CO_3 to obtain the desired derivatives **(6a-l)** as final products in moderate to good yields [266, 270].

Synthesized 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives were characterized by spectral analysis such as, IR, ^{1}H NMR, Mass and elemental analysis. IR spectra of the synthesized compounds showed *N-H* stretching at 3394 to 3282

cm^{-1}, *C= O* absorption at 1698 to 1682 cm^{-1}, *C-C* aromatic stretching around 1481 to 1456, *C-O* absorption (methoxy) band at 1249 to 1246 cm^{-1}, *C-Cl* absorption band at 766 to 744 cm^{-1} and in nitro derivative *N-O* asymmetric stretching was observed around 1519 cm^{-1}. ^1H NMR spectrum of the compounds showed singlet peak of one hydrogen at δ 5.14 to 4.83 due to position-1 methine proton of the β-carboline ring, two protons of phenacyl methylene group appeared as two doublets, each of one proton at δ 4.14 to 4.03 and 3.92 to 3.71 with high coupling constant values of ~17 Hz, due to geminal hydrogen coupling. Four aliphatic hydrogens of two methylene groups of tetrahydro-β-carboline were appeared as four multiplets around δ 3.35-3.27, 3.23-3.11, 3.03-2.97 and 2.92-2.89, respectively, methoxy protons appeared as singlet at δ ~3.80 and methyl protons as singlet at δ ~2.4. Indole *NH* proton position was varied with δ value 7.96 to 7.30. In mass spectral analysis, M+1 peak appeared as parent ion peak. HPLC purity of the compounds (6a, 6b, 6c & 6d) was determined using C18 column, methanol and phosphate buffer (pH 7) as mobile phase and purity of the compounds was found to be 97, 96, 98 and 97%, respectively. Elemental analysis data showed that, the calculated and observed values are within the acceptable limits (±0.05%).

5.3.1.2. Synthesis of *N*-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl) acetamide derivatives (10a-p)

Synthesis of *N*-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetamide derivatives (**10a-p**) was carried out by following the conditions showed in scheme 2. 2-chloro-*N*-(substituted)phenylacetamides (**9a-p**) were prepared from substituted anilines (**7a-p**) and chloroacetylchloride (**8**) using triethylamine as base in dichloromethane as solvent with good to excellent yields [120]. These 2-chloro-*N*-(substituted)phenylacetamides (**9a-p**) were treated with key intermediate 1-phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**3**) in presence of base K_2CO_3 and dimethylformamide as solvent to obtain final compounds (**10a-p**) in moderate to good yields [270].

Structures of the synthesized compounds were confirmed by IR, ^1H NMR, Mass and Elemental spectral data. IR spectra of the synthesized compounds showed characteristic peaks such as *N-H* stretching at 3323 to 3234 cm^{-1}, *C=O* absorption around 1693 to 1658

cm^{-1}, *C-C* aromatic stretching at 1537 to 1442 cm^{-1}, nitro group *N-O* asymmetric stretching at 1535 to 1506 and 1356 to 1342 cm^{-1}, *C-O* absorption (methoxy) band around 1248 to 1246 cm^{-1} and *C-Cl* absorption band at 756 to 746 cm^{-1}. ^{1}H NMR spectrum of the compounds showed singlet peak of one hydrogen at δ 4.85 to 4.61 due to position-1 methine proton of the β-carboline ring, naphthyl derivative showed more downfield (δ 4.85) and 3-trifluoromethyl derivative showed more up field (δ 4.61). Acetamide linker methylene protons are appeared as two doublets, each of one proton at δ 3.62 to 3.24 and 3.32 to 2.99 with high coupling constant values (~17 Hz) due to geminal hydrogen coupling. Four aliphatic hydrogens of two methylene groups of tetrahydro-β-carboline were appeared as three multiplets around δ 3.89-3.09, 3.26-2.86 and 3.09-2.79, methoxy protons appeared as singlet at δ ~3.80, methyl protons appeared as singlet at δ ~2.3. Indole *NH* proton was appeared as broad singlet at δ 9.91 to 9.12 and amide *NH* was also appeared as broad singlet at δ ~7.30, but most of the times, it is appeared as multiplet by merging with other aromatic protons. Mass spectral data showed that, M+1 peak appeared as parent ion peak. Elemental analysis data showed that, the calculated and observed values are within the acceptable limits (±0.05%).

5.3.1.3. Synthesis of *N*'-benzylidene-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl) acetohydrazide derivatives (13a-o)

Synthesis of *N*'-benzylidene-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl) acetohydrazide derivatives (**13a-o**) was done by using the protocol illustrated in scheme 3. Key intermediate 1-phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**3**) was treated with ethyl 2-chloroacetate in acetonitrile in presence of base K_2CO_3 to acquire ethyl 2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetate (**11**) [286] as an intermediate followed by reaction with hydrazine hydrate in ethanol to obtain carbohydrazide derivative (**12**) and finally hydrazone formation by the condensation reaction of carbohydrazide derivative and substituted aromatic aldehydes to get the desired derivatives (**13a-o**) as final products in moderate to good yield [160, 270, 272].

Synthesized compounds were characterized by spectral analysis such as IR, ^{1}H NMR, Mass and Elemental analysis. IR spectra of the synthesized compounds showed *N-H* stretching at

3408 to 3284 cm^{-1}, *C≡N* group stretching at 2220 cm^{-1}, *C=O* absorption of amide around 1697 to 1682 cm^{-1}, *C=N* around 1552 to 1513cm^{-1}, nitro derivative *N-O* asymmetric stretching at 1538 to 1532 and 1388 to 1372 cm^{-1}, *C-O* absorption (methoxy) band at 1267 to 1238 cm^{-1}, and *C-Cl* absorption band at 748 to 724 cm^{-1}. ^{1}H NMR spectrum of the compounds showed singlet peak of position-1 methine proton δ 4.75 to 4.73. Acetyl linker methylene protons were appeared as two doublets at δ 3.50 to 3.48 and 3.27 to 3.25 because geminal hydrogen coupling with high coupling constant values (~17 Hz). Four aliphatic hydrogens of two methylene groups of tetrahydro-β-carboline were appeared as three multiplets around δ 3.38-3.28, 3.16-3.07 and 3.01-2.96; methoxy protons appeared as singlet at δ ~3.93 and methyl protons appeared as singlet at δ ~2.40. Indole *NH* proton was appeared as broad singlet around δ 10.32 to 10.05, and hydrazone CH appeared as singlet at δ 8.68 to 7.94. In mass spectral analysis, M+1 peak appeared as parent ion peak. HPLC purity of the compounds (13a & 13b) was found to be 98 and 96% respectively. Elemental analysis data showed that, the calculated and observed values are within the acceptable limits (±0.05%).

5.3.2 Synthesis of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

The synthetic protocol of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives was illustrated in scheme 4. The compounds were synthesized from the starting material, DL-Tryptophan **(14)** in a sequence of reactions. Initial esterification of DL-Tryptophan using thionylchloride in ethanol to obtain the ethyl ester of tryptophan (**15**), was followed by the pictet-spengler reaction in the presence of trifluoroacetic acid to acquire 1-substituted tetrahydro-β-carboline-3-ethyl ester intermediates (**16a-d**), oxidation with potassium permanganate to 1-substituted β-carboline ethyl ester intermediates (**17a-d**) [270] and continued by alkaline ester hydrolysis to afford 1-substituted β-carboline-3-carboxylic acid derivatives (**18a-d**) as key intermediates. The carboxylic acid group containing key intermediates were finally treated with 1-substituted piperazines (**19a-p**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and hydroxybenzotriazole (HOBt) to obtain the desired products **[20(a-d)(a-o)**] in good to moderate yields [274].

IR spectra of the synthesized compounds showed *N-H* stretching at 3398 to 3192 cm^{-1}, *C=O* absorption around 1654 to 1612 cm^{-1}, *C-O* absorption (methoxy) band at 1262 to 1238 cm^{-1}, nitro derivative *N-O* asymmetric stretching at 1568 to 1492 and 1338 to 1317 cm^{-1}, and *C-Cl* absorption band at 786 to 736 cm^{-1}. The ^{1}H NMR spectrum of the reported compounds showed, *NH* proton of indole nucleus as broad singlet and δ value was varied from 11.8 to 8.70, characteristic singlet around δ ~8.50 was observed due to position-4 proton of β-carboline ring. Eight piperazine protons appeared as two multiplets around δ value 4.30 to 3.70 and 3.40 to 3.00, piperazine protons were shifted to down field (δ ~3.65) in nitro and 2-pyridyl derivatives whereas shifted up field in benzyl derivatives (δ ~2.62). Methoxy protons were appeared as singlet at δ ~3.8, aromatic methyl protons as singlet at ~2.3. In mass spectral analysis, M+1 peak appeared as parent ion peak. Analytical purity of the compounds was determined using HPLC (>95%) as well as Elemental analysis (±0.05%) and values were found to be within the acceptable limits.

5.4. Biological evaluation

5.4.1. Cytotoxicity

Initially all the synthesized β-carboline derivatives were evaluated for cytotoxicity against vero cell line at 50 µg/mL concentration using MTT assay method. Most of these reported derivatives were non-cytotoxic to vero cells at tested concentration with few exceptions like 13a, 13g, 13j, 13k, 13l, 20bn, 20cm, 20cn and 20dm. These derivatives were further evaluated using different concentrations, to calculate CC_{50} values and CC_{50} values were used to calculate selectivity index.

5.4.2. HIV-1 RT inhibition activity

Synthesized β-carboline derivatives were evaluated for *in-vitro* HIV-1 reverse transcriptase inhibition activity at 100 µM concentration in duplicates using colorimetric assay method (Elisa kit supplied by Roche diagnostics, Switzerland). First generation NNRTI drug, efavirenz (100 µM) was used as standard drug to compare the inhibitory potency of the tested β-carboline derivatives.

5.4.2.1. 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

Three different series (6, 10 and 13) of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives were designed by varying the length of hydrophilic body (NCH_2CO, NCH_2CONH, $NCH_2CONHNCH$) and with different electron donating, withdrawing group substitutions on phenyl ring (wing-2). All the synthesized 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives were screened against HIV-1 reverse transcriptase. *In-vitro* HIV-1 RT inhibition assay results of these tetrahydro-β-carboline derivatives were showed in table 5.8.

Table 5.8: *In-vitro* HIV-1 RT inhibition activity of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

Comp. code	substitution	% RT inhibition[a]	Comp. code	substitution	% RT inhibition[a]
6a	H	25	10n	3-CF_3	21
6b	**4-OCH_3**	**55**	**13a**	**ph**	**55**
6c	4-CH_3	23	**13b**	**4-OCH_3ph**	**72**
6d	4-Cl	40	13c	4-CH_3ph	25
6e	4—NO_2	20	**13d**	**4-Clph**	**64**
6l	3,4-di-OCH_3	32	13e	4-NO_2ph	28
10a	H	44	13f	4-Brph	39
10b	**4-OCH_3**	**61**	13g	4-OHph	22
10c	**4-CH_3**	**54**	13i	3-OCH_3ph	34
10d	4-Cl	46	13m	2-Clph	42
10f	4-F	27	13o	2-thiophenyl	47
Efavirenz		97.7			

a: Average value of at least duplicate measurements (SD±5%).

Significant inhibition (≥50%), moderate inhibition (50-30%), weak inhibition (≤30%)

In 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives (6a-l) having keto (C=O) functional group as linker, compound 6b exhibited significant

inhibition, 6d, 6l displayed moderate and compounds 6a, 6c, 6e showed weak inhibition of HIV-1 RT. Among these derivatives, un-substituted proto type compound 6a displayed weak inhibitory (25%) activity, substitution on *para* position of the phenyl ring showed considerable effect on inhibition potency, especially *ortho-para* directing groups such as methoxy (6b), chloro (6d) substitution increased the activity (55 and 40%) while methyl substitution (6c) did not showed any effect on inhibition activity (23%). However, substitution of these groups on other positions of the phenyl ring resulted in complete loss of activity. Para substitution of *meta* directing nitro group (6e) did not been shown any effect on inhibition activity (20%) while halogens like fluoro and bromo ruined the activity and 3,4-di-methoxy substitution (6l) retained the activity (32%). In conclusion, substitution of *ortho-para* directing groups such as, methoxy and chloro group on *para* position favored the HIV-1 RT inhibition activity of these derivatives.

Among the series of *N*-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetamide derivatives (10a-p) containing NCH_2CONH group as hydrophilic body, compounds 10b, 10c displayed significant inhibition, compounds 10a, 10d showed moderate and derivatives 10f, 10n exhibited weak inhibitory activity against HIV-1 RT. Among these reported derivatives, un-substituted prototype compound 10a displayed moderate activity (44%), while compounds 10b, 10c with electron donating groups on *para* position displayed significant increase (61, 54% respectively) in activity and chloro group substitution on *para* position (10d, 46%) did not alter the inhibitory activity although strong electron withdrawing fluoro (10f), nitro (10e) group substitution resulted in complete loss of activity. Substitution on *ortho* and *meta* position with either electron donating or electron withdrawing group diminished the activity. Replacement of aromatic phenyl ring with bulky naphthyl ring led to complete ruin of activity. HIV-1 RT inhibition activity results of this series of molecules disclosed that, *ortho-para* directing groups on *para* position favored the activity, substitution of electron donating groups on *ortho, meta* position, electron withdrawing groups on phenyl ring and replacement of phenyl ring had the opposite effect on RT inhibitory activity.

In the final series of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives (13a-o), compounds 13a, 13b, 13d showed significant activity, derivatives 13f, 13i, 13m, 13o exhibited moderate activity and compounds 13c, 13e and 13g displayed weak inhibition activity against HIV-1 RT. Among these compounds, un-substituted phenyl derivative exhibited significant (13a, 55%) activity, compounds with *ortho-para* directing methoxy (13b, 72%), chloro substituents (13d, 64%) on *para* position increased the potency while bromo (13f, 39%), methyl (13c, 25%) and hydroxy (13g, 22%) substitution decreased the activity radically. *Para* substitution of *meta* directing nitro (13e, 28%) showed significant decrease in activity while cyano (13h) group resulted in complete loss of activity. Although *meta* methoxy derivative (13i, 34%) showed moderate activity, other substitutions were not advisable for RT inhibition activity whereas *ortho* chloro substitution (13m, 42%) led to minute reduction in potency. Replacement of phenyl ring with hetero cyclic thiophene ring (13o, 47%) almost retained the activity. From these results, we observed that, substitution of methoxy, chloro groups on *para* position favored RT inhibition and replacement of phenyl ring with heterocyclic ring retained the potency.

From the structure activity relationship study of these three series of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives [6(a-l), 10(a-p) and 13(a-o)], we observed that, hydrazone derivatives exhibited better activity than amide and keto derivatives, suggesting that, distance between two hydrophobic wings and polarity of the hydrophilic body might played crucial role in RT inhibition activity. Substitution on *para* position with *ortho-para* directing groups, which were able to form electrostatic interactions are favorable and substitutions on other position of phenyl ring (wing-2) were not advisable for RT inhibition activity. Replacement of phenyl ring with bulkier naphthyl group resulted in complete loss of activity while replacement with heterocyclic thiophene ring retained the potency, indicating that, replacement with other hetero cyclic rings may results in beneficial effect on inhibition activity.

5.4.2.2. 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

We have synthesized four series (20a, 20b, 20c and 20d) of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives by exploring position-1 of the β-carboline ring, phenyl ring attached to piperazine moiety and evaluated for HIV-1 RT inhibition activity. We have designed molecules by making substitutions like phenyl, substituted phenyl, thiophene ring on position-1 of β-carboline ring and different electron donating, withdrawing group substitution/replacement on/of phenyl ring attached to piperazine moiety. HIV-1 RT inhibition activity results of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives were showed in table 5.9.

Table 5.9: *In-vitro* HIV-1 RT inhibition activity of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

Comp. code	Wing-2	% RT inhibition[a]	Comp. code	Wing-2	% RT inhibition[a]
20aa	**-ph**	**66**	20bf	4-Fph	33
20ab	**4-OCH_3ph**	**53**	20bi	2-OCH_3ph	46
20ac	**4-CH_3ph**	**56**	20bj	2-CH_3ph	37
20ad	**4-Clph**	**62**	20bm	4-pyridyl	43
20ae	4-NO_2ph	26	20bn	2-pyridyl	22
20af	4-Fph	22	20bo	-CH_2ph	45
20ag	3-OCH_3ph	38	20cb	4-OCH_3ph	24
20ai	**2-OCH_3ph**	**60**	20ce	4-NO_2ph	21
20aj	2-CH_3ph	23	20cm	4-pyridyl	27
20ak	2-Clph	20	**20da**	**-ph**	**63**
20am	**4-pyridyl**	**52**	20db	4-OCH_3ph	36
20an	2-pyridyl	42	20dc	4-CH_3ph	48

20ao	**-CH_2ph**	**56**	20dd	4-Clph	34
20ba	-ph	23	20di	2-OCH_3ph	22
20bb	4-OCH_3ph	36	**20dm**	**4-pyridyl**	**62**
20bc	**4-CH_3ph**	**53**	**20do**	**-CH_2ph**	**53**
20bd	4-Clph	35	Efavirenz	-	97.7

a: Average value of at least duplicate measurements (SD±5%).

Significant inhibition (≥50%), moderate inhibition (50-30%), weak inhibition (≤30%)

Among the (1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives (20aa-ao), compounds 20aa, 20ab, 20ac, 20ad, 20ai, 20am and 20ao displayed significant activity, compounds 20ag and 20an exhibited moderate and derivatives 20ae, 20af, 20aj and 20ak showed weak activity against HIV-1 reverse transcriptase. In these derivatives, un-substituted proto type compound 20aa exhibited significant activity with 66% inhibition of HIV-RT. Although, substitutions on piperazine attached phenyl ring showed significant effect on inhibition activity, most of these substitutions produced decrease in activity. *Para* substitution on phenyl ring with *ortho-para* directing (20ab, 20ac, 20ad) groups retained the potency (53, 56 62% respectively) with minute decrease, while electron withdrawing groups like nitro (20ae, 26%) and fluoro (20af, 22%) produced considerable decrease in inhibition activity. *Meta* substitution on phenyl ring either with electron donating/withdrawing groups resulted in drastic reduction of potency. Substitution of methoxy group (20ai, 60%) on *ortho* position preserved the potency whereas other groups like methyl (20aj, 23%) and chloro (20ak, 20%) groups rigorously reduced the activity and replacement of phenyl ring with heterocyclic 4-pyridyl (20am, 52%) and benzyl ring (20ao, 56%) faintly affected the inhibition potency.

In these (1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives (20ba-bo), compound 20bc exhibited significant activity, compounds 20bb, 20bd, 20bf, 20bi, 20bj, 20bm, 20bo showed moderate and derivatives 20ba, 20bn displayed weak inhibition of HIV-1 RT. Among this series of compounds, un-

substituted phenyl (20ba, 23%) derivative exhibited weak activity, *para* substitution of methyl group (20bc, 53%) increased inhibition potency significantly while methoxy (20bb, 36%), chloro (20bd, 35%), fluoro (20bf, 33%) groups showed little increment and nitro (20be) substitution led to devoid of activity. As previous series of molecules, *meta* substitution of phenyl ring was not favorable for inhibition activity. Even though, electron donating groups such as methoxy (20bi, 46%) and methyl (20bj, 37%) on o*rtho* position increased the potency moderately but electron withdrawing groups like chloro (20bk), fluoro (20bl) substitution resulted in complete loss of activity. Replacement of phenyl ring with benzyl (20bo, 45%) and 4-pyridyl (20bm, 43%) ring produced reasonable increase in HIV-1 RT inhibition potency.

From the (1-(4-chlorophenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives (20ca-co), few compounds such as 20cb, 20ce and 20cm displayed weak inhibition (24, 21, 27% respectively) of HIV-1 RT at tested concentration. Surprisingly, *para* substitution with electron withdrawing chloro group on position-1 phenyl ring produced radical reduction in inhibition potency and moreover, substitutions on piperazine attached phenyl ring have not shown any beneficial effect on RT inhibition activity.

In the final series of *9H-pyrido[3,4-b]indol-3-yl)(piperazin-1-yl)methanone* derivatives (20da-do), position-1 phenyl ring has been replaced with aromatic thiophene-2-yl scaffold. Among these derivatives, compounds 20da, 20dm, 20do exhibited significant inhibition, compounds 20db, 20dc, 20dd displayed moderate inhibition and compound 20di showed weak inhibition of HIV-1 RT. In these 1-(thiophen-2-yl)-3-piperazinoyl-β-carboline derivatives, un-substituted phenyl derivative (20da) exhibited significant inhibition (63%) of HIV-1 RT. *Para* substitution of *ortho-para* directing (20dc, 20db, 20dd) groups resulted moderate decrease in inhibition activity (48, 36, 34% respectively) while substitution on other positions (*meta* and *ortho*) resulted considerable decrease in potency. Electron withdrawing groups like fluoro, nitro group substitution on phenyl produced drastic decline in HIV-1 RT inhibition activity. Interestingly, replacement of phenyl ring with 4-pyridyl ring (20dm, 62%) and benzyl (20do, 53%) retained RT inhibition potency.

From the structure activity relationship study of these 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives [20(a-d)(a-o)], we observed that, phenyl ring on position-1 of β-carboline favored RT inhibition activity. Substitutions on position-1 phenyl ring were not advised for HIV-1 RT inhibition activity, as derivatives with methoxy and chloro substitution produced considerable reduction in potency. Molecular docking analysis study revealed that, decreased electrostatic interactions of these derivatives might be the plausible reason for their decreased activity. Replacement of position-1 phenyl ring with heterocyclic 2-thiophenyl retained the potency with minute decrease. Phenyl ring attached to piperazine ring was also explored with various electron donating and withdrawing substituents. *Ortho* and *meta* positions on phenyl ring were highly sensitive for RT inhibitory activity as *ortho* and *meta* substituted derivatives showed severe decline in potency with one exception of 2-methoxy substitution (20ai) retained the potency. Substitution of *ortho-para* directing groups on *para* position of phenyl ring had nominal effect on inhibition potency as derivatives with methoxy, methyl, chloro substitution showed minute decrease in activity and even in few occasions increased the potency considerably, might be due to enhanced hydrophobic interactions with extended wing-2. Substitution of electron withdrawing groups like fluoro and nitro on phenyl ring radically decreased the inhibition potency. Moreover, retention in RT inhibition activity was observed with replacement of phenyl ring with pyridine and benzyl ring, enhanced the scope to develop derivatives with other different heterocyclic moieties for HIV-1 RT inhibition activity.

5.4.3. Anti-leishmanial activity

As leishmaniasis is one of the major opportunistic infections in HIV infected patients, these β-carboline derivatives were evaluated for *in-vitro* anti-leishmanial activity against promastigote form of *Leishmania infantum.* Anti-leishmanial drug miltefosine was used as standard drug to compare the anti-leishmanial potency of these compounds.

5.4.3.1. 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

In-vitro cytotoxicity and anti-leishmanial activity assay results of tetrahydro-β-carboline (6, 10 and 13 series) derivatives were showed in table 5.10.

Table 5.10: Anti-leishmanial activity of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

Comp. Code	R	EC_{50} (μM)[a]	CC_{50} (μM)[b] Vero	SI[c]	Comp. Code	R	EC_{50} (μM)[a]	CC_{50} (μM)[b] Vero	SI[c]
6a	H	24.45	>136.6	>5.6	10m	2-Cl	20.69	>120.2	>5.8
6b	4-OCH_3	23.00	>126.3	>5.5	10n	3-CF_3	45.18	>111.4	>2.4
6c	4-CH_3	30.04	>131.6	>4.4	**13a**	**-H**	**3.50**	**57.2**	**16.3**
6d	**4-Cl**	**12.21**	**>124.7**	**>10.2**	**13b**	**-4-OCH_3**	**2.07**	**>113.9**	**>55.0**
6h	3-CH_3	48.90	>131.6	>2.7	13c	4-CH_3	20.10	>118.2	>5.9
6i	2-OCH_3	36.9	>126.3	>3.4	13d	4-Cl	12.80	>112.9	>8.8
6j	2-CH_3	19.60	>131.6	>6.7	**13e**	**4-NO_2**	**5.98**	**>110.4**	**>18.5**
6k	2-Cl	27.40	>124.7	>4.5	13f	4-Br	15.10	>102.7	>6.8
10a	**H**	**12.37**	**>131.2**	**>10.6**	**13g**	**4-OH**	**10.10**	**75.3**	**7.5**
10c	4-CH_3	19.25	>126.3	>6.6	13h	4-CN	18.00	>115.2	>6.4
10d	4-Cl	15.13	>120.2	>7.9	13i	3-OCH_3	21.40	>113.9	>5.3
10e	**4-NO_2**	**8.10**	**>117.4**	**>14.5**	13j	3-NO_2	37.50	49.1	1.3
10f	4-F	15.10	>125.3	>8.3	13k	3-Br	23.30	47.2	2.0
10g	3-OCH_3	14.58	>121.4	>8.3	13l	3-OH	12.70	39.1	3.1
10h	**3-CH_3**	**7.63**	**>126.3**	**>16.6**	**13m**	**2-Cl**	**9.83**	**>112.9**	**>11.5**
10i	**3-Cl**	**6.68**	**>120.2**	**>18.0**	**13n**	**3,4-OCH_3**	**1.93**	**>106.6**	**>55.2**
10k	**2-OCH_3**	**1.99**	**>121.4**	**>61.0**	**13o**	**2-thiophenyl**	**11.90**	**>120.5**	**>10.1**
10l	**2-CH_3**	**10.43**	**>126.3**	**>12.1**	miltefosine		12.6		

a: Average value of at least duplicate measurements (SD±2%); b: Average value of at least duplicate measurements (SD±5%); c: selectivity index = CC_{50}/EC_{50}.

Potent anti-leishmanial activity (<12.6 μM), significant activity (12.6-25.0 μM), moderate activity (25.0-50.0 μM), weak activity (>50 μM).

In the series of 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives (6a-l), compound 6d exhibited potent anti-leishmanial activity (EC_{50} 12.21 µM) and is comparable with standard drug miltefosine (12.6 µM). Compounds 6a, 6b, and 6j displayed significant activity (24.45, 23.00 and 19.60 µM, respectively) and derivatives 6c, 6h, 6i and 6k showed moderate (30.04, 48.90, 36.9 and 27.40 µM, respectively) anti-leishmanial activity. Moreover, these molecules were not showed any cytotoxicity against vero cell line at 50 µg/mL concentration (µM concentration showed in table 5.10). Among these derivatives, un-substituted proto type compound (6a) displayed significant activity (24.45 µM), *para* substitution on phenacyl ring with electron donating groups such as methoxy (6b, 23.00 µM), methyl (6c, 30.04 µM) groups were not showed any beneficial effect on potency. Although, *para* substitution of electron withdrawing chloro group increased the potency extensively, other electron withdrawing groups like nitro (6e), fluoro (6f) and bromo (6g) substitution resulted in complete loss of anti-leishmanial activity. Substitution on *ortho-meta* position of phenacyl with methoxy (6i), methyl (6j, 6h) and chloro (6k) groups produced decrease in potency. From this short SAR study, we concluded that, *para* chloro substitution favored the anti-leishmanial activity and *ortho-meta* substitutions were not advisable for the same.

N-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetamide derivatives (10a-p), displayed anti-leishmanial activity with EC_{50} values ranges from 1.99 to 45.18 µM. Among these derivatives, compounds 10a, 10e, 10h, 10i, 10k, 10l exhibited potent activity (12.37, 8.10, 7.63, 6.68, 1.99 and 10.43 µM, respectively) than standard drug miltefosine. More interestingly, compound 10k displayed six times potent anti-leishmanial activity than miltefosine. Compounds 10c, 10d, 10f, 10g and 10m showed significant activity (19.25, 15.13, 15.10, 14.58 and 20.61 respectively) and 10n exhibited moderate (45.18 µM) anti-leishmanial activity. In these molecules, un-substituted proto type compound 10a showed potent activity (12.37 µM) and substitutions on phenyl (phenacetamide) showed significant effect on anti-leishmanial activity. *Para* substitution of phenyl ring with *ortho-para* directing groups such as methyl (10c, 19.25 µM), chloro (10d, 15.13 µM) and fluoro (10f, 15.10 µM) produced little decrease in potency while methoxy group led to drastic decline in activity

and *meta* directing nitro group (10e, 8.10 µM) increased the potency marginally. Anti-leishmanial potency of these derivatives is inversely proportional to *ortho-para* directing effect of substituents on *para* position. *Meta* substitution with methyl (10h, 7.63 µM) and chloro (10i, 6.68 µM) groups, reasonably increased the potency while methoxy group (10g, 14.58 µM) faintly decreased the potency and nitro group decreased extensively led to complete loss of activity. Substitution on *ortho* position with methoxy (10k, 1.99 µM) group increased the potency six times and methyl (10l, 10.43 µM) group increased the potency marginally while chloro (10m, 20.69 µM) and trifluoromethyl (10n, 45.18 µM) groups decreased the potency. Anti-leishmanial potency of these derivatives is directly proportional to *ortho-para* directing effect of substituents on *ortho* position. In other extreme, replacement of phenyl ring with naphthyl ring resulted in complete loss of activity. Additionally, these derivatives displayed good selectivity towards *Leishmania* parasite, as these molecules were not showed cytotoxicity against vero cell line at 50 µg/mL concentration. Perusal of the above mentioned small SAR study, we concluded that, *meta* and *ortho-para* directing groups substitution favored anti-leishmanial potency on *para* and *ortho* positions of phenacetamide ring respectively.

In these *N*'-benzylidene-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetohydrazide (13a-o) derivatives, compounds 13a, 13b, 13e, 13g, 13m, 13n, 13o displayed potent (3.50, 2.07,5.98, 10.10, 9.83, 1.93 and 11.90 µM, respectively) anti-leishmanial activity than standard drug miltefosine. In particular, two compounds 13b and 13n exhibited six times potent anti-leishmanial activity than miltefosine with good selectivity index. Compounds 13c, 13d, 13f, 13h, 13i, 13k and 13l exhibited significant (20.10, 12.80, 15.10, 18.00, 21.40, 23.30 and 12.70 µM, respectively) activity and compound 13j showed moderate anti-leishmanial (37.50 µM) activity. Among these derivatives, proto type compound 13a displayed more potent activity (3.50 µM) than miltefosine with selectivity index 16.3, substitution on *para* position showed marginal effect on anti-leishmanial potency with significant reduction of cytotoxicity. On *para* substitution of *ortho-para* directing hydroxyl group (13g), marginal decrease in activity (10.10 µM) as well as cytotoxicity was observed, while methoxy group (13b) showed considerable increase in

potency (2.07 µM) without cytotoxicity at 50 µg/mL concentration and substitution of chloro (13d, 12.80 µM), bromo (13f, 15.10 µM), methyl (13c, 20.10 µM) groups resulted in moderate decrease in potency. *Para* substitution of *meta* directing nitro group (13e, 5.98 µM) showed marginal decrease in potency whereas cyano (13h, 18.00 µM) group produced considerable decrease in activity, with complete loss of cytotoxicity. Un-fortunately *meta* substitution on phenyl ring with hydroxy (13l, 12.70 µM), nitro (13j, 37.50 µM) and bromo (13k, 23.30 µM) groups led to increased cytotoxicity while *ortho* chloro (13m, 9.83 µM) substitution, replacement of phenyl ring with thiophene (13o, 11.90 µM) decreased the activity. Surprisingly, 3,4-dimethoxy (13n) substitution produced the most potent (1.93 µM) compound of the series, with high selectivity index. From the SAR study of this series of compounds, we observed that, substitution on *para* position is favorable for potent anti-leishmanial activity with good selectivity index.

Among these reported 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives, irrespective to their substitution on phenyl ring, carbohydrazide derivatives exhibited potent anti-leishmanial activity followed by acetamide and ketone derivatives.

5.4.3.2. 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

In-vitro anti-leishmanial activity and cytotoxicity assay results of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone (20a, 20b, 20c and 20d) derivatives were showed in table 5.11.

Table 5.11: Anti-leishmanial activity of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

Comp. Code	Wing-2	EC_{50} (µM)[a]	CC_{50} (µM)[b] Vero	SI[c]	Comp. Code	Wing-2	EC_{50} (µM)[a]	CC_{50} (µM)[b] Vero	SI[c]
20aa	**-ph**	**12.50**	**>115.5**	**>9.2**	**20bl**	**-2-Fph**	**11.90**	**>103.9**	**>8.7**
20ab	**-4-OCH_3ph**	**9.07**	**>108.0**	**>11.9**	20bm	-4-pyridyl	16.30	>107.8	>6.6
20ac	**-4-CH_3ph**	**9.63**	**>111.9**	**>11.6**	20bn	-2-pyridyl	20.00	81.6	4.1
20ad	-4-Clph	33.0	>107.1	>3.2	20bo	-CH_2ph	15.30	>104.8	>6.8

20ae	-4-NO_2ph	19.80	>104.6	>5.3	20ca	-ph	23.90	>107.1	>4.5
20af	-4-Fph	14.10	>110.9	>7.9	20cc	-4-CH_3ph	29.90	>103.9	>3.5
20ag	**-3-OCH_3ph**	**2.89**	**>108.0**	**>37.4**	**20ci**	**-2-OCH_3ph**	**9.39**	**>100.6**	**>10.7**
20ah	-3-Clph	15.90	>107.1	>6.7	**20cj**	**-2-CH_3ph**	**10.90**	**>103.9**	**>9.5**
20ai	**-2-OCH_3ph**	**3.35**	**>108.0**	**>32.2**	20ck	-2-Clph	53.20	>99.8	>1.9
20aj	-2-CH_3ph	36.2	>111.9	>3.1	20cl	-2-Fph	84.90	>103.1	>1.2
20ak	-2-Clph	15.70	>107.1	>6.8	20cm	-4-pyridyl	29.60	50.0	1.7
20al	-2-Fph	14.90	>110.9	>7.4	20cn	-2-pyridyl	34.10	37.4	1.1
20am	-4-pyridyl	66.70	>115.2	>1.7	20co	-CH_2ph	17.20	>103.9	>6.0
20an	-2-pyridyl	34.00	>115.2	>3.4	20db	-4-OCH_3ph	15.50	>106.6	>6.9
20ao	-CH_2ph	24.60	>111.9	>4.5	**20dc**	**-4-CH_3ph**	**7.70**	**>110.4**	**>14.3**
20ba	**-ph**	**11.70**	**>108.0**	**>9.2**	**20df**	**-4-Fph**	**7.00**	**>109.4**	**>15.6**
20bb	-4-OCH_3ph	46.20	>101.4	>2.2	**20dg**	**-3-OCH_3ph**	**3.80**	**>106.6**	**>28.1**
20bc	**-4-CH_3ph**	**6.38**	**>104.8**	**>16.4**	20dh	-3-Cl-ph	26.80	>105.7	>3.9
20bd	-4-Clph	39.10	>100.6	>2.6	**20di**	**-2-OCH_3ph**	**7.10**	**>106.6**	**>15.0**
20bf	-4-Fph	15.50	>103.9	>6.7	**20dj**	**-2-CH_3ph**	**9.25**	**>110.4**	**>11.9**
20bg	-3-OCH_3ph	32.90	>101.4	>3.1	20dl	-2-Fph	71.05	>109.4	>1.5
20bh	-3-Clph	32.70	>100.6	>3.1	**20dm**	**-4-pyridyl**	**3.10**	**43.4**	**14.0**
20bi	-2-OCH_3ph	33.90	>101.4	>3.0	20dn	-2-pyridyl	37.90	>113.6	>3.0
20bj	-2-CH_3ph	39.70	>104.8	>2.6	**20do**	**-CH_2ph**	**4.85**	**>110.4**	**>22.8**
20bk	**-2-Clph**	**4.28**	**>100.6**	**>23.5**	miltefosine		12.6	-	-

a: Average value of at least duplicate measurements (SD±2%); b: Average value of at least duplicate measurements (SD±5%); c: selectivity index = CC_{50}/EC_{50}.

Potent anti-leishmanial activity (<12.6 μM), significant activity (12.6-25.0 μM), moderate activity (25.0-50.0 μM), weak activity (>50 μM).

In (1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone derivatives (20aa-ao), five compounds 20aa, 20ab, 20ac, 20ag and 20ai displayed potent anti-leishmanial (12.50, 9.07, 9.63, 2.89 and 3.35 µM, respectively) activity than standard drug miltefosine. Particularly, compound 20ag exhibited four times potent activity than miltefosine with good selectivity index. Compounds 20ae, 20af, 20ah, 20ak, 20al, 20ao showed significant activity (19.80, 14.10, 15.90, 15.70, 14.90, 24.60 µM, respectively), derivatives 20ad, 20aj, 20an displayed moderate anti-leishmanial activity (33.00, 36.2, 34.00 µM, respectively) and compound 20am exhibited weak activity (66.70 µM). Among these derivatives, un-substituted proto type compound (20aa) displayed equipotent anti-leishmanial activity (12.50 µM) with miltefosine and derivatives with different substitutions on piperazine attached phenyl ring were explored for their inhibition activity. Substitution on *para* position with electron donating groups such as, methoxy (20ab, 9.07 µM) and methyl (20ac, 9.63 µM) produced marginal increase in potency while electron withdrawing groups like fluoro (20af, 14.10 µM), nitro (20ae, 19.80 µM) and chloro (20ad, 33.00 µM) decreased the activity. Electron donating methoxy group substitution on *meta* (20ag, 2.89 µM) and *ortho* (20ai, 3.35 µM) position led to considerable increase in potency, whereas ortho substitution of fluoro (20al, 14.90 µM), chloro (20ak, 15.70 µM) and methyl (20aj, 36.20 µM) group decreased the activity respectively and replacement of phenyl ring with benzyl (20ao, 24.60 µM), 2-pyridyl (20an, 34.00 µM) and 4-pyridyl (20am, 66.70 µM) resulted in drastic decline in potency. From this little SAR study, we identified that, electron donating groups on *para* position as well as methoxy group on *ortho* and *meta* position is favorable while electron withdrawing groups and phenyl ring replacement is not advisable for anti-leishmanial activity.

Among these (1-(4-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone (20ba-bo) derivatives, four compounds 20ba, 20bc, 20bk, 20bl showed potent anti-leishmanial (11.70, 6.38, 4.28, 11.90 µM, respectively) activity than standard drug miltefosine with good selectivity index. Compounds 20bf, 20bm, 20bn, 20bo displayed significant (15.30, 16.30, 20.00, 15.30 µM, respectively) and derivatives 20bb, 20bd, 20bg, 20bh, 20bi, 20bj exhibited moderate (46.20, 39.10, 32.90, 32.70, 33.90, 39.70 µM,

respectively) inhibition of *Leishmania* parasite in comparison with miltefosine. In this series of molecules, un-substituted proto compound 20ba displayed comparable potent activity (11.70 µM) as that of standard drug miltefosine, *para* substitution of phenyl ring with fluoro (20bf, 15.50 µM), chloro (20bd, 39.10 µM), methoxy (20bb, 46.20 µM) produced considerable decrease in activity while methyl (20bc, 6.38 µM) increased the potency and nitro substitution resulted in complete loss of activity. *Ortho* substitution of electron withdrawing groups like chloro (20bk, 4.28 µM) and fluoro (20bl, 11.90 µM) amplified the potency but electron donating substitutents methoxy (20bi, 33.90 µM), methyl (20bj 39.70 µM) decreased the activity. Regardless of the nature of substitutions on *meta* position, all *meta* derivatives displayed reduced activity. Replacements of phenyl ring with benzyl (20bo, 15.30 µM), heterocyclic pyridyl ring (20bm, 16.30 µM; 20bn, 20.00 µM) resulted in marginal decrease in potency. With this little SAR study, we concluded that, electron withdrawing group substitution on ortho position and methyl group on *para* position is beneficial for activity but electron donating groups are not advisable and replacement with benzyl, pyridyl ring retained the anti-leishmanial activity.

From the series of (1-(4-chlorophenyl)-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone (20ca-co) derivatives, compounds 20ci, 20cj exhibited potent (9.39, 10.90 µM, respectively) anti-leishmanial activity than miltefosine. Compounds 20ca, 20co displayed significant activity (23.90, 17.20 µM, respectively) with good selectivity index. In-addition, derivatives 20cc, 20cm, 20cn showed moderate (29.90, 29.60, 34.10 µM, respectively) and 20ck, 20cl displayed weak (53.20, 84.90 µM, respectively) anti-leishmanial activity. In these derivatives, un-substituted proto compound (20ca) showed significant inhibition activity (23.90 µM) and substitutions on *para* and *meta* position further decreased the activity. *Ortho* substitution with electron donating methoxy (20ci, 9.39 µM) and methyl (20cj, 10.90 µM) groups showed increase in potency whereas, electron withdrawing groups (20ck, 53.20 µM) caused significant decrease in activity. Replacement of phenyl ring with benzyl ring (20co, 17.20 µM) resulted in little increment in potency. However, replacement with pyridyl ring retained the activity but it might be due to increased cytotoxicity than selective parasite inhibition. SAR study of these derivatives

suggested that, *ortho* position substitution with electron donating group, replacement with benzyl is advisable, *para* and *meta* substitutions are not suitable for anti-leishmanial activity.

Final series of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives (20da-do), exhibited anti-leishmanial activity with EC_{50} values ranges 3.80 to 71.05 μM. Among this series, compound 20dc, 20df, 20dg, 20di, 20dj, 20dm, 22do displayed potent anti-leishmanial (7.70, 7.00, 3.80, 7.10, 9.25, 3.10, 4.85 μM, respectively) activity than standard drug miltefosine. In particular, compound 20dm exhibited four times potent activity. Compound 20db showed significant activity (15.50 μM), derivatives 20dh, 20dn displayed moderate activity (26.80, 37.90 μM, respectively) and compound 20dl possessed weak anti-leishmanial activity (71.05 μM). In these compounds, un-substituted phenyl derivative (20da) was in-active against promastigotes of *Leishmanial* parasite, surprisingly *para* substitution with fluoro (20df, 7.00 μM), methyl (20dc, 7.70 μM), methoxy (20db, 15.50 μM) groups raised the inhibition potency impulsively but chloro (20dd) and nitro (20de) derivatives were devoid of activity. Electron donating methoxy on *meta* (20dg, 3.80 μM), *ortho* (20di, 7.10 μM) substitution increased the potency exceptionally and methyl substitution on *ortho* (20dj, 9.25 μM) also increased the activity while, ortho substitution with fluoro (20dl, 71.05 μM) group was not shown much beneficial effect on activity. Replacement of phenyl ring with benzyl group (20do, 4.85 μM) resulted in incredible increase in potency without any toxicity, while replacement with 4-pyridyl (20dm) ring produced considerable increment in both activity (3.10 μM) and cytotoxicity (43.4 μM). In conclusion, this SAR study suggested that, *ortho-para* directing groups like methoxy, methyl and fluoro substitutions as well as replacement of benzyl is advisable for anti-leishmanial activity.

Among these four series of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives, compounds with thiophene-2-yl, un-substituted phenyl ring on position-1 of the β-carboline ring exhibited potent anti-leishmanial activity followed by 4-methoxyphenyl and 4-cholorphenyl derivatives. Anti-leishmanial activity results of these derivatives suggested that, un-substituted phenyl on position-1 is valuable for activity than 4-mehtoxy or 4-

chlorophenyl derivatives. Interestingly, order of decreased activity is directly proportional to the bulkiness of substituent. Moreover, replacement of phenyl ring with heterocyclic thiophene-2-yl ring had increased the anti-leishmanial potency remarkably, even though, un-substituted derivative remained inactive and it increases the scope to develop new derivatives with other heterocyclic rings as anti-leishmanial agents.

5.4.4. Anti-tubercular activity

Tuberculosis is more prevalent in immune compromised patients and is one of the leading causes for mortality of HIV infection. Development of anti-HIV drugs with potent anti-tubercular activity is highly desirable, as they decreases pill burden and increases patient compliance. Hence in the present study, we also evaluated the anti-tubercular activity of these anti-HIV β-carboline derivatives against *Mycobacterium tuberculosis* H37Rv strain using Microplate Alamar Blue Assay (MABA). Minimum inhibitory concentration (MIC) is the minimum concentration of inhibitor required to complete inhibition of bacterial growth. Anti-tubercular drugs, isoniazid and rifampicin were used as standards for the comparison of anti-tubercular potency of these β-carboline derivatives.

5.4.4.1. 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

In-vitro anti-tubercular activity results of 1-phenyl tetrahydro-β-carboline derivatives [6(a-l), 10(a-p) and 13(a-o)], against H37Rv strain of *Mycobacterium tuberculosis* were shown in table 5.12.

Table 5.12: Anti-tubercular activity of 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

Comp. Code	R	MIC (µg/mL)[a]	MIC (µM)[a]	CC_{50} (µM)[b]	SI[c]
6e	4-NO_2	46.8	113.9	>121.7	>1.1
6l	3,4-OCH_3	24.3	56.9	>117.1	>2.1
10a	H	19.4	50.9	>131.2	>2.6
10m	**2-Cl**	**4.3**	**10.3**	**>120.2**	**>11.6**
10o	2,4-OCH_3	23.0	51.1	>111.1	>2.2
13a	H	17.57	42.9	57.2	1.3
13c	4-CH_3	40.00	94.56	>118.2	>1.25
13e	4-NO_2	24.10	53.2	>110.4	>2.1
13f	4-Br	7.04	14.5	>102.7	>7.1
13g	4-OH	22.96	54.0	75.3	1.4
13j	**3-NO_2**	**6.24**	**13.8**	**49.1**	**3.6**
13k	3-Br	22.71	46.6	47.2	1.01
13l	3-OH	17.45	41.1	39.1	1.04
13n	3,4-OCH_3	20.14	42.9	>106.6	>2.5
Isoniazid		0.21	-	-	-
Rifampicin		0.12	-	-	-

a: Average value of at least duplicate measurements (SD±2%); b: Average value of at least duplicate measurements (SD±5%); c: Selectivity index = CC_{50}/EC_{50}.

Significant anti-tubercular activity (MIC <10µg/mL), moderate activity (10-25 µg/mL), weak activity (>25µg/mL)

These reported 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives exhibited anti-tubercular activity with MIC value ranges from 4.3 to 46.8 µg/mL. Twelve compounds (6l, 10a, 10m, 10o, 13a, 13e, 13f, 13g, 13j, 13k, 13l, 13n) displayed MIC value below 25 µg/mL, among them two compounds (10m, 13j) showed MIC values <6.25 µg/mL, is postulated as

an upper threshold value of new anti-tubercular drugs for the evaluation of new *tuberculosis* therapy. Hence, for easy comparison, anti-tubercular potency of the derivatives was expressed in µg/mL concentration, even though µM units were used to calculate selectivity index. Among these reported tetrahydro-β-carboline derivatives, carbohydrazide derivatives displayed comparatively more potent anti-tubercular activity than acetamide and keto derivatives. Among keto derivatives, one compound 6l showed moderate activity (24.3 µg/mL) and 6e displayed weak anti-tubercular activity (46.8 µg/mL). Whereas, in acetamide derivatives, compound 10m showed potent (4.3 µg/mL) activity, 10a, 10o displayed moderate anti-tubercular activity (19.4, 23.0 µg/mL) respectively. Among the carbohydrazide linker derivatives, two compounds (13f and 13j) exhibited potent (7.04 and 6.24 µg/mL) activity, six derivatives (13a, 13e, 13g, 13k, 13l and 13n) showed moderate (17.57, 24.10, 22.96, 22.71, 17.45 and 20.14 µg/mL, respectively) and one compound (13c) displayed weak (40.00 µg/mL) anti-tubercular activity. With the limited number of active compounds from keto (6) and acetamide (10) derivatives (2 and 3 respectively), we were unable to conclude appropriate structure activity relationship of the same. Anti-tubercular activity results of carbohydrazide derivatives suggested that, *para* substitution with electron withdrawing groups is favorable anti-tubercular activity.

5.4.4.2. 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

In-vitro M. tuberculosis (H37Rv) inhibition assay results of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives [20(a-d)(a-o)], were showed in table 5.13.

Table 5.13: Anti-tubercular activity of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

Comp. Code	Wing-2	MIC (µg/mL)[a]	MIC (µM)[a]	CC_{50} (µM)[b]	SI[c]
20aa	**Ph**	**5.9**	**13.6**	**>115.5**	**>8.5**
20ac	4-CH_3ph	11.5	25.7	>111.9	>4.3
20aj	-2-CH_3-ph	22.7	50.7	>111.9	>2.2
20am	-4-pyridyl	23.4	53.9	>115.2	>2.1
20ao	**-CH_2ph**	**5.5**	**12.3**	**>111.9**	**>9.1**
20bn	-2-pyridyl	16.7	36.0	81.6	2.3
20bo	**-CH_2ph**	**5.9**	**12.4**	**>104.8**	**>8.5**
20cm	-4-pyridyl	23.4	50.0	50.0	1.0
20cn	-2-pyridyl	11.87	25.4	37.4	1.5
20dm	-4-pyridyl	12.08	27.5	43.4	1.6
20do	-CH_2ph	21.7	47.9	>110.4	>2.3
Isoniazid		0.21	-	-	-
Rifampicin		0.12	-	-	-

a: Average value of at least duplicate measurements (SD±2%); b: Average value of at least duplicate measurements (SD±5%); c: Selectivity index = $CC_{50}/EC_{50.}$

Significant anti-tubercular activity (MIC <10µg/mL), moderate activity (10-25 µg/mL), weak activity (>25µg/mL)

Anti-tubercular activity of these *9H-pyrido[3,4-b]indol-3-yl)(piperazin-1-yl)methanone* derivatives ranges from MIC 5.5 to 23.4 µg/mL. In these derivatives, three compounds 20aa (5.9 µg/mL), 20ao (5.5 µg/mL) and 20bo (5.9 µg/mL) displayed significant anti-tubercular activity against *M. tuberculosis* with MIC <6.25 µg/mL. Among these reported four series of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives, 20a series compounds with un-substituted phenyl substitution on position-1 of the β-carboline ring displayed significant anti-tubercular activity and substitution on phenyl ring, 2-thiophenyl replacement produced drastic reduction in activity. Compound 20aa with phenyl ring

attached to piperazine exhibited significant activity but substitution on phenyl ring led to decrease in inhibition potency and replacement with benzyl ring 20ao (5.5 µg/mL) retained the activity. Among these un-substituted phenyl derivatives (20a series), compounds 20ac, 20aj and 20am displayed moderate activity (11.5, 22.7 and 23.4 µg/mL, respectively). In 4-methoxyphenyl substituted derivatives (20b series), compounds 20bo (5.9 µg/mL) and 20bn (16.7 µg/mL) showed significant and moderate anti-tubercular activity respectively. Among 4-chlorophenyl derivatives (20c series), compounds 20cn and 20cm displayed moderate activity (11.87 and 23.4 µg/mL). In thiophene derivatives (20d series), compounds 20dm and 20do (12.08 and 21.7 µg/mL) exhibited moderate anti-tuber activity. Anti-tubercular activity study of these 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives suggested that, un-substituted position-1 phenyl ring is favorable for anti-tubercular activity and replacement of piperazine attached phenyl ring with benzyl ring is also beneficial for activity. Although, replacement with heterocyclic pyridyl had increased the anti-tubercular activity and few derivatives were showed cytotoxicity at tested concentration.

5.5. Molecular docking

Molecular docking was performed to study their exact binding mode and binding interactions of reported compounds in non-nucleoside inhibitory binding pocket (NNIBP) of HIV-1 RT (PDB ID: 3MEE) using molecular modeling suite Schrodinger 2014. Wild type HIV-1 RT with following experimental details (Resolution: 2.40 Å, R-value: 0.224 and R-free 0.261) was retrieved from protein data bank (http://www.rcsb.org/pdb/home) and further purified using protein preparation wizard. Purified protein was prepared for docking by generating the docking grid around co-crystallized ligand using glide grid generation application of Glide module of maestro 9.4 [297, 300]. Ramachandran plot of the prepared protein was showed in fig. 5.4.

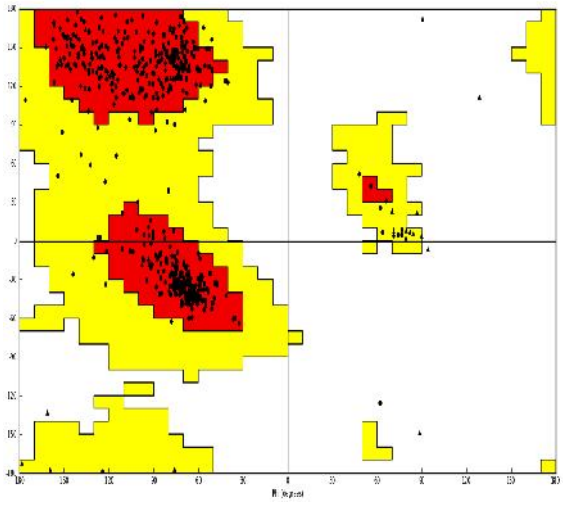


Fig. 5.4: Ramachandran plot of the prepared protein 3MEE

Validation of docking

Validation of docking protocol was done by calculating the root mean square deviation (RMSD) value of super imposed structure of co-crystallized ligand (rilpivirine) and re-docked ligand (rilpivirine). RMSD value was found to be 0.38 Å between co-crystallized and re-docked rilpivirine (Glide score: 14.3) (fig. 5.5). Second generation NNRTIs etravirine (Glide score: 14.0) (fig. 5.6) and first generation NNRTI efavirenz (Glide score: 13.0) (fig. 5.7) were used as standard drugs for comparison purpose.

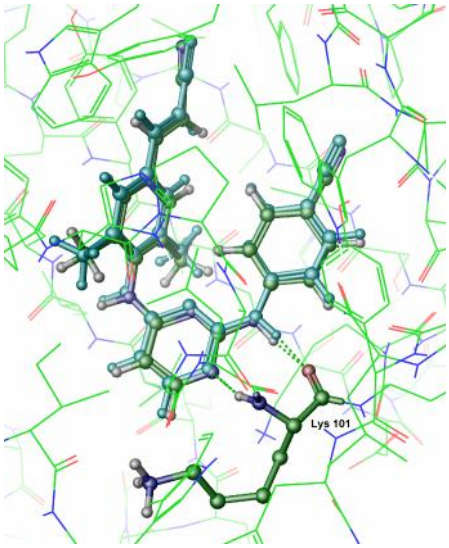


Fig. 5.5: Superimposed structure of co-crystallized rilpivirine (green color) and re-docked rilpivirine (turquoise) in NNIBP of HIV-1 RT

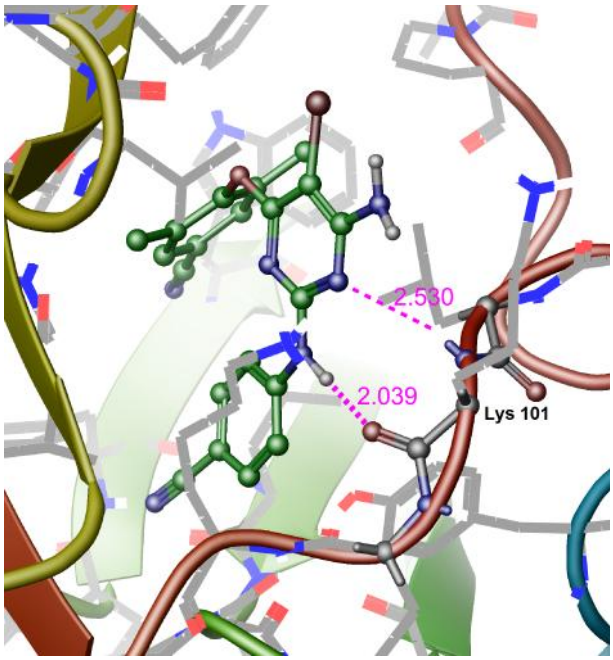


Fig. 5.6: Docking orientation and hydrogen bond interaction (2.039 Å, 2.530 Å) of etravirine with amino acid Lys101

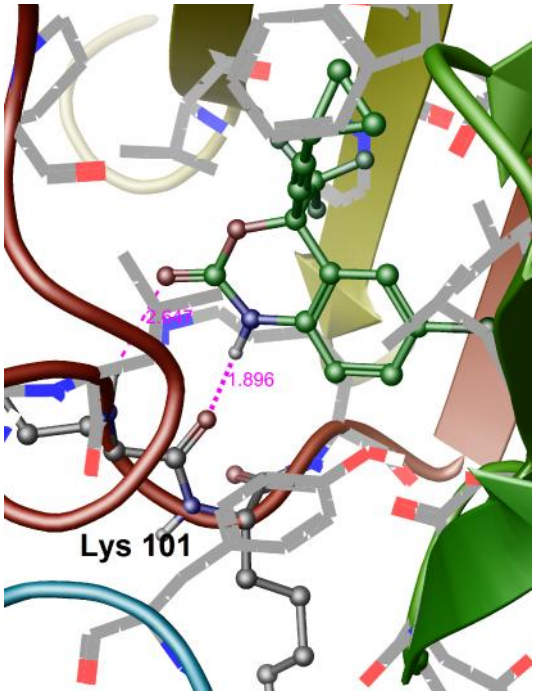


Fig. 5.7: Docking orientation and hydrogen bond interaction (1.896 Å, 2.647 Å) of efavirenz with amino acid Lys101

5.5.1. 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives

Molecular docking analysis of tetrahydro-β-carboline derivatives revealed that, interactions like electrostatic (pi-pi, pi-cationic), vanderwaals, hydrogen bond with NNIBP aminoacids played vital role in *in-vitro* inhibition activity. Hydrophobic wings (tetrahydro-β-carboline and substituted aromatic rings) were involved in electrostatic, hydrophobic interactions with amino acids Leu100, Val106, Tyr181, Tyr188, Phe227, Trp229, Leu234, Tyr318 and Hydrophilic linker displayed hydrogen bond interactions with Lys101, Lys103 of HIV-1 RT. More interestingly, these derivatives also showed vanderwaals interactions with conserved aminoacids Phe227, Trp229 and Tyr318 of HIV-1 RT.

Among the 1-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)ethanone derivatives, *para* methoxy derivative (6b) exhibited significant *in-vitro* RT inhibition activity. Docking results suggested that, tetrahydro-β-carboline ring and para methoxy substituted phenyl displayed electrostatic interactions, hydrophobic interactions with hydrophobic aminoacids like Tyr181, Tyr188, Phe227, Trp229, Leu234, Tyr318 of HIV-1 RT 6b (Glide score: 9.2, fig. 5.8). Especially *para* substitution with electrondonating methoxy group showed increased electrostatic interactions with NNIBP aminoacids than their respective *meta* and *ortho* derivatives. These increased interactions with NNIBP aminoacids, with its extended wing 2 might be the plausible reason for significant inhibition activity of *para* derivative than respective *ortho*, *meta* analogues. This postulation was also supported by *para* chloro derivative 6d (Glide score: 8.6) which showed moderate activity than its respective *meta* and *ortho* analogues. Hydrophilic linker (carbonyl oxygen) of these derivatives (6a-l) showed hydrogen bond interaction (fig. 5.9) with amino acid Lys101. Docking results analysis of *meta* and *ortho* derivatives, was further supported the above mentioned postulation, extended wing-2 reponsible for increased aminoacid interactions. Docking orientation of in-active compound (6i; glide score: 5.6; fig. 5.10), demonstrated that, *meta* substitution of methoxy group, displayed decreased hydrophobic interactions with NNIBP aminoacids than respective *para* analogue. Moreover, hydrophilic body of these *meta* and *ortho* derivatives was unable to show hydrogen bond interaction, it might be because of increased steric hinderence effect on linker due to *meta* or *ortho* substitution.

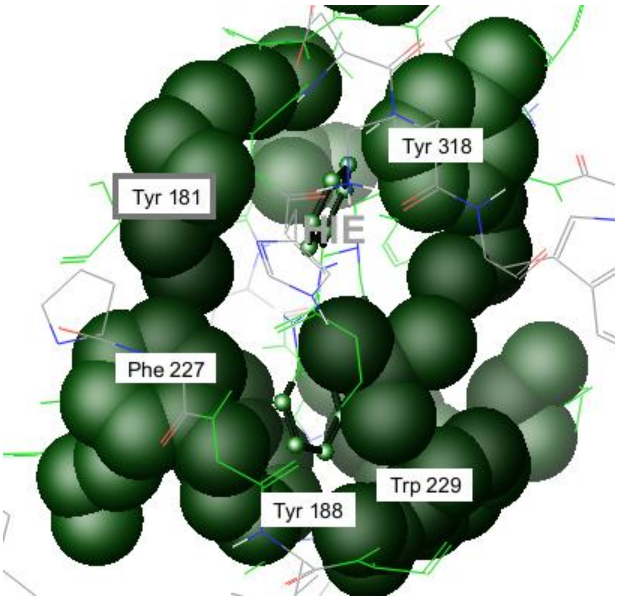


Fig. 5.8: Hydrophobic interactions of compound 6b in NNIBP of HIV-1 RT

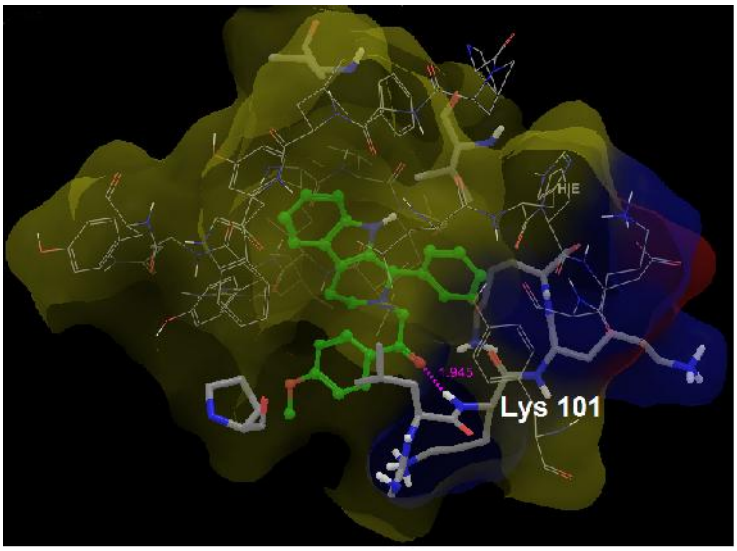


Fig. 5.9: Hydrogen bond interaction (1.945 Å) of compound 6b with Lys101 in NNIBP of HIV-1 RT

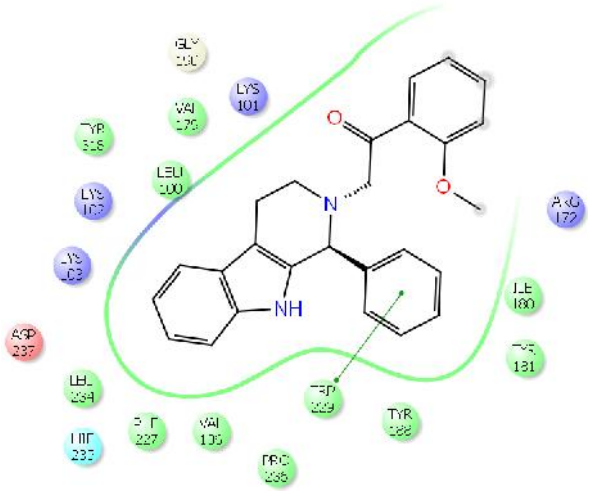


Fig. 5.10: Docking orientation of compound 6i in NNIBP of HIV-1 RT

In *N*-phenyl-2-(1-phenyl-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)acetamide derivatives, *para* methoxy derivative (10b) displayed significant *in-vitro* HIV-1 RT inhibition activity followed by *para* methyl (10c) and *para* chloro (10d) derivatives. Docking study analysis of this series of compounds (10a-p) also supported the previously mentioned postulation as increased electrostatic and hydrophobic interactions of derivatives with their extended hydrophobic wing-2 could be responsible for their significant *in-vitro* activity. Docking results revealed that, tetrahydro-β-carboline (wing-1) ring and substituted phenyl (wing-2), showed electrostatic interactions, hydrophobic interactions with NNIBP aminoacids of HIV-1 RT. Molecular orientation and hydrophobic interactions of compound 10c (Glide score: 8.8) showed in fig. 5.11. Hydrophilic body of these acetamide derivatives exhibited hydrogen bonding interactions with aminoacid Lys101 (10b, Glide score: 9.4, fig. 5.12) of HIV-1 RT, where oxygen atom of carbonyl group acted as hydrogen bond acceptor. Docking analysis of in-active *ortho* methoxy derivative 10k (Glide score: 5.6), illustrated that, wing-2 (*ortho* methoxy phenyl portion oriented outside the non-nucleoside binding pocket and was unable to interact with NNIBP aminoacids HIV-1 RT (fig. 5.13). Docking studies revealed that, wing-2 displacement from NNIBP of HIV-1 RT was observed with both *meta* and *ortho* derivatives and it could influenced their *in-vitro* HIV-1 RT inhibition activity effectively.

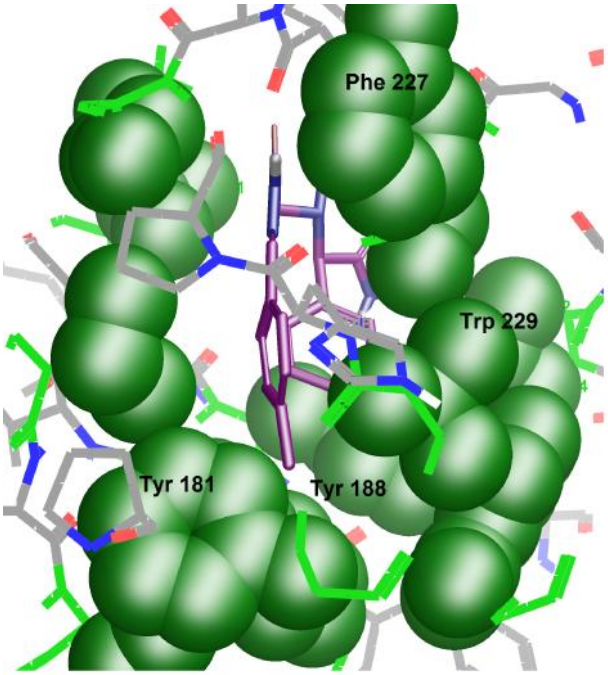


Fig. 5.11: Hydrophobic interactions of compound 10c in NNIBP of HIV-1 RT

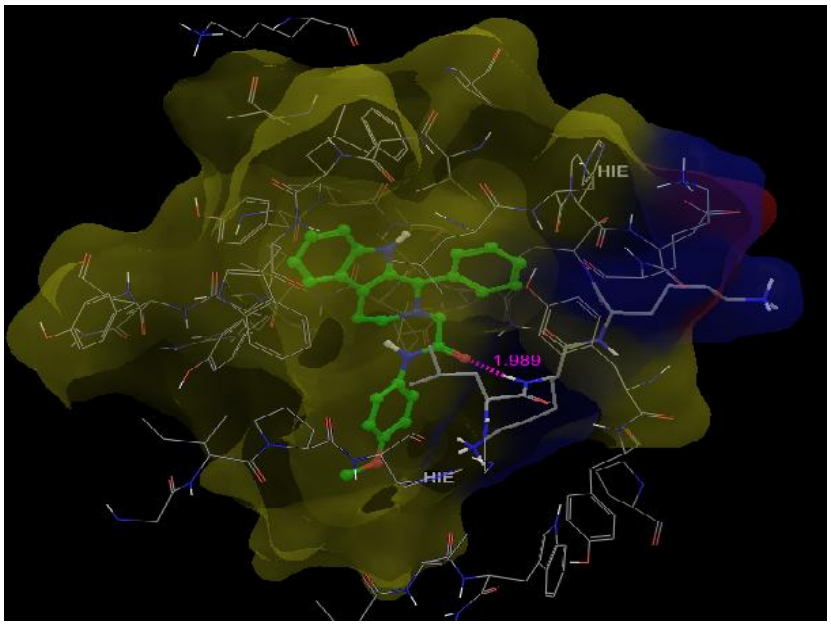


Fig. 5.12: Hydrogen bond interaction (1.989 Å) of compound 10b with Lys101 in NNIBP of HIV-1 RT

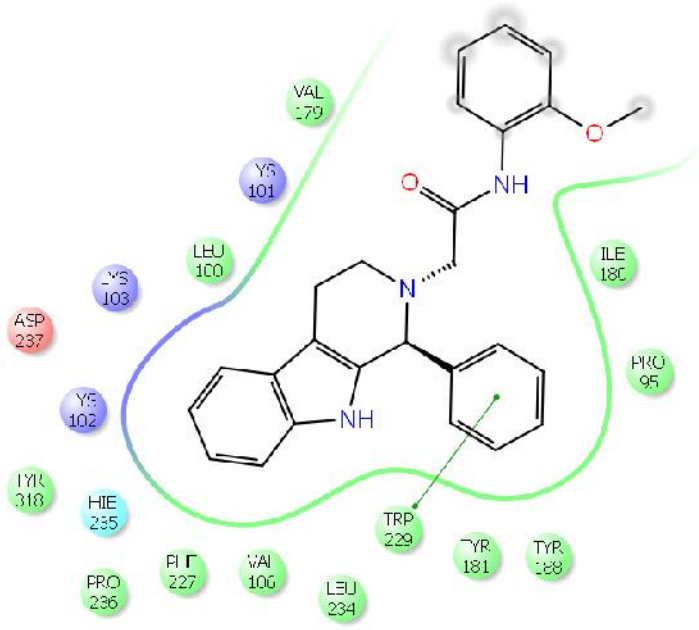


Fig. 5.13: Docking orientation of compound 10k in NNIBP of HIV-1 RT

Final series of tetrahydro-β-carboline derivatives with carbohydrazide linker exhibited increased *in-vitro* HIV-1 RT inhibition activity than keto and acetamide linkers. Docking results suggested that, with the increased distance between hydrophobic wings, they became more flexible to interact with more number of aminoacids and carbohydrazide linker is more polar, to establish hydrogen bond interactions with NNIBP aminoacids. Compounds with carbohydrazide linker displayed two hydrogen bond interactions with aminoacid Lys101. In these derivatives (13a-o), oxygen atom of carbonyl carbon acts as hydrogen bond acceptor and NH carbohydrazide group as hydrogen bond donors to form interaction with Lys101. The increased number of electrostatic and hydrophobic interactions with flexible hydrophobic wings and hydrogen bond interaction might play important role in increased HIV-1 RT inhibition activity than other tetrahydro-β-carboline derivatives. Hydrophobic interactions of 13b (Glide score: 9.9) and hydrogen bond interaction of 13d (Glide score: 9.4) in NNIBP of HIV-1 RT showed in fig. 5.14 and 5.15 respectively. Docking orientation of in-active *meta* methoxy derivative 13i (Glide score: 5.9) in HIV-1 RT (fig. 5.16) demonstrated that, wing-2 phenyl ring oriented ouside the NNIBP and unable to form interactions with aminoacids. Decreased number of interactions of these in-active derivatives with NNIBP aminoacids affected their *in-vitro* inhibition activity.

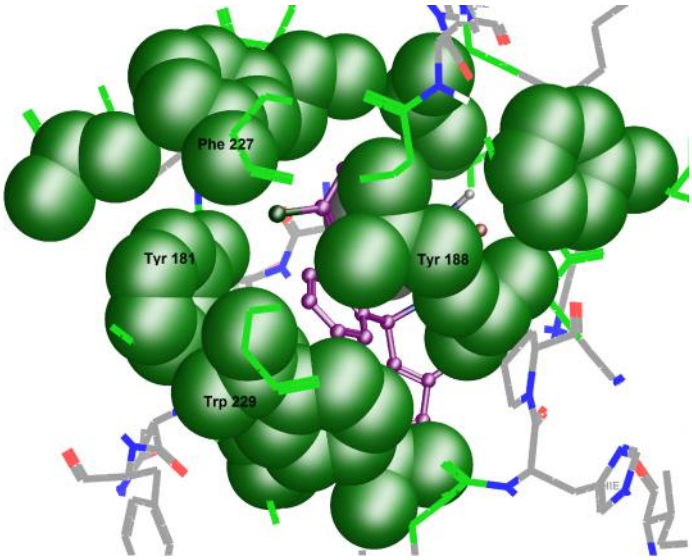


Fig. 5.14: Hydrophobic interactions of compound 13b in NNIBP of HIV-1 RT

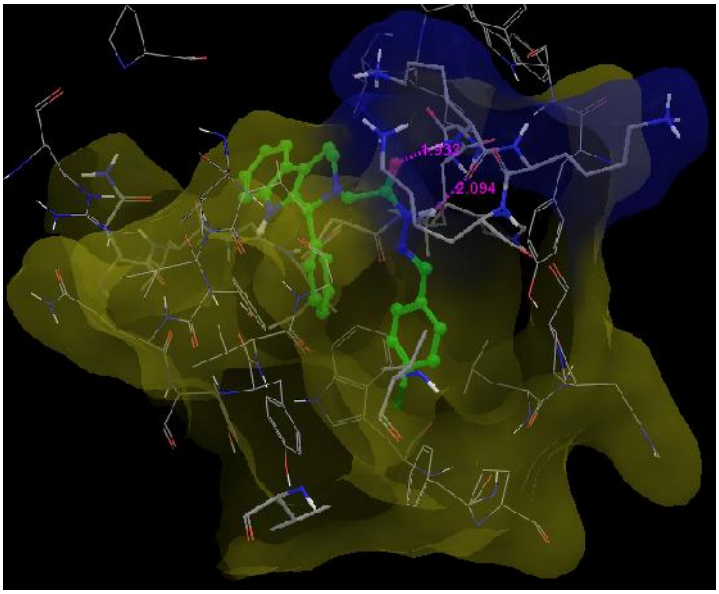


Fig. 5.15: Hydrogen bond interaction of compound 13d with Lys101 (1.93 Å) and Lys103 (2.09 Å) in NNIBP of HIV-1 RT

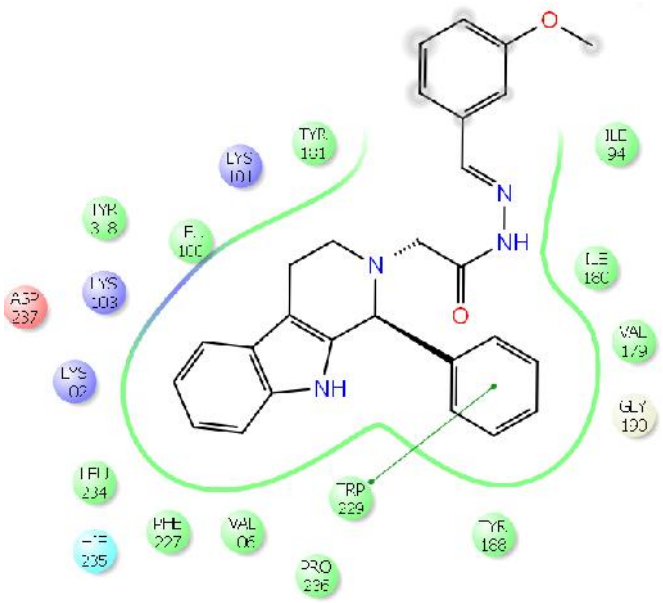


Fig. 5.16: Docking orientation of compound 13i in NNIBP of HIV-1 RT

Docking analysis of 1-phenyl-2,3,4,9-tetrahydro-β-carboline revealed that, electrostatic, hydrophobic interactions of tetrahydro-β-carboline, phenyl rings and hydrogen bond interactions of hydrophilic linker were played vital role in HIV-1 RT inhibition activity.

5.5.2. 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives

Molecular docking study of 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives suggested that, these derivatives displayed hydrogen bond interactions with Lys101, Lys103, electrostatic, hydrophobic interactions with aminoacids like Leu100, Ser105, Val106, Val179, Tyr181, Tyr188, Pro225, Phe227, Trp229, Leu234, Pro236, Tyr318 and ionic interactions with Lys101, Lys102, Lys 103, Lys 104 His 235 of HIV-1 RT. In these derivatives, oxygen atom of carbonyl group was mainly involved in hydrogen bond interactions as hydrogen bond acceptor with Lys101 and Lys103 aminoacids. Indole ring of the β-carboline moiety and position-1 phenyl ring are responsible for electrostatic and hydrophobic interactions, which has major contribution in RT inhibition activity of these derivatives. As tetrahydro-β-carboline derivatives, these 3-piperazinoyl-β-carboline derivatives were also showed interactions with conserved aminoacids like Phe227, Trp229 and Tyr318 of HIV-1 RT.

Among the (1-phenyl-9*H*-pyrido[3,4-*b*]indol-3-yl)(4-phenylpiperazin-1-yl)methanone (20aa-ao) derivatives, un-substituted phenyl derivative displayed significant *in-vitro* inhibition of HIV-1 RT, followed by *para* derivatives with chloro, methoxy and methyl substitutions respectively. Un-substituted phenyl ring on position-1 displayed electrostatic interactions with aminoacids like Tyr181, Tyr188, Phe227, Trp229 and Tyr318 of HIV-1 RT. *Para* position substitution on phenyl ring attached piperazine with hydrophobic groups like methyl and chloro displayed hydrophobic interactions, while fluoro, nitro derivatives might failed to show and it might be the plausible reason for their decreased potency. These electrostatic interactions of β-carboline ring and position-1 phenyl ring might be strongly responsible for activity of 1-phenyl 3-piperazinoyl-β-carboline derivatives and molecular interactions of 20aa (Glide score: 10.7), 20ac (Glide score: 9.6) and 20ad (Glide score: 10.1) with NNIBP aminoacids were showed in fig. 5.17 and 5.18 and 5.19 respectively. Docking orientation of *meta* chloro derivative 20ah (Glide score: 6.4) in NNIBP of HIV-1 RT (fig. 5.20) disclosed that, meta substitution on piperazinyl phenyl ring is not suggested for RT inhibition activity, as it oriented outside NNIBP of HIV-1 RT and unable to show interactions with aminoacids.

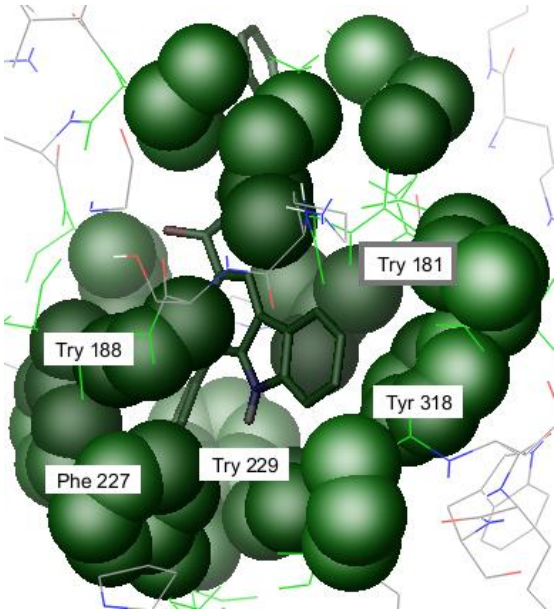


Fig. 5.17: Hydrophobic interactions of compound 20aa in NNIBP of HIV-1 RT

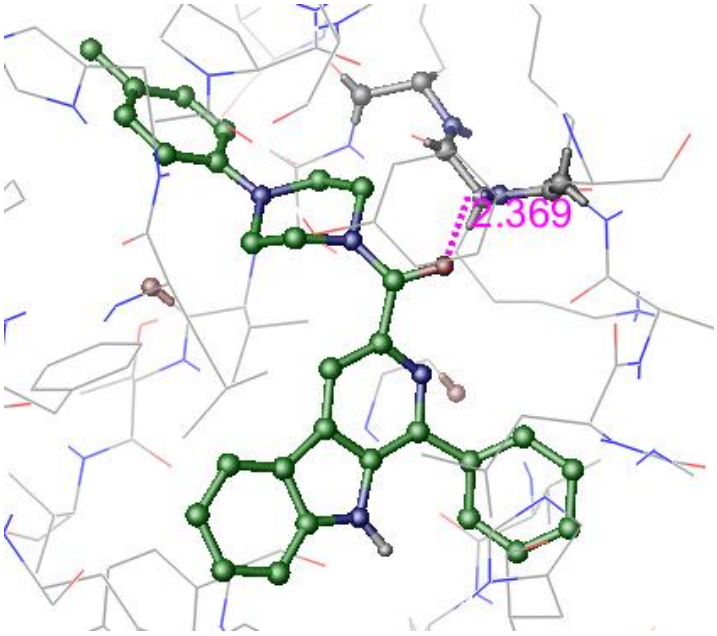


Fig. 5.18: Hydrogen bond interaction (2.369 Å) of compound 20ac with Lys 101 of HIV-1 RT

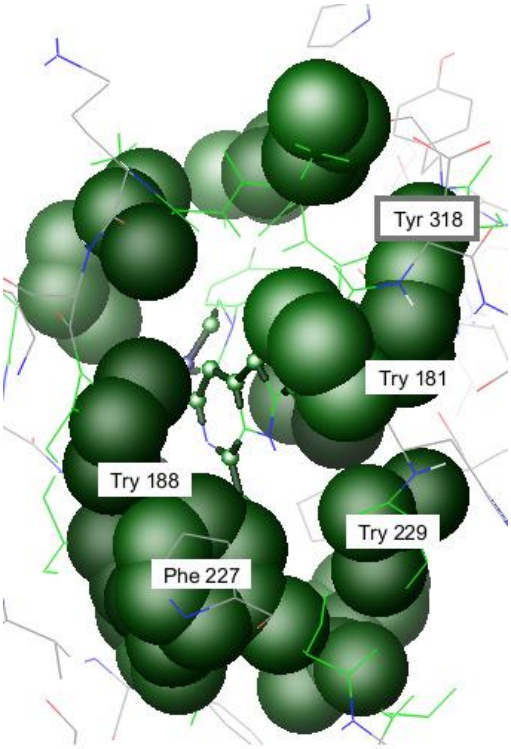


Fig. 5.19: Hydrophobic interactions of compound 20ad in NNIBP of HIV-1 RT

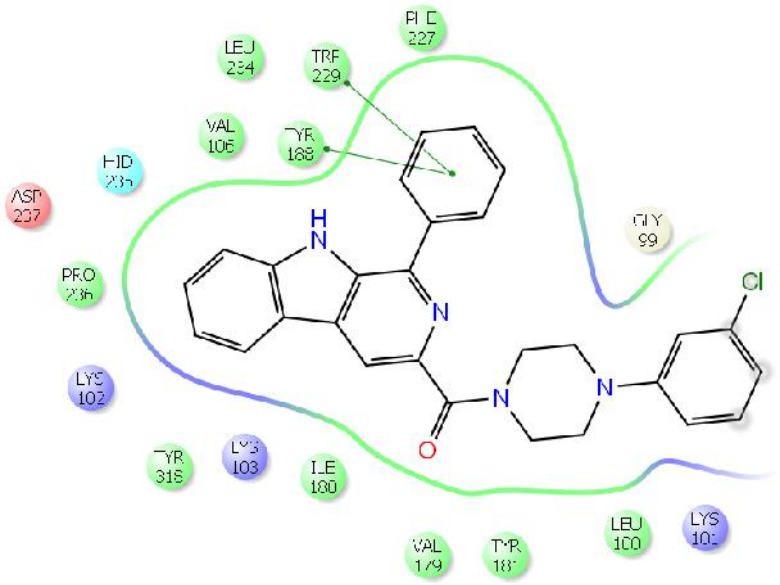


Fig. 5.20: Docking orientation of compound 20ah in NNIBP of HIV-1 RT

Among the 1-(4-methoxyphenyl)-3-piperazinoyl-β-carboline (20ba-bo) derivatives, position-1, 4-methoxyphenyl ring showed hydrophobic interaction, π-cationic interactions with Lys101, Lys 102, Lys 103, but unable to show electrostatic interactions as un-substituted phenyl ring. Methoxy substitution on position-1 phenyl ring decreased the inihibition activity considerably in comparison with un-substituted analogues. The exact reason for sudden activity decrease is still un-clear, but as docking studies suggested, the presence of polar and steric methoxy showed weak π-cationic interactions rather than strong π-π interactions with NNIBP aminoacids. The decreased number of electrostatic interactions, might influenced their RT inhibition activity significantly. Among these molecules, *para* methyl derivative exhibited significant *in-vitro* HIV-1 RT inhibition activity. As docking results suggested that, hydrophobic interaction of *para* methyl substitution with NNIBP aminoacids might play considerable role in *in-vitro* inhibition activity (20bc, Glide score: 10.9, fig. 5.21) of HIV-1 RT. Docking orientation of *meta* chloro derivative 20bh (Glide score: 6.7) (fig. 5.22), indicated that, *meta* substitution slightly displaced from NNIBP of HIV-1 RT. We assumed that, these decreased interactions of *meta* and *ortho* derivatives due to phenyl displacement from NNIBP, might showed depress effect on *in-vitro* RT inhibition activity.

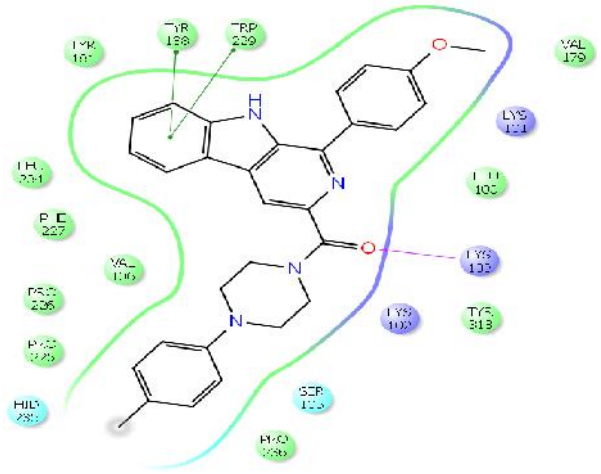


Fig. 5.21: Molecular interactions of compound 20bc in NNIBP of HIV-1 RT, electrosatatic interactions showed as green bars, hydrogen bond (2.245 Å) as purple arrow and hydrophobic interactions as green line

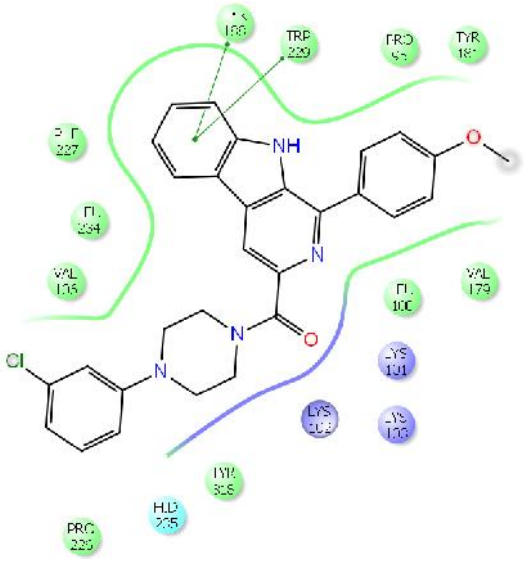


Fig. 5.22: Docking orientation of compound 20bh in NNIBP of HIV-1 RT

In *in-vitro* HIV-1 RT inhibition assay, 1-(4-chlorophenyl) 3-piperazinoyl-β-carboline (20ca-co) derivatives displayed marginal effect on RT activity. Docking analysis of these derivatives became more valuable to understand this drastic decline in RT inhibition activity. Docking studies of these derivatives revealed that, chloro substitution on position-1 phenyl ring, strongly influenced the orientation and molecular interactions of these analogues in NNIBP of HIV-1 RT. β-carboline skeleton with 1-(4-chlorophenyl) was displaced from NNIBP and

unable to show electrostatic, hydrophobic and hydrogen bond interactions with NNIBP amino acids and it might effected their *in-vitro* RT inhibition activity severely. This complete displacement of β-carboline skeleton from NNIBP of HIV-1 RT, might be due to bulkier substitution and adverse steric interactions with NNIBP aminoacids. Molecular orientation of compound 20cb (glide score: 5.6) was showed in fig. 5.23.

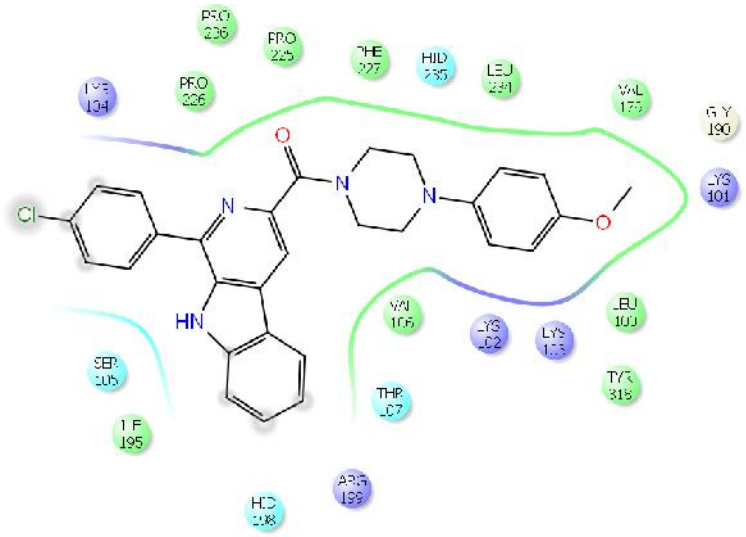


Fig. 5.23: Docking orientation of compound 20cb in NNIBP of HIV-1 RT

In molecular docking studies of 1-thiophenyl-3-piperazinoyl-β-carboline derivatives (20da-do), β-carboline and thiophene ring displayed electrostatic and hydrophobic interactions with NNIBP aminoacids of HIV-1 RT. Among these derivatives, piperazine attached phenyl ring and β-carboline ring displayed electrostatic, hydrophobic, polar interactions and keto group exhibited hydrogen bond interaction with Lys 103. Interestingly, thiophene-2-yl derivatives also displayed electrostatic interactions with NNIBP aminoacids, while substituted phenyl derivatives (20b, 200c) were failed to show the same. Molecular interaction of 20da (Glide score: 9.8) and 20dm (Glide score: 9.2) were showed in fig 5.24 and 5.25 respectively, as these results suggested electrostatic interactions of these derivatives had considerable effect on their *in-vitro* HIV-1 RT inhibition activity. Docking orientation of *meta* chloro derivative 20dh (Glide score: 5.8, fig 5.26), suggested that, displacement of phenyl ring from NNIBP, decreased interactions with NNIBP aminoacids might be responsible for their decreased *in-vitro* RT inhibition activity.

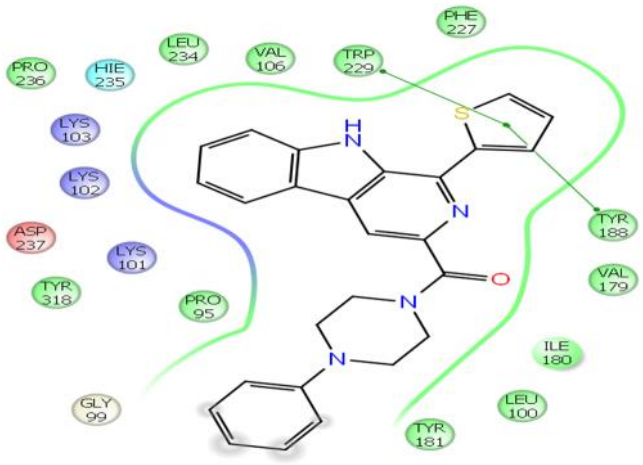


Fig. 5.24: Molecular interactions of compound 20da in NNIBP of HIV-1 RT, elecrostatic interactions showed as green bars, and hydrophobic interactions as green line

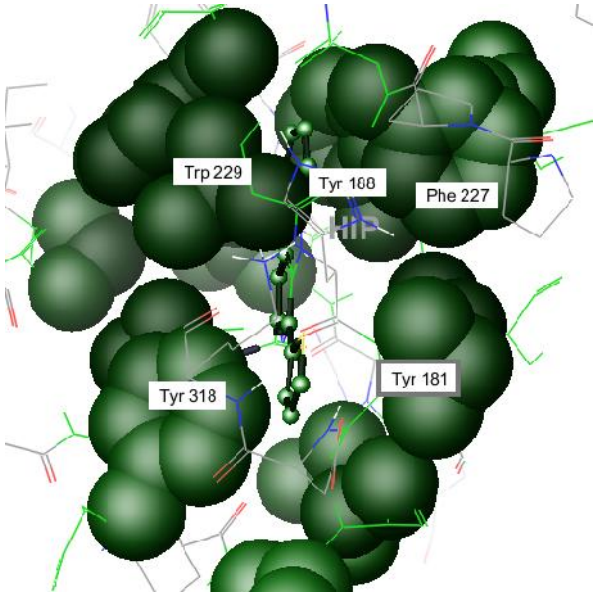


Fig. 5.25: Hydrophobic interactions of compound 20dm in NNIBP of HIV-1 RT

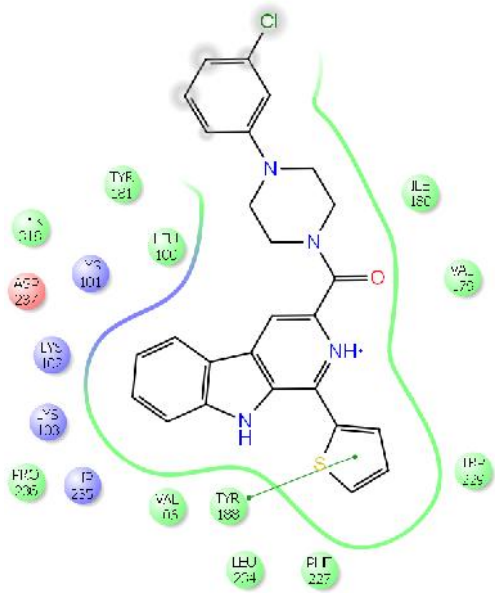


Fig. 5.26: Docking orientation of compound 20dh in NNIBP of HIV-1 RT

Docking results suggested that, derivatives with un-substituted phenyl ring and thiophene-2-yl ring on position-1 displayed electrostatic interactions such as π-π, π-cationic, hydrophobic and hydrogen bond with NNIBP aminoacids whereas 4-methoxy and 4-chloro derivatives were unable to show electrostatic interactions. These electrostatic interactions of 20a and 20d derivatives had significant effect on their *in-vitro* RT inhibition activity.

6. SUMMARY AND CONCLUSION

The present dissertation work was engaged towards the design, synthesis and biological evaluation of β-carboline derivatives against HIV-1 RT, HIV assosciated infections like leishmaniasis and tuberculosis.

- Novel β-carboline derivatives were designed as HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors based on butterfly shaped pharmacophore.
- *In-silico* molecular properties study revealed that, these designed β-carboline derivatives predicted to have to good pharmacokinetic profile.
- Most of these designed β-carboline derivatives showed encouraging drug likeness and drug score values.
- Designed 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives were synthesized from the starting material tryptamine and benzaldehyde via pictet-spengler reaction, followed by nucleophilic substitution reaction on pyridine nitrogen under alkaline condition.
- 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives were synthesized using starting material DL-tryptophan in a sequence of reactions. Reactions involved are, initial esterification of DL-tryptophan, pictet-spengler reaction with aromatic aldehydes, followed by oxidation with potassium permanganate, continued by alkaline ester hydrolysis and finally acid-amide coupling reaction to obtain the designed β-carboline derivatives.
- Structures of all the synthesized final compounds were elucidated using IR, ^{1}H NMR, Mass and elemental analytical data.
- Cytotoxicity of these reported β-carboline derivatives against vero cell line was determined using MTT assay method.
- Most of these reported β-carboline derivatives were non-cytotoxic to vero cell line at tested 50 µg/mL concentration.
- All these designed β-carboline derivatives were evaluated for their *in-vitro* inhibition activity against HIV-1 RT using colorimetric assay method.

- Among these reported 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives, six compounds such as 6b, 10b, 10c, 13a, 13b and 13d showed significant inhibition (≥ 50%) of HIV-1 RT at tested concentration.
- In general, tetrahydro-β-carboline derivatives with hydrophilic body $NCH_2CONHNCH$ displayed comparatively better activity than NCH_2CONH and NCH_2CO derivatives.
- Hydrophilic linker with 5-6 carbon length and *para* substitution on wing-2 of these designed tetrahydro-β-carboline derivatives favored the anti-HIV activity.
- In 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives, eleven compounds 20aa, 20ab, 20ac, 20ad, 20ai, 20am, 20ao, 20bc, 20da, 20dm and 20do exhibited significant inhibitory (≥ 50%) activity against HIV-1 RT.
- HIV-1 RT inhibition potency of these 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives was followed in the order of phenyl, thiophenyl, 4-methoxyphenyl and 4-chlorophenyl substitution on position-1 suggested that, substitution on position-1 phenyl ring is not favorable for anti-HIV activity.
- In general, among these synthesized compounds, 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives with un-substituted phenylpiperazine ring displayed potent activity, however para-substitution on phenyl ring, replacement with benzyl ring retained the potency with minute decrease while substitutions on other position of phenyl ring resulted in drastic reduction of inhibitory activity.
- Molecular docking studies were performed to understand the exact molecular level interactions of β-carboline derivatives with NNIBP aminoacids of HIV-1 RT using molecular modeling suite Schrödinger 2014.
- Molecular modeling studies revealed that, electrostatic interactions of hydrophobic wings (β-carboline and phenyl) had significant effect on anti-HIV activity of these designed β-carboline derivatives.
- These final compounds were screened for their anti-leishmanial activity against promastigotes of *Leishmania infantum* using MTT assay method.

- In *in-vitro* anti-leishmanial activity screening, fourteen tetrahydro-β-carboline (6d, 10a, 10e, 10h, 10i, 10k, 10l, 13a, 13b, 13e, 13g, 13m, 13n, 13o) and eighteen β-carboline derivatives (20aa, 20ab, 20ac, 20ag, 20ai, 20ba, 20bc, 20bk, 20bl, 20ci, 20cj, 20dc, 20df, 20dg, 20di, 20dj, 20dm, 22do) showed potent inhibition of *L. infantum* promastigotes than standard drug miltefosine.
- In 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives, irrespective to the substitutions on wing-2 phenyl ring, carbohydrazide derivatives exhibited potent activity against *Leishmania* parasite followed by amide and keto derivatives respectively.
- Among the 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives, comparatively thiophene-2-yl derivatives exhibited potent anti-leishmanial activity followed un-substituted phenyl, 4-methoxyphenyl and 4-chlorophenyl derivatives.
- Anti-leishmanial activity results and SAR study of these β-carboline derivatives emphasized the effect of heterocyclic ring at position-1 and it increased the scope of development of β-carboline derivatives with other heterocyclic ring substitution.
- All the β-carboline derivatives were also subjected to anti-tubercular activity screening against *Mycobacterium tuberculosis* H37Rv strain using Microplate Alamar Blue Assay (MABA) method.
- In anti-tubercular activity evaluation, two 1-phenyl-2,3,4,9-tetrahydro-β-carboline derivatives (10m, 13j) and three piperazinoyl-β-carboline derivatives (20aa, 20ao, 20bo) exhibited significant inhibition of *M. tuberculosis* with MIC values <6.25 µg/mL.
- As following the anti-HIV and anti-leishmanial potency pattern, carbohydrazide derivatives showed promising anti-tubercular activity than the respective tetrahydro-β-carboline derivatives.
- Whereas, in 9*H*-pyrido[3,4-*b*]indol-3-yl)(piperazin-1-yl)methanone derivatives, un-substituted phenyl derivative (20aa) displayed comparatively better inhibitory activity followed by 4-methoxyphenyl, 4-chlorophenyl and thiophenyl derivatives.

- In contrast to anti-HIV, anti-leishmanial activity pattern, thiophene-2-yl ring substitution on position-1 resulted in decreased anti-tubercular potency of 3-piperazinoyl-β-carboline derivatives.

7. FUTURE PERSPECTIVE

From this study, we have identified the molecules with significant activity against HIV-1 RT as well opportunistic infections like leishmaniasis and tuberculosis. Biological evaluation of these identified lead molecules (6b, 10b, 10c, 13a, 13b, 13d, 20aa, 20ab, 20ac, 20ad, 20ai, 20am, 20ao, 20bc, 20da, 20dm and 20do) against HIV-1, HIV-2 cell lines are in-process, and these molecules needs to be evaluated further against various drug resistant strains. Moreover, these identified reverse transcriptase inhibitors may also act as useful lead molecules to develop inhibitors against other RT containing viruses like Human T-lymphotropic virus (HTLV-1 HTLV-2, 3, 4), Simian immunodeficiency virus (SIV), Feline immunodeficiency virus (FIV), Bovine leukemia virus, etc.

Surprisingly, these designed anti-HIV molecules showed promising results in anti-leishmanial activity evaluation and more interestingly large number of compounds (29 Nos) displayed potent inhibition of *Leishmania* parasite with good selectivity index than standard drug miltefosine. These identified hit molecules required further studies against other parasite forms and *in-vivo* evaluation to become promising candidates in anti-leishmanial drug discovery pipeline. Extensive studies will be required to identify the exact mechanism of action and molecular target for their potent anti-leishmanial activity.

Anti-HIV agents with potent anti-tubercular activity are the ideal candidates to use in HIV chemotherapy as these dual activity molecules decreases the pill burden in HIV-TB co-infection. As few of our molecules displayed both anti-HIV and anti-tubercular activity, hence further development of this class of molecules may generate potent anti-HIV drugs with promising anti-tubercular activity.

8. REFERENCES

[1] L. Ratner, HIV life cycle and genetic approaches, Perspect. Drug Discov. Des., 1 (1993) 3-22.

[2] D. Robertson, J. Anderson, J. Bradac, J. Carr, B. Foley, R. Funkhouser, F. Gao, B. Hahn, M. Kalish, C. Kuiken, HIV-1 nomenclature proposal, Science, 288 (2000) 55-55.

[3] A. Alaeus, T. Leitner, K. Lidman, J. Albert, Most HIV-1 genetic subtypes have entered Sweden, AIDS, 11 (1997) 199-202.

[4] N. Vidal, M. Peeters, C. Mulanga Kabeya, N. Nzilambi, D. Robertson, W. Ilunga, H. Sema, K. Tshimanga, B. Bongo, E. Delaporte, Unprecedented degree of human immunodeficiency virus type 1 (HIV-1) group M genetic diversity in the democratic republic of congo suggests that the HIV-1 pandemic originated in central africa, J. Virol., 74 (2000) 10498-10507.

[5] O.T. Campbell Yesufu, R.T. Gandhi, Update on human immunodeficiency virus (HIV)-2 Infection, Clin. Infect. Dis., 52 (2011) 780-787.

[6] A.L. Cunningham, H. Donaghy, A.N. Harman, M. Kim, S.G. Turville, Manipulation of dendritic cell function by viruses, Curr. Opin. Microbiol., 13 (2010) 524-529.

[7] E. De Clercq, HIV-chemotherapy and prophylaxis: New drugs, leads and approaches, Int. J. Biochem. Cell Biol., 36 (2004) 1800-1822.

[8] R. Detels, P. Tarwater, J.P. Phair, J. Margolick, S.A. Riddler, A. Munoz, Effectiveness of potent antiretroviral therapies on the incidence of opportunistic infections before and after AIDS diagnosis, AIDS, 15 (2001) 347-355.

[9] A.K. Ghosh, S. Leshchenko Yashchuk, D.D. Anderson, A. Baldridge, M. Noetzel, H.B. Miller, Y. Tie, Y.F. Wang, Y. Koh, I.T. Weber, H. Mitsuya, Design of HIV-1 protease inhibitors with pyrrolidinones and oxazolidinones as novel P1'-ligands to enhance backbone-binding interactions with protease: Synthesis, biological evaluation, and protein-ligand X-ray studies, J. Med. Chem., 52 (2009) 3902-3914.

[10] M. Hamann, D. Alonso, E. Martín Aparicio, A. Fuertes, M.J. Perez Puerto, A. Castro, S. Morales, M.L. Navarro, M. del Monte Millan, M. Medina, H. Pennaka, A. Balaiah, J. Peng, J. Cook, S. Wahyuono, A. Martinez, Glycogen synthase kinase-3 (GSK-3) inhibitory activity and structure-activity relationship (SAR) studies of the manzamine alkaloids. Potential for Alzheimer's disease, J. Nat. Prod., 70 (2007) 1397-1405.

[11] UNAIDS report on the global AIDS epidemic. (http://www.unaids.org/en/resources/ campaigns/ World-AIDS-Day-Report-2014) Accessed on 2nd Dec, 2014.

[12] A.A. Haqqani, J.C. Tilton, Entry inhibitors and their use in the treatment of HIV-1 infection, Antiviral Res., 98 (2013) 158-170.

[13] J. He, Y. Chen, M. Farzan, H. Choe, A. Ohagen, S. Gartner, J. Busciglio, X. Yang, W. Hofmann, W. Newman, C.R. Mackay, J. Sodroski, D. Gabuzda, CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia, Nature, 385 (1997) 645-649.

[14] S. Sierra, B. Kupfer, R. Kaiser, Basics of the virology of HIV-1 and its replication, J. Clin. Virol., 34 (2005) 233-244.

[15] P. Bean, New drug targets for HIV, Clin. Infect. Dis., 41 (2005) S96-S100.

[16] J.C. Boyer, K. Bebenek, T.A. Kunkel, Unequal human immunodeficiency virus type 1 reverse transcriptase error rates with RNA and DNA templates, Proc. Natl. Acad. Sci., 89 (1992) 6919-6923.

[17] K. Bebenek, J. Abbotts, J.D. Roberts, S.H. Wilson, T. Kunkel, Specificity and mechanism of error-prone replication by human immunodeficiency virus-1 reverse transcriptase, J. Biol. Chem., 264 (1989) 16948-16956.

[18] N. Tsurutani, M. Kubo, Y. Maeda, T. Ohashi, N. Yamamoto, M. Kannagi, T. Masuda, Identification of critical amino acid residues in human immunodeficiency virus type 1 IN required for efficient proviral DNA formation at steps prior to Integration in dividing and nondividing cells, J. Virol., 74 (2000) 4795-4806.

[19] O. Delelis, K. Carayon, A. Saib, E. Deprez, J.F. Mouscadet, Integrase and integration: Biochemical activities of HIV-1 integrase, Retrovirology, 5 (2008) 1-14.

[20] A. Brik, C.H. Wong, HIV-1 protease: Mechanism and drug discovery, Org. Biomol. Chem., 1 (2003) 5-14.

[21] H. Yang, J. Nkeze, R.Y. Zhao, Effects of HIV-1 protease on cellular functions and their potential applications in antiretroviral therapy, Cell Biosci., 4 (2012) 2-32.

[22] L.E. Henderson, M. Bowers, R.d. Sowder, S. Serabyn, D. Johnson, J. Bess, L. Arthur, D. Bryant, C. Fenselau, Gag proteins of the highly replicative MN strain of human immunodeficiency virus type 1: Posttranslational modifications, proteolytic processings, and complete amino acid sequences, J. Virol., 66 (1992) 1856-1865.

[23] D.M. Lowe, A. Aitken, C. Bradley, G.K. Darby, B.A. Larder, K.L. Powell, D.J. Purifoy, M. Tisdale, D.K. Stammers, HIV-1 reverse transcriptase: Crystallization and analysis of domain structure by limited proteolysis, Biochemistry, 27 (1988) 8884-8889.

[24] A. Agopian, E. Gros, G. Aldrian Herrada, N. Bosquet, P. Clayette, G. Divita, A new generation of peptide-based inhibitors targeting HIV-1 reverse transcriptase conformational flexibility, J. Biol. Chem., 284 (2009) 254-264.

[25] L. Kohlstaedt, J. Wang, J. Friedman, P. Rice, T. Steitz, Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor, Science, 256 (1992) 1783-1790.

[26] S. Huang, J. Smith, L. Moen, Contribution of the p51 subunit of HIV-1 reverse transcriptase to enzyme processivity, Biochem. Biophys. Res. Commun., 184 (1992) 986-992.

[27] T. Cihlar, A.S. Ray, Nucleoside and nucleotide HIV reverse transcriptase inhibitors: 25 years after zidovudine, Antiviral Res., 85 (2010) 39-58.

[28] M. Bethune, Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery, development and use in the treatment of HIV-1 infection: A review of the last 20 years (1989-2009), Antiviral Res., 85 (2010) 75 - 90.

[29] R. Goody, B. Muller, T. Restle, Factors contributing to the inhibition of HIV reverse transcriptase by chain-terminating nucleotides *in vitro* and *in vivo*, FEBS Lett., 291 (1991) 1-5.

[30] B.D. Herman, N. Sluis Cremer, Molecular pharmacology of nucleoside and nucleotide HIV-1 reverse transcriptase inhibitors, Pharmacology, 10 (2012) 63-81.

[31] A.A. Johnson, A.S. Ray, J. Hanes, Z. Suo, J.M. Colacino, K.S. Anderson, K.A. Johnson, Toxicity of antiviral nucleoside analogs and the human mitochondrial DNA polymerase, J. Biol. Chem., 276 (2001) 40847-40857.

[32] D. Rai, W. Chen, Y. Tian, X. Chen, P. Zhan, E. De Clercq, C. Pannecouque, J. Balzarini, X. Liu, Design, synthesis and biological evaluation of 3-benzyloxy-linked pyrimidinylphenylamine derivatives as potent HIV-1 NNRTIs, Biorg. Med. Chem., 21 (2013) 7398-7405.

[33] E.D. Clercq, New anti-HIV agents and targets, Med. Res. Rev., 22 (2002) 531-565.

[34] Y. Hsiou, J. Ding, K. Das, A. Clark Jr, S. Hughes, E. Arnold, Structure of unliganded HIV-1 reverse transcriptase at 2.7 Å resolution: Implications of conformational changes for polymerization and inhibition mechanisms, Structure, 4 (1996) 853-860.

[35] X. Wang, J. Zhang, Y. Huang, R. Wang, L. Zhang, K. Qiao, L. Li, C. Liu, Y. Ouyang, W. Xu, Design, synthesis, and biological evaluation of 1-[(2-benzyloxyl/alkoxyl)methyl]-5-halo-6-aryluracils as potent HIV-1 non-nucleoside reverse transcriptase inhibitors with an improved drug resistance profile, J. Med. Chem., 55 (2012) 2242-2250.

[36] J.R.M. Boyer, E. Arnoult, M. Medebielle, J.R.M. Guillemont, J. Unge, D. Jochmans, Difluoromethylbenzoxazole pyrimidine thioether derivatives: A novel class of potent non-nucleoside HIV-1 reverse transcriptase inhibitors, J. Med. Chem., 54 (2011) 7974-7985.

[37] P.A.J. Janssen, P.J. Lewi, E. Arnold, F. Daeyaert, M. de Jonge, J. Heeres, L. Koymans, M. Vinkers, J. Guillemont, E. Pasquier, M. Kukla, D. Ludovici, K. Andries, M.P. de Bethune, R. Pauwels, K. Das, A.D. Clark, Y.V. Frenkel, S.H. Hughes, B. Medaer, F. De Knaep, H. Bohets, F. De Clerck, A. Lampo, P. Williams, P. Stoffels, In search of a novel anti-HIV drug: Multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1*E*)-2-Cyanoethenyl]-2,6-dimethylphenyl]amino]-2- pyrimidinyl]amino]benzonitrile (R278474, Rilpivirine), J. Med. Chem., 48 (2004) 1901-1909.

[38] A. Penta, S. Ganguly, S. Murugesan, Design and synthesis of tetrahydrophthalimide derivatives as inhibitors of HIV-1 reverse transcriptase, Org. Med. Chem. Lett., 3 (2013) 1-8.

[39] D. Li, P. Zhan, E. De Clercq, X. Liu, Strategies for the design of HIV-1 non-nucleoside reverse transcriptase inhibitors: Lessons from the development of seven representative paradigms, J. Med. Chem., 55 (2012) 3595-3613.

[40] J. Guillemont, E. Pasquier, P. Palandjian, D. Vernier, S. Gaurrand, P.J. Lewi, J. Heeres, M.R. de Jonge, L.M.H. Koymans, F.F.D. Daeyaert, M.H. Vinkers, E. Arnold, K. Das, R. Pauwels, K. Andries, M.P. de Bethune, E. Bettens, K. Hertogs, P. Wigerinck, P. Timmerman, P.A.J. Janssen, Synthesis of novel diarylpyrimidine analogues and their antiviral activity against human immunodeficiency virus type 1, J. Med. Chem., 48 (2004) 2072-2079.

[41] A. Astulla, K. Zaima, Y. Matsuno, Y. Hirasawa, W. Ekasari, A. Widyawaruyanti, N. Zaini, H. Morita, Alkaloids from the seeds of *Peganum harmala* showing antiplasmodial and vasorelaxant activities, J. Nat. Med., 62 (2008) 470-472.

[42] A.I. Maïga, D. Descamps, L. Morand Joubert, I. Malet, A. Derache, M. Cisse, V. Koita, A. Akonde, B. Diarra, M. Wirden, Resistance-associated mutations to etravirine (TMC-125) in antiretroviral-naive patients infected with non-B HIV-1 subtypes, Antimicrob. Agents Chemother., 54 (2010) 728-733.

[43] J.D. Winkler, J.M. Axten, The first total syntheses of ircinol A, ircinal A, and manzamines A and D, J. Am. Chem. Soc., 120 (1998) 6425-6426.

[44] R. Silvestri, M. Artico, G. De Martino, G. La Regina, R. Loddo, M. La Colla, P. La Colla, Simple, short peptide derivatives of a *S*(L-737,126) active *in vitro* against HIV-1 wild type and variants carrying non-nucleoside reverse transcriptase inhibitor resistance mutations, J. Med. Chem., 47 (2004) 3892-3896.

[45] J.M. Tronchet, M. Seman, Nonnucleoside inhibitors of HIV-1 reverse transcriptase: From the biology of reverse transcription to molecular design, Curr. Top. Med. Chem., 3 (2003) 1496-1511.

[46] S. Martins, M.J. Ramos, P.A. Fernandes, The current status of the NNRTI family of antiretrovirals used in the HAART regime against HIV infection, Curr. Med. Chem., 15 (2008) 1083-1095.

[47] S.G. Deeks, S.R. Lewin, D.V. Havlir, The end of AIDS: HIV infection as a chronic disease, The Lancet, 382 (2013) 1525-1533.

[48] J.A. Esté, T. Cihlar, Current status and challenges of antiretroviral research and therapy, Antiviral Res., 85 (2010) 25-33.

[49] P.L. Vernazza, L. Troiani, M.J. Flepp, R.W. Cone, J. Schock, F. Roth, K. Boggian, M.S. Cohen, S.A. Fiscus, J.J. Eron, Potent antiretroviral treatment of HIV-infection results in suppression of the seminal shedding of HIV, AIDS, 14 (2000) 117-121.

[50] A. Telenti, G. Paolo Rizzardi, Limits to potent antiretroviral therapy, Rev. Med. Virol., 10 (2000) 385-393.

[51] J. Lange, Strategies for rescue therapy, Antivir. Ther., 3 (1997) 75-77.

[52] M.R. Sadaie, R. Mayner, J. Doniger, A novel approach to develop anti-HIV drugs: Adapting non-nucleoside anticancer chemotherapeutics, Antiviral Res., 61 (2004) 1-18.

[53] R. Sakai, T. Higa, C.W. Jefford, G. Bernardinelli, Manzamine A, a novel antitumor alkaloid from a sponge, J. Am. Chem. Soc., 108 (1986) 6404-6405.

[54] S.R. Patpi, L. Pulipati, P. Yogeeswari, D. Sriram, N. Jain, B. Sridhar, R. Murthy, A.D. T, S.V. Kalivendi, S. Kantevari, Design, synthesis, and structure-activity correlations of novel bibenzo[*b,d*]furan, dibenzo[*b,d*]thiophene, and *N*-methylcarbazole clubbed 1,2,3-triazoles as potent inhibitors of *Mycobacterium tuberculosis*, J. Med. Chem., 55 (2012) 3911-3922.

[55] WHO, UNAIDS report on the global AIDS epidemic 2013.

[56] A. Kwara, T. Flanigan, E. Carter, Highly active antiretroviral therapy (HAART) in adults with tuberculosis: Current status, Int. J. Tuberc. Lung Dis., 9 (2005) 248-257.

[57] C. Padmapriyadarsini, G. Narendran, S. Swaminathan, Diagnosis and treatment of tuberculosis in HIV co-infected patients, Indian J. Med. Res., 134 (2011) 850-865.

[58] A.J. Wood, S.C. Piscitelli, K.D. Gallicano, Interactions among drugs for HIV and opportunistic infections, New Engl. J. Med., 344 (2001) 984-996.

[59] P.O.A.G.F. Adults, Adolescents, Department of Health and Human Services, Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents, (2009) 1-161.

[60] M. Narita, D. Ashkin, E.S. Hollender, A.E. Pitchenik, Paradoxical worsening of tuberculosis following antiretroviral therapy in patients with AIDS, Am. J. Respir. Crit. Care Med., 158 (1998) 157-161.

[61] M.J. Thompson, J.C. Louth, S.M. Little, M.P. Jackson, Y. Boursereau, B. Chen, I. Coldham, Synthesis and evaluation of 1-amino-6-halo-β-carbolines as antimalarial and antiprion agents, ChemMedChem., 7 (2012) 578-586.

[62] D. Stærk, E. Lemmich, J. Christensen, A. Kharazmi, C.E. Olsen, J.W. Jaroszewski, Leishmanicidal, antiplasmodial and cytotoxic activity of indole alkaloids from *Corynanthe pachyceras*, Planta Med., 66 (2000) 531-536.

[63] S.N. Suryawanshi, S. Kumar, R. Shivahare, S. Pandey, A. Tiwari, S. Gupta, Design, synthesis and biological evaluation of aryl pyrimidine derivatives as potential leishmanicidal agents, Bioorg. Med. Chem. Lett., 23 (2013) 5235-5238.

[64] R. Reithinger, J.C. Dujardin, H. Louzir, C. Pirmez, B. Alexander, S. Brooker, Cutaneous leishmaniasis, Lancet Infect. Dis., 7 (2007) 581-596.

[65] T.P. Barbosa, S.C.O. Sousa, F.M. Amorim, Y.K.S. Rodrigues, P.A.C. de Assis, J.P.A. Caldas, M.R. Oliveira, M.L.A.A. Vasconcellos, Design, synthesis and antileishmanial *in vitro* activity of new series of chalcones-like compounds: A molecular hybridization approach, Biorg. Med. Chem., 19 (2011) 4250-4256.

[66] R. Brokamp, B. Bergmann, I.B. Müller, S. Bienz, Stereoselective preparation of pyridoxal 1,2,3,4-tetrahydro-β-carboline derivatives and the influence of their absolute and relative configuration on the proliferation of the malaria parasite *Plasmodium falciparum*, Bioorg. Med. Chem., 22 (2014) 1832-1837.

[67] M.J. Chan Bacab, L.M. Pena Rodriguez, Plant natural products with leishmanicidal activity, Nat. Prod. Rep., 18 (2001) 674-688.

[68] A. Chattopadhyay, M. Jafurulla, A novel mechanism for an old drug: Amphotericin B in the treatment of visceral leishmaniasis, Biochem. Biophys. Res. Commun., 416 (2011) 7-12.

[69] T.S. Kam, K.M. Sim, T. Koyano, K. Komiyama, Leishmanicidal alkaloids from *Kopsia griffithii*, Phytochemistry, 50 (1999) 75-79.

[70] S. Ganguly, N.K. Das, J.N. Barbhuiya, M. Chatterjee, Post-kala-azar dermal leishmaniasis - An overview, Int. J. Dermatol., 49 (2010) 921-931.

[71] A.D.L. Loma, J. Alvar, E.M. Galiano, J. Blazquez, A.A. Munoz, R. Najera, Leishmaniasis or AIDS?, Trans. R. Soc. Trop. Med. Hyg., 79 (1985) 421-422.

[72] World Health Organization, Leishmania/HIV co-infection. Epidemiological analysis of 692 retrospective cases, Wkly. Epidemiol. Rec., 72 (1997) 49-54.

[73] I. Cruz, J. Nieto, J. Moreno, C. Canavate, P. Desjeux, J. Alvar, Leishmania/HIV co-infections in the second decade, Indian J. Med. Res., 123 (2006) 357-388.

[74] J.R. Allen, B.R. Holmstedt, The simple β-carboline alkaloids, Phytochemistry, 19 (1980) 1573-1582.

[75] J. Ishida, H.K. Wang, M. Oyama, M.L. Cosentino, C.Q. Hu, K.H. Lee, Anti-AIDS agents. 46.1 Anti-HIV activity of harman, an anti-HIV principle from *Symplocos setchuensis*, and its derivatives, J. Nat. Prod., 64 (2001) 958-960.

[76] J. Adachi, Y. Mizoi, T. Naito, Y. Ogawa, Y. Uetani, I. Ninomiya, Identification of tetrahydro-β-carboline-3-carboxylic acid in foodstuffs, human urine and human milk, J. Nutr., 121 (1991) 646-652.

[77] A. Brossi, A. Focella, S. Teitel, Alkaloids in mammalian tissues. 3. Condensation of L-tryptophan and L-5-hydroxytryptophan with formaldehyde and acetaldehyde, J. Med. Chem., 16 (1973) 418-420.

[78] R. Cao, W. Peng, Z. Wang, A. Xu, β-Carboline alkaloids: Biochemical and pharmacological functions, Curr. Med. Chem., 14 (2007) 479-500.

[79] F. Goebel, Ueber das Harmalin, Justus Liebigs Ann. Chem., 38 (1841) 363-366.

[80] A. Chatterjee, M. Ganguly, Alkaloidal constituents of *Peganum harmala* and synthesis of the minor alkaloid deoxyvascinone, Phytochemistry, 7 (1968) 307-311.

[81] J. Asgarpanah, F. Ramezanloo, Chemistry, pharmacology and medicinal properties of *Peganum harmala* L, Afr. J. Pharm. Pharmacol, 6 (2012) 1573-1580.

[82] J. Callaway, Various alkaloid profiles in decoctions of *Banisteriopsis caapi*, J. Psychoactive Drugs, 37 (2005) 151-155.

[83] N. Valin, D. Haybron, L. Groves, H. Mower, The nitrosation of alcohol-induced metabolites produces mutagenic substances, Mutat. Res. Genet. Tox., 158 (1985) 159-168.

[84] N.S. Buckholtz, Neurobiology of tetrahydro-β-carbolines, Life Sci., 27 (1980) 893-903.

[85] G. Farrel, W.M. McIsaac, Adrenoglomerulotropin, Arch. Biochem. Biophys., 94 (1961) 543-544.

[86] J.J. Maresh, L.A. Giddings, A. Friedrich, E.A. Loris, S. Panjikar, B.L. Trout, J. Stockigt, B. Peters, S.E. O'Connor, Strictosidine synthase: Mechanism of a pictet-spengler catalyzing enzyme, J. Am. Chem. Soc., 130 (2007) 710-723.

[87] A.E. Laine, C. Lood, A.M. Koskinen, Pharmacological importance of optically active tetrahydro-β-carbolines and synthetic approaches to create the C1 stereocenter, Molecules, 19 (2014) 1544-1567.

[88] M. Radwan, A. Hanora, S. Khalifa, S.H.A.E. Ela, Manzamines: A potential for novel cures, Cell Cycle, 11 (2012) 1765-1772.

[89] K.V. Rao, M.S. Donia, J. Peng, E. Garcia Palomero, D. Alonso, A. Martinez, M. Medina, S.G. Franzblau, B.L. Tekwani, S.I. Khan, S. Wahyuono, K.L. Willett, M.T. Hamann, Manzamine B and E and Ircinal A related alkaloids from an indonesian *Acanthostrongylophora s*ponge and their activity against infectious, tropical parasitic, and Alzheimer's diseases, J. Nat. Prod., 69 (2006) 1034-1040.

[90] K.V. Rao, B.D. Santarsiero, A.D. Mesecar, R.F. Schinazi, B.L. Tekwani, M.T. Hamann, New manzamine alkaloids with activity against infectious and tropical parasitic diseases from an Indonesian sponge, J. Nat. Prod., 66 (2003) 823-828.

[91] S.A. Snyder, D.A. Vosburg, M.G. Jarvis, J.H. Markgraf, Intramolecular hetero diels–alder routes to γ-carboline alkaloids, Tetrahedron, 56 (2000) 5329-5335.

[92] F. Soriano Agatón, D. Lagoutte, E. Poupon, F. Roblot, A. Fournet, J.C. Gantier, R. Hocquemiller, Extraction, hemisynthesis, and synthesis of canthin-6-one analogues. Evaluation of their antifungal activities, J. Nat. Prod., 68 (2005) 1581-1587.

[93] P.C. Kuo, L.S. Shi, A.G. Damu, C.R. Su, C.H. Huang, C.H. Ke, J.B. Wu, A.J. Lin, K.F. Bastow, K.H. Lee, T.S. Wu, Cytotoxic and antimalarial β-carboline alkaloids from the roots of *Eurycoma longifolia*, J. Nat. Prod., 66 (2003) 1324-1327.

[94] N. Keawpradub, G.C. Kirby, J.C.P. Steele, P.J. Houghton, Antiplasmodial activity of extracts and alkaloids of three *Alstonia* species from Thailand, Planta Med., 65 (1999) 690-694.

[95] M. Frederich, M. Tits, M.P. Hayette, V. Brandt, J. Penelle, P. DeMol, G. Llabres, L. Angenot, 10'-Hydroxyusambarensine, a new antimalarial bisindole alkaloid from the roots of *Strychnos usambarensis*, J. Nat. Prod., 62 (1999) 619-621.

[96] C. Jimenez, E. Quinoa, M. Adamczeski, L.M. Hunter, P. Crews, Novel sponge-derived amino acids. 12. Tryptophan-derived pigments and accompanying sesterterpenes from *Fascaplysinopsis reticulata*, J. Org. Chem., 56 (1991) 3403-3410.

[97] D.M. Roll, C.M. Ireland, H.S.M. Lu, J. Clardy, Fascaplysin, an unusual antimicrobial pigment from the marine sponge *Fascaplysinopsis sp*, J. Org. Chem., 53 (1988) 3276-3278.

[98] G. Kirsch, G.M. Konig, A.D. Wright, R. Kaminsky, A new bioactive sesterterpene and antiplasmodial alkaloids from the marine sponge *Hyrtios cf. erecta*, J. Nat. Prod., 63 (2000) 825-829.

[99] J.C.P. Steele, N.C. Veitch, G.C. Kite, M.S.J. Simmonds, D.C. Warhurst, Indole and β-carboline alkaloids from *Geissospermum sericeum*, J. Nat. Prod., 65 (2002) 85-88.

[100] M. Frederich, M.J. Jacquier, P. Thépenier, P. De Mol, M. Tits, G. Philippe, C. Delaude, L. Angenot, M. Zeches Hanrot, Antiplasmodial activity of alkaloids from various *Strychnos species*, J. Nat. Prod., 65 (2002) 1381-1386.

[101] J. Sun, H. Lou, S. Dai, H. Xu, F. Zhao, K. Liu, Indole alkoloids from *Nauclea officinalis* with weak antimalarial activity, Phytochemistry, 69 (2008) 1405-1410.

[102] H. Huang, Y. Yao, Z. He, T. Yang, J. Ma, X. Tian, Y. Li, C. Huang, X. Chen, W. Li, S. Zhang, C. Zhang, J. Ju, Antimalarial β-carboline and indolactam alkaloids from *Marinactinospora thermotolerans*, A deep sea isolate, J. Nat. Prod., 74 (2011) 2122-2127.

[103] R.A. Davis, S. Duffy, V.M. Avery, D. Camp, J.N.A. Hooper, R.J. Quinn, (+)-7-Bromotrypargine: An antimalarial β-carboline from the Australian marine sponge *Ancorina sp.*, Tetrahedron Lett., 51 (2010) 583-585.

[104] S.T.S. Chan, A.N. Pearce, M.J. Page, M. Kaiser, B.R. Copp, Antimalarial β-carbolines from the New Zealand ascidian *Pseudodistoma opacum*, J. Nat. Prod., 74 (2011) 1972-1979.

[105] Y. Boursereau, I. Coldham, Synthesis and biological studies of 1-amino β-carbolines, Bioorg. Med. Chem. Lett., 14 (2004) 5841-5844.

[106] K. Takasu, T. Shimogama, C. Saiin, H.S. Kim, Y. Wataya, M. Ihara, π-Delocalized β-carbolinium cations as potential antimalarials, Bioorg. Med. Chem. Lett., 14 (2004) 1689-1692.

[107] S. Van Miert, S. Hostyn, B.U.W. Maes, K. Cimanga, R. Brun, M. Kaiser, P. Mátyus, R. Dommisse, G. Lemière, A. Vlietinck, L. Pieters, Isoneocryptolepine, a synthetic indoloquinoline alkaloid, as an antiplasmodial lead compound, J. Nat. Prod., 68 (2005) 674-677.

[108] J.D. Winkler, A.T. Londregan, M.T. Hamann, Antimalarial activity of a new family of analogues of manzamine A, Org. Lett., 8 (2006) 2591-2594.

[109] J.D. Winkler, A.T. Londregan, J.R. Ragains, M.T. Hamann, Synthesis and biological evaluation of manzamine analogues, Org. Lett., 8 (2006) 3407-3409.

[110] J.D. Winkler, A.T. Londregan, M.T. Hamann, Synthetic modification of manzamine A via Grubbs metathesis. Novel structures with enhanced antibacterial and antiprotozoal properties, Org. Lett., 9 (2007) 4467-4469.

[111] A.G. Shilabin, N. Kasanah, B.L. Tekwani, M.T. Hamann, Kinetic studies and bioactivity of potential manzamine prodrugs, J. Nat. Prod., 71 (2008) 1218-1221.

[112] M.A. Ibrahim, A.G. Shilabin, S. Prasanna, M. Jacob, S.I. Khan, R.J. Doerksen, M.T. Hamann, 2-*N*-methyl modifications and SAR studies of manzamine A, Bioorg. Med. Chem., 16 (2008) 6702-6706.

[113] L. Gupta, K. Srivastava, S. Singh, S.K. Puri, P.M.S. Chauhan, Synthesis of 2-[3-(7-Chloro-quinolin-4-ylamino)-alkyl]-1-(substituted phenyl)-2,3,4,9-tetrahydro-1*H*-β-carbolines as a new class of antimalarial agents, Bioorg. Med. Chem. Lett., 18 (2008) 3306-3309.

[114] A.E. Wahba, J. Peng, S. Kudrimoti, B.L. Tekwani, M.T. Hamann, Structure-activity relationship studies of manzamine A: Amidation of positions 6 and 8 of the β-carboline moiety, Bioorg. Med. Chem., 17 (2009) 7775-7782.

[115] J. Peng, S. Kudrimoti, S. Prasanna, S. Odde, R.J. Doerksen, H.K. Pennaka, Y.M. Choo, K.V. Rao, B.L. Tekwani, V. Madgula, S.I. Khan, B. Wang, A.M.S. Mayer, M.R. Jacob, L.C. Tu, J.r. Gertsch, M.T. Hamann, Structure-activity relationship and mechanism of action studies of manzamine analogues for the control of neuroinflammation and cerebral infections, J. Med. Chem., 53 (2009) 61-76.

[116] S. Bonazzi, D. Barbaras, L. Patiny, R. Scopelliti, P. Schneider, S.T. Cole, M. Kaiser, R. Brun, K. Gademann, Antimalarial and antitubercular nostocarboline and eudistomin derivatives: Synthesis, *in vitro* and *in vivo* biological evaluation, Bioorg. Med. Chem., 18 (2010) 1464-1476.

[117] B.K.S. Yeung, B. Zou, M. Rottmann, S.B. Lakshminarayana, S.H. Ang, S.Y. Leong, J. Tan, J. Wong, S. Keller-Maerki, C. Fischli, A. Goh, E.K. Schmitt, P. Krastel, E. Francotte, K. Kuhen, D. Plouffe, K. Henson, T. Wagner, E.A. Winzeler, F. Petersen, R. Brun, V. Dartois, T.T. Diagana, T.H. Keller, Spirotetrahydro β-carbolines (spiroindolones): A new class of potent and orally efficacious compounds for the treatment of malaria, J. Med. Chem., 53 (2010) 5155-5164.

[118] M. Rottmann, C. McNamara, B.K.S. Yeung, M.C.S. Lee, B. Zou, B. Russell, P. Seitz, D.M. Plouffe, N.V. Dharia, J. Tan, S.B. Cohen, K.R. Spencer, G.E. Gonzalez Paez, S.B. Lakshminarayana, A. Goh, R. Suwanarusk, T. Jegla, E.K. Schmitt, H.P. Beck, R. Brun, F. Nosten, L. Renia, V. Dartois, T.H. Keller, D.A. Fidock, E.A. Winzeler, T.T. Diagana, Spiroindolones, a potent compound class for the treatment of malaria, Science, 329 (2010) 1175-1180.

[119] N.J. White, S. Pukrittayakamee, A.P. Phyo, R. Rueangweerayut, F. Nosten, P. Jittamala, A. Jeeyapant, J.P. Jain, G. Lefevre, R. Li, Spiroindolone KAE609 for *falciparum* and *vivax*, *malaria*, N. Engl. J. Med., 371 (2014) 403-410.

[120] T.B. Beghyn, J. Charton, F. Leroux, G. Laconde, A. Bourin, P. Cos, L. Maes, B. Deprez, Drug to genome to drug: Discovery of new antiplasmodial compounds, J. Med. Chem., 54 (2011) 3222-3240.

[121] T.B. Beghyn, J. Charton, F. Leroux, A. Henninot, I. Reboule, P. Cos, L. Maes, B. Deprez, Drug-to-genome-to-drug, step 2: Reversing selectivity in a series of antiplasmodial compounds, J. Med. Chem., 55 (2011) 1274-1286.

[122] A. Gellis, A. Dumetre, G. Lanzada, S. Hutter, E. Ollivier, P. Vanelle, N. Azas, Preparation and antiprotozoal evaluation of promising β-carboline alkaloids, Biomed. Pharmacother., 66 (2012) 339-347.

[123] T.S. Kam, K.M. Sim, Alkaloids from *Kopsia griffithii*, Phytochemistry, 47 (1998) 145-147.

[124] C. Di Giorgio, F. Delmas, E. Ollivier, R. Elias, G. Balansard, P. Timon David, *In vitro* activity of the β-carboline alkaloids harmane, harmine, and harmaline toward parasites of the species *Leishmania infantum*, Exp. Parasitol., 106 (2004) 67-74.

[125] K.V. Rao, N. Kasanah, S. Wahyuono, B.L. Tekwani, R.F. Schinazi, M.T. Hamann, Three new manzamine alkaloids from a common Indonesian sponge and their activity against Infectious and tropical parasitic diseases, J. Nat. Prod., 67 (2004) 1314-1318.

[126] M. Leboeuf, A. Cave, P. Forgacs, J. Provost, A. Chiaroni, C. Riche, Alkaloids of the annonaceae. Part 33. Annomontine and methoxyannomontine, two new pyrimidine-[small β]-carboline-type alkaloids from *Annona montana*, J. Chem. Soc., Perkin Trans. 1, (1982) 1205-1208.

[127] E.V. Costa, M.L.B. Pinheiro, C.M. Xavier, J.R.A. Silva, A.C.F. Amaral, A.D.L. Souza, A. Barison, F.R. Campos, A.G. Ferreira, G.M.C. Machado, L.L.P. Leon, A pyrimidine-β-carboline and other alkaloids from *Annona foetida* with antileishmanial activity, J. Nat. Prod., 69 (2006) 292-294.

[128] A. Kumar, S.B. Katiyar, S. Gupta, P.M.S. Chauhan, Syntheses of new substituted triazino tetrahydroisoquinolines and β-carbolines as novel antileishmanial agents, Eur. J. Med. Chem., 41 (2006) 106-113.

[129] R. Kumar, S. Khan, A. Verma, S. Srivastava, P. Viswakarma, S. Gupta, S. Meena, N. Singh, J. Sarkar, P.M.S. Chauhan, Synthesis of 2-(pyrimidin-2-yl)-1-phenyl-2,3,4,9-tetrahydro-1*H*-β-carbolines as antileishmanial agents, Eur. J. Med. Chem., 45 (2010) 3274-3280.

[130] S.S. Chauhan, L. Gupta, M. Mittal, P. Vishwakarma, S. Gupta, P.M.S. Chauhan, Synthesis and biological evaluation of indolyl glyoxylamides as a new class of antileishmanial agents, Bioorg. Med. Chem. Lett., 20 (2010) 6191-6194.

[131] L.T.D. Tonin, M.R. Panice, C.V. Nakamura, K.J.P. Rocha, A.O.D. Santos, T. Ueda Nakamura, W.F.D. Costa, M.H. Sarragiotto, Antitrypanosomal and antileishmanial activities of novel *N*-alkyl-(1-phenylsubstituted-β-carboline)-3-carboxamides, Biomed. Pharmacother., 64 (2010) 386-389.

[132] R.B. Pedroso, L.T.D. Tonin, T.U. Nakamura, B.P.D. Filho, M.H. Sarragiotto, C.V. Nakamura, β-carboline-3-carboxamide derivatives as promising antileishmanial agents, Ann. Trop. Med. Parasitol., 105 (2011) 549-557.

[133] T. Stefanello, M. Panice, T. Ueda-Nakamura, M. Sarragiotto, R. Auzely-Velty, C. Nakamura, *N*-butyl-[1-(4-Methoxy) phenyl-9*H*-β-carboline]-3-carboxamide prevents cytokinesis in *Leishmania amazonensis*, Antimicrob. Agents Chemother., 58 (2014) 7112-7120.

[134] V.M. Gohil, K.G. Brahmbhatt, P.M. Loiseau, K.K. Bhutani, Synthesis and anti-leishmanial activity of 1-aryl-β-carboline derivatives against *Leishmania donovani*, Bioorg. Med. Chem. Lett., 22 (2012) 3905-3907.

[135] H. Volpato, V. Desoti, J. Cristina, J. Cogo, M.R. Panice, M.H. Sarragiotto, S.D.O. Silva, T. Ueda Nakamura, C.V. Nakamura, The effects of *N*-butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide against *Leishmania amazonensis* are mediated by mitochondrial dysfunction, J. Evid. Based Complementary Altern. Med., 2013 (2013) 1-7.

[136] S. Manda, S.I. Khan, S.K. Jain, S. Mohammed, B.L. Tekwani, I.A. Khan, R.A. Vishwakarma, S.B. Bharate, Synthesis, antileishmanial and antitrypanosomal activities of *N*-substituted tetrahydro-β-carbolines, Bioorg. Med. Chem. Lett., 24 (2014) 3247-3250.

[137] S.S. Chauhan, S. Pandey, R. Shivahare, K. Ramalingam, S. Krishna, P. Vishwakarma, M. Siddiqi, S. Gupta, N. Goyal, P.M.S. Chauhan, Novel β-carboline-quinazolinone hybrid as an inhibitor of *Leishmania donovani* trypanothione reductase: Synthesis, molecular docking and bioevaluation, MedChemComm., 6 (2015) 351-356.

[138] P. Rivas, B.K. Cassels, A. Morello, Y. Repetto, Effects of some β-carboline alkaloids on intact *Trypanosoma cruzi* epimastigotes, Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol., 122 (1999) 27-31.

[139] M.E. Ferreira, H. Nakayama, A.R. de Arias, A. Schinini, N.V. de Bilbao, E. Serna, D. Lagoutte, F. Soriano Agaton, E. Poupon, R. Hocquemiller, A. Fournet, Effects of canthin-6-one alkaloids from *Zanthoxylum chiloperone* on *Trypanosoma cruzi*-infected mice, J. Ethnopharmacol., 109 (2007) 258-263.

[140] L.T. Düsman Tonin, V.A. Barbosa, C.C. Bocca, E.R.F. Ramos, C.V. Nakamura, W. Ferreira da Costa, E.A. Basso, T.U. Nakamura, M.H. Sarragiotto, Comparative study of the trypanocidal activity of the methyl 1-nitrophenyl-1,2,3,4-9*H*-tetrahydro-β-carboline-3-carboxylate derivatives and benznidazole using theoretical calculations and cyclic voltammetry, Eur. J. Med. Chem., 44 (2009) 1745-1750.

[141] R.H. Valdez, L.T.D. Tonin, T. Ueda Nakamura, B.P.D. Filho, J.A. Morgado Diaz, M.H. Sarragiotto, C.V. Nakamura, Biological activity of 1,2,3,4-tetrahydro-β-carboline-3-carboxamides against *Trypanosoma cruzi*, Acta Trop., 110 (2009) 7-14.

[142] R.H. Valdez, L.T.D. Tonin, T. Ueda-Nakamura, S.O. Silva, B.P. Dias Filho, E.N. Kaneshima, S.F. Yamada Ogatta, L.M. Yamauchi, M.H. Sarragiotto, C.V. Nakamura, *In vitro* and *In vivo* trypanocidal synergistic activity of *N*-butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide associated with benznidazole, Antimicrob. Agents Chemother., 56 (2012) 507-512.

[143] E.V. Costa, M.L.B. Pinheiro, A.D.L.D. Souza, A. Barison, F.R. Campos, R.H. Valdez, T. Ueda Nakamura, C.V. Nakamura, Trypanocidal activity of oxoaporphine and pyrimidine-β-carboline alkaloids from the branches of *Annona foetida* Mart.(Annonaceae), Molecules, 16 (2011) 9714-9720.

[144] K.A. El Sayed, M. Kelly, U.A.K. Kara, K.K.H. Ang, I. Katsuyama, D.C. Dunbar, A.A. Khan, M.T. Hamann, New manzamine alkaloids with potent activity against infectious diseases, J. Am. Chem. Soc., 123 (2001) 1804-1808.

[145] M.L. Alomar, F.A. Rasse Suriani, A. Ganuza, V.M. Coceres, F.M. Cabrerizo, S.O. Angel, *In vitro* evaluation of β-carboline alkaloids as potential anti-toxoplasma agents, BMC Res. Notes, 6 (2013) 193.

[146] M.R. Prinsep, J.W. Blunt, M.H.G. Munro, New cytotoxic β-carboline alkaloids from the marine bryozoan, *Cribricellina cribraria*, J. Nat. Prod., 54 (1991) 1068-1076.

[147] H. Aassila, M. Bourguet Kondracki, S. Rifai, A. Fassouane, M. Guyot, Identification of harman as the antibiotic compound produced by a tunicate-associated bacterium, Mar. Biotechnol., 5 (2003) 163-166.

[148] G. Nenaah, Antibacterial and antifungal activities of (β)-carboline alkaloids of *Peganum harmala* (L) seeds and their combination effects, Fitoterapia, 81 (2010) 779-782.

[149] Z. Saify, J. Farhad, N. Mushtaq, F. Noor, S. Akhtar, M. Arif, B.S. Naqvi, M.H. Shoaib, Antibacterial activity of 1-methyl-7-methoxy-β-carboline and its phenacyl and coumarine analogues, Pak. J. Pharm. Sci., 18 (2005) 39-41.

[150] Z. Li, C. Haimin, H. Xiaotian, L. Wei, Y. Xiaojun, Antimicrobial screening and active compound isolation from marine bacterium NJ6-3-1 associated with the sponge *Hymeniacidon perleve*, World J. Microbiol. Biotechnol., 21 (2005) 201-206.

[151] Y. Iinuma, S. Kozawa, H. Ishiyama, M. Tsuda, E. Fukushi, J. Kawabata, J. Fromont, J.I. Kobayashi, Gesashidine A, A β-carboline alkaloid with an imidazole ring from a *Thorectidae* sponge, J. Nat. Prod., 68 (2005) 1109-1110.

[152] M. Tsuda, D. Watanabe, J.I. Kobayashi, Ma'eganedin A, A new manzamine alkaloid from Amphimedon sponge, Tet. Lett., 39 (1998) 1207-1210.

[153] J.F. Blom, T. Brutsch, D. Barbaras, Y. Bethuel, H.H. Locher, C. Hubschwerlen, K. Gademann, Potent algicides based on the cyanobacterial alkaloid nostocarboline, Org. Lett., 8 (2006) 737-740.

[154] K.L. Rinehart, J. Kobayashi, G.C. Harbour, R.G. Hughes, S.A. Mizsak, T.A. Scahill, Eudistomins C, E, K, and L, potent antiviral compounds containing a novel oxathiazepine ring from the Caribbean tunicate *Eudistoma olivaceum*, J. Am. Chem. Soc., 106 (1984) 1524-1526.

[155] J. Kobayashi, G.C. Harbour, J. Gilmore, K.L. Rinehart, Eudistomins A, D, G, H, I, J, M, N, O, P, and Q, bromo, hydroxy, pyrrolyl and iminoazepino β-carbolines from the antiviral Caribbean tunicate *Eudistoma olivaceum*, J. Am. Chem. Soc., 106 (1984) 1526-1528.

[156] W. Wang, S.J. Nam, B.C. Lee, H. Kang, β-carboline alkaloids from a Korean tunicate *Eudistoma sp*, J. Nat. Prod., 71 (2008) 163-166.

[157] M. Till, M.R. Prinsep, 5-Bromo-8-methoxy-1-methyl-β-carboline, an alkaloid from the New Zealand marine bryozoan *Pterocella vesiculosa*, J. Nat. Prod., 72 (2009) 796-798.

[158] T.H. Won, J.E. Jeon, S.H. Lee, B.J. Rho, K.B. Oh, J. Shin, β-carboline alkaloids derived from the ascidian *Synoicum sp.*, Bioorg. Med. Chem., 20 (2012) 4082-4087.

[159] K.L. Rinehart, J. Kobayashi, G.C. Harbour, J. Gilmore, M. Mascal, T.G. Holt, L.S. Shield, F. Lafargue, Eudistomins A-Q, β-carbolines from the antiviral Caribbean tunicate *Eudistoma olivaceum*, J. Am. Chem. Soc., 109 (1987) 3378-3387.

[160] A.S. Nazari Formagio, P.R. Santos, K. Zanoli, T. Ueda-Nakamura, L.T. Dusman Tonin, C.V. Nakamura, M.H. Sarragiotto, Synthesis and antiviral activity of β-carboline derivatives bearing a substituted carbohydrazide at C-3 against poliovirus and herpes simplex virus (HSV-1), Eur. J. Med. Chem., 44 (2009) 4695-4701.

[161] J.F. Miller, E.M. Turner, R.G. Sherrill, K. Gudmundsson, A. Spaltenstein, P. Sethna, K.W. Brown, R. Harvey, K.R. Romines, P. Golden, Substituted tetrahydro-β-carbolines as potential agents for the treatment of human papillomavirus infection, Bioorg. Med. Chem. Lett., 20 (2010) 256-259.

[162] H. Song, Y. Liu, Y. Liu, L. Wang, Q. Wang, Synthesis and antiviral and fungicidal activity evaluation of β-carboline, dihydro-β-carboline, tetrahydro-β-carboline alkaloids, and their derivatives, J. Agric. Food Chem., 62 (2014) 1010-1018.

[163] Z. Xu, F.R. Chang, H.K. Wang, Y. Kashiwada, A.T. McPhail, K.F. Bastow, Y. Tachibana, M. Cosentino, K.H. Lee, Anti-HIV agents 451 and antitumor agents 205.2 Two new sesquiterpenes, leitneridanins A and B, and the cytotoxic and anti-HIV principles from *Leitneria floridana*, J. Nat. Prod., 63 (2000) 1712-1715.

[164] M. Yousaf, N.L. Hammond, J. Peng, S. Wahyuono, K.A. McIntosh, W.N. Charman, A.M.S. Mayer, M.T. Hamann, New manzamine alkaloids from an Indo-Pacific sponge. Pharmacokinetics, oral availability, and the significant activity of several manzamines against HIV-I, AIDS opportunistic infections, and inflammatory diseases, J. Med. Chem., 47 (2004) 3512-3517.

[165] X. Yu, W. Lin, J. Li, M. Yang, Synthesis and biological evaluation of novel β-carboline derivatives as Tat–TAR interaction inhibitors, Bioorg. Med. Chem. Lett., 14 (2004) 3127-3130.

[166] P.W. Hsieh, F.R. Chang, K.H. Lee, T.L. Hwang, S.M. Chang, Y.C. Wu, A new anti-HIV alkaloid, drymaritin, and a new C-glycoside flavonoid, diandraflavone, from *Drymaria diandra*, J. Nat. Prod., 67 (2004) 1175-1177.

[167] Y.H. Wang, J.G. Tang, R.R. Wang, L.M. Yang, Z.J. Dong, L. Du, X. Shen, J.K. Liu, Y.T. Zheng, Flazinamide, a novel β-carboline compound with anti-HIV actions, Biochem. Biophys. Res. Commun., 355 (2007) 1091-1095.

[168] J.G. Tang, Y.H. Wang, R.R. Wang, Z.J. Dong, L.M. Yang, Y.T. Zheng, J.K. Liu, Synthesis of analogues of flazin, in particular, flazinamide, as promising anti-HIV agents, Chem. Biodivers., 5 (2008) 447-460.

[169] K.G. Brahmbhatt, N. Ahmed, S. Sabde, D. Mitra, I.P. Singh, K.K. Bhutani, Synthesis and evaluation of β-carboline derivatives as inhibitors of human immunodeficiency virus, Bioorg. Med. Chem. Lett., 20 (2010) 4416-4419.

[170] S.F. Li, Y.T. Di, H.P. He, Y. Zhang, Y.H. Wang, J.L. Yin, C.J. Tan, S.L. Li, X.J. Hao, Trigonoines A and B, two novel alkaloids from the leaves of *Trigonostemon lii*, Tetrahedron Lett., 52 (2011) 3186-3188.

[171] M. Yousaf, K.A. El Sayed, K.V. Rao, C.W. Lim, J.F. Hu, M. Kelly, S.G. Franzblau, F. Zhang, O. Peraud, R.T. Hill, M.T. Hamann, 12,34-Oxamanzamines, novel biocatalytic and natural products from manzamine producing Indo-Pacific sponges, Tetrahedron, 58 (2002) 7397-7402.

[172] K.A.E. Sayed, A.A. Khalil, M. Yousaf, G. Labadie, G.M. Kumar, S.G. Franzblau, A.M.S. Mayer, M.A. Avery, M.T. Hamann, Semisynthetic studies on the manzamine alkaloids, J. Nat. Prod., 71 (2008) 300-308.

[173] J. Peng, J.F. Hu, A.B. Kazi, Z. Li, M. Avery, O. Peraud, R.T. Hill, S.G. Franzblau, F. Zhang, R.F. Schinazi, S.S. Wirtz, P. Tharnish, M. Kelly, S. Wahyuono, M.T. Hamann, Manadomanzamines A and B: A novel alkaloid ring system with potent activity against mycobacteria and HIV-1, J. Am. Chem. Soc., 125 (2003) 13382-13386.

[174] B. Baruah, K. Dasu, B. Vaitilingam, P. Mamnoor, P.P. Venkata, S. Rajagopal, K.R. Yeleswarapu, Synthesis and cytotoxic activity of novel quinazolino-β-carboline-5-one derivatives, Bioorg. Med. Chem., 12 (2004) 1991-1994.

[175] P.C. Kuo, A.G. Damu, K.H. Lee, T.S. Wu, Cytotoxic and antimalarial constituents from the roots of *Eurycoma longifolia*, Bioorg. Med. Chem., 12 (2004) 537-544.

[176] M. Laronze, M. Boisbrun, S. Léonce, B. Pfeiffer, P. Renard, O. Lozach, L. Meijer, A. Lansiaux, C. Bailly, J. Sapi, J.Y. Laronze, Synthesis and anticancer activity of new pyrrolocarbazoles and pyrrolo-β-carbolines, Bioorg. Med. Chem., 13 (2005) 2263-2283.

[177] N. Sunder Plassmann, V. Sarli, M. Gartner, M. Utz, J. Seiler, S. Huemmer, T.U. Mayer, T. Surrey, A. Giannis, Synthesis and biological evaluation of new tetrahydro-β-carbolines as inhibitors of the mitotic kinesin Eg5, Bioorg. Med. Chem., 13 (2005) 6094-6111.

[178] M. Zhao, L. Bi, W. Wang, C. Wang, M. Baudy Floch, J. Ju, S. Peng, Synthesis and cytotoxic activities of β-carboline amino acid ester conjugates, Bioorg. Med. Chem., 14 (2006) 6998-7010.

[179] R. Cao, W. Yi, Q. Wu, X. Guan, M. Feng, C. Ma, Z. Chen, H. Song, W. Peng, Synthesis and cytotoxic activities of 1-benzylidine substituted β-carboline derivatives, Bioorg. Med. Chem. Lett., 18 (2008) 6558-6561.

[180] A.S.N. Formagio, L.T.D. Tonin, M.A. Foglio, C. Madjarof, J.E. de Carvalho, W.F. da Costa, F.P. Cardoso, M.H. Sarragiotto, 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, Bioorg. Med. Chem., 16 (2008) 9660-9667.

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

[182] P.A. Barsanti, W. Wang, Z.J. Ni, D. Duhl, N. Brammeier, E. Martin, D. Bussiere, A.O. Walter, The discovery of tetrahydro-β-carbolines as inhibitors of the kinesin Eg5, Bioorg. Med. Chem. Lett., 20 (2010) 157-160.

[183] Z. Chen, R. Cao, B. Shi, W. Yi, L. Yu, H. Song, Z. Ren, W. Peng, Synthesis of novel β-carbolines with efficient DNA-binding capacity and potent cytotoxicity, Bioorg. Med. Chem. Lett., 20 (2010) 3876-3879.

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

[185] Z. Chen, R. Cao, B. Shi, W. Yi, L. Yu, H. Song, Z. Ren, Synthesis and biological evaluation of novel β-carbolines as potent cytotoxic and DNA intercalating agents, Chem. Pharm. Bull., 58 (2010) 901-907.

[186] Z. Chen, R. Cao, L. Yu, B. Shi, J. Sun, L. Guo, Q. Ma, W. Yi, X. Song, H. Song, Synthesis, cytotoxic activities and DNA binding properties of β-carboline derivatives, Eur. J. Med. Chem., 45 (2010) 4740-4745.

[187] C. Ma, R. Cao, B. Shi, X. Zhou, Q. Ma, J. Sun, L. Guo, W. Yi, Z. Chen, H. Song, Synthesis and cytotoxic evaluation of 1-carboxamide and 1-amino side chain substituted β-carbolines, Eur. J. Med. Chem., 45 (2010) 5513-5519.

[188] C. Ma, R. Cao, B. Shi, S. Li, Z. Chen, W. Yi, W. Peng, Z. Ren, H. Song, Synthesis and cytotoxic evaluation of *N*2-benzylated quaternary β-carboline amino acid ester conjugates, Eur. J. Med. Chem., 45 (2010) 1515-1523.

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

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

[191] J. Kobayashi, J.F. Cheng, T. Ohta, S. Nozoe, Y. Ohizumi, T. Sasaki, Eudistomidins B, C, and D: Novel antileukemic alkaloids from the Okinawan marine tunicate *Eudistoma glaucus*, J. Org. Chem., 55 (1990) 3666-3670.

[192] Y. Takahashi, H. Ishiyama, T. Kubota, J.I. Kobayashi, Eudistomidin G, a new β-carboline alkaloid from the Okinawan marine tunicate *Eudistoma glaucus* and structure revision of eudistomidin B, Bioorg. Med. Chem. Lett., 20 (2010) 4100-4103.

[193] G. Dong, C. Sheng, S. Wang, Z. Miao, J. Yao, W. Zhang, Selection of evodiamine as a novel topoisomerase I inhibitor by structure-based virtual screening and hit optimization of evodiamine derivatives as antitumor agents, J. Med. Chem., 53 (2010) 7521-7531.

[194] G. Dong, S. Wang, Z. Miao, J. Yao, Y. Zhang, Z. Guo, W. Zhang, C. Sheng, 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., 55 (2012) 7593-7613.

[195] C. Tan, S. Lai, S. Wu, S. Hu, L. Zhou, Y. Chen, M. Wang, Y. Zhu, W. Lian, W. Peng, L. Ji, A. Xu, Nuclear permeable Ruthenium(II) β-carboline complexes induce autophagy to antagonize mitochondrial-mediated apoptosis, J. Med. Chem., 53 (2010) 7613-7624.

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

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

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

[199] R. Ikeda, T. Kimura, T. Tsutsumi, S. Tamura, N. Sakai, T. Konakahara, Structure-activity relationship in the antitumor activity of 6-, 8- or 6,8-substituted 3-benzylamino-β-carboline derivatives, Bioorg. Med. Chem. Lett., 22 (2012) 3506-3515.

[200] P. Mayser, U. Schäfer, H.J. Kramer, B. Irlinger, W. Steglich, Pityriacitrin - An ultraviolet-absorbing indole alkaloid from the yeast *Malassezia furfur*, Arch. Dermatol. Res., 294 (2002) 131-134.

[201] L.P. Liew, J.M. Fleming, A. Longeon, E. Mouray, I. Florent, M.L. Bourguet-Kondracki, B.R. Copp, Synthesis of 1-indolyl substituted β-carboline natural products and discovery of antimalarial and cytotoxic activities, Tetrahedron, 70 (2014) 4910-4920.

[202] P. Zhang, X. Sun, B. Xu, K. Bijian, S. Wan, G. Li, M. Alaoui-Jamali, T. Jiang, Total synthesis and bioactivity of the marine alkaloid pityriacitrin and some of its derivatives, Eur. J. Med. Chem., 46 (2011) 6089-6097.

[203] T. Suzuki, T. Kubota, J.I. Kobayashi, Eudistomidins H-K, new β-carboline alkaloids from the Okinawan marine tunicate *Eudistoma glaucus*, Bioorg. Med. Chem. Lett., 21 (2011) 4220-4223.

[204] S.P.B. Ovenden, J.L. Nielson, C.H. Liptrot, R.H. Willis, D.M. Tapiolas, A.D. Wright, C.A. Motti, Callophycin A, a cytotoxic tetrahydro-β-carboline from the red alga *Callophycus oppositifolius*, Phytochem. Lett., 4 (2011) 69-71.

[205] Y.C. Shen, Y.T. Chang, C.L. Lin, C.C. Liaw, Y.H. Kuo, L.C. Tu, S.F. Yeh, J.W. Chern, Synthesis of 1-substituted carbazolyl-1, 2, 3, 4-tetrahydro-and carbazolyl-3, 4-dihydro-β-carboline analogs as potential antitumor agents, Mar. Drugs, 9 (2011) 256-277.

[206] S.F. Li, Y. Zhang, Y. Li, X.R. Li, L.M. Kong, C.J. Tan, S.L. Li, Y.T. Di, H.P. He, X.J. Hao, β-Carboline alkaloids from the leaves of *Trigonostemon lii*, Bioorg. Med. Chem. Lett., 22 (2012) 2296-2299.

[207] R.l. Freedeerick, C.l. Bruyere, C. Vancraeynest, J.R.M. Reniers, C.l. Meinguet, L. Pochet, A. Backlund, B. Masereel, R. Kiss, J. Wouters, Novel trisubstituted harmine derivatives with original *in vitro* anticancer activity, J. Med. Chem., 55 (2012) 6489-6501.

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

[209] B. Shi, R. Cao, W. Fan, L. Guo, Q. Ma, X. Chen, G. Zhang, L. Qiu, H. Song, Design, synthesis and *in vitro* and *in vivo* antitumor activities of novel bivalent β-carbolines, Eur. J. Med. Chem., 60 (2013) 10-22.

[210] R. Cao, W. Fan, L. Guo, Q. Ma, G. Zhang, J. Li, X. Chen, Z. Ren, L. Qiu, Synthesis and structure-activity relationships of harmine derivatives as potential antitumor agents, Eur. J. Med. Chem., 60 (2013) 135-143.

[211] R. Chaniyara, S. Tala, C.W. Chen, X. Zang, R. Kakadiya, L.F. Lin, C.H. Chen, S.I. Chien, T.C. Chou, T.H. Tsai, T.C. Lee, A. Shah, T.L. Su, Novel antitumor indolizino[6,7-*b*]indoles with multiple modes of action: DNA cross-linking and topoisomerase I and II inhibition, J. Med. Chem., 56 (2013) 1544-1563.

[212] P. de Oliveira Figueiredo, R.T. Perdomo, F.R. Garcez, M. de Fatima Cepa Matos, J.E. de Carvalho, W.S. Garcez, Further constituents of galianthe thalictroides (Rubiaceae) and inhibition of DNA topoisomerases I and IIα by its cytotoxic β-carboline alkaloids, Bioorg. Med. Chem. Lett., 24 (2014) 1358-1361.

[213] B. Bai, X.Y. Li, L. Liu, Y. Li, H.J. Zhu, Synthesis of novel β-carboline derivatives and the activities against five tumor-cell lines, Bioorg. Med. Chem. Lett., 24 (2014) 96-98.

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

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

[216] C. Zheng, Y. Fang, W. Tong, G. Li, H. Wu, W. Zhou, Q. Lin, F. Yang, Z. Yang, P. Wang, Y. Peng, X. Pang, Z. Yi, J. Luo, M. Liu, Y. Chen, 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., 57 (2014) 600-612.

[217] K.B. Wang, Y.T. Di, Y. Bao, C.M. Yuan, G. Chen, D.H. Li, J. Bai, H.P. He, X.J. Hao, Y.H. Pei, Peganumine A, a β-carboline dimer with a new octacyclic scaffold from *Peganum harmala*, Org. Lett., 16 (2014) 4028-4031.

[218] K. Fang, G.Q. Dong, H. Gong, N. Liu, Z.G. Li, S.P. Zhu, Z.Y. Miao, J.Z. Yao, W.N. Zhang, C.Q. Sheng, Design, synthesis and biological evaluation of E-ring modified evodiamine derivatives as novel antitumor agents, Chinese Chem. Lett., 25 (2014) 978-982.

[219] S.S. Chauhan, A.K. Singh, S. Meena, M. Lohani, A. Singh, R.K. Arya, S.H. Cheruvu, J. Sarkar, J.R. Gayen, D. Datta, Synthesis of novel β-carboline based chalcones with high cytotoxic activity against breast cancer cells, Bioorg. Med. Chem. Lett., 24 (2014) 2820-2824.

[220] I. Filali, J. Bouajila, M. Znati, F. Bousejra-El Garah, H. Ben Jannet, Synthesis of new isoxazoline derivatives from harmine and evaluation of their anti-alzheimer, anti-cancer and anti-inflammatory activities, J. Enzyme Inhib. Med. Chem., (2014) 1-6.

[221] W.H. Jiao, G.D. Chen, H. Gao, J. Li, B.B. Gu, T.T. Xu, H.B. Yu, G.H. Shi, F. Yang, X.S. Yao, (±)-Quassidines I and J, two pairs of cytotoxic bis-β-carboline alkaloid enantiomers from *Picrasma quassioides*, J. Nat. Prod., 78 (2014) 125-130.

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

[223] F.C. Savariz, M.A. Foglio, A.L.T.G. Ruiz, W.F. da Costa, M. de Magalhaes Silva, J.C.C. Santos, I.M. Figueiredo, E. Meyer, J.E. de Carvalho, M.H. Sarragiotto, Synthesis and antitumor activity of novel 1-substituted phenyl 3-(2-oxo-1, 3, 4-oxadiazol-5-yl) β-carbolines and their mannich bases, Bioorg. Med. Chem., 22 (2014) 6867-6875.

[224] P. Srihari, B. Padmabhavani, S. Ramesh, Y.B. Kumar, A. Singh, R. Ummanni, PMA-SiO_2 catalyzed synthesis of indolo [2, 3-*c*] quinolines as potent anti cancer agents, Bioorg. Med. Chem. Lett., 25 (2015) 2360-2365.

[225] J. Chatwichien, S. Basu, M.E. Murphy, M.T. Hamann, J.D. Winkler, Design, synthesis and biological evaluation of β-carboline dimers based on the structure of neokauluamine, Tet. Lett., 56 (2015) 3515-3517.

[226] C. Meinguet, C. Bruyere, R. Frederick, V. Mathieu, C. Vancraeynest, L. Pochet, J. Laloy, J. Mortier, G. Wolber, R. Kiss, 3D-QSAR, design, synthesis and characterization of trisubstituted harmine derivatives with *in-vitro* antiproliferative properties, Eur. J. Med. Chem., 94 (2015) 45-55.

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

[228] N. Lin, M. Zhao, C. Wang, S. Peng, Synthesis and antithrombotic activity of carbolinecarboxyl RGD sequence, Bioorg. Med. Chem. Lett., 12 (2002) 585-587.

[229] M. Zhao, L. Bi, W. Bi, C. Wang, Z. Yang, J. Ju, S. Peng, Synthesis of new class dipeptide analogues with improved permeability and antithrombotic activity, Bioorg. Med. Chem., 14 (2006) 4761-4774.

[230] J. Liu, X. Jiang, M. Zhao, X. Zhang, M. Zheng, L. Peng, S. Peng, A class of 3*S*-2-aminoacyltetrahydro-β-carboline-3-carboxylic Acids: Their facile synthesis, inhibition for platelet activation, and high *in vivo a*nti-thrombotic potency, J. Med. Chem., 53 (2010) 3106-3116.

[231] K. Yao, M. Zhao, X. Zhang, Y. Wang, L. Li, M. Zheng, S. Peng, A class of oral *N*-[(1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carbonyl]-*N*'-(amino-acid-acyl)hydrazine: Discovery, synthesis, *in vitro* anti-platelet aggregation/*in vivo* anti-thrombotic evaluation and 3D QSAR analysis, Eur. J. Med. Chem., 46 (2011) 3237-3249.

[232] C. Li, X. Zhang, M. Zhao, Y. Wang, J. Wu, J. Liu, M. Zheng, S. Peng, A class of novel *N*-(1-methyl-β-carboline-3-carbonyl)-*N*'-(aminoacid-acyl)-hydrazines: Aromatization leaded design, synthesis, *in vitro* anti-platelet aggregation/*in vivo* anti-thrombotic evaluation and 3D QSAR analysis, Eur. J. Med. Chem., 46 (2011) 5598-5608.

[233] S.K. Srivastava, A. Agarwal, P.M.S. Chauhan, S.K. Agarwal, A.P. Bhaduri, S.N. Singh, N. Fatima, R.K. Chatterjee, Potent 1,3-disubstituted-9*H*-pyrido[3,4-*b*]indoles as new lead compounds in antifilarial chemotherapy, J. Med. Chem., 42 (1999) 1667-1672.

[234] S.K. Srivastava, A. Agarwal, P.M.S. Chauhan, S.K. Agarwal, A.P. Bhaduri, S.N. Singh, N. Fatima, R.K. Chatterjee, Potent 1,3-disubstituted-9*H*-pyrido[3,4-*b*]indoles as new lead compounds in antifilarial chemotherapy, Bioorg. Med. Chem., 7 (1999) 1223-1236.

[235] A. Daugan, P. Grondin, C. Ruault, A.C. Le Monnier de Gouville, H. Coste, J. Kirilovsky, F. Hyafil, R. Labaudinière, The discovery of tadalafil: A novel and highly selective PDE5 inhibitor. 1: 5,6,11,11a-tetrahydro-1*H*-imidazo[1',5':1,6]pyrido[3,4-*b*]indole-1,3(2*H*)-dione analogues, J. Med. Chem., 46 (2003) 4525-4532.

[236] A. Daugan, P. Grondin, C. Ruault, A.C. Le Monnier de Gouville, H. Coste, J.M. Linget, J. Kirilovsky, F. Hyafil, R. Labaudiniere, 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., 46 (2003) 4533-4542.

[237] N.S. Ahmed, B.D. Gary, H.N. Tinsley, G.A. Piazza, S. Laufer, A.H. Abadi, Design, synthesis and structure-activity relationship of functionalized tetrahydro-β-carboline derivatives as novel PDE5 inhibitors, Arch. Pharm., 344 (2011) 149-157.

[238] A.H. Abadi, B.D. Gary, H.N. Tinsley, G.A. Piazza, M. Abdel-Halim, Synthesis, molecular modeling and biological evaluation of novel tadalafil analogues as phosphodiesterase 5 and colon tumor cell growth inhibitors, new stereochemical perspective, Eur. J. Med. Chem., 45 (2010) 1278-1286.

[239] H.A. Mohamed, N.M.R. Girgis, R. Wilcken, M.R. Bauer, H.N. Tinsley, B.D. Gary, G.A. Piazza, F.M. Boeckler, A.H. Abadi, Synthesis and molecular modeling of novel tetrahydro-β-carboline derivatives with phosphodiesterase 5 inhibitory and anticancer properties, J. Med. Chem., 54 (2010) 495-509.

[240] S.O. Ochiana, A. Gustafson, N.D. Bland, C. Wang, M.J. Russo, R.K. Campbell, M.P. Pollastri, Synthesis and evaluation of human phosphodiesterases (PDE) 5 inhibitor analogs as trypanosomal PDE inhibitors. Part 2. Tadalafil analogs, Bioorg. Med. Chem. Lett., 22 (2012) 2582-2584.

[241] W. Bi, Y. Bi, P. Xue, Y. Zhang, X. Gao, Z. Wang, M. Li, M.l. Baudy Floch, N. Ngerebara, K.M. Gibson, L. Bi, Synthesis and characterization of novel indole derivatives reveal improved therapeutic agents for treatment of ischemia/reperfusion (I/R) Injury, J. Med. Chem., 53 (2010) 6763-6767.

[242] M.L. Yang, P.C. Kuo, T.L. Hwang, W.F. Chiou, K. Qian, C.Y. Lai, K.H. Lee, T.S. Wu, 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, Bioorg. Med. Chem., 19 (2011) 1674-1682.

[243] L. Zeng, J. Zhang, Design, synthesis, and evaluation of a novel class of 2,3-disubstituted-tetrahydro-β-carboline derivatives, Bioorg. Med. Chem. Lett., 22 (2012) 3718-3722.

[244] A. Pasternak, Z. Feng, R. de Jesus, Z. Ye, S. He, P. Dobbelaar, S.A. Bradley, G.G. Chicchi, K.L. Tsao, D. Trusca, G.J. Eiermann, C. Li, Y. Feng, M. Wu, Q. Shao, B.B. Zhang, R. Nargund, S.G. Mills, A.D. Howard, L. Yang, Y.P. Zhou, Stimulation of glucose-dependent insulin secretion by a potent, selective SST3 antagonist, ACS Med. Chem. Lett., 3 (2012) 289-293.

[245] S. He, Z. Lai, Z. Ye, P.H. Dobbelaar, S.K. Shah, Q. Truong, W. Du, L. Guo, J. Liu, T. Jian, Investigation of cardiovascular effects of tetrahydro-β-carboline SSTR3 antagonists, ACS Med. Chem. Lett., 5 (2014) 748-753.

[246] D. Minehira, D. Takeda, H. Urata, A. Kato, I. Adachi, X. Wang, Y. Matsuya, K. Sugimoto, M. Takemura, S. Endo, T. Matsunaga, A. Hara, J. Koseki, K. Narukawa, S. Hirono, N. Toyooka, Design, synthesis, and biological evaluation of novel (1-thioxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids as selective inhibitors for AKR1B1, Bioorg. Med. Chem., 20 (2012) 356-367.

[247] C.L. Cardoso, I. Castro Gamboa, D.H.S. Silva, M. Furlan, R.d.A. Epifanio, A.D.C. Pinto, C. Moraes de Rezende, J.A. Lima, V.D.S. Bolzani, Indole glucoalkaloids from *Chimarrhis turbinata* and their evaluation as antioxidant agents and acetylcholinesterase inhibitors, J. Nat. Prod., 67 (2004) 1882-1885.

[248] P.G. Becher, J. Beuchat, K. Gademann, F. Jüttner, Nostocarboline: Isolation and synthesis of a new cholinesterase inhibitor from *Nostoc* 78-12A, J. Nat. Prod., 68 (2005) 1793-1795.

[249] Y. Schott, M. Decker, H. Rommelspacher, J. Lehmann, 6-Hydroxy- and 6-methoxy-β-carbolines as acetyl- and butyrylcholinesterase inhibitors, Bioorg. Med. Chem. Lett., 16 (2006) 5840-5843.

[250] Y. Rook, K.U. Schmidtke, F. Gaube, D. Schepmann, B. Wunsch, J.R. Heilmann, J. Lehmann, T. Winckler, Bivalent β-carbolines as potential multitarget anti-Alzheimer agents, J. Med. Chem., 53 (2010) 3611-3617.

[251] R. Otto, R. Penzis, F. Gaube, T. Winckler, D. Appenroth, C. Fleck, C. Trankle, J. Lehmann, C. Enzensperger, Beta and gamma carboline derivatives as potential anti-Alzheimer agents: A comparison, Eur. J. Med. Chem., 87 (2014) 63-70.

[252] J.S. Lan, S.S. Xie, S.Y. Li, L.F. Pan, X.B. Wang, L.Y. Kong, Design, synthesis and evaluation of novel tacrine-(β-carboline) hybrids as multifunctional agents for the treatment of alzheimer's disease, Bioorg. Med. Chem., 22 (2014) 6089-6104.

[253] S.P. Hollinshead, M.L. Trudell, P. Skolnick, J.M. Cook, Structural requirements for agonist actions at the benzodiazepine receptor: Studies with analogs of 6-(benzyloxy)-4-(methoxymethyl) β-carboline-3-carboxylic acid ethyl ester, J. Med. Chem., 33 (1990) 1062-1069.

[254] E.D. Cox, H. Diaz Arauzo, Q. Huang, M.S. Reddy, C. Ma, B. Harris, R. McKernan, P. Skolnick, J.M. Cook, Synthesis and evaluation of analogues of the partial agonist 6-(propyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ester (6-PBC) and the full agonist 6-(Benzyloxy)-4-(methoxymethyl)-β- carboline-3-carboxylic acid ethyl ester (ZK 93423) at wild type and recombinant GABA-A Receptors, J. Med. Chem., 41 (1998) 2537-2552.

[255] H.A. Robertson, G.B. Baker, R.T. Coutts, A. Benderly, R.A. Locock, I.L. Martin, Interactions of β-carbolines with the benzodiazepine receptor: Structure-activity relationships, Eur. J. Pharmacol., 76 (1981) 281-284.

[256] M.S. Allen, T.J. Hagen, M.L. Trudell, P.W. Codding, P. Skolnick, J.M. Cook, Synthesis of novel 3-substituted β-carbolines as benzodiazepine receptor ligands: Probing the benzodiazepine receptor pharmacophore, J. Med. Chem., 31 (1988) 1854-1861.

[257] M.L. Trudell, A.S. Basile, H.E. Shannon, P. Skolnick, J.M. Cook, Synthesis of 7,12-dihydropyrido[3,4-b:5,4-*b*']diindoles. A novel class of rigid, planar benzodiazepine receptor ligands, J. Med. Chem., 30 (1987) 456-458.

[258] T.J. Hagen, P. Skolnick, J.M. Cook, Synthesis of 6-substituted β-carbolines that behave as benzodiazepine receptor antagonists or inverse agonists, J. Med. Chem., 30 (1987) 750-753.

[259] B. Grella, M. Teitler, C. Smith, K. Herrick Davis, R.A. Glennon, Binding of β-carbolines at 5-HT2 serotonin receptors, Bioorg. Med. Chem. Lett., 13 (2003) 4421-4425.

[260] J.E. Audia, D.A. Evrard, G.R. Murdoch, J.J. Droste, J.S. Nissen, K.W. Schenck, P. Fludzinski, V.L. Lucaites, D.L. Nelson, M.L. Cohen, Potent, selective tetrahydro-β-carboline antagonists of the serotonin 2B (5HT2B) contractile receptor in the rat stomach fundus, J. Med. Chem., 39 (1996) 2773-2780.

[261] R.A. Glennon, B. Grella, R.J. Tyacke, A. Lau, J. Westaway, A.L. Hudson, Binding of β-carbolines at imidazoline I2 receptors: A structure-affinity investigation, Bioorg. Med. Chem. Lett., 14 (2004) 999-1002.

[262] M. Abou Gharbia, U.R. Patel, J.A. Moyer, E.A. Muth, Psychotropic agents: Synthesis and antipsychotic activity of substituted β-carbolines, J. Med. Chem., 30 (1987) 1100-1105.

[263] T. Herraiz, D. Gonzalez, C. Ancin Azpilicueta, V.J. Aran, H. Guillen, β-carboline alkaloids in *Peganum harmala* and inhibition of human monoamine oxidase (MAO), Food Chem. Toxicol., 48 (2010) 839-845.

[264] J. Reniers, S. Robert, R. Frederick, B. Masereel, S. Vincent, J. Wouters, Synthesis and evaluation of β-carboline derivatives as potential monoamine oxidase inhibitors, Bioorg. Med. Chem., 19 (2011) 134-144.

[265] Small-Molecule Drug Discovery Suite 2014-4: QikProp, Schrödinger, LLC, New York, NY, 2014.

[266] V. Singh, S. Hutait, S. Biswas, S. Batra, Versatility of substituted 1-formyl-9*H*-β-carbolines for the synthesis of new fused β-carbolines via intramolecular 1,3-dipolar cycloaddition, Eur. J. Org. Chem., 2010 (2010) 531-539.

[267] V. Singh, S. Hutait, S. Batra, Baylis–Hillman reaction of 1-formyl-β-carboline: One-step synthesis of the canthin-6-one framework by an unprecedented cascade cyclization reaction, Eur. J. Org. Chem., 2009 (2009) 6211-6216.

[268] E.D. Cox, J.M. Cook, The pictet-spengler condensation: A new direction for an old reaction, Chem. Rev., 95 (1995) 1797-1842.

[269] W. Jiang, J. Guan, M.J. Macielag, S. Zhang, Y. Qiu, P. Kraft, S. Bhattacharjee, T.M. John, D. Haynes-Johnson, S. Lundeen, Pyrroloquinolone PDE5 inhibitors with improved pharmaceutical profiles for clinical studies on erectile dysfunction, J. Med. Chem., 48 (2005) 2126-2133.

[270] B. Das, K. Venkateswarlu, G. Mahender, I. Mahender, A simple and efficient method for α-bromination of carbonyl compounds using *N*-bromosuccinimide in the presence of silica-supported sodium hydrogen sulfate as a heterogeneous catalyst, Tet. Lett., 46 (2005) 3041-3044.

[271] S. Hutait, M. Nayak, A. Penta, S. Batra, Convenient synthesis of a library of lactam-fused β-carbolines via the Ugi reaction, Synthesis, 3 (2011) 419-430.

[272] N.M. Raghavendra, A. Jyothsna, A. Venkateswara Rao, C. Subrahmanyam, Synthesis, pharmacological evaluation and docking studies of *N*-(benzo [*d*] thiazol-2-yl)-2-(piperazin-1-yl) acetamide analogs as COX-2 inhibitors, Bioorg. Med. Chem. Lett., 22 (2012) 820-823.

[273] R. Cao, H. Chen, W. Peng, Y. Ma, X. Hou, H. Guan, X. Liu, A. Xu, Design, synthesis and *in vitro* and *in vivo* antitumor activities of novel β-carboline derivatives, Eur. J. Med. Chem., 40 (2005) 991-1001.

[274] I. Kucukguzel, E. Tatar, S.G. Kucukguzel, S. Rollas, E. De Clercq, Synthesis of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy) methyl]-4-alkyl/aryl-2, 4-dihydro-3*H*-1, 2, 4-triazole-3-thiones and evaluation as antiviral/anti-HIV and anti-tuberculosis agents, Eur. J. Med. Chem., 43 (2008) 381-392.

[275] R. Mahesh, T. Devadoss, D.K. Pandey, S. Bhatt, Discovery of new anti-depressants from structurally novel 5-HT3 receptor antagonists: Design, synthesis and pharmacological evaluation of 3-ethoxyquinoxalin-2-carboxamides, Bioorg. Med. Chem. Lett., 21 (2011) 1253-1256.

[276] R. Mahesh, T. Devadoss, D.K. Pandey, S. Bhatt, S.K. Yadav, Design, synthesis and structure-activity relationship of novel quinoxalin-2-carboxamides as 5-HT3 receptor antagonists for the management of depression, Bioorg. Med. Chem. Lett., 20 (2010) 6773-6776.

[277] K. Falzari, Z. Zhu, D. Pan, H. Liu, P. Hongmanee, S.G. Franzblau, *In vitro* and *in vivo* activities of macrolide derivatives against *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother., 49 (2005) 1447-1454.

[278] C.L. Cantrell, T. Lu, F.R. Fronczek, N.H. Fischer, L.B. Adams, S.G. Franzblau, Antimycobacterial cycloartanes from *Borrichia frutescens*, J. Nat. Prod., 59 (1996) 1131-1136.

[279] W. Chen, P. Zhan, E. De Clercq, C. Pannecouque, J. Balzarini, X. Jiang, X. Liu, Design, synthesis and biological evaluation of *N*2,*N*4-disubstituted-1,1,3-trioxo-2*H*,4*H*-pyrrolo[1,2-*b*][1,2,4,6]thiatriazine derivatives as HIV-1 NNRTIs, Biorg. Med. Chem., 21 (2013) 7091-7100.

[280] M. De Rycker, I. Hallyburton, J. Thomas, L. Campbell, S. Wyllie, D. Joshi, S. Cameron, I.H. Gilbert, P.G. Wyatt, J.A. Frearson, A.H. Fairlamb, D.W. Gray, Comparison of a high-throughput high-content intracellular *Leishmania donovani a*ssay with an axenic amastigote assay, Antimicrob. Agents Chemother., 57 (2013) 2913-2922.

[281] L. Collins, S.G. Franzblau, Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*, Antimicrob. Agents Chemother., 41 (1997) 1004-1009.

[282] E.B. Lansdon, K.M. Brendza, M. Hung, R. Wang, S. Mukund, D. Jin, G. Birkus, N. Kutty, X. Liu, Crystal structures of HIV-1 reverse transcriptase with etravirine (TMC125) and rilpivirine (TMC278): Implications for drug design, J. Med. Chem., 53 (2010) 4295-4299.

[283] G.M. Sastry, M. Adzhigirey, T. Day, R. Annabhimoju, W. Sherman, Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments, J. Comput. Aided Mol. Des., 27 (2013) 221-234.

[284] Schrödinger Release 2014-4: LigPrep, Schrödinger, LLC, New York, NY, 2014.

[285] R.A. Friesner, R.B. Murphy, M.P. Repasky, L.L. Frye, J.R. Greenwood, T.A. Halgren, P.C. Sanschagrin, D.T. Mainz, Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes, J. Med. Chem., 49 (2006) 6177-6196.

[286] S. Murugesan, S. Ganguly, G. Maga, Synthesis, evaluation and molecular modelling studies of some novel 3-(3, 4-dihydroisoquinolin-2 (1*H*)-yl)-*N*-(substitutedphenyl) propanamides as HIV-1 non-nucleoside reverse transcriptase inhibitors, J. Chem. Sci., 122 (2010) 169-176.

[287] P.A. Janssen, P.J. Lewi, E. Arnold, F. Daeyaert, M. de Jonge, J. Heeres, L. Koymans, M. Vinkers, J. Guillemont, E. Pasquier, In search of a novel anti-HIV drug: Multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1 *E*)-2-cyanoethenyl]-2, 6-dimethylphenyl] amino]-2-pyrimidinyl] amino] benzonitrile (R278474, rilpivirine), J. Med. Chem., 48 (2005) 1901-1909.

[288] A.L. Hopkins, J. Ren, R.M. Esnouf, B.E. Willcox, E.Y. Jones, C. Ross, T. Miyasaka, R.T. Walker, H. Tanaka, D.K. Stammers, Complexes of HIV-1 reverse transcriptase with inhibitors of the HEPT series reveal conformational changes relevant to the design of potent non-nucleoside inhibitors, J. Med. Chem., 39 (1996) 1589-1600.

[289] P. Zhan, X. Chen, D. Li, Z. Fang, E. Clercq, X. Liu, HIV-1 NNRTIs: Structural diversity, pharmacophore similarity, and impliations for drug design, Med. Res. Rev., 33 (2013) E1-E72.

[290] K. Asokkumar, L.T. Prathyusha, M. Umamaheshwari, T. Sivashanmugam, V. Subhadradevi, P. Jagannath, A. Madeswaran, F. Salesheir, Design, admet and docking studies on some novel chalcone derivatives as soluble epoxide hydrolase enzyme inhibitors, J. Chil. Chem. Soc., 57 (2012) 1442-1446.

[291] Molinspiration Cheminformatics: Available at (http://www.molinspiration.com/cgi-bin/properties), Accessed on 23rd Mar, 2013.

[292] Organic Chemistry Portal, Molecular property explorer, Available at (http://www.organic-chemistry.org/prog/peo/), Accessed on 18th Jan, 2014.

[293] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Del. Rev., 64 (2012) 4-17.

[294] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Del. Rev., 46 (2001) 3-26.

[295] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, J. Med. Chem., 45 (2002) 2615-2623.

[296] T. Hou, J. Wang, W. Zhang, X. Xu, ADME evaluation in drug discovery. 6. Can oral bioavailability in humans be effectively predicted by simple molecular property-based rules?, J. Chem. Inform. Model., 47 (2007) 460-463.

[297] S. Tian, Y. Li, J. Wang, J. Zhang, T. Hou, ADME evaluation in drug discovery. 9. Prediction of oral bioavailability in humans based on molecular properties and structural fingerprints, Mol. Pharm., 8 (2011) 841-851.

[298] P. Daisy, S. Suveena, M. Rajalakshmi, V. Lilly, Ligand based Virtual screening on natural compounds for discovering active ligands, Der Pharm. Chem., 3 (2011) 15-22.

[299] J.C. Lee, Y.H. Bae, S.K. Chang, Efficient alpha-halogenation of carbonyl compounds by *N*-bromosuccinimide and *N*-chlorosuccinimde, Bull. Korean Chem. Soc., 24 (2003) 407-408.

[300] R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M.P. Repasky, E.H. Knoll, M. Shelley, J.K. Perry, Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, J. Med. Chem., 47 (2004) 1739-1749.

List of Publications

1. Penta Ashok, Subhash Chander, Jan Balzarini, Christophe Pannecouque, Sankaranarayanan Murugesan. Design, synthesis of new β-carboline derivatives and their selective anti-HIV-2 activity, **Bioorganic & Medicinal Chemistry Letters**, 25 (6), 2015, 1232-1235.
2. Penta Ashok, Cui-Lin Lu, Subhash Chander, Yong-Tang Zheng, Sankarnarayanan Murugesan. Design, Synthesis, and biological evaluation of 1-(thiophen-2-yl)-9*H*-pyrido[3,4-*b*]indole derivatives as anti-HIV-1 agents, **Chemical Biology & Drug Discovery**, 85 (6), 2015, 722-728.
3. Subhash Chander, **Penta Ashok**, Sankaranarayanan Murugesan. Structure-based virtual screening and docking studies for the identification of novel inhibitors against wild and drug resistance strains of HIV-1 RT, **Medicinal Chemistry Research**, 24 (5), 2015, 1869-1883.
4. **Penta Ashok**, Subhash Chander, Hiren Lathiya, Hardik Sharma, Kriti Goyal, Sankaranarayanan Murugesan. De-novo design and *in-silico* studies of novel bis-arylpiperazine derivatives as non-nucleoside inhibitors of HIV-1 reverse transcriptase, **Journal of Pharmaceutical Chemistry,** 1 (2), 2014, 22-27.
5. **Penta Ashok**, Hiren Lathiya, Sankarnarayanan Murugesan. Manzamine alkaloids as antileishmanial agents - A review, **European Journal Medicinal Chemistry,** 97 (5), 2015, 928-936.
6. **Penta Ashok**, Swastika Ganguly and Sankaranarayanan Murugesan. Manzamine alkaloids: isolation, cytotoxicity, antimalarial activity and SAR studies, **Drug Discovery Today**, 19 (11), 2014 1781-1791.
7. **Ashok Penta**, Subhash Chander, S. Ganguly, S. Murugesan. De novo design and in-silico studies of novel 1-phenyl-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-b]indole-3-carboxylic acid derivatives as HIV-1 reverse transcriptase inhibitors, **Medicinal Chemistry Research**, 23 (8), 2014, 3662-3670.

8. **Penta Ashok**, Hardik Sharma, Hiren Lathiya, Subhash Chander, Sankaranarayanan Murugesan. In-silico design and study of novel Piperazinyl β-carbolines as inhibitor of HIV-1 reverse transcriptase, **Medicinal Chemistry Research,** 24 (2), 2015, 513-522.
9. **Penta Ashok**, S. Ganguly and S. Murugesan. Review on In-Vitro anti-Malarial activity of Natural β-carboline alkaloids, **Mini Reviews in Medicinal Chemistry**, 13(12), 2013, 1778-1791.

List of Presentations

1. **Ashok Penta**, Subhash Chander and Sankaranarayanan Murugesan. Paper entitled "Design and In-Silico study of novel 2,3,4,9-tetrahydro-1-phenyl-*1H*-pyrido[3,4-*b*]indole derivatives as Inhibitor of HIV-1 Reverse Transcriptase" presented at World Conference on Infectious Diseases(WCID-2013) organized by Jayaa Charitable and Educational Trust, Tamil Nadu. **December 18-22nd, 2013.**

2. Subhash Chander, **Ashok Penta** and Sankaranarayanan Murugesan. Paper entitled "In-Silico design and study of novel Benzopiperidines as HIV-1 Reverse Transcriptase Inhibitor" presented at Recent Advances in Computational Drug Design (RACDD-2013) organized by IISC, Bangalore. **September 16-17th, 2013.**

3. **Ashok. P**, Subhash Chander, S. Ganguly, Rafael Balana-Fouce and S. Murugesan. Paper entitled "Synthesis and Evaluation of β-carboline derivatives as Anti-Leishmanial agents" presented at 2nd UK-India Medicinal Chemistry Congress organized by IICT, Hyderabad. **March 22-23rd, 2013.**

4. **Ashok. P**, S. Murugesan and S. Ganguly. Paper entitled "Design and Comparative Docking studies of novel 1-Phenyl-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole-3-carboxylic acid analogs as Inhibitors of HIV-1 Reverse Transcriptase" presented at World Conference on HIV and AIDS organized by Institute For Holistic Medical Sciences, Kottayam and Ayurveda-Und Venen-Klink, Austria. **November 09-11th, 2012. (Got third prize in poster presentation)**

5. **P. Ashok**, S. Murugesan and S. Ganguly. Paper entitled "Design and Docking studies of some novel 1-Phenyl-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-*b*]indole-3-carboxylic acids as Inhibitors of Human Immunodeficiency Virus Type-1 Reverse Transcriptase" presented at International Conference on Global Trends in Pure and Applied Chemical Sciences organized by Asian Journal of Chemistry, Udaipur. **March 3-4th, 2012.**

6. **Ashok. P**, Kakumanu Kishore Babu, S. Murugesan and S. Ganguly. Paper entitled "Design and docking studies of some novel 2-Substituted Tetrahydro-β-carbolines as Human Immunodeficiency Virus type-1 Reverse Transcriptase Inhibitors" presented at Antivirals for the Developing World, 10th Annual Symposium of International Consortium on Anti-Virals in collaboration with International Centre for Genetic Engineering & Biotechnology, New Delhi, INDIA. **February 7-10, 2012**.

Biographies

Dr. S. Murugesan graduated in Pharmacy from Dr. M.G.R Medical University, Tamilnadu, India in the year 1999. He obtained his Master in Pharmacy degree from BITS, Pilani, India in the year 2002. Later on, in the year 2006, he joined with Dr. Swastika Ganguly's research group in the area of Computer Aided Drug Design along with drug synthesis against infectious diseases and acquired his Ph. D in the year 2009. Then he joined in the department of Pharmacy, BITS, Pilani as assistant professor and pursuing his teaching for both B. Pharmacy and M. Pharmacy programmes as well as research in the area of Molecular Modeling along with drug synthesis against HIV and allied opportunistic infections. He had more than 9 years of teaching and research experience. He has guided more than 6 B. Pharm, 3 M. Pharm students in the fulfillment of their dissertation and guiding 2 full time Ph. D scholars. He has been related in 3 research projects and has published more than 40 research papers in various peer reviewed journals of international and national reputes. He also presented his research findings in more than 30 conferences at national and international levels.

Ashok Penta graduated in Pharmacy from Kakatiya University, Telangana, India in the year 2009. He received his MS degree in Medicinal Chemistry from National Institute of Pharmaceutical Education and Research, India in 2011. In the same year, he has joined with Dr. S. Murugesan group, BITS-Pilani, Pilani Campus to pursue his doctorial research work. He has been awarded senior research Fellowship from Council of Scientific & Industrial Research, New Delhi, in the year 2013. His area of interest is computer-aided drug design, and development of novel heterocyclic agents as anti-infective agents. He has published 12 research and 3 review articles in peer-reviewed journals.