Multicomponent Reactions in the Synthesis of Some Biologically Potent Heterocyclic Compounds

THESIS

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DOCTOR OF PHILOSOPHY

by

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Under the supervision of

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CERTIFICATE

This is to certify that the thesis entitled **Multicomponent Reactions in the Synthesis of Some Biologically Potent Heterocyclic Compounds** and submitted by **Mr. Buchi Reddy Vaddula** ID No **2005PHXF012** for award of Ph.D. Degree of the Institute embodies the original work done by him under my supervision.

Signature in full of the Supervisor:
Name in capital block letters: **Dr. DALIP KUMAR**
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Date:

Dedicated to My Family

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ABSTRACT

Multicomponent reactions (MCRs) can rapidly deliver combinatorial libraries of molecules with greater efficiency, atom economy, and tunable pharmacophores thus fulfilling the requirements of drug discovery. The thesis describes the development of novel MCRs and construction of biologically active heterocycles such as 2-aminochromenes, 4*H*-pyrans, 1,2,3-triazoles, 1,3,4-thiadiazoles and indolylthiazoles. Efforts were made to develop facile protocols under catalytic conditions using benign reaction solvents or without any solvents to cater bioactive compounds.

The first chapter gives an introduction to the MCRs and its classification schemes. The strategies for developing novel MCRs and parameters that make the MCRs the choice of reactions are also described. The role played by the MCRs in drug discovery and total synthesis of natural products is discussed with some examples.

A one-pot synthesis of 2-aminochromenes catalysed by nanosized MgO in good yields is described in chapter two. The characterisation of nanosized MgO and its catalytic activity in comparison to commercially available MgO was also studied. The catalyst was reused reproducing the product yields. The commercially available MgO was also successfully used in the one-pot preparation of 4*H*-pyrans which is described in third chapter. The synthesis involves grinding the arylaldehyde, malononitrile and β-diketone in presence of MgO under solvent-free conditions. The synthesised compounds exhibited moderate antibacterial activity in all the three standard strains of bacteria.

The preparation of an array of 1,2,3-triazoles under Click conditions and their Src kinase inhibitory evaluation is reported in chapter four. The advantages of the protocol are the short reaction time, simple workup, benign and reusable reaction media. The structure-activity relationship of the synthesised 1,2,3-triazoles was studied to optimize the activity. The 1,2,3-triazoles with aryl substitutions at positions 1 and 4 were also synthesised regioselectively and is reported in chapter five. The preparation was carried out by reaction of diaryliodonium salts, sodium azide and terminal alkynes. The reaction media was successfully reused without any loss of product yield.

The sixth chapter comprises the synthesis and *in vivo* anticonvulsant evaluation of 2,5-disubstituted-1,3,4-thiadiazoles in one-pot by the reaction of arylaldehyde, hydrazine and arylisothiocyanate followed by oxidative cyclization. The compounds showed moderate anticonvulsant activity in subcutaneous picrotoxin induced seizures in swiss albino mice.

The synthesis of 5-(2'-indolyl)thiazoles in one-pot from thioamides, 3-tosyloxypentane-2,4-dione and arylhydrazines is accounted in chapter seven describing the effects of various substitutions on the reactivity.

In chapter eight, the work reported in the thesis is summarised and the future scope of the research work undertaken is also highlighted.

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LIST OF ABBREVIATIONS / SYMBOLS

Abbreviation/Symbol	Description
%	Percentage
3CR	Three component reaction
3-MATIDA	(+)- and (-)-3-methyl-5-carboxythien-2-ylglycine
A°	Angstrom
ATP	Adenosine triphosphate
CA	Carbonic anhydrase
CCR5	C-C Chemokine Receptor 5
CD45	Leukocyte common antigen
CML	Chronic myelogenous leukemia
CNS	Central nervous system
COX	Cyclooxygenase
d	doublet
DCM	Dichloromethane
DMF	*N,N*-Dimethylformamide
DMSO-d_6	Deuterated dimehtylsulfoxide
E	Exploratory power
EC_{50}	Maximal effective concentration
ED50	Effective Dose 50%
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EI	Electron ionisation
EP_3	Extracelluar protein 3
ESI	Electrospray ionisation
FAS	Ferric ammonium sulfate
G6PT	Glucose-6-phosphate translocase
GABA	γ-amino-4-butyric acid
GHS	Growth hormone secretagogue
GIST	Gastrointestinal stromal tumors
GPCR	G-protein coupled receptors
h	hours
HCN	Hydrogen cyanide
HRMS	High resolution mass spectra

HTMAB	Hexadecyltrimethyl ammonium bromide
HTS	High-throughput screening
IC_{50}	half maximal inhibitory concentration
IR	Infrared
J	Coupling constant
JAK	Janus kinase
KF-Al_2O_3	Ketjenfine - Al_2O_3
LAR phosphatase	leukocyte–antigen-related phosphatase
Lit.	Literature
M.p.	Melting point
MAP	Mitogen activated protein
MAPKAP	MAP kinase activated protein
MCH1	melaninconcentrating hormone 1
mCPBA	*m*-Cl perbenzoic acid
MCRs	Multicomponent reactions
MES	Maximum electroshock
mg	milligram
mGluR	metabotropic glutamate receptors
MgO	Magnesium Oxide
MHz	Mega hertz
MIC	Minimum inhibitory concentration
min	minutes
mL	millilitre
mmol	millimole
NH3	Ammonia
NMR	Nuclear magnetic resonance
NPFF	Neuropeptide FF
^oC	centigrade
PADAM	Passerini reaction/deprotection/acyl migration
PDB	Protein data base
PEG	Polyethylene glycol
PPA	Polyphosphoric acid
PPAR	Peroxisome proliferator-activated receptor
PTK	Protein tyrosine kinase
PTP1B	Protein-Tyrosine Phosphatase 1B
p-TsOH	*p*-Toluene sulfonic acid

PTZ	Pentylenetetrazole
s	Singlet
SAR	Structure-activity realtionship
scPIC	Subcutaneous picrotoxin
SEM	Scanning Electron Micrograph
SEM	Standard error of the mean
SH2	Src homology domain 2
SRR	Single reactant replacement
SRS-A	Slow-reacting substance of anaphylaxis
t	triplet
t-Bu	tertiary butyl
TCPTP	T-Cell Protein Tyrosine Phosphatase
TEBAC	Triethylbenzylammonium chloride
TEM	Transmission Electron Micrograph
TFA	Trifluoroacetic acid
TfOH	Triflic acid
THF	Tetrahyrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TMSCl	Trimethyl silyl chloride
TNF α	Tumor necrosis factor α
TOSMIC	Tosylmethylisocyanide
U-4CR	Ugi-four component reaction
UV	Ultraviolet
vL-3CR	van Leussen three component reaction
XRD	X-ray diffraction
Δ	Delta
δ	Parts per million
θ	Theta
μM	Micromolar

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CHAPTER - I

INTRODUCTION

Chapter 1

1.1 Introduction

Synthesis of diversely substituted products with minimal effort in a short time, and involving at least three reactants in one-pot are called multicomponent reactions (MCRs). The final product from multicomponent reaction displays features of all reactants thus improving the atom economy of the chemical transformation (Figure 1.1). The sequence of transformations in a MCR follows a programmed fashion. The intermediates formed during the process, reacts with further functionalities yielding the products.

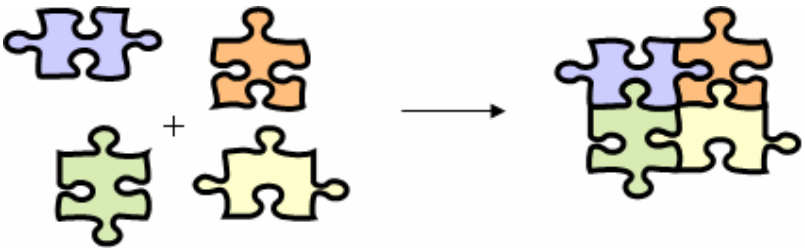


Figure 1.1 General idea of multicomponent reactions

MCRs constitute an especially attractive synthetic strategy since they provide easy and rapid access to large libraries of organic compounds with diverse substitution patterns. As MCRs are one-pot reactions, they are easier to carry out than multi-step syntheses. Coupled with high-throughput library screening, this strategy is an important development in the drug discovery in the context of rapid identification and optimization of biologically active lead compounds. Libraries of small-molecule organic compounds are perhaps the most desired class of potential drug candidates, because standard peptides and oligonucleotides have limitations as bioavailable therapeutics.[1-3] With a small set of starting materials, very large libraries can be built up within a short time, which can then be used for research on medicinal substances.

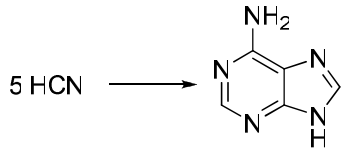
In spite of the significant useful attributes of MCRs for modern organic chemistry and their suitability for building up large compound libraries these reactions were of limited interest in the past fifty years. However, in the last decade, with the introduction of high-throughput biological screening, the importance of MCRs for drug discovery has been recognized and considerable efforts from both academic and industrial researchers have been focussed especially on the design and development of multicomponent procedures for the generation of libraries of heterocyclic compounds.[4-5] This growing interest is stimulated by the significant therapeutic potential that is associated with many

heterocycles. Furthermore, the utility of the rigid well-defined structures of heterocycles was demonstrated in many detailed structure activity relationship (SAR) studies.[6]

The art of carrying out efficient chemical transformations is a major concern in modern organic synthesis. Selectivity and efficiency (optimization of yields) are two aspects of utmost importance when considering the outcome of a reaction. Utilization of catalysts in the synthetic organic chemistry helps to explore variety of new reactions, which are selective and otherwise difficult to produce in good yields. Employing efficient catalysts in MCRs gives better yields and also decreases the waste production. Development of MCRs that involves heterogeneous catalysts and ecofriendly solvents would be an ideal synthesis. MCRs are also reported in environmentally benign solvents like water.[7]

1.2 History of Multicomponent Chemistry

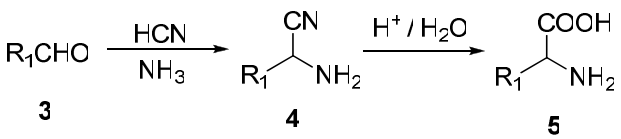
The concept of MCRs is not new in nature, it is important especially in evolution. It seems that adenine **2**, one of the major constituents of DNA and RNA, was prebiotically formed by the condensation of five molecules of HCN (**1**), a plentiful component of prebiotic atmosphere, in a reaction catalysed by NH_3 (Scheme 1.1).[8-11] The other nucleic bases have been generated in similar reactions involving HCN and H_2O.



1 2

Scheme 1.1 Synthesis of adenine **2** by the nature

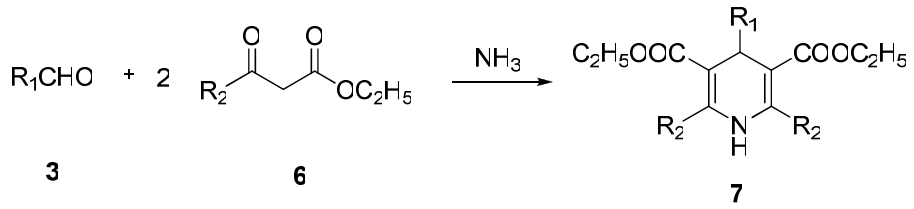
The first modern contribution to the development of MCRs was made by Strecker in 1850.[12] The crucial step in the well-known Strecker synthesis of α-amino acids is the formation of α-amino nitriles **4** from HCN **1**, aldehydes **3** and NH_3 in one-pot. Subsequent hydrolysis of these synthetically valuable intermediate α-amino nitriles **4** results in the amino acids **5** (Scheme 1.2).



3 4 5

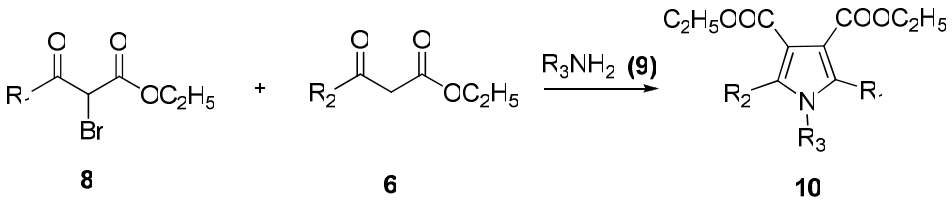
Scheme 1.2 Synthesis of α-amino acids **5**

Further progress of multicomponent chemistry can be attributed to the Hantzsch synthesis of dihydropyridines **7** from NH_3, aldehydes **3** and two equivalents of β-ketoesters **6** (Scheme 1.3).[13]



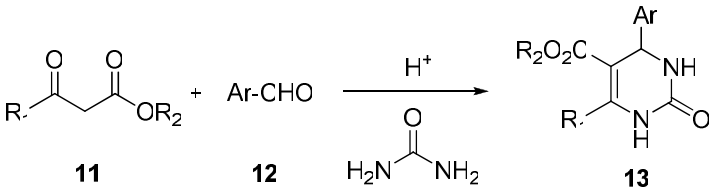
Scheme 1.3 Synthesis of dihydropyridines **7**

Another contribution made by Hantzsch to MCRs in 1890 was the synthesis of pyrroles **10** by reacting β-ketoesters **6**, α-bromo β-ketoesters **8** and primary amines **9** .[13]



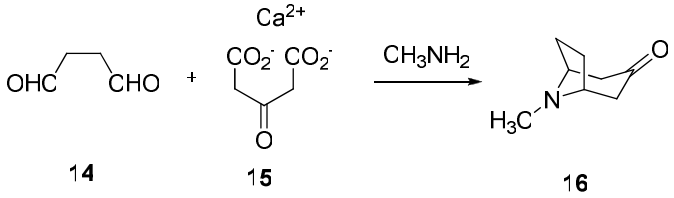
Scheme 1.4 One-pot synthesis of pyrroles **10**

The Biginelli reaction first described in 1893 represents multicomponent synthesis of substituted dihydropyrimidinones **13** by acid-catalysed cyclocondensation of β-ketoesters **11**, aromatic aldehydes **12** and urea (Scheme 1.5).[14-17]



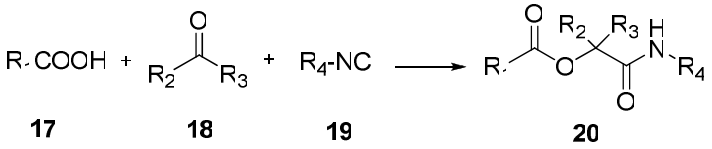
Scheme 1.5 One-pot synthesis of dihydropyrimidines **13**

In 1917, Robinson[18] reported the first important application of MCRs for the preparation of naturally occurring alkaloid, tropinone **16** from succinaldehyde **14**, methylamine and calcium salt of acetonedicarboxylic acid **15** (Scheme 1.6).



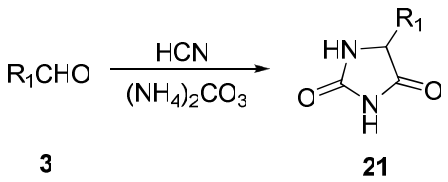
Scheme 1.6 One-pot synthesis of tropinones **16**

The first MCR involving isocyanides was discovered in 1921 by Passerini.[8] One-pot reaction of carboxylic acids **17**, carbonyl compounds **18** and isocyanides **19** afforded α-acyloxy carboxamides **20** in good yields (Scheme 1.7).[9,19]



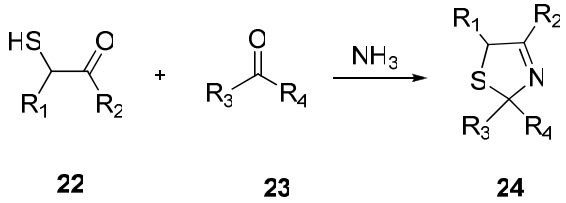
Scheme 1.7 One-pot synthesis of α-acyloxy carboxamides **20**

In 1934, Bucherer and Bergs described a four-component reaction for the synthesis of hydantoins **21**. One-pot reaction of HCN, aldehydes **3**, NH_3 and CO_2 afforded hydantoins **21**,[20] which can be easily transformed into α-amino acids by simple hydrolysis.[21-22]



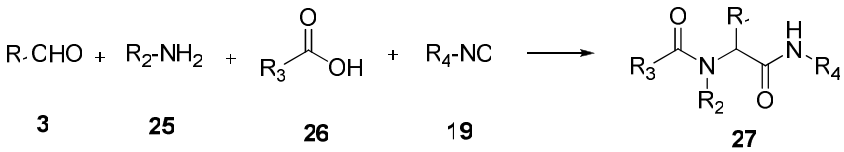
Scheme 1.8 Multicomponent synthesis of hydantoins **21**

The next important example is the Asinger reaction reported in 1958. The reaction of thiols **22** (generated *in situ* from α-halo ketones and sodium hydrogen sulfide) with carbonyl compounds **23** and ammonia afforded thiazolines **24** (Scheme 1.9).[23]



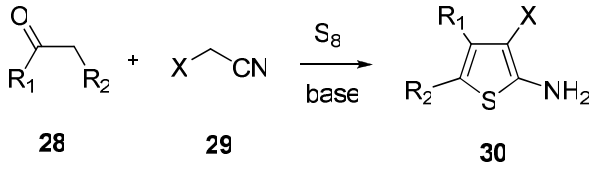
Scheme 1.9 Multicomponent synthesis of thiazolines **24**

One of the most utilized MCRs was discovered in 1959 by Ugi et al.[9] Synthesis of α-acylamino amides **27** was achieved by reacting aldehydes **3**, primary amines **25**, carboxylic acids **26** and isocyanides **19**.[9-11,19,24]



Scheme 1.10 One-pot preparation of α-acylamino amides **27**

In 1961, Gewald and coworkers described the synthesis of polysubstituted thiophenes with electron withdrawing substituents such as cyano, carboethoxy and carboxamido at the third position and alkyl, aryl, cycloalkyl and heteroaryl groups at the fourth and fifth positions. Three major modifications of this method were described in literature which give access to various 2-aminothiophenes **30** (Scheme 1.11).[25]



28 **29** **30**

Scheme 1.11 One-pot synthesis of substituted thiophenes **30**

A large and important class of MCRs are the isocyanide based multicomponent reactions, first of them was introduced in 1921 by Passerini.[26-27] One of the most significant advantages of isocyanide based MCRs are their compatibility with a range of ancillary functional groups not taking part in the initial MCR. Of particular relevance to complex synthesis is the planned possibility that such functional groups can then be utilized in a secondary reaction through various methodologies.

The prominence attached to the isocyanide based MCRs (Ugi and Passerini) are due to the isocyanide functionality which is an extraordinary functional group and unusual valence structure and reactivity. Isocyanides represented for a long time the only class of stable organic compounds with a formally divalent carbon. Owing to its reactivity the isocyanide group differs fundamentally from other functional groups. The chemistry of isocyanides is characterized by three properties: the α-acidity, the α-addition and the easy formation of radicals. Though there are several advantages associated with isocyanides, it suffers from a lack of commercial availability.

1.3 Classification of MCRs

Over the years, there have been various classification systems for MCRs, e.g. according to the components involved, the type of reaction or the reversibility of reactions leading to intermediary products or their intrinsic variability. In terms of the components involved, MCRs that utilise, for example, three components (3CR) can be further divided into three different categories depending on the nature of the starting materials. For instance, ABC designates a multicomponent reaction involving three different reagents; ABB (or AB2), a reaction that involves one molecule of reagent A and two molecules of

reagent B; and finally, AAA, a reaction that involves three molecules of the same reagent. It appears reasonable that in order to achieve the highest possible complexity and diversity in the final products, the ideal multicomponent reaction would fall into the first category (ABC) although this is not strictly necessary as evidenced by a few examples that have appeared in the literature over the last decade. The key to this particular category of MCRs that can be designated ABB' is the chemo-differentiating incorporation of the component B in two distinct manners (B and B'), which ensures the complexity and functional diversity of the final product.[28-29]

Table 1.1 Classification of MCRs on the basis of components involved

S.No.	Type	Examples
1.	Three component reactions	(i) Strecker's synthesis of α-amino acids (ii) Hantzsch synthesis of dihydropyridines and pyrroles (iii) Biginelli synthesis of dihydropyrimidones (iv) Petasis reaction (v) MCRs involving click chemistry
2.	Four component reactions	(i) Ugi reaction (ii) Asinger reaction (iii) Miscellaneous four component reactions[7]
3.	Union of MCRs /Multiple MCRs[30] / Tandem MCRs	(i) Tandem Ugi – Ugi reaction (ii) Tandem Ugi – Passerini reaction

The classification of MCRs on the basis of reaction mechanism depends on the reversibility and stability of starting materials, intermediates and products. They are classified into three categories.

Category I:

• **IA:** The isolation of products, in general, is not feasible because all the states (starting materials, intermediates and final products) of the reaction have drifting equilibria.

• **IB:** The isolation of the products is possible because of their stability though the starting materials, intermediates and final products are in equilibrium.

Category II:

• **IIA:** The pre-final products of type IB react with further multifunctional components and form irreversibly heterocyclic products, while the formal number of bonds does not change.

• **IIB:** The intermediate products of type I react with other reactants and form irreversibly pre-final or final products, so that the formal number of bonds increases.

Category III:

The reactants of MCR form one-pot products in a sequence of irreversible intermediate reactions.

Table 1.2 Classification of MCRs on the basis of reaction mechanism

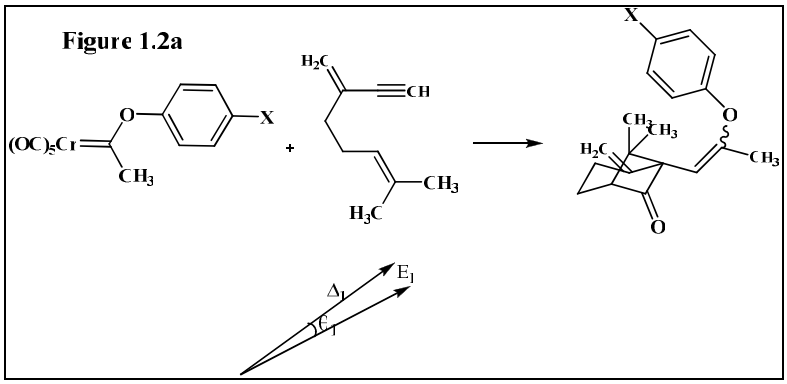
S. No.	Category	Sub category	Depiction
1.	I	IA, IB	A + B ⇌ C ⇌ D ⇌ E
2.	II	IIA, IIB	A + B ⇌ C ⇌ D → E
3.	III	…	A + B → C → D → E

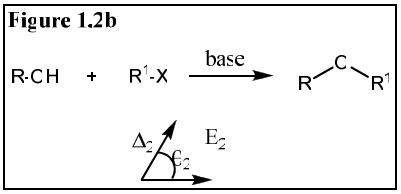
1.4 MCRs as the New Avenue for Ameliorating Synthetic Efficiency

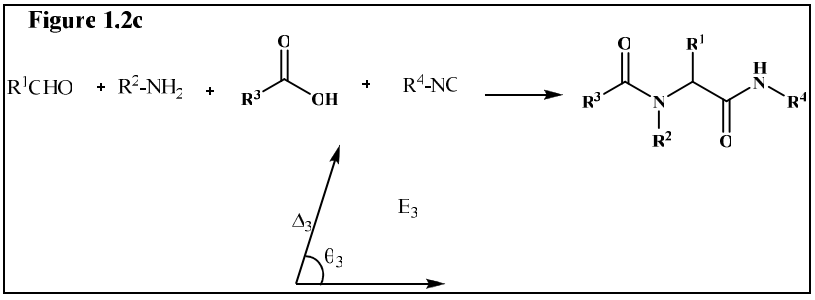
A rapid look at the literature will convince the most skeptical that MCRs are becoming increasingly popular. It is reasonable to believe that this field will continue to grow as both the academic and industrial sectors are directing their efforts towards more efficient drug-discovery processes. MCRs have gained a special place in the arsenal of the synthetic chemist to deal with the new paradigm of drug discovery. Following are some of the attributes of MCR that make them highly venerable:

Reactions with high exploratory power: The chemical pool, which contains all the structures, is more like a n-dimensional space with n being a user-defined number of parameters representing the best of our affordable qualities and physico-chemical properties of a drug candidate. Chemical reactions are our medium of travel from place to place in this n-dimensional space and, already mentioned, they are not all equal in doing their job. In choosing an efficient reaction/vehicle, one would prefer a reaction that starts from simple synthons to produce an optimum complexity product[31-33] and a reaction versatile enough to allow a maximum number of new structures to be reached by systematic variation of reactants. A reaction that induces a large increase of structural complexity from starting materials to products and which possess a high degree of versatility would be said to have a high exploratory power. By standing with our image of the chemical pool depicted as a plane, one could fully describe a chemical reaction as a vector with a length proportional to the increase of structural complexity it produces from

reactants to products, in which the angle (θ) is related to the actual reagents being used. A reaction with a high versatility, or scope, would have a high degree of tolerance of, or compatibility with, the initial reagents and thus many different values of Δ would be feasible. The exploratory power of a reaction could then be defined as the surface area described by the vector as Δ varies within the tolerable θΔ range. By using this classification the one-pot transformation of electron poor phenoxy complexes into a Taxol derivatives would be associated with very large increase of molecular complexity, but the structural constraints imposed on the reactants would be so great that only a very few derivatives would be attainable (Figure 1.2a).[34]







Figures 1.2a-c Exploratory power (E) = Complexity (Δ) x Reagents (θ). The exploratory power of multicomponent reaction $E_3 > E_2$ & E_1

The corresponding surface area would be rather small despite a quite long associated vector. On the other hand the formation of ether from an alcohol and an alkyl halide would offer a large θΔ as many alcohols and alkyl halides are available for that reaction.

However, the corresponding increase of molecular complexity would only be modest as it is only related to the complexity of two reagents and the formation of a single bond. For the combinatorial chemist, both of these reactions would be considered as having a low to medium exploratory power and be probably disregarded if used in a single-step process (Figure 1.2b). In contrast, an average MCR would be associated with a long vector as at least three reagents and several newly created bonds would contribute to the product molecular complexity, and a large $\theta\Delta$ as many reagents combinations would be allowed (Figure 1.2c).

In addition to their high exploratory power, MCRs possess several attributes of synthetic efficiency.

- ✓ **Selectivity:** even though diversity is a goal, one expects to form only a single product from a given set of inputs.
- ✓ **Atom economy:** the valuable property of transformations in which adducts are composed of the exact sum of the reactants.[35-38] Maximizing atom economy avoids wasteful atom loss from the starting materials.[35]
- ✓ **Convergence:** a synthetic route is said to be convergent when it tends to maximize the overall yield by minimizing the number of consecutive steps for each building block.[39-40] Accordingly, an MCR, as a synthetic route, is the ultimate in maximal convergence.

From a practical standpoint, understanding why these transformations have found such deep interest by the combinatorial community is simple: a single-step transformation is needed to synthesise a target compound. In most cases the reactions will be robust enough to give high yields by simply mixing reagent solutions at room temperature; this allows large molecular collections to be prepared with minimal man power. Therefore, together with a high exploratory power, MCR may be defined as productive according to industrial standards.

Finally, as is clear from the previous discussion, exploratory powerful reactions require a combination of qualities that makes their discovery and development a difficult goal to achieve (Figure 1.3). Nevertheless, the intensive research effort currently undertaken towards the discovery of new MCRs is driven by the considerable economical value attached to them.

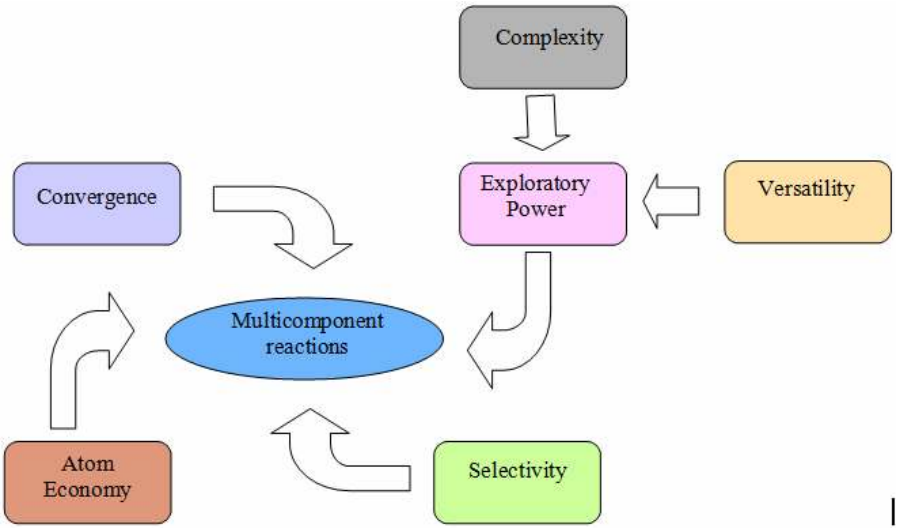


Figure 1.3 The important aspects of MCRs that make them highly efficient and paramount to the synthetic organic chemists.

MCRs in practice: Combinatorial collections are typically composed of 10^2 —10^3 members (for focused or targeted libraries)[41] and 10^4 —10^5 members (for generic libraries). Many generic drugs synthesis is established using MCRs.[42] Powerful transformations should allow facile and rapid access to both such directed and large generic libraries. Combinatorial chemists, today, are focusing on heterocyclic low-molecular-weight molecules (i.e., the so-called "drug-like" molecules). The movement from oligomeric libraries is clearly motivated by several reasons, specifically the generally poor bioavailability and biostability of peptides and their derivatives, and the difficulty in optimizing these flexible structures once a hit has been identified. On the other hand, constrained structures provide more workable "structure-activity relationship" (SAR) data about protein/ligand interactions. These considerations are at the basis of a recent upsurge in the interest of powerful heterocyclic MCRs, as will be shown below.

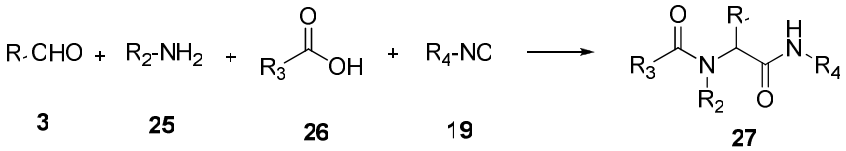
Exploratory power is not the sole advantage of such reactions. Other benefits of these approaches are highlighted below:

- ✓ The time devoted to chemistry development (often the major constituent of overall library generation) will be shorter than with a multi-step process.
- ✓ The mean library purity will be higher, as side-products will not accumulate with the number of steps: that is, the shorter, the better.
- ✓ These one-step or "multi-step, one-pot" procedures are often compatible with a solution-phase approach to library generation. In these cases, common drawbacks associated with the use of resins (e.g., troublesome monitoring of the reaction

advancement, requirement of anchoring functions that limit the input diversity, linker and resin compatibility, etc.) are avoided.

- ✓ The "hit to lead" transition (i.e., the rapid acquisition of primary SAR), or the lead optimization (vs. various pharmacological parameters) may be shortened, if the same chemistry is used throughout the process.
- ✓ Ultimately, one might speculate that an MCR-based industrial process would be more cost-effective than a multi-step route owing to the higher degree of convergence associated with the corresponding synthetic pathway.

Multicomponent and cascade processes:[43-46] The most famous, and truly powerful reaction is the Ugi four-component reaction (U-4CR, Scheme 1.12). It proceeds via a cascade of processes to form the final product.



Scheme 1.12 Ugi's one-pot reaction of α-acylamino amides **27**

Its uniqueness was recognized early on, even well before the introduction of combinatorial methods: "If, for example, 40 each of the different components are reacted with one another, the result is 40^4 = 25,60,000 reaction products".[47]

1.5 Strategies for Innovation in MCR Design

Figure 1.4 depicts a general approach to improving known MCRs that takes advantage of a detailed knowledge of reaction mechanism. A typical four-component reaction of interest in combinatorial synthesis would employ inputs A, B, C, and D, each representing a family of compounds. The overall transformation might be visualized as a linear series of individual bimolecular reactions successively producing the symbolic intermediates A-D and A-D-C on the way to the final MCR product, A-D-C-B. [Note: The progression from two interlocking puzzle pieces to three interlocking puzzle pieces is only meant to connote increasing molecular complexity and is not meant to imply or designate specific connectivity between inputs.]

Applying retrosynthetic analysis to intermediates A-D-C and A-D might identify independent routes to those intermediates from X + Y or from Q + R + S, respectively, as indicated. It follows that combining X + Y with B (and likewise combining Q + R + S

with C and B) would constitute new three- and five-component routes, respectively, to the same product of the cognate reaction of A + B + C + D but from a more diverse set of reactants, thus broadening the potential scope of chemical library synthesis. If Q, R, and S are readily available commercial compounds, the enhanced dimensionality of the five-component route exponentially increases the potential size of the synthetic library.

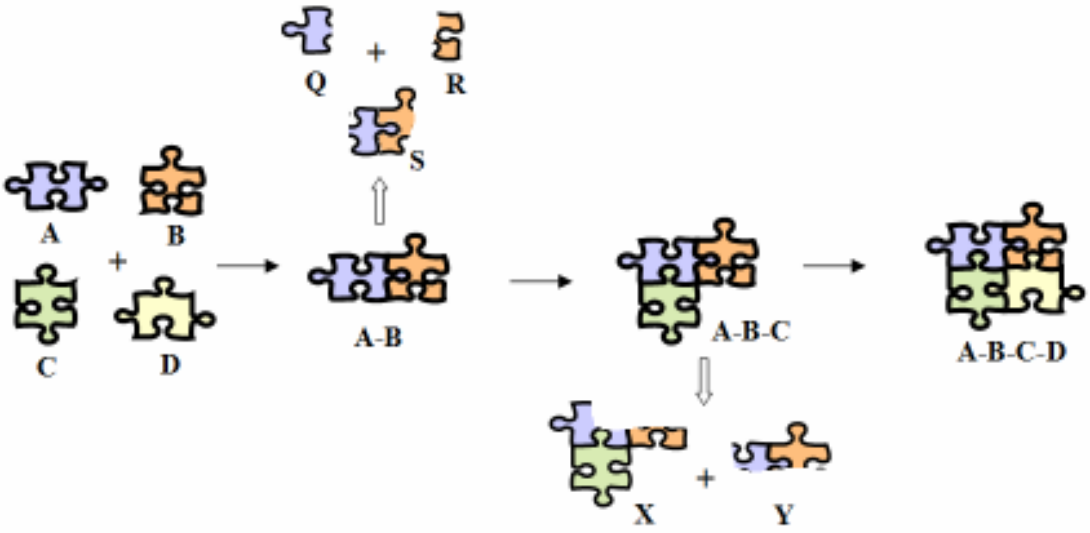


Figure 1.4 Employing retrosynthetic approach for improving the MCRs

Thus, logical ways to reengineer (and thus improve) a known MCR can be described. However, the notion that the creative or serendipitous elements involved in inventing or discovering new MCRs might also be guided, somehow, by a rationally designed process seems far less obvious. Nevertheless, Figure 1.5 depicts a general strategy whereby mechanistic insights into a known MCR might serve as an innovation platform for finding new MCRs using a process nicknamed the *single reactant replacement* (SRR) approach. This approach begins with a systematic assessment of the mechanistic or functional role of each reactant in a known MCR. Based on the resulting chemical insights, one input (A, in this case) is then replaced with a different input W that mimics the key chemical reactivity or property necessary for condensation to occur with B, C and D. By embedding additional reactivity or functionality (either explicit or latent) into W, the resulting MCR might be directed to a different outcome, for example, either a new structural framework or ring system. Thus, the chemist‘s mechanistic insight into a known MCR can serve as an innovation platform from which to design or create imaginative SRR substitutions. While the newly developed MCR might resemble the cognate reaction, the SRR process is iterative and can be applied again, this time

replacing one of the other components in the same fashion. After one or two SRR cycles, the new MCRs that emerge are likely to be quite distinctive and bear little resemblance to their progenitors.

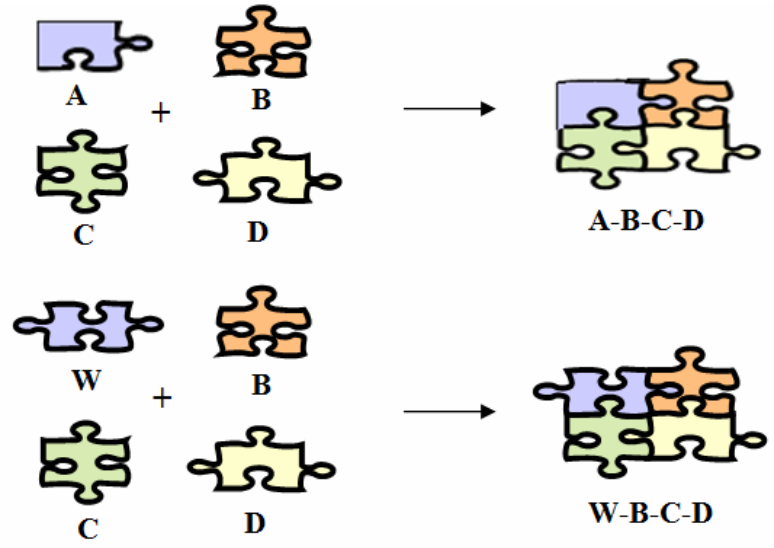


Figure 1.5 Innovation of new MCRs by single reactant replacement approach. This strategy lies in the replacement of the component A with a component W having reactivity groups.

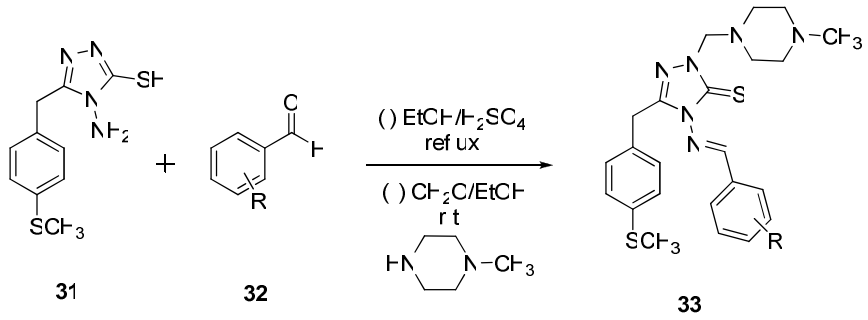
1.6 Applications of MCRs in Drug Discovery

Applications of MCRs described until today arise from the area of drug discovery. Potentially, the ease of performance, the time-saving aspect, the versatility and diversity of scaffolds,[48] and the very large chemical space will attract chemists in pharmaceutical companies to use MCRs for their projects. Another interesting aspect of MCRs in the context of drug discovery is the effective assembly of scaffolds out of smaller fragments. The hurdles involved in the total synthesis of natural products is alleviated using MCRs.[49] Isocyanide based MCRs are turning to be an impetus to drug discovery.[50]

1.6.1 Antibacterial Compounds

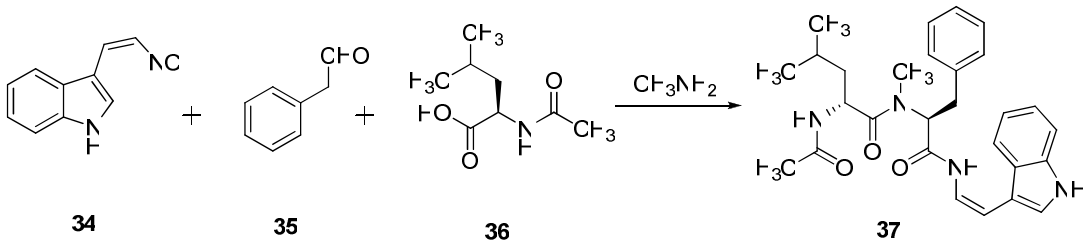
MCRs play a vital role in the rapid exploration of the novel potent antibacterial molecules. A three-component Mannich reaction involving 3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-5-mercapto-1,2,4-triazoles **31**, substituted benzaldehyde **32**, formaldehyde and morpholine/N-methylpiperazine have been employed for developing two new series of Mannich bases, namely, 1-(morpholino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-triazol-5-thiones and 1-(N-methylpiperazino)methyl-3-(4-methylthiobenzyl)-4-(substituted arylidene)amino-1,2,4-

triazol-5-thiones **33** (Scheme 1.13). These newly developed compounds when screened against various bacterial strains exhibited appreciable potency.[51]



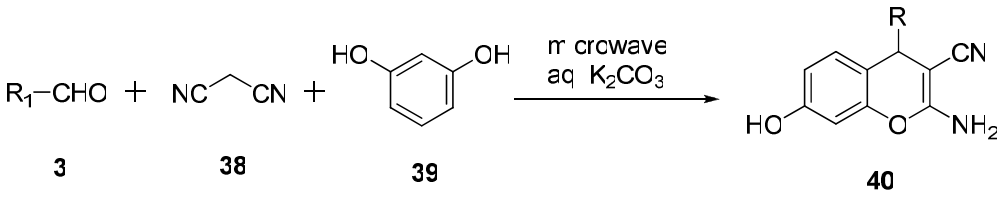
Scheme 1.13 One-pot synthesis of 1,2,4-triazol-5-thiones **33**

A one-pot total synthesis employing Ugi MCR has also been used to develop a library of Aspergillamide based analogues which were later discovered to have potent antibacterial activity (Scheme 1.14).[52]



Scheme 1.14 Ugi four component reaction in the synthesis of Aspergillamide **37**

A one-pot protocol using K_2CO_3 as catalyst in water under microwave irradiation for the synthesis of potent antibacterial 2-aminochromenes **40** also serves as an example to illustrate the crucial role played by the MCRs in the designing of libraries of biologically active moieties (Scheme 1.15).[53]

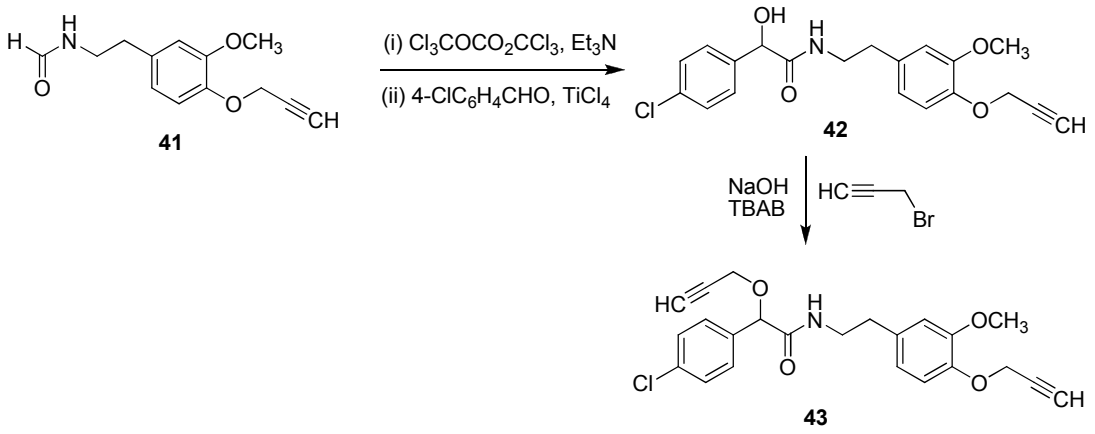


Scheme 1.15 Synthesis of antibacterial 2-aminochromenes **40**

1.6.2 Antifungal Compounds

Ugi- and Passerini-type isocyanide-based MCRs accelerated the rapid exploration and screening of novel chemical moieties of phenylglycinamides and mandelamides with high

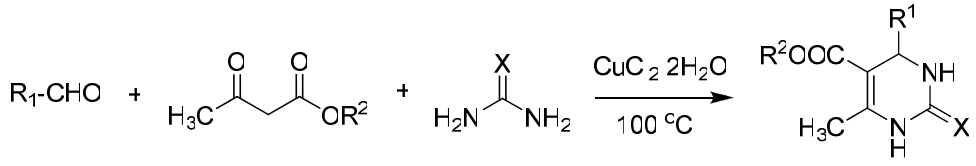
antifungal potency. The discovery of Mandipropamid **43**, a potent antifungal molecule, involves the Seebach's variation of Passerini reaction (Scheme 1.16).[54]



41 **42** **43**

Scheme 1.16 Synthesis of Mandiopropamid **43**

Singh and coworkers[55] synthesised dihydropyrimidinones **46** using the multicomponent approach and demonstrated their antifungal activities. The three component Biginelli reaction was carried out using aromatic aldehyde **3**, β-ketoester **44** and urea/thiourea **45** in presence of $CuCl_2$ under solvent free conditions (Scheme 1.17).



3 **44** **45** **46**

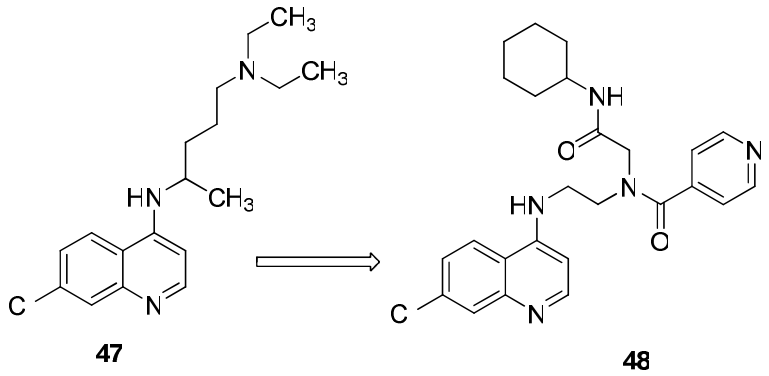
Scheme 1.17 $CuCl_2.2H_2O$ catalysed synthesis of dihydropyrimidones **46**

1.6.3 Antimalaria Drugs

The incorporation of the pharmacophore scaffold into the MCR backbone is done conceptually in two ways, either the incorporation of the pharmacophore element or scaffold is done through one of the starting materials or it is formed during the course of the MCR through the atom and bond skeletal rearrangement. The former process possesses obvious limitations regarding the number of starting materials available and the "weight" of the product library. Moreover, the pharmacophoric structure is only variable at one or a few connection points, whereas the *in situ* assembly of the pharmacophore during the MCR allows for many more variations all around the core structure and there

by leaving a possibility for the occurrence of scaffold hopping, a phenomenon wherein a change from one scaffold to another scaffold retains the biological potency.

Discovery of novel 4-aminoquinoline antimalarial drugs by using Ugi chemistry serves as an illustration for the former "ligand-introduced pharmacophore approach".[56] The authors synthesised an amine building block, 4-(3-aminopropyl)amino chloroquine, and produced a library of antimalarial compounds using the structure of chloroquine **47** as a template. Analogue of chloroquine, **48**, was found to be slightly less active than **47** in one of the three tested *P. falciparum* strains but with superior resistance index (Scheme 1.18).

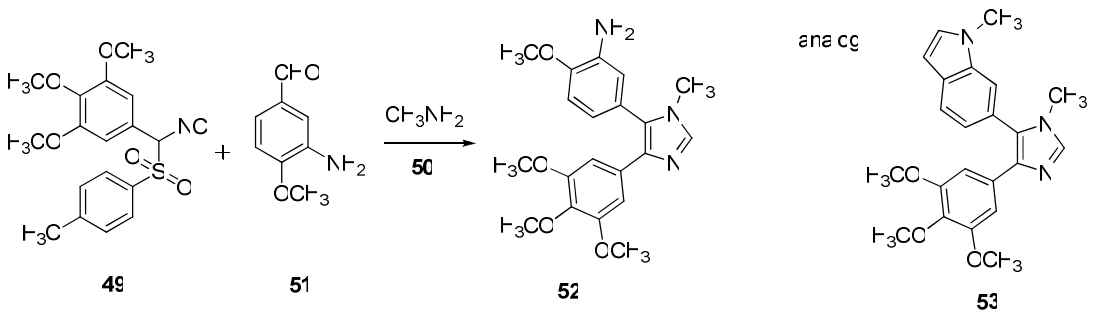


Scheme 1.18 Potent antimalarial drug discovery by incorporation of pharmacophore motifs of the marketed drugs.

The drug discovery by incorporation of pharmacophore motifs of the marketed drugs poses several advantages such as the introduction of an already known and validated pharmacophore precludes the need for the synthesis of large and costly libraries. In addition, the authors further described the possibility of combining two different modes-of-action in one molecule made by an MCR. Further, the emergence of drug resistance can also be overcome by these dual or multiple action molecules. Thus, this approach would be similar to a combination therapy, but combined in one drug. Finally economic production of drugs for diseases highly prevalent in the third world, such as malaria, is often possible by employing MCRs for the synthesis of drug molecules.

1.6.4 Tubulin Inhibitors

Exploration of orally available tubulin inhibitors with potential applications in cancer therapy has also been carried out using MCR techniques.[57] The imidazole MCR of van Leussen (vL-3CR) was used in this approach wherein, 1,4,5-trisubstituted imidazoles were synthesised by reacting α-substituted tosylmethyl isocyanides (TOSMIC) **49** with primary amines **50** and aldehydes **51** (Scheme 1.19).[58]



Scheme 1.19 Synthesis of tubulin inhibitors (**52** & **53**) using van Leussen MCR.

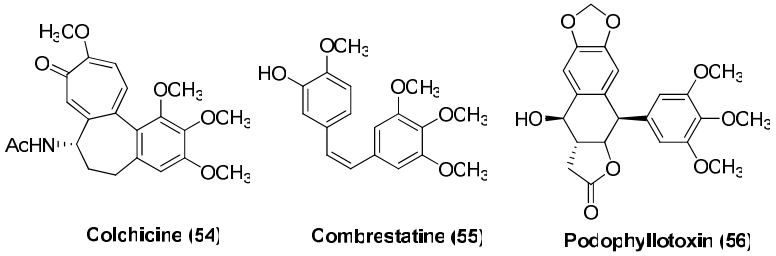


Figure 1.6 Natural tubulin inhibitors **54-56**

These novel tubulin inhibitors **52** and **53** with IC_{50} values of 170 and 32 nM in the cancer cell line NCI-H460 and oral bioavailabilities of 82 and 36%, respectively nicely fit into the pharmacophore model (Figure 1.6). Mice xenograft models further revealed remarkable oral efficacy of both these inhibitors **52** and **53** against the solid murine M5076 reticulum sarcoma cell line.

1.6.5 Phosphatase Inhibitors

A new class of specific PTP1B inhibitors, expected to enhance insulin sensitivity and act as therapeutics for the treatment of Type II diabetes, insulin resistance, and obesity were synthesised utilizing the Groebcke reaction (Scheme 1.20), a one-pot MCR wherein nonhydrolyzable phosphate mimetics were introduced via the benzaldehyde component (Figure 1.7). The inhibitor molecules synthesised using the above stated technique exhibited low micromolar activity and remarkable selectivity versus similar phophatases such as TCPTP, LAR, and CD45. The molecule **57,** an outcome of above technique, possessed an IC_{50} (PTP1B) of 700 nM, versus 44 μM (TCPTP), 118 μM (LAR), and 58 μM (CD45).[59]

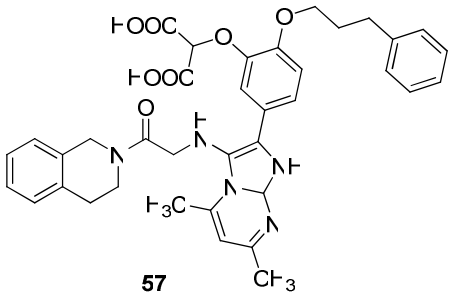
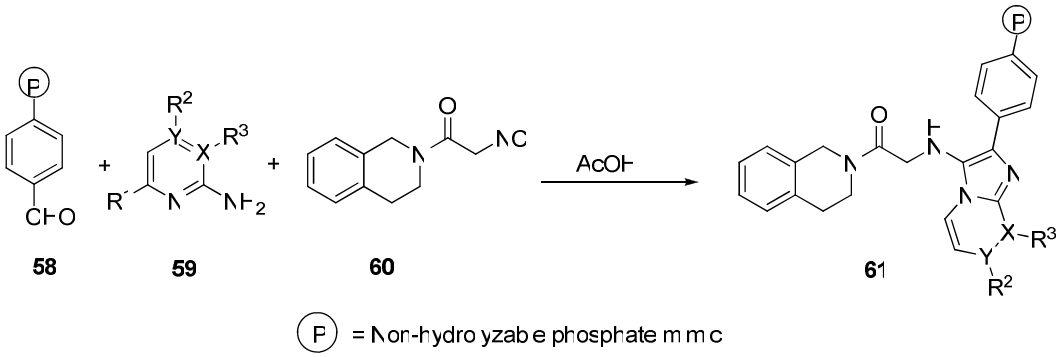


Figure 1.7 Novel PTP1b inhibitor synthesised using Groebcke MCR.



Scheme 1.20 Schematic diagram of Groebcke reaction

Glucose-6-phosphate translocase (G6PT), a multienzyme complex involving a phosphatase activity catalyses the hydrolysis of glucose-6-phosphate into glucose and phosphate as a final step in both glucose producing pathways in the liver namely, gluconeogenesis and glycogenolysis and hence emerged as a potential target useful in treatment of Type 2 diabetes.

Morphochem scientists described the use of a genetic algorithm driven discovery of potent G6PT inhibitors involving vL-3CR and other scaffolds (Figure 1.8).[60]

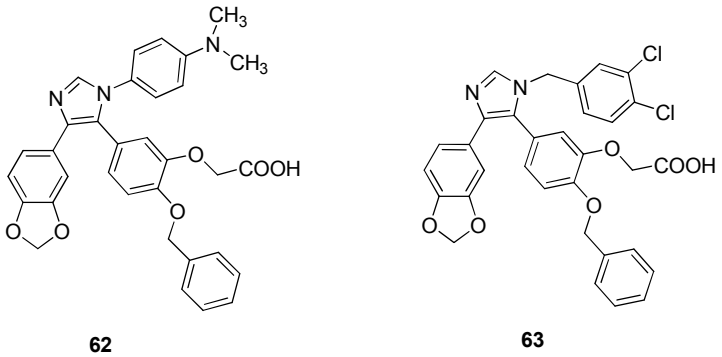


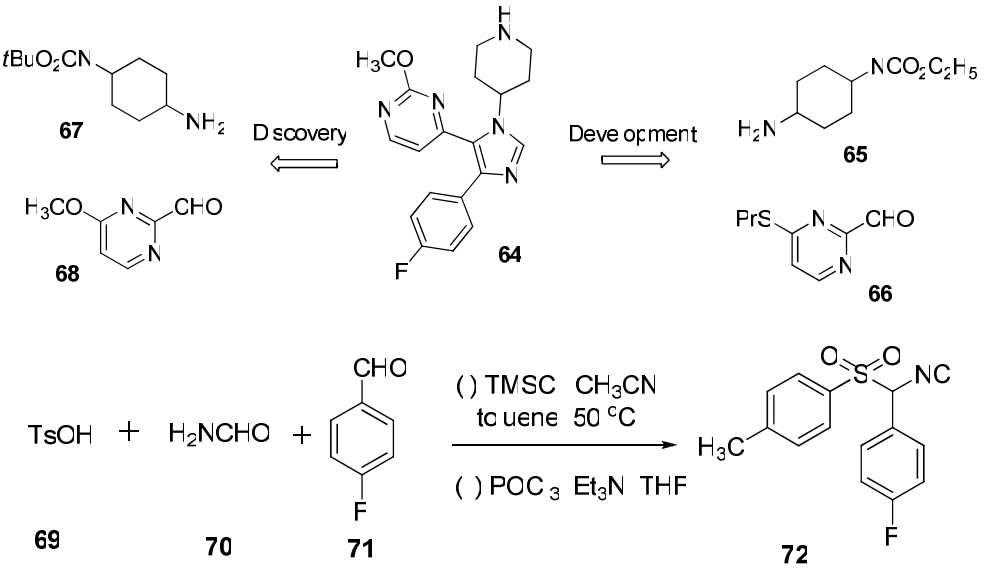
Figure 1.8 G6PT inhibitors **62** and **63** by vL-3CR chemistry

An array of 88 compounds was synthesised with four different aldehydes, 22 amines and one isonitrile. Activities below IC_{50}-10 μM were found among these imidazoles, most compounds being also selective against disrupted microsomes (IC_{50} disrupted > 200 μM), e.g. **62** and **63**.

1.6.6 Kinase Inhibitors

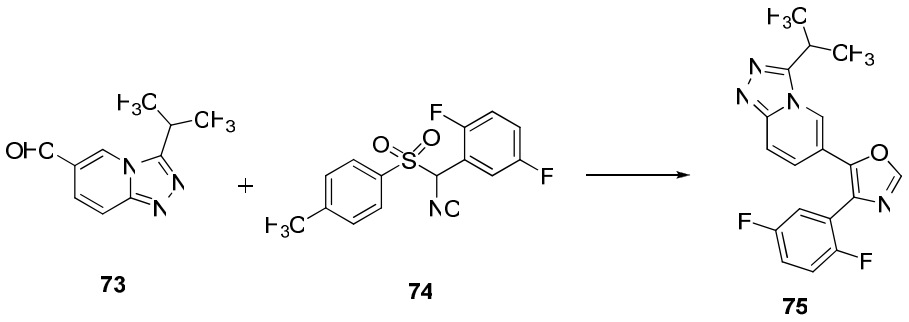
The molecules with p38 kinase inhibitory activity are potentially useful for treating inflammation, osteoarthritis, rheumatoid arthritis, cancer, reperfusion or ischemia in stroke or heart attack, autoimmune diseases, and other disorders. Several highly substituted imidazoles that have been investigated as potential therapies for the treatment of rheumatoid arthritis has spawned the need for a general synthetic method for their preparation on a multi-kilogram scale.

The vL-3CR approach has been adopted for the synthesis of p38 inhibitor molecule **64**, that was previously in phase III clinical trials (Scheme 1.21).[61]



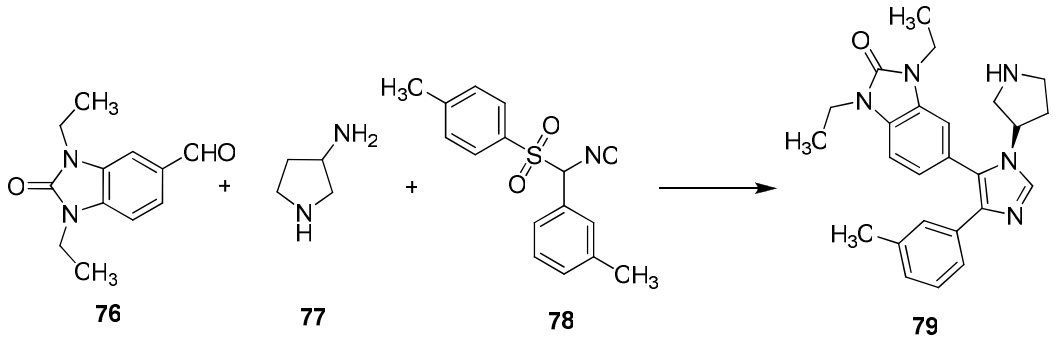
Scheme 1.21 Synthesis of imidazole **64** by vL 3CR approach

Synthesis of novel libraries of potent p38 kinase inhibitors utilized the oxazole variant of van Leussen TOSMIC chemistry, wherin TOSMIC derivative **74** reacts with aldehyde **73** under basic conditions to form an oxazole ring (Scheme 1.22). The resulting molecules had an IC_{50} < 10 μM in the TNFα and MAPKAP *in vitro* assays, and an EC_{50} < 50 mg/kg in the *in vivo* TNF-α assay.[62]



Scheme 1.22 Synthesis of p38 kinase inhibitor **75**

Pfizer chemists further disclosed van Leussen-type trisubstituted imidazoles, e.g. **79**, as MAP kinase inhibitors that can serve as potent antiinflammatory agents (Scheme 1.23).[63]



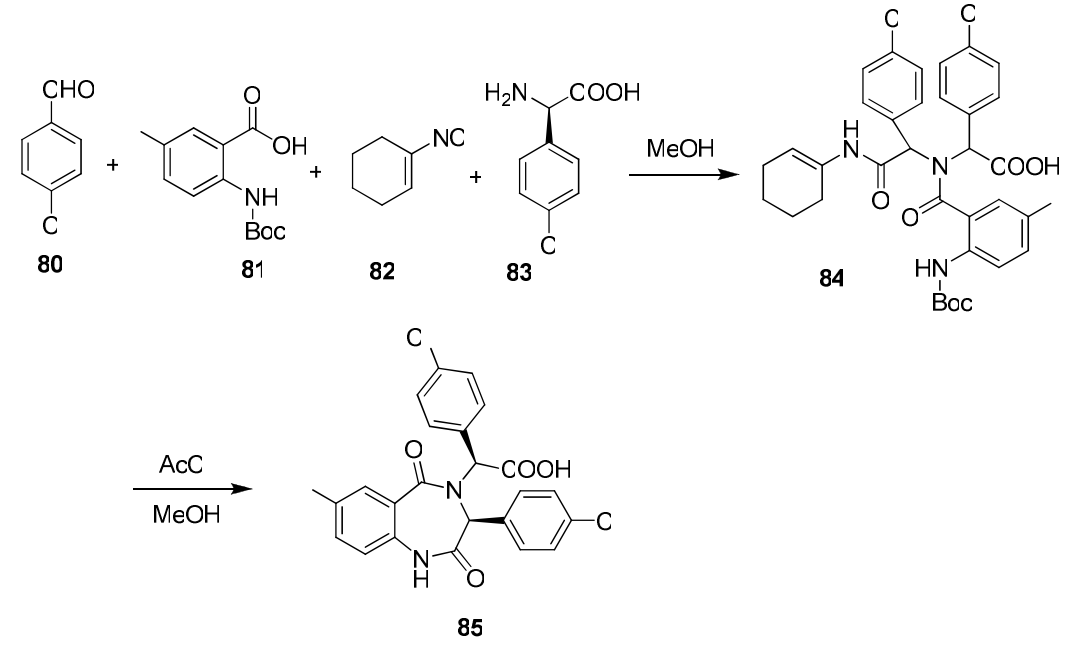
Scheme 1.23 A chiral MAP kinase inhibitor formed by vL-3CR

1.6.7 Protein-Protein Interaction Inhibitors by MCR

The failure of the p53 gene to function is implicated in 50% of the human cancer diseases. It was also reported that abnormalities in this gene are among the most common molecular events correlated with neoplasia. Through direct binding to the *N*-terminal transactivation domain of p53, the HDM2 oncogene product suppresses its transcriptional activity. The X-ray determined structure (PDB ID: 1YCR) also revealed the fact that a small piece of α-helix mediated the protein-protein interaction between HDM2 and p53. The crystal structure and mutational studies further explored the importance of the hydrophobic pockets occupied by Phe-19, Trp-23, and Leu- 26 of the p53 peptide.

The discovery and optimization of a series of 1,4-benzodiazepine-2,5-diones (BDPs) as potent antagonists of the HDM2-p53 interaction has been reported.[64] The highly effcient and versatile U-4CR technique has been employed in synthesising the library of designed benzodiazepines, wherein combination of anthranilic acid, an amine, an

aldehyde, and 1-isocyanocyclohexene was followed by acid catalysed cyclisation, thereby yielding the desired BDPs in good yield and purity. The authors synthesised twenty-two thousand BDPs using the above scheme and then screened in a fluorescence polarization (FP) peptide displacement assay. Compound **85** exhibited a binding constant (K_i) of 67 nM and also potent cell based activity (Scheme 1.24).



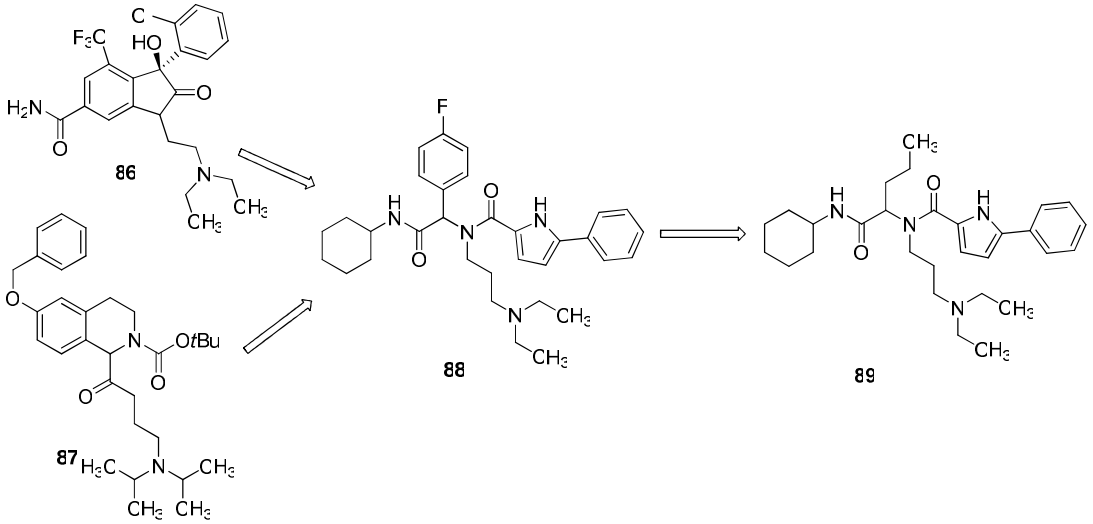
Scheme 1.24 Synthesis of benzodiazepine p53-HDM2 inhibitor **85**

1.6.8 GPCR Ligands

Ever since their discovery, G-protein coupled receptors (GPCRs) are the most prominent targets in the field of drug discovery and development. A refined analysis on the drug statistics indicate that around 200 out of 500 identified druggable biomolecules are formed from GPCRs. Due to the vagueness of information available in the field of GPCRs, High-throughput screening (HTS) poses an effective path for initiation of chemistry based research.

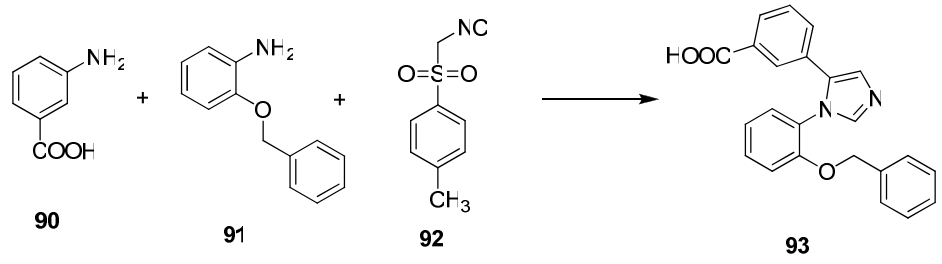
Two growth hormone secretagogue (GHS) agonists, **92** and **93,** were used as templates to computationally screen an in-house library using Tanimoto similarity (Scheme 1.25).[65] Out of 108 compounds selected from computational screening, five were found to be active in both cell based and direct binding assays in the low-micromolar range. The α-aminoacylamides **94** was taken as a template for preparing a small library of analogues rapidly. One-pot U-4CR was employed for synthesising 40 compounds, of which the

molecule **95** exhibited K_i of 0.22 μM. Thus, compounds that were structurally diverse and significantly different from known GHS agonists were developed. This approach depicts the value of MCR chemistry in rapid hit to lead conversion and a ready establishment of SAR. Moreover, this approach could be nicely translated to use the very large virtual MCR chemistry space to computationally and finally physically discover potent drug molecules.



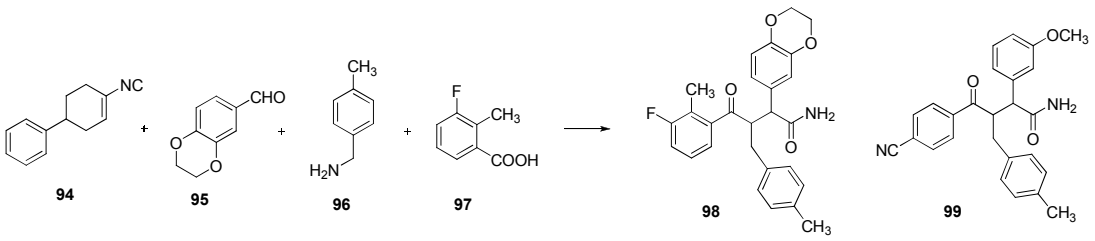
Scheme 1.25 Templates **86** & **87** used for carrying out a computational screening

Scientists from GSK employed vL3-CR of TOSMIC, 3-aminobenzoic acids, and 2-*O*-benzyl anilines for synthesising a library of compounds, eg. **93**, binding to the 7-transmembrane receptor EP_1, which is associated with pain, renal regulation, inflammation, gastric or enteric mucus secretion, allergic activities and smooth muscle contraction (Scheme 1.26). The screened compounds had an antagonist pIC_{50} value between 7.0 and 9.5 at EP_1 receptors while exhibiting a pIC_{50} value of <6.0 at EP_3 receptors.



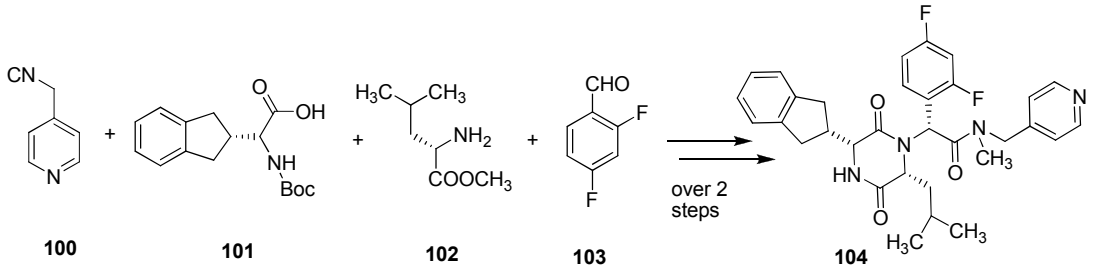
Scheme 1.26 Example of an EP_1 Inhibitor, imidazole **93,** synthesised by vL3-CR

Chemists from Pfizer reported the application of U-4CR for synthesis of potent oxytocin inhibitors with low nanomolar activity, e.g. **98** and **99,** where in 4-phenylcylohex-1-ene isocyanide (**94**) has been employed for synthesising large arrays of compounds. (Scheme 1.27).[66]



Scheme 1.27 Oxytocin agonists **98** & **99** synthesised by U-4CR.

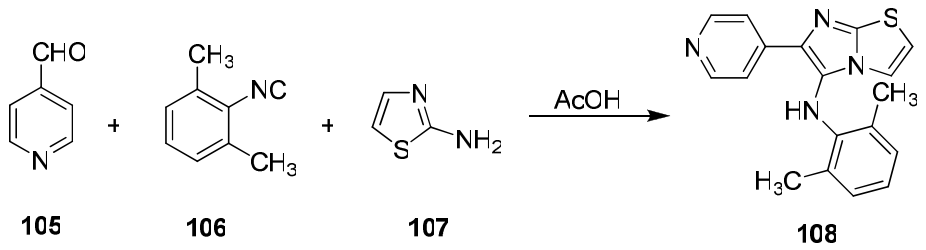
Another group of chemists from GSK employed U-4CR of 4-picolylisocyanide **100**, H-D-Leu-OMe HCl **102**, *N*-Boc-D-indanylglycine **101**, and aldehyde **103**, subsequent Boc deprotection and cyclisation (UDC) to yield piperazinedione **104** which is used for treatment or prevention of disease conditions mediated through oxytocin action (Scheme 1.28).[67]



Scheme 1.28 U-4CR in the synthesis of piperazinedione **104**

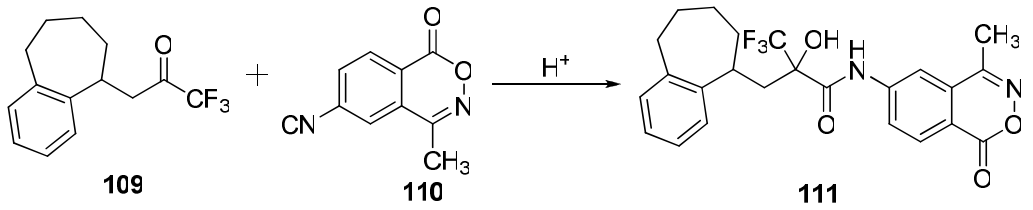
Liddle and coworkers[68] synthesised GSK221149 that has nanomolar affinity for the oxytocin receptor with >1400-fold selectivity over the closely related vasopressin receptors. GSK221149A has a good pharmacokinetic profile in rats, low human microsomal clearance and has been shown to inhibit Oxytocin-induced contraction *in vivo* in the anaesthetized rat. The synthesis was initiated using U-4CR.

Utilizing the catalytic properties of $HClO_4$, Groebcke chemistry has been applied to prepare libraries of bicyclic imidazopyridineamines and analogues e.g. **108** that were further discovered as potent analgesics (Scheme 1.29).[69]



Scheme 1.29 Synthesis of analgesic imidazopyridineamine **108**

Glaxo Chemists disclosed the synthesis of *N*-benzoxazinylpropanamides, e.g. **111**, as binders and agonists of glucocorticoid receptor and are used for the treatment of allergic, inflammatory, and skin diseases. Passerini reaction has been employed for the synthesis of α-hydroxy-α-trifluoromethylcarboxamide pharmacophore (Scheme 1.30).[70]



Scheme 1.30 Synthesis of glucocorticoid agonist *N*-benzoxazinylpropanamide **111**

A further application of U-4CR has been enclosed by Ono chemists to prepare nitrogen-containing heterocyclic derivatives like *N*-Butyl-*N*-[1-[4-[4-(methylsulfonylamino)-phenoxy]benzyl]piperidin-4-yl]cyclohexanecarboxamide hydrochloride **112**, that inhibited the human RANTES-induced temporary increase in cellular Ca^{2+} ion concentration with an IC_{50} of 0.077 μM in CHO cells stably expressing excess human CCR5 receptor(Figure 1.9).[71]

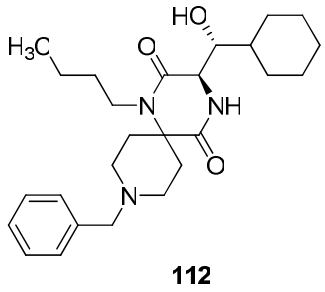
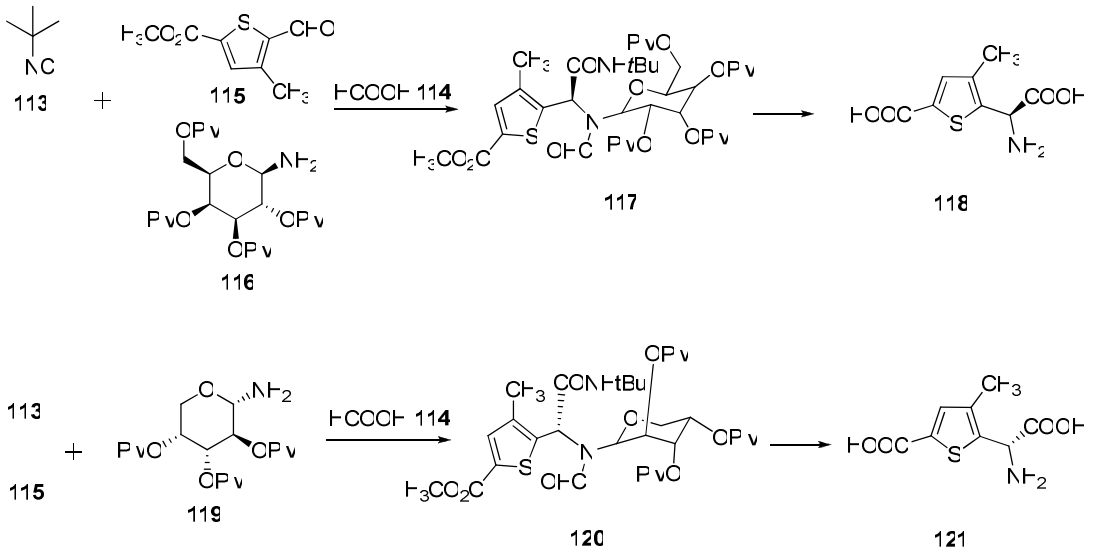


Figure 1.9 Preparation of CCR5 antagonist **112** by U-4CR

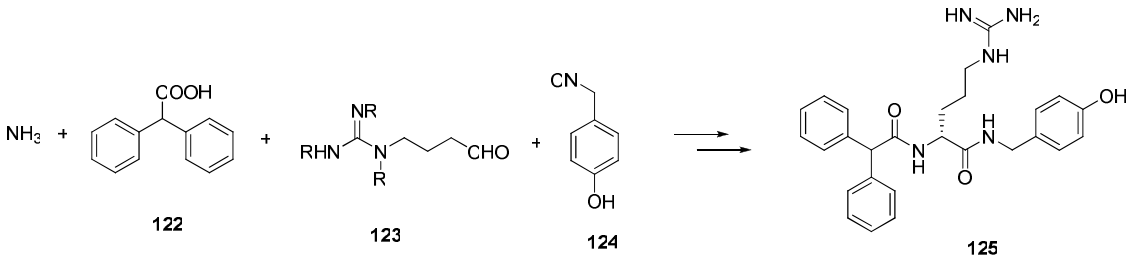
Type I GTP-binding metabotropic glutamate receptors (mGluR) are of great medicinal interest because of their involvement in processes leading to excitotoxic neuronal death after ischemia. Stereoselective synthesis and preliminary evaluation of (+)- and (-)-3-

methyl-5-carboxythien-2-ylglycine (3-MATIDA), **118** and **121**, and the subsequent identification of (+)-3-MATIDA, **121**, as a novel mGluR1 competitive antagonist were reported by Costantino et al. (Scheme 1.31) They have used chiral sugar based auxiliaries **116** and **119** for synthesisizing enantiomerically pure unnatural amino acids.[72]



Scheme 1.31 Enantioselective synthesis of novel mGluR1 antagonists (**118** & **121**)

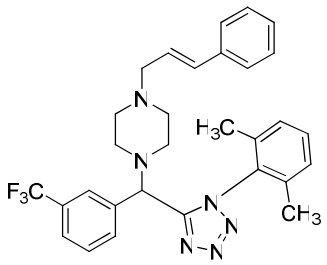
Arginine, involved in specific interactions of endogenous neuropeptides with their specific receptors, such as neuropeptide FF, neuropeptide Y, or neurotensine results in most non-peptidic receptor (ant-)agonists constitute the hydrogen donating network of a guanidine functional group. Guery et al., chose a U-4CR for rapidly synthesising a library of *N*-terminal and *C*-terminal arginine derivatives e.g BIBP 3226 **125**, that possesses a good affinity for NPFF receptors (Scheme 1.32).[73]



Scheme 1.32 U-4CR in the preparation of NPFF receptor agonist **125**

A library of about 10,000 tetrazole based compounds were developed by Amgen scientists, from which several compounds exhibited sub-micromolar level antagonist activity of the GPCR melaninconcentrating hormone 1 (MCH1), an important

obesity target. The compound **126** exhibited an acceptable pharmacokinetic profile (Figure 1.10).[74]



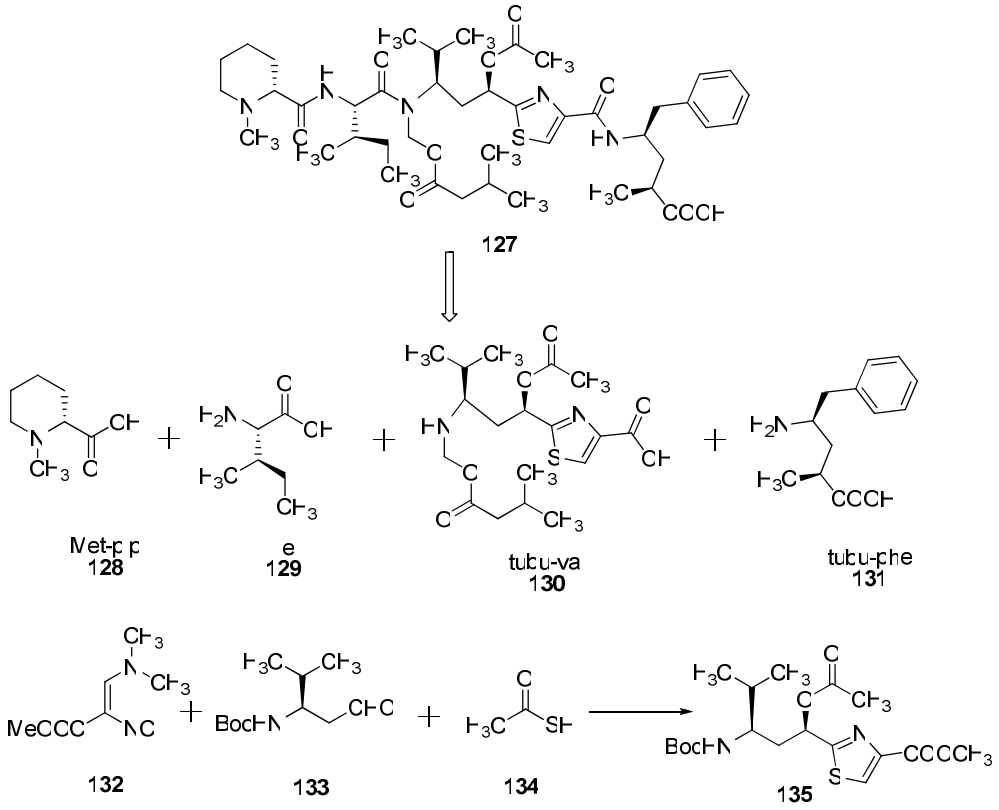
126

Figure 1.10 MCH1 receptor antagonist tetrazole **126**

1.7 MCRs in Natural Product Synthesis

1.7.1 Tubulysin

Tubulysin D, **127**, an extremly potent antiangiogenic tetrapeptide is composed of the unnatural amino acids *N*-methylpipecolinic acid **128**, natural Ile, **129**, tubuval **130** and tubuphe **131**.

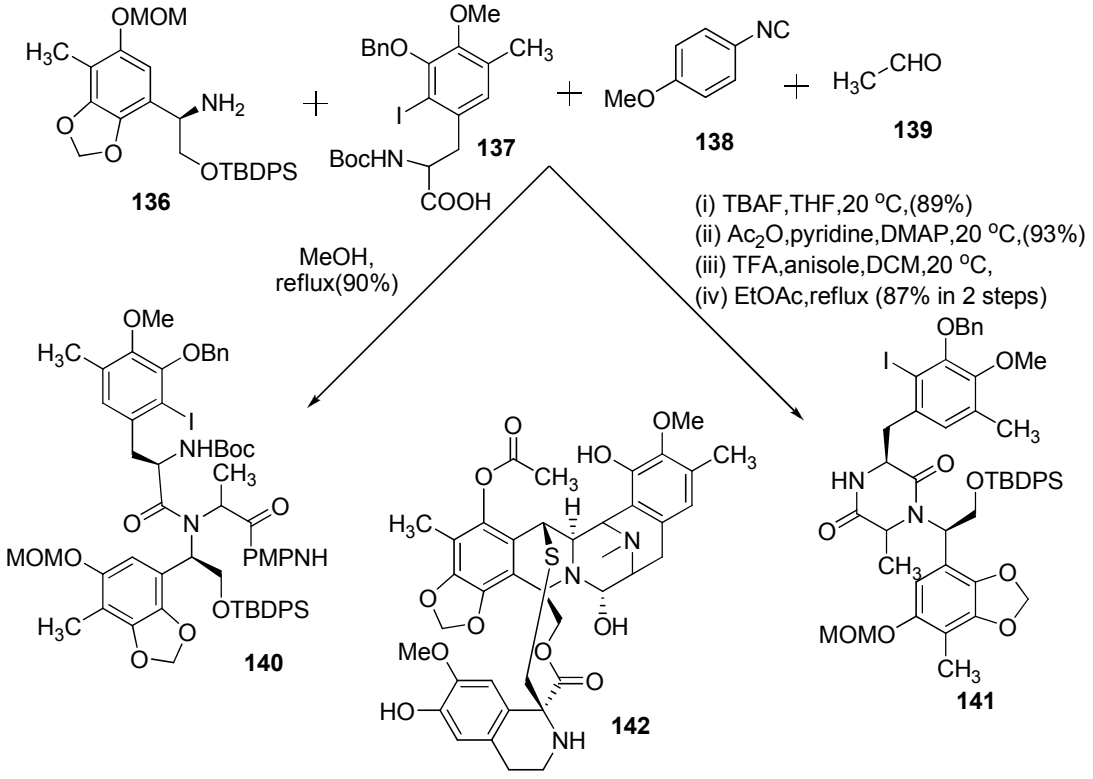


Scheme 1.33 MCR in the total synthesis of Tubulysin **127**

It was discovered in a fermentation broth of Myxobacteria by Hofle and Reichenbach. A convergent and stereoselective thiazole multicomponent reaction has been described for synthesising tubuval **130**, synthetically most demanding part of tubulysin (Scheme 1.33). The central core of tubulysin is amenable from its precursors, multifunctional isocyanide **132**, protected homo-Val carbaldehyde **133** and thioacetic acid **134** in a diasteromeric ratio of 3:1 favoring the desired diastereomer, **135** in one-step in 40% yield.

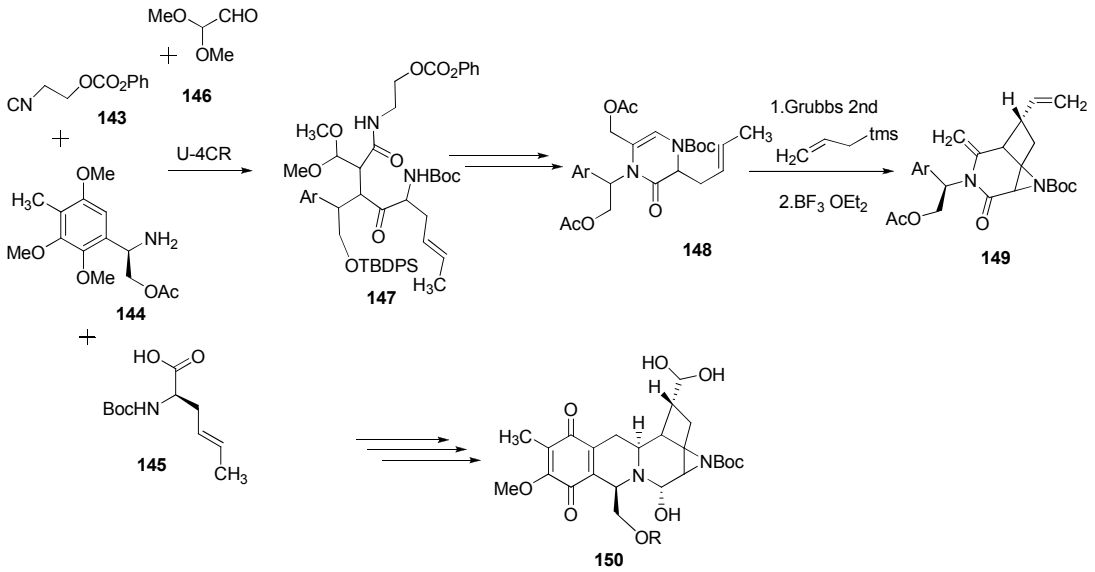
1.7.2 Ecteinascidine

A total synthesis of the antitumor antibiotic Ecteinascidine **142**, currently undergoing advanced clinical trials has been developed. The key reaction in this approach is the convergent assembly of several parts of this molecule into **140** that constitutes 66% of the atoms of the final molecule in a U-4CR (Scheme 1.34). Using the convertible *p*-methoxyphenylisocyanide **138** allows for the mild conversion into a diketopiperazine **141** in the subsequent steps.[75]



Scheme 1.34 U-4CR in total synthesis of the antitumor antibiotic Ecteinascidine **142**

1.7.3 (-)-Lemonomycin



Scheme 1.35 MCR in the total synthesis of (-)-Lemonomycin **150**

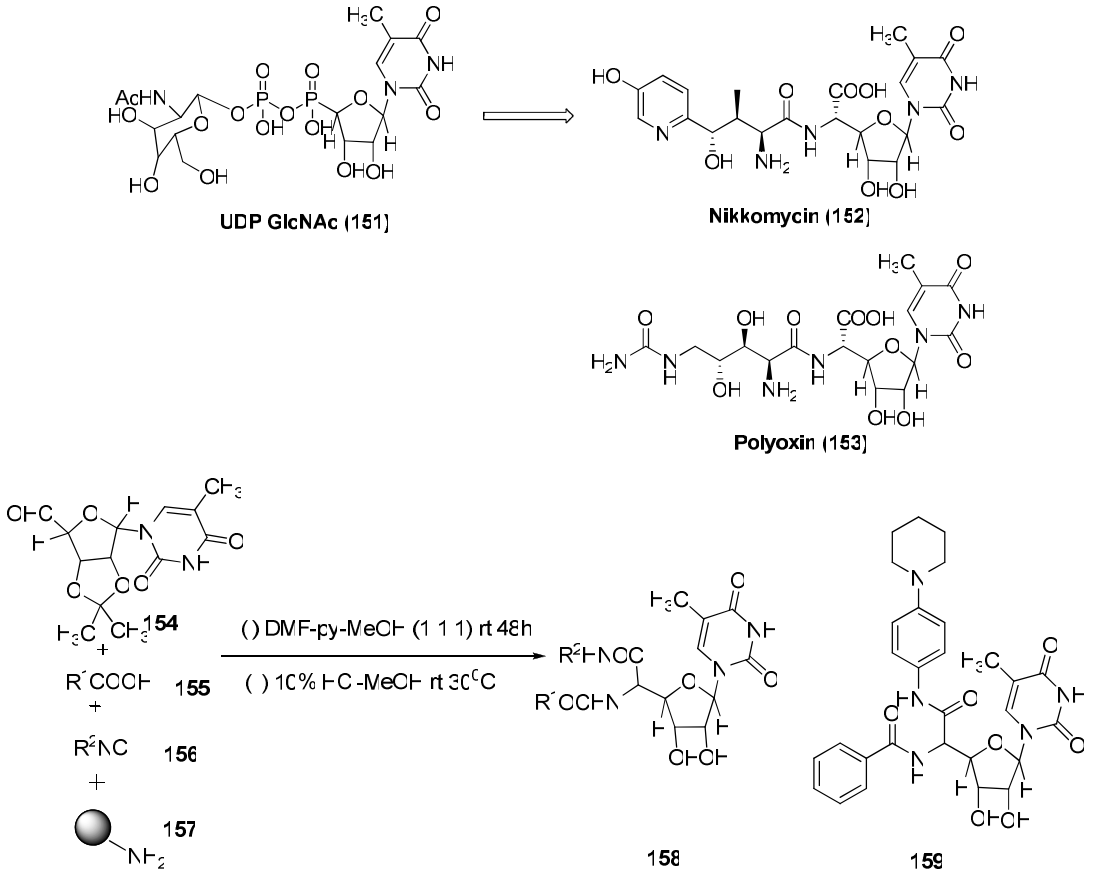
Fukuyama et al. performed synthetic studies toward the class of naphthyridin antibiotics using a U-4CR and a subsequent regioselective diketopiperazine formation.[76] A stereocontrolled construction of the 3,8-diazabicyclo[3.2.1] skeleton, an imminent part of the tetrahydroisoquinoline alkaloid Lemonomycin **150**, has been published (Scheme 1.35).[77]

Once more employing the efficient synthetic strategy for the synthesis of the tetrahydroisoquinoline alkaloids via the U-4CR, **144** was used as an amine component and converted to the ketopiperazine **148**. U-4CR of Ugi's convertible carbonate isocyanide **143**, amine **144**, amino acid **145**, and glyoxyaldehyde dimethylacetal **146** in trifluoroethanol was more suitable for an efficient preparation of **147** than using *p*-methoxyphenyl isocyanide. Thus, upon treatment of the amidocarbonate **147** with *t*-BuOK, the oxazolidinone formation proceeded smoothly with release of phenol to provide **148** after reduction and protection. Later cross-metathesis and Lewis acid catalysed cyclisation affords **149**, which can be further elaborated to yield the target natural product.

1.7.4 Nikkomycin

Nikkomycin **152** and Polyoxin **153,** naturally occurring peptidyl nucleoside antibiotics that target fungal chitin cell wall assembly have structural similarity with UDP-*N*-

acetylglucosamine **151**; a substrate for chitin synthases. This structural similarity is speculated to be the reason for their biological activity. Chemists from Roche prepared libraries of Nikkomycin analogues on solid support by U-4CR by reacting protected uracil aldehyde building block **154** with an array of isocyanides and carboxylic acids on Rink amide resin **157** and obtained after acidic deprotection and cleavage the screening compounds. A diverse library of 450 different derivatives were prepared and of them One derivative, **159**, showed activity comparable to Nikkomycin.[78]

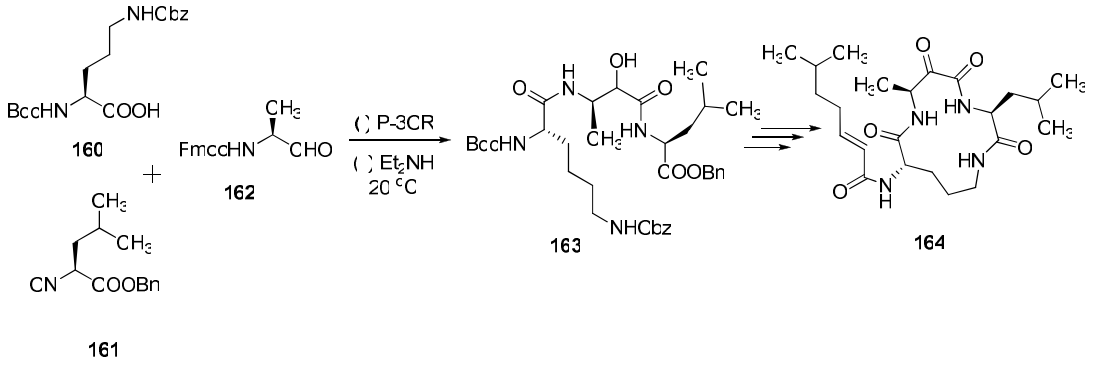


Scheme 1.36 Library synthesis of Nikkomycin analogues **158** & **159**

1.7.5 Eurystatin A

Eurystatins A and B, 13-membered macrocyclic natural products **164** isolated from *Streptomyces eurythermus* R353-21 are reported to be potent inhibitors of the serine protease prolyl endopeptidase (PEP). They feature leucine, ornithine, and α-ketoalaninamide subunits and they serve as attractive targets for the development of new α-hydroxy-*β*-amino amide and α-ketoamide methodologies due to their relative structural simplicity.

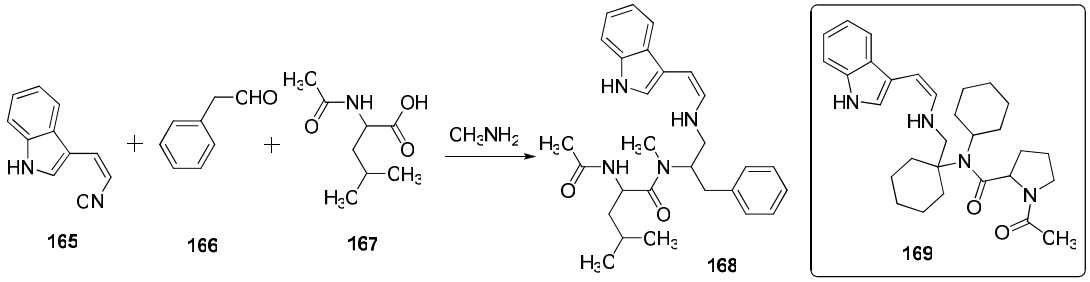
A concise total synthesis of the eurystatine, a peptidase inhibitor by employing the PADAM (Passerini reaction/deprotection/acyl migration) strategy as a key step has been developed (Scheme 1.37).[79] Here, reaction of orthogonally protected ornithine **160**, chiral Ile derived isocyanide **161**, and chiral Ala derived α-aminoaldehyde **162** involves a P-3CR. Base induced Fmoc deprotection and subsequent *O*,*N*-transacylation yielded **163** in 60% over the two steps.



Scheme 1.37 The key step involving MCR in Semple's synthesis of Eurystatine **164**

1.7.6 Aspergillamide

Aspergillamides are cytotoxic natural products isolated from *Aspergillus sp*. with unknown mode-of-action. A one-pot U-4CR protocol for total synthesis of Aspergillamide A by utilization of easily available starting materials has been developed and then employed for synthesis of a library of hundreds of Aspergillamide derivatives. (Scheme 1.38).[52] The mixture of *E*/*Z* isomers was separated by chromatography. A one-pot synthesis of Aspergillamide **168** can be achieved by U-4CR of isocyanide **165**, phenylacetaldehyde **166**, methylamine, and *N*-acetylleucine **167**. Several analogues of Aspergillamide, e.g. **169**, showed similar or better activity in cell based assays.



Scheme 1.38 A one-pot U-4CR in the synthesis of Aspergillamide A **168**

1.8 Conclusions and Present Work

The advent of several new techniques/technologies into the organic chemistry lab made the life of a chemist much simpler. Today's research sees a great scope for the role of MCRs in organic transformations. The MCRs demand for atom economic, waste minimized and labour free protocols makes them centerstage. It was demonstrated in many cases the breakthrough that MCRs can bring to the table of drug discovery. Though, initially not much focus was given to the MCRs, the interest upsurged with the various modifications on Ugi four component reactions. The diversity and complexity that can be achieved with simple starting materials can be understood by the role they play in total synthesis of natural products. Most of the putative drugs are delivered by the isocyanide based MCRs which are nothing but the ramifications of Ugi, Broecke, van Leussen and Passerini reactions. Several strategies were designed to invent new MCRs. The tandem MCR chemistry has evidenced one-pot reactions of up to eight components making products with several new bonds and points of diversity. The complexity and the chirality that can be generated in one-pot can be delineated to several steps in conventional synthesis. The literature witnesses the pioneering work carried out in the last decade employing MCR in drug discovery. The huge combinatorial libraries that can be generated with simple versatile starting materials provide an impetus to high throughput screening. MCR approach coupled with green chemistry would be an impeccable strategy.

The present work focuses on developing new protocols for the synthesis of various biologically potent heterocycles. Relentless efforts were invested to develop ecofriendly protocols that require simple workup, inexpensive solvents and easily available starting materials which are non-toxic and greener. The synthesised molecules were screened for various activities including Src kinase, antibacterial and anticonvulsant.

1.9 Conspectus of the Thesis

The thesis is segregated into eight chapters including a brief introduction to MCRs and conclusion of the work. The first chapter introduces the MCRs and clearly elaborates its classification schemes and its scope in drug discovery and natural product synthesis focusing on the capability of MCRs in diverse heterocyclic scaffold synthesis. A brief light was shed on the history of MCRs which concomitantly gives an idea of the named

reactions involving MCRs. The strategies for innovation of new MCRs and parameters that make the MCRs the choice of reactions are also outlined.

The second chapter describes the environmentally benign synthesis of 2-aminochromenes using the multicomponent approach in presence of nanosized MgO. The preparation of nanosized MgO and its employment in the synthesis of 2-aminochromenes under various solvent systems is reported. The characterization of nanosized MgO and its catalytic activity is studied in comparison to commercially available MgO.

The third chapter unveils a noteworthy protocol for the synthesis of 4*H*-pyran derivatives. The reaction involves mechanochemical mixing of three components in presence of commercially available MgO under solvent-free conditions affording the products in good yields. The antibacterial screening of the synthesised compounds is elaborately described in terms of the MIC and zone of inhibition.

The fourth chapter comprises the synthesis of an array of 1,2,3-triazole derivatives under Click conditions starting from α-bromo ketones / α-tosyloxy ketones and studies on their their Src kinase inhibitory activity. Various parameters followed in establishing this green protocol is described. The Src kinase inhibitory activity is discussed with the structure-activity relationship of the 1,2,3-triazoles on the basis of their IC_{50} values.

The chapter five deals with a facile, efficient and regioselective synthesis of 1,4-diaryl-1*H*-1,2,3-triazole derivatives. This multicomponent reaction is achieved from diaryliodonium salts in short time affording high yields of 1,4-diaryl-1,2,3-triazoles. Variety of salts were employed with differently substituted terminal acetylenes to yield 1,2,3-triazoles. This beguiling reaction encompasses the recyclability aspect of the reaction media.

The sixth chapter emphasizes on synthesis and *in vivo* anticonvulsant evaluation study of 2,5-disubstituted-1,3,4-thiadiazoles. The synthesis of these molecules was achieved in one-pot by the reaction of arylaldehyde, hydrazine and arylisothiocyanate followed by oxidative cyclisation. The anticonvulsant screening showed that some of the 1,3,4-thiadiazoles were moderately active and the structure-activity relationship studies revealed that the presence of bulky substituents on the C-5 aryl ring leads to diminished activity, whereas, bulky substituents on the C-2 arylamino ring elevates the activity.

The work described in this chapter reveals a facile and expeditious one-pot synthesis of 5-(2′-indolyl)thiazoles, analogues oxazolyl indole alkaloids. The reaction of thioamides with 3-tosyloxypentane-2,4-dione led to *in situ* formation of 5-acetylthiazole which upon treatment with arylhydrazines in polyphosphoric acid results in exclusive formation of 5-(2′-indolyl)thiazoles. A library of 5-(2′-indolyl)thiazoles was synthesised by using various thioamides and arylhydrazines in moderate yields. The advantages of the protocol include the use of readily available starting materials and simple experimentation.

The eighth chapter gives a comprehensive overview and concluding remarks of the thesis. The results of the work carried out and the future scope of the work is detailed.

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CHAPTER - II

Nanosized MgO Catalysed Synthesis of 2-Aminochromenes

The present chapter deals with the synthesis of 2-aminochromenes with nanosized magnesium oxide (MgO) as catalyst by the three-component condensation of aldehyde, malononitrile, and α-naphthol. The 2-aminochromenes possess significant biological activity as therapeutics and as agrochemicals. The nanosized MgO was successfully synthesised using precipitation technique from magnesium nitrate and liquid ammonia. The size and nature of the magnesium oxide was analysed using XRD, SEM and TEM and it was in the range of 100-200 nm. The catalytic activity of the synthesised nanosized MgO and commercially available MgO was compared and found higher yields and shorter reaction time with the former. Among various solvents, aqueous PEG-400 was found be the solvent of choice for the rapid formation of 2-aminochromenes at room temperature.The greener protocol was found to be fairly general and the catalyst was reused in subsequent reactions with consistent activity.

Chapter 2

2.1 Introduction

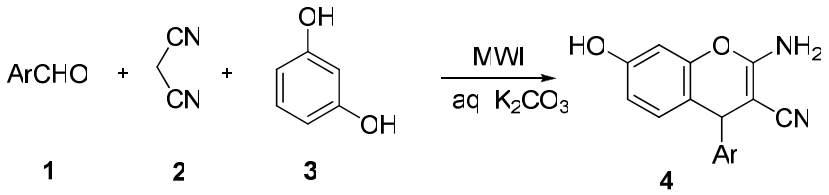
Organic transformations involving benign reaction media are of considerable interest in synthesis; especially multicomponent reactions (MCR) in which two or more steps are completed without isolation of any intermediate.[1-4] The use of many toxic and volatile organic solvents, particularly chlorinated hydrocarbons, as reaction media contributes pollution to the environment, and it is highly desirable to develop environmentally benign processes that can be conducted in green solvents. The low cost of water and polyethylene glycol (PEG), along with their non-toxic nature, renders them as attractive media for chemical synthesis and transformations. Moreover, in many cases these solvents can be recycled and reused to alleviate the problem of solvent disposal. In general, PEG is non-toxic, being used in cosmetics and food products, is potentially recyclable and is water-miscible, which facilitates its removal from reaction products.[5] Reactions in aqueous media offer many advantages such as simple operation and high efficiency in many organic reactions that involve water soluble substrates and reagents.[1-4] Further, the potential for cost savings is tremendous from the point of raw material, energy, cost, and time, by using MCR approach. These advantages become even more attractive if such reactions can be conducted using heterogeneous recyclable catalysts.

Heterogeneous catalysts are advantageous over conventional homogeneous catalysts as they can be easily recovered from the reaction mixture by simple filtration and can be reused after activation, thereby making the process economically viable. Among the heterogeneous basic catalysts, magnesium oxide is a versatile material used as catalyst for several base-catalysed organic transformations,[6] toxic waste remediation, and as additive in refractory, paint, and superconductor products.[7-9] It is a stable, non-volatile, non-hygroscopic, odourless, and white crystalline solid. Further, nanosized particles are considered to be particularly attractive as catalysts for their high reactivity, due to larger surface area.[7-9] They can be easily recovered from the reaction mixture and recycled after activation, thereby rendering the process economically viable.

2.1.1 Biological Significance of 2-Aminochromenes

The 2-aminochromenes are widely employed as pigments, cosmetics, potential agrochemicals, and represent an important class of chemical entities being the main constituents of many natural products.[10-14]

A set of 2-aminochromenes showed *in vitro* antibacterial activity against standard reference strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923), and their MICs ranged between 64 and 128 μg/mL. The synthesis of these compounds was achieved using K_2CO_3 as a green catalyst in water under microwave irradiation.



Scheme 2.1 Synthesis of 2-amino-4*H*-chromene derivatives in aqueous medium

The antioxidant activity of a series of indolyl chromenes has been described by Shanthi and coworkers.[15] All of the compounds exhibited excellent free radical scavenging activity (upto 99%) and found to be more potent than the standard, 2-tert-butyl-4-methoxyphenol (84%) and 2,6-ditert-butyl-4-methoxyphenol (83%). The compounds **5** and **6** exhibited 99% and 94% activity respectively (Figure 2.1). The synthesis of these compounds was achieved by the one-pot three-component reaction of salicylaldehyde, malononitrile and indole, catalysed by L-proline in water.

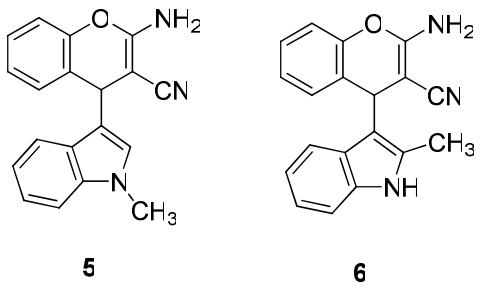


Figure 2.1 Antioxidant indolyl chromenes **5** and **6**

A series of 4-aryl-4*H*-chromenes have induced apoptosis in cell- and caspase-based HTS assay. SAR studies of the 4-position of 4*H*-chromenes showed that trisubstituted phenyl and pyridyl were the preferred groups. Compounds **7** and **8** were about as potent as or slightly more potent than Colchicine, Vinblastin, and Paclitaxel in the caspase activation assay (Figure 2.2). These 4-aryl-4*H*-chromenes, **7** and **8,** were found to inhibit tubulin polymerisation, which might be the main mechanism of action of this series of apoptosis

inducers. Compound **7** retains activity in cells resistant toward current antimitotic agents, taxanes and vinca alkaloids.

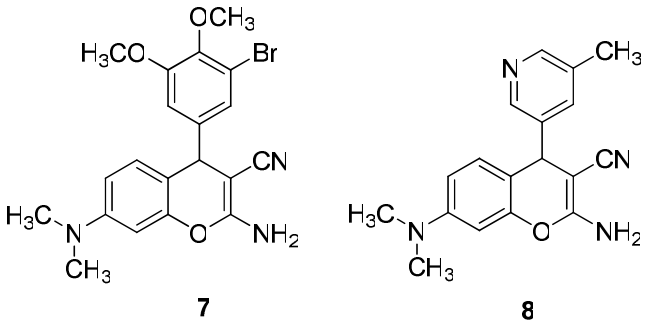


Figure 2.2 Apoptosis inducing 4-Aryl-4*H*-chromenes **7** and **8**

Two novel chromene dimers were evaluated for the activity on *Aspergillus* spp. growth and on ochratoxin A production.[16] The results of the bioassays indicate that the chromene dimer **9** inhibited mycelia growth by approximately 50% (EC_{50}) at 140.1 µmol/L for *A. niger*, 384.2 µmol/L for *A. carbonarius*, 69.1 µmol/L for *A. alliaceus* and 559.1 µmol/L for *A. ochraceus* (Figure 2.3). When applied at concentrations of 2 mmol/L, **9** totally inhibited the growth of all *Aspergillus* spp. tested. Furthermore, Ochratoxin A production by *A. alliaceus* was reduced by about 94% with a 200 µmol/L solution of this compound. A moderate inhibitory effect was observed for the analogous structure **10** on Ochratoxin A production but not in mycelia growth.

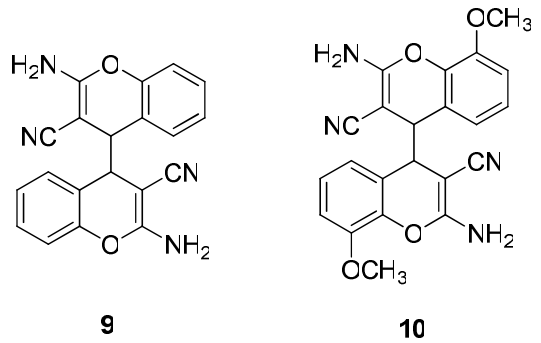


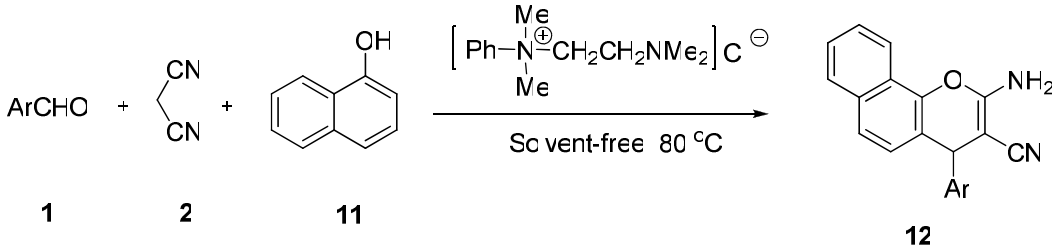
Figure 2.3 Chromene dimers **9** and **10** as Aspergillus growth inbhitors

2.1.2 Reported Protocols for the Synthesis of 2-Aminochromenes

Thus the synthesis of 2-aminochromenes is of much importance to organic chemists. The most straightforward synthesis of this heterocyclic nucleus involves the MCR of aldehyde, malononitrile, and an activated phenol in the presence of piperidine using

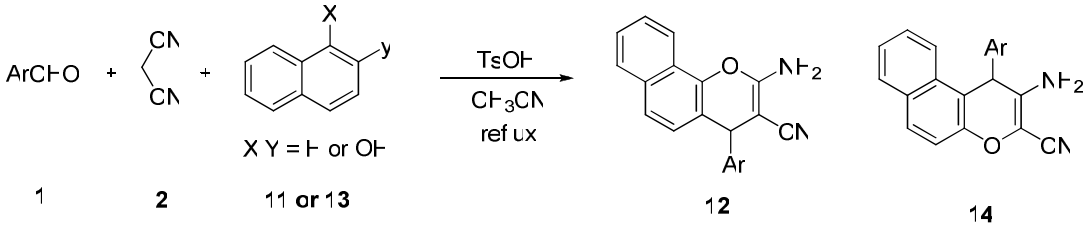
acetonitrile or ethanol as a reaction solvent.[17-19] Recently, relatively benign reagents such as cetyltrimethylammonium chloride and basic alumina in water have also been used.[20] The following are some of the reported protocols for the synthesis of 2-aminochromenes.

Lu Chen and coworkers[21] described a simple, clean, and environmentally benign three-component process for the synthesis of 2-amino-4*H*-chromenes **12** using a basic ionic liquid, N,N-dimethylaminoethylbenzyldimethylammoniumchloride, as an efficient catalyst under solvent-free conditions. A wide variety of aromatic aldehydes **1** were employed with α-naphthol **11** and malononitrile **2** under solvent-free condition to afford the desired products in good purity and yield (Scheme 2.2). Taking into account environmental and economical considerations, the protocol has the merits of environmentally benign, simple operation, convenient work-up and good yields.



Scheme 2.2 One-pot synthesis of 2-aminochromenes **12** in ionic liquid

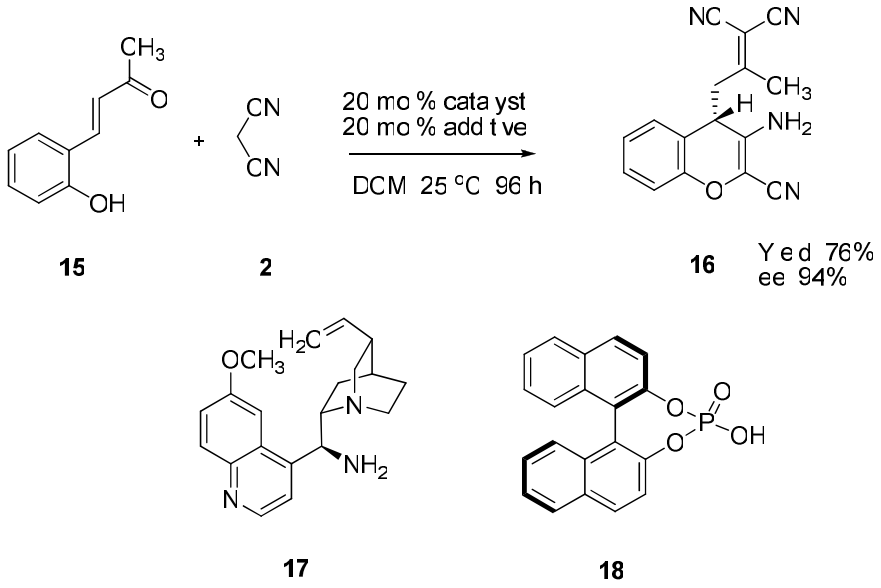
Baghernejad and coworkers[22] employed *p*-toluenesulfonic acid (TsOH) as a facile catalyst for the synthesis of 2-amino-4*H*-chromenes. The three component reaction involved refluxing aromatic aldehyde, malononitrile and α-naphthol/β-naphthol in acetonitrile in presence of TsOH as a catalyst to afford 2-aminochromenes in excellent yields (Scheme 2.3).



Scheme 2.3 TsOH catalysed one-pot synthesis of 2-aminochromenes **12** or **14**

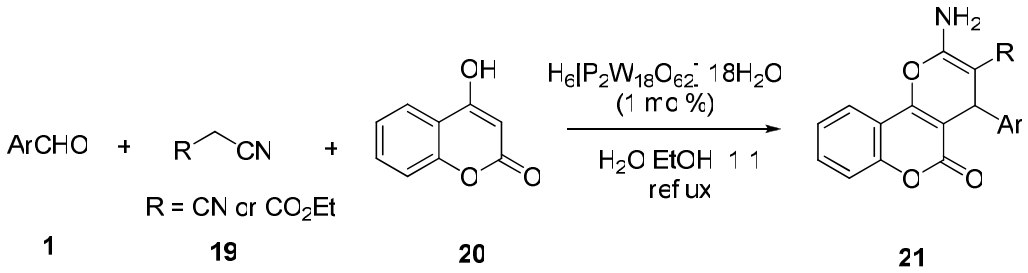
A research group[23] at Zhejiang Normal University reported for the first time the asymmetric synthesis of functionalised 2-aminochromenes **16** with high

enantioselectivities via one-pot tandem reactions of functionlised α,β-unsaturated ketones **15** with malononitrile **2** catalysed by 9-amino-9-deoxyepiquinine **17** in combination with (R)-1,1'-binaphth-2,2'-diylhydrogen phosphate **18.**



Scheme 2.4 Synthesis of optically active 2-aminochromenes **16**

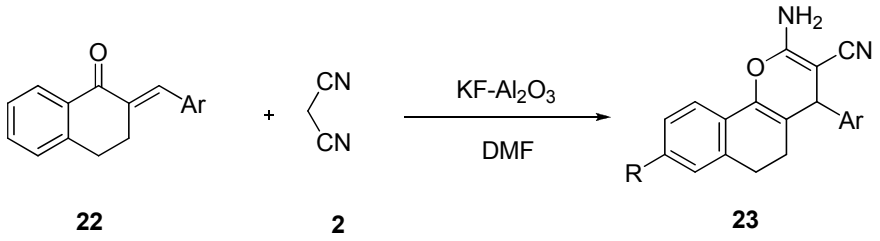
An elegant protocol was demonstrated[24] for the synthesis of substituted pyrano[3,2-c]chromene-5-ones **21** using $H_6P_2W_{18}O_{62}.18H_2O$, which proceeds efficiently in aqueous ethanol under heating conditions. Also the use of green, non toxic, inexpensive and reusable catalyst, $H_6P_2W_{18}O_{62}.18H_2O$, renders this method ecofriendly, with a very simple isolation procedure that entails the filtration of the precipitated products.



Scheme 2.5 Heteropolyacid catalysed synthesis of 2-aminochromenes **21**

Wang and his coworkers described[25] the synthesis of a series of new 2-aminochromenes **23** by the reaction of malononitrile with 2-arylmethylidene-3,4-dihydro-1(2*H*)-

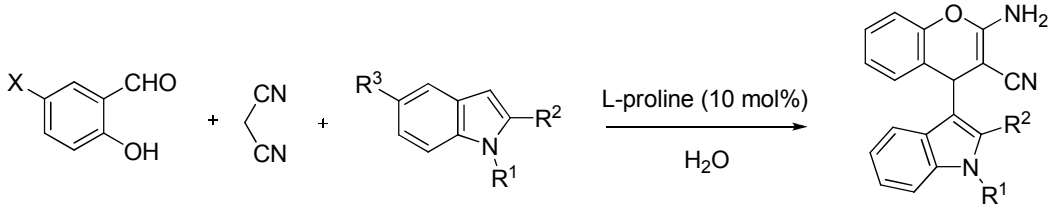
naphthalenone **22** in *N,N*-dimethylformamide (DMF) at 80°C catalysed by KF-Al_2O_3 (Scheme 2.6).



22 **2** **23**

Scheme 2.6 Ketjenfine (KF)-Al_2O_3 catalysed synthesis of 2-aminochromenes **23**

The synthesis of a series of indolyl 2-aminochromenes **26** was demonstrated by the one-pot three-component reaction of substituted salicylaldehyde **24**, malononitrile **2**, and substituted indole **25** catalysed by L-proline in water.

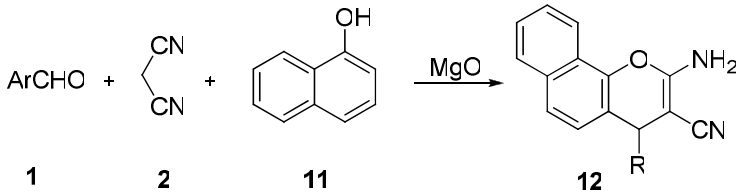


24 **2** **25** **26**

Scheme 2.7 L-proline catalysed synthesis indolyl 2-aminochromenes **26**

2.2 The Present Study

Most of the reported methods require expensive catalysts, prolonged reaction time, reagents in stoichiometric amount, toxic solvents, and generate moderate yields of the product. We report herein the synthesis of 2-aminochromenes **12** with nanosized magnesium oxide that efficiently catalyses three-component reaction of aromatic aldehyde **1**, malononitrile **2**, and α-naphthol **11** in methanol, water, or PEG–water (Scheme 2.8).



1 **2** **11** **12**

Scheme 2.8 MgO catalysed one-pot synthesis of 2-aminochromenes **12**

2.3 Results and Discussion

2.3.1 Chemistry

A simple and high yielding protocol is described for the synthesis of 2-aminochromenes involving the three-component, one-pot reaction of aldehyde, malononitrile, and α-naphthol using nanosized magnesium oxide as a novel and ecofriendly heterogeneous catalyst. The MgO catalyst is generally prepared by the decomposition of various magnesium salts or magnesium hydroxide ($Mg(OH)_2$, brucite). However, the MgO formed by this method usually exhibits relatively large grain sizes, inhomogeneous morphologies, and small surface areas. These structural and textural features limit its application as a catalyst. Several novel methods such as controlled precipitation, sol–gel route, sol–gel followed by hypercritical drying, amorphous citrate method, and preparation under hydrothermal conditions, have been reported in literature for preparation of nanosized MgO particles.[26-28] These methods are highly advantageous in terms of the crystallite size and shape, surface area, and surface basic characteristics of the synthesised MgO particles. The particle size and surface morphology of synthesised MgO by wet chemical procedure depend upon several factors such as the rate of hydrolysis of magnesium salts, temperature, type of base, concentration of the salt, and drying and calcination steps. Proper choice of these parameters can lead to particles of uniform morphology and size. In this study, we have prepared nanosized MgO catalyst and used it successfully in the synthesis of 2-aminochromenes. The XRD pattern of the synthesised MgO particles in the 2θ range of 30–65^o is shown in Figure 2.4. Broad and intense peaks were observed at 2θ values of 36.8, 42.7 and 62.1^o, corresponding to the d values of 2.43, 2.11, and 1.49 A°, respectively. These peaks indicate the presence of face centered cubic structure of the synthesised MgO particles.

The scanning electron micrograph (SEM) of the synthesised MgO particles is shown in Figure 2.5a. Small agglomerated particles of disordered surface morphology are observed in the SEM picture of the MgO samples. The transmission electron micrograph (TEM) of the MgO particles is shown in Figure 2.5b. The particle size of the MgO sample is typically in the range of 100–200 nm. The small crystallites are irregular in shape and are attached to each other along the grain boundaries. Similar particle sizes have been reported earlier for MgO particles prepared by hydrolysis of $Mg(NO_3)_2.6H_2O$ using aqueous ammonia solution under hydrothermal conditions.

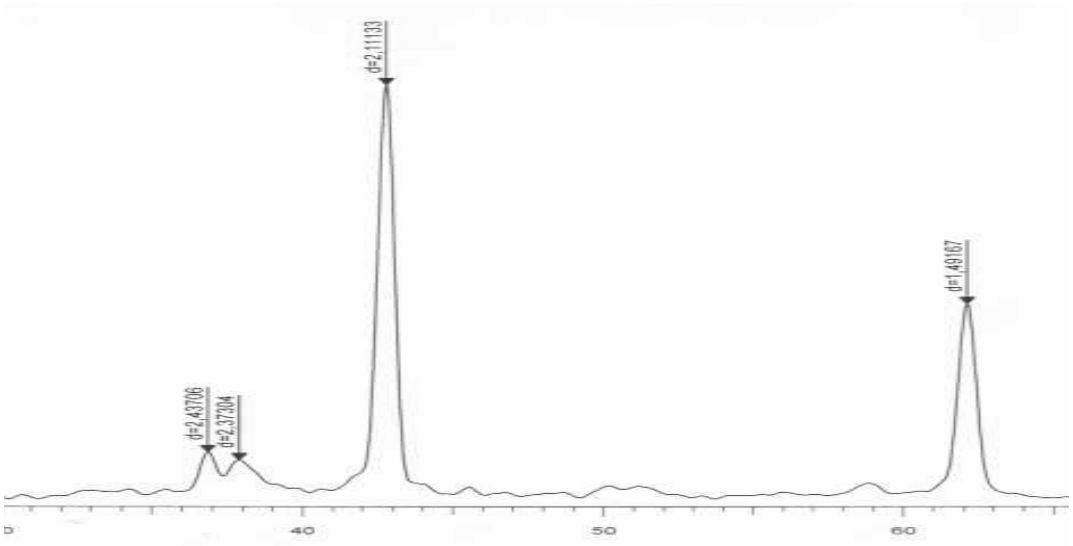
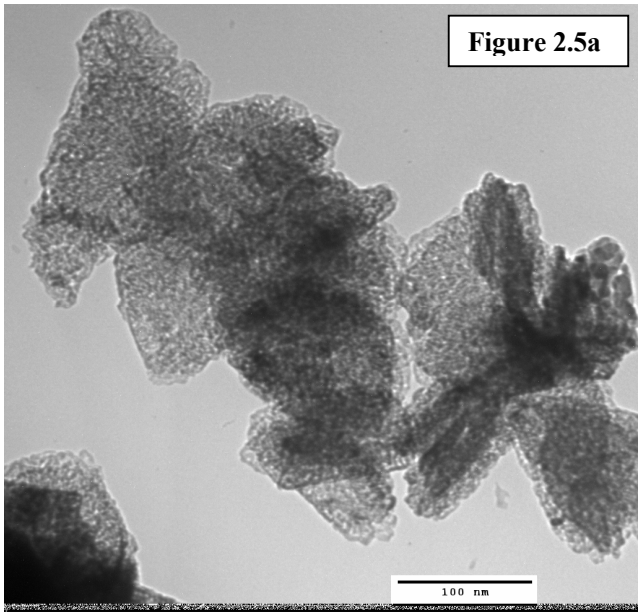


Figure 2.4 X-ray diffraction pattern of the synthesised MgO samples

However, unlike in the earlier case, lamellar structure was not observed for the particles synthesised by this method. This is probably due to the difference in the calcination procedure used for the preparation of these materials. Ding et al. have used stepwise calcination with special precautions to retain the lamellar structure of the intervening brucite phase.[26] In the present study, the temperature was linearly raised to 500 ^{o}C and maintained at that temperature for 3 h. Under the prevailing condition, it is likely that the lamellar structure of the intervening brucite phase was destroyed as it is being transformed to MgO phase.

The treatment of benzaldehyde, malononitrile, and α-naphthol in presence of magnesium oxide in methanol under refluxing conditions resulted in the formation of 2-aminochromene in 96% yield (Table 2.1, Compd **12a**). Under similar reaction condition, various substituted 2-aminochromenes were obtained (Table 2.1, Compds **12b–f**). The catalytic activity of the synthesised nanosized MgO particles was compared with the commercially available MgO catalyst. The reactions were carried out in methanol for both the catalysts for 1 h at 40 ^{o}C. The products were collected periodically at a time interval of 5 min and analysed by gas chromatography. Figure 2.6 shows the variation of the percentage conversion of benzaldehyde to the product in the reaction mixture with time. It was observed that the initial activity of the nanosized MgO was much faster as compared to the commercial MgO catalyst. After 30 min of the reaction, the nanosized

MgO shows 55% conversion of the benzaldehyde to the product as compared to the 35% observed in case of commercial MgO catalyst.



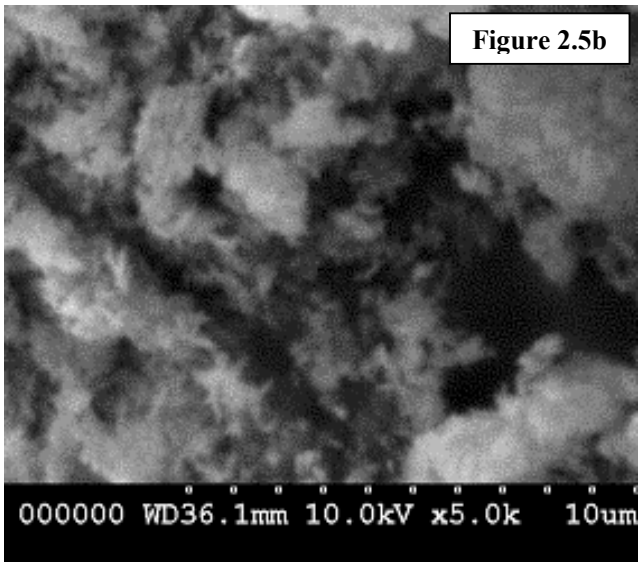
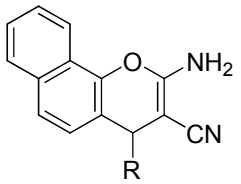


Figure 2.5 (a) Transmission electron micrograph
(b) Scanning electron micrograph and of the synthesised MgO

In this study, the initial activity clearly indicates that the particle size in nanoregime helps to expedite the reaction. This is in agreement with a recent report where an increase in the initial activity was observed in case of nanoparticles as compared to the bulk catalyst.[29] In order to examine the solvent effect and in our quest for the deployment of a benign reaction medium, the three-component condensation was explored in water. The

nanosized magnesium oxide catalysed condensation of three-component reaction proceeded smoothly in water under refluxing condition while affording substituted 2-aminochromenes in moderate yield (Table 2.1, Compds **12a–f**). The three-component condensation in water required relatively longer reaction time and afforded moderate yields of the product, probably because of the poor solubility of the reactants in comparison with methanol. After the completion of reaction in water, the magnesium oxide was recovered by extraction of the organic compounds followed by filtration of the aqueous mixture.

Table 2.1 Synthesis of 2-aminochromenes **12** in methanol and water



12a - f

Compd	R	Yield (%)[a]		M.p. (^{o}C)
		Methanol	Water	
12a	C_6H_5	96	86	178-180
12b	4-OMeC_6H_4	95	85	176-179
12c	3-$NO_2C_6H_4$	96	92	208-211
12d	4-NO_2 C_6H_4	97	93	231-234
12e	4-Cl C_6H_4	89	86	230-232
12f	2-Furyl	87	84	169-172

[a] Yield refers to the pure isolated products.

The initial formation of benzylidenemalononitrile by the Knoevenagel addition of malononitrile to the aldehyde has been reported without catalysis in polar protic solvent.[30] In view of the importance of polar protic solvent in the formation of benzylidenemalononitrile and substrate solubility, the reaction was conducted in PEG 400. We noticed that the MgO catalysed condensation of benzaldehyde, malononitrile, and α-naphthol in PEG also required the same reaction time without any appreciable improvement in reaction yield. Because of the poor solubility of aldehyde, malononitrile, and α-naphthol in water at room temperature, we decided to explore three-component condensation in a mixture of PEG-400 and water.

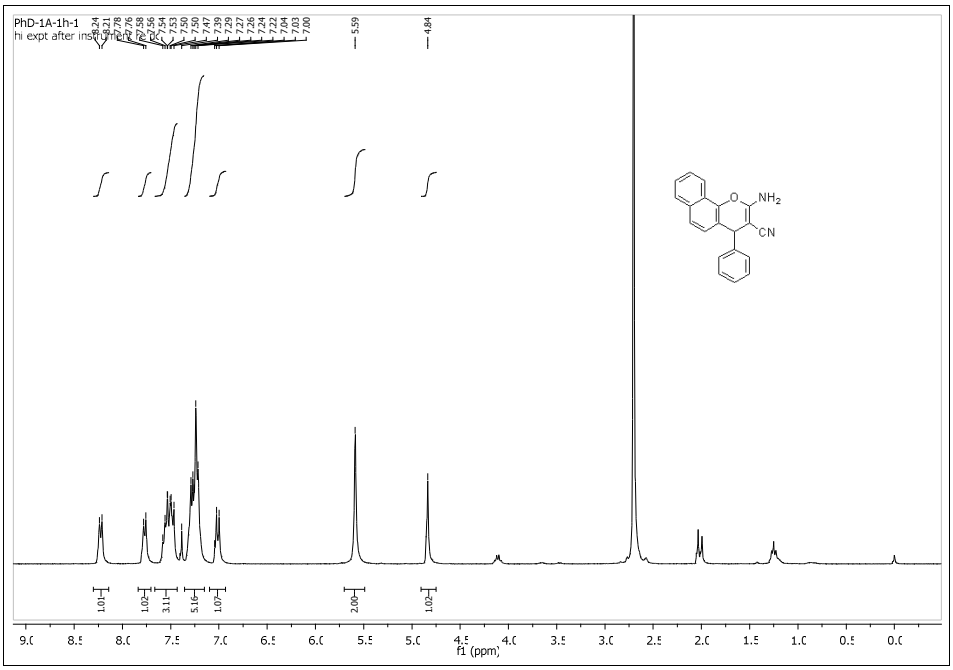


Figure 2.6 ^{1}H NMR spectrum of of 2-aminochromene **12a**

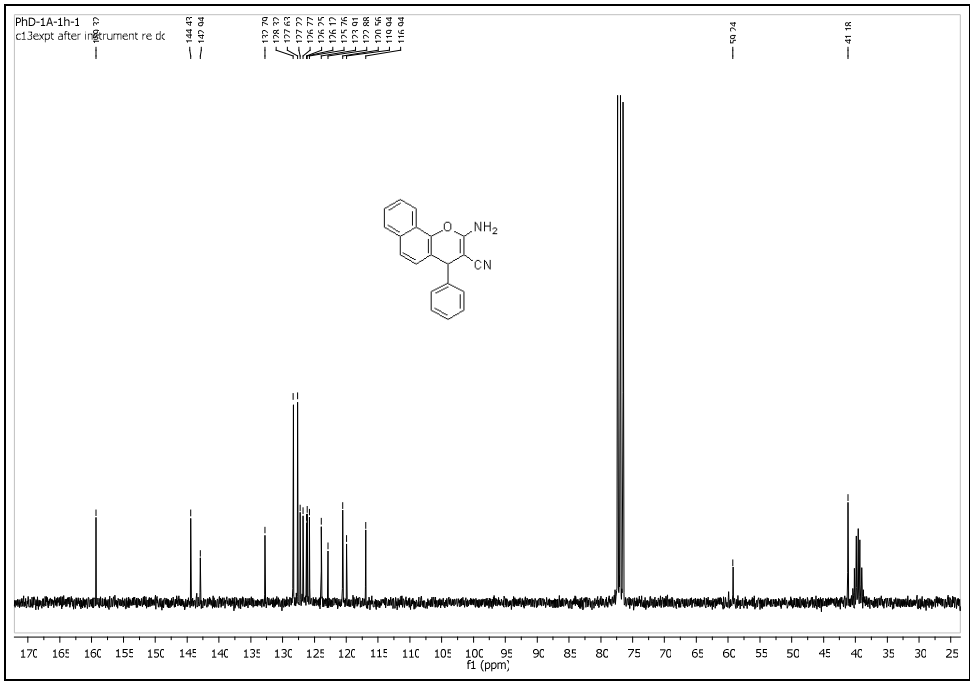


Figure 2.7 ^{13}C NMR spectrum of 2-aminochromene **12a**

Surprisingly, the reaction in 1:1 mixture of PEG-400 and water was completed within 15 min at ambient temperature. Different combinations of PEG-400 and water led us to conclude that equal amounts of both solvents are ideal for the efficient and rapid condensation of benzaldehyde, malononitrile, and α-naphthol. The formation of the product is confirmed by its ^{1}H NMR (Figure 2.6) and ^{13}C NMR (Figure 2.7).

To study the generality of this protocol, syntheses of various substituted 2-aminochromenes were accomplished (Table 2.2). Aromatic aldehydes with electron-withdrawing or donating groups were employed to afford the corresponding 2-aminochromenes in good to excellent yields. In the case of aldehyde bearing electron donating group, condensation was found to be relatively slow (Table 2.2, entry 2). Reaction in PEG–water mixture generated almost similar yields of the products;

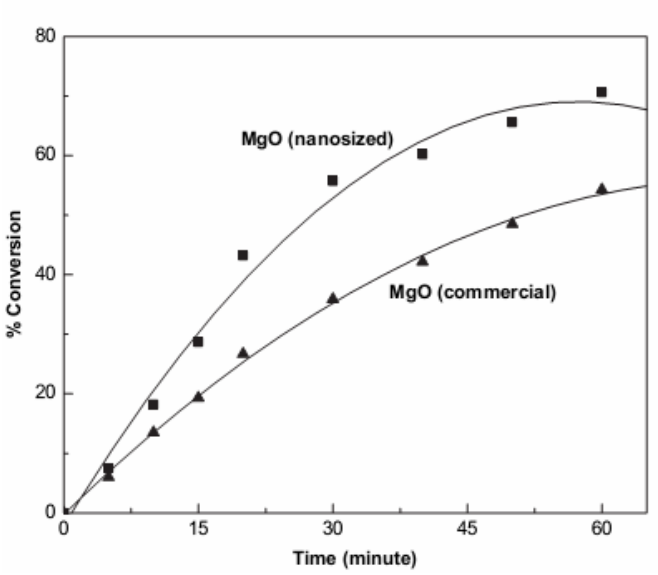
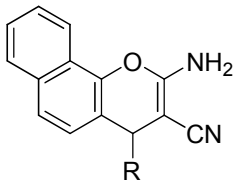


Figure 2.8 Comparison of the catalytic activity of nanosized MgO with commercial MgO

However, three-component condensation was rapid and occurred at room temperature. The role of MgO was found to be crucial in the success of reaction as no product was detected in absence of the catalyst.

After conducting the reaction with varying amounts of MgO, it was concluded that for 2 mmol of the substrates, 50 mg of the catalyst was sufficient for formation of the products. Further increasing the amount of the catalyst did not improve the results significantly.

Table 2.2 Synthesis of 2-aminochromenes in PEG–water (1:1)



12a - f

Compd.	R	Yield (%)[a] PEG 400-Water (1:1, v/v)	Time (min)
12a	C_6H_5	96	15
12b	4-$OMeC_6H_4$	82	90
12c	3-$NO_2C_6H_4$	84	15
12d	4-NO_2 C_6H_4	70	15
12e	4-Cl C_6H_4	98	20
12f	2-Furyl	95	15

[a] Yield refers to the pure isolated products.

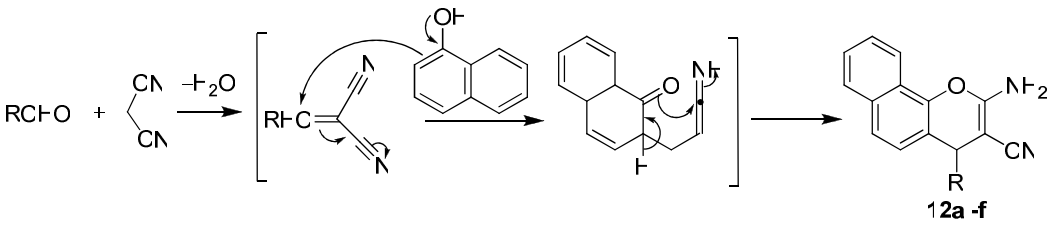
After completion of the reaction, the catalysts were regenerated using two procedures. In the first case, the catalyst was washed three times with 10 mL portions of methanol and dried at 150 ^{o}C overnight. The other method employed for regeneration was simply heat treatment at 450 ^{o}C in air for 2 h. Both the methods were effective to regenerate the catalytic activity of the MgO sample. The reactivated catalyst was reused for second and third consecutive cycles without any significant loss in catalytic activity (Table 2.3).

Table 2.3 Recyclability studies of nanosized MgO in the synthesis of compound **12a**

Cycle	Solvent	Time (min)	Yield (%)
1	PEG 400-Water (1:1)	15	96
2	PEG 400-Water (1:1)	15	92
3	PEG 400-Water (1:1)	15	94

2.3.2 Plausible Mechanism

Mechanistically, the reaction occurs via initial formation of benzylidenemalononitrile in quantitative yield by the Knoevenagel addition of malononitrile to the aldehyde and followed by loss of water molecules. Subsequently, ortho C-alkylation of α-naphthol and nucleophilic addition of hydroxyl moiety to the nitrile produce 2-amino-chromenes (Scheme 2.9).



Scheme 2.9 Mechanistic pathway for the formation of chromenes

2.4 Conclusions

The nanosized magnesium oxide has been employed for the first time as a novel and efficient catalyst for the benign synthesis of various substituted 2-aminochromenes in a three-component condensation approach. The nanosized magnesium oxide was successfully synthesised using precipitation technique with $Mg(NO_3)_2$ salt and liquid ammonia as the precipitating agent. The size and nature of the magnesium oxide was analysed using XRD, SEM and TEM and it was in the range of 100-200 nm. It efficiently catalysed the three-component condensation. The kinetic studies showed the yields are greater with nanosized MgO in comparision to commercially available MgO. The reaction was explored in methanol, water and PEG-400:water as solvents. Astoundingly, the reaction proceeded to completion in shorter time at room temperature and high yields with PEG-400:water. The attractive features of this protocol are: simple experimentation procedure, use of benign reaction solvents, cost effectiveness, the recyclability of catalysts, and its adaptability for the synthesis of a diverse set of 2-aminochromenes.

2.5 Experimental Details

2.5.1 General

Melting points were measured on a Micro Scientific Works apparatus and are uncorrected. IR spectra were recorded on a JASCO IR spectrophotometer using KBr pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker NMR spectrometer at 400 MHz using TMS as internal standard. Reactions were monitored by thin layer chromatography (TLC) on 0.2 mm silica gel F_{254} plates. All the products are known compounds and are characterized by comparing their IR, ^1H NMR, and melting points with those reported in literature. The XRD pattern of the MgO sample was recorded in the 2θ range of 30–700^o using a Shimadzu XD-D1 diffractometer employing Cu Kα radiation (λ = 1.5418 A°). Scanning electron micrograph pictures were taken using Jeol JSM-5300 microscope (acceleration voltage10 kV). The sample powder was deposited on a

carbon tape before mounting on a sample holder. In order to reduce the charge developed on the sample, gold sputtering was done for 3 min. The transmission electron micrographs (TEM) were obtained with a Jeol-1200EX microscope. The MgO sample for TEM was prepared by dispersing the powdered sample in ethanol by sonication and then drop drying on a copper grid (400 mesh) coated with carbon film.

2.5.2 Synthesis of Nanosized MgO

The MgO nanoparticles were synthesised by precipitation of the magnesium hydroxide gels in aqueous solution using $Mg(NO_3)_2$ as salt and liquid ammonia as the precipitating agent. Initially, the pH of 200 mL of distilled water was adjusted to 10.5 by addition of liquid ammonia. To this solution, 0.1 M magnesium nitrate solution was added dropwise with continuous stirring. The rate of addition of the salt solution was kept at 20 mL/h. During the addition, the pH of the mixture decreased due to hydrolysis of the salt. The pH was maintained at 10.5 by controlled addition of liquid ammonia solution. After completion of the precipitation procedure, the mixture was stirred at room temperature for 12 h, filtered, repeatedly washed with distilled water, dried at 120 ^{o}C, and calcined at 500 ^{o}C for 2 h. The calcination step was carried out in a temperature programmable Muffle furnace in flowing air. The temperature of the furnace was linearly raised from room temperature to 500 ^{o}C with an increment of 10 ^{o}C/min. At 500 ^{o}C, the temperature was maintained for 2 h to yield the final material.

2.5.3 Synthesis of 2-Aminochromenes

2.5.3.1 Synthesis of 2-Aminochromenes in Methanol

A mixture of aromatic aldehyde **1** (2 mmol), malononitrile **2** (2 mmol), α-naphthol **11** (2 mmol), and MgO (50 mg) in methanol (15 mL) was refluxed for 1 h. After completion of the reaction, as indicated by TLC, MgO was removed by filtration and excess methanol was distilled off. The crude product so obtained was recrystallised from methanol to afford the pure product **12**.

2.5.3.2 Synthesis of 2-Aminochromenes in Water

A mixture of aromatic aldehyde **1** (2 mmol), malononitrile **2** (2 mmol), α-naphthol **11** (2 mmol), and MgO (50 mg) in water (15 mL) was refluxed for 1 h. After completion of the reaction, as indicated by TLC, the mixture was extracted with ethyl acetate (3 x 5 mL). The organic phase was dried, filtered, and excess ethyl acetate was distilled off

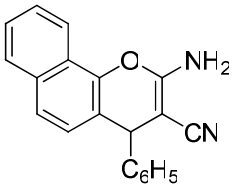
(filtration of both organic and aqueous phases led to the recovery of solid magnesium oxide). The residue was recrystallised from methanol to afford the pure product in 86% yield (Table 2.1, entry 1).

2.5.3.3 Synthesis of 2-Aminochromenes in PEG-Water Mixture

A mixture of aromatic aldehyde **1** (2 mmol), malononitrile **2** (2 mmol), α-naphthol **11** (2 mmol), and MgO (50 mg) in PEG–water (1 mL; 1:1) was stirred at room temperature for 15 min. On completion of the reaction, as indicated by TLC, the reaction mixture was diluted with water and the solid product was removed by filtration. The product was separated from catalyst by dissolving into hot methanol followed by simple filtration. The filtrate was concentrated to afford the pure product in 96% yield (Table 2.2, entry 1).

Analytical data of the synthesised 2-aminochromenes (**12a-f**):

2-Amino-4-phenyl-4*H*-benzo[*h*]chromene-3-carbonitrile (12a)



Yield: 96%

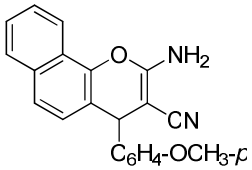
M.p.: 178-180 ^{o}C .

IR (KBr, *v* cm^{-1}): 3449, 3304, 2205, 1651, 1373, 1099.

^{1}H NMR (300 MHz, CDCl$_3$+DMSO-*d*$_6$): δ = 8.23 (d, *J* = 7.7 Hz, 1H), 7.77 (d, *J* = 6.2 Hz, 1H), 7.61 – 7.44 (m, 3H), 7.33 – 7.18 (m, 5H), 7.02 (t, *J* = 7.0 Hz, 1H), 5.59 (s, 2H), 4.84 (s, 1H).

^{13}C NMR (75 MHz, CDCl$_3$+DMSO- *d*$_6$): δ = 159.3, 144.4, 142.9, 132.8, 128.3, 127.6, 127.2, 126.8, 126.2, 126.1, 125.7, 123.9, 122.9, 120.6, 119.9, 116.9, 59.2, 41.2.

2-Amino-4-(4-methoxyphenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (12b)

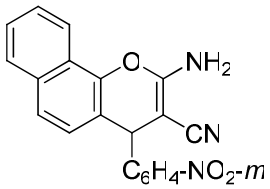


Yield: 82%

M.p.: 176-179 ^{o}C (Lit. M.p.[21]: 180-182 ^{o}C).

IR (KBr, *v* cm^{-1}): 3418, 3321, 2193, 1666, 1375, 1105.

2-Amino-4-(3-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (12c)

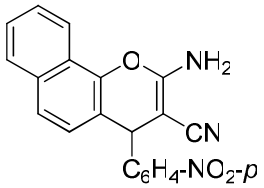


Yield: 84%

M.p.: 208-211 °C (Lit. M.p.[21] 208-211 °C).

IR (KBr, *v* cm^{-1}): 3460, 3362, 2185, 1659, 1348, 1103.

2-Amino-4-(4-nitrophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (12d)

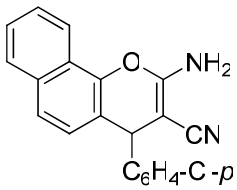


Yield: 70%

M.p.: 231-234 °C (Lit. M.p.[21]: 231-234 °C).

IR (KBr, *v* cm^{-1}): 3458, 3360, 2187, 1660, 1344, 1103.

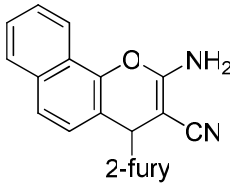
2-Amino-4-(4-chlorophenyl)-4*H*-benzo[*h*]chromene-3-carbonitrile (12e)



Yield: 98%

M.p.: 230-232 °C (Lit. M.p.[21]: 231-232 °C).

2-Amino-4-(furan-2-yl)-4*H*-benzo[*h*]chromene-3-carbonitrile (12f)



Yield: 95%

M.p.: 169-172 °C.

IR (KBr, *v* cm^{-1}): 3381, 3319, 2191, 1666, 1370, 1105.

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CHAPTER - III

One-pot Synthesis and Antibacterial Activity of 4*H*-Pyran Derivatives

The 4*H*-pyran derivatives are known for a wide range of biological activities. This chapter deals with the facile one-pot expeditious synthesis of 2-amino-4*H*-pyrans in very good yields has been described under solvent-free conditions by the sequential reaction of aromatic aldehyde, malononitrile and ethyl acetoacetate/dimedone using magnesium oxide as a catalyst. The reaction was carried out by grinding the reactants in a mortar followed by a simple workup. The reaction catalyst, magnesium oxide was reused and recycled without any loss of activity and product yield. All the synthesised compounds were screened for *in vitro* antibacterial activity against three bacterial strains, namely *Escherichia coli* (MTCC 41), *Staphylococcus aureus* (MTCC 1144) and *Pseudomonas putida* (MTCC 1072). Eight compounds showed complete inhibition of bacterial growth at 128 μg/mL or less and the rest of the compounds exhibited incomplete inhibition. The compounds with electron-rich substituents on the C-4 aryl ring of pyran imparted good antibacterial activity.

Chapter 3

3.1 Introduction

The development of environmentally benign, efficient and economical methods for the synthesis of biologically interesting compounds remains a significant challenge in synthetic chemistry. The chemical industry is one of the major contributors to environment pollution, owing to the use of hazardous chemicals and in particular, large amounts of flammable, volatile and often toxic organic solvents. Green chemistry emphasizes the need for environmentally clean synthesis, which involves improvement in selectivity, high atom efficiency, elimination of hazardous reagents, and easy separation with recovery and reuse of reagents. As a result, volatile organic solvents are being replaced by non-toxic, non-volatile media such as ionic liquids, polyethylene glycol, and water. Alternatively, the reactions are carried out under solvent-free conditions. The phenomenal response, as evident from the growing number of publications, in order to achieve this goal is overwhelming. It is more advantageous to carry out reactions under solvent-free conditions.[1]

3.1.1 Solvent-free Reactions: Importance and Advantages

Many organic solvents, especially halogenated ones, are harmful. Consequently, minimum usage of toxic reagents and volatile solvents is central towards the development of benign chemical processes. Organic reactions under solvent-free conditions are attractive because of their improved selectivity and efficiency, ease of manipulation, pure product formation, avoidance of toxic or volatile solvents.[2-4]

Particularly, in recent years, reactions under solvent-free conditions have continuously attracted the attention of researchers both from academia and industry. This is due to the fact that without solvent, reactions usually need shorter reaction time, simpler reactors, and require simple and efficient workup procedures.

The toxic waste removal in the design, manufacture and applications of chemical products can be achieved using the principles of green chemistry.[5-6] Nowadays, the replacement of hazardous solvents with relatively benign solvents is one of the main considerations of any chemical industry. Attempts have been made to develop solvent-free chemistry, which to some extent have been successful in few transformations.

3.1.2 Biological Significance of Pyran Derivatives

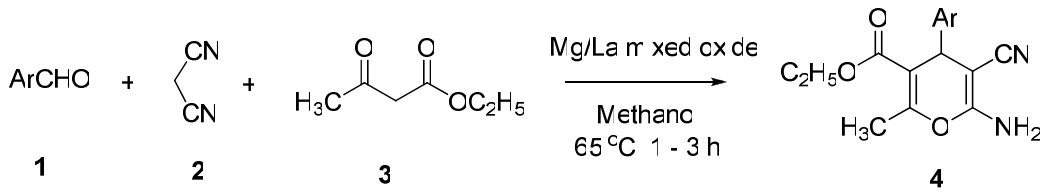
2-Amino-4*H*-pyran derivatives represent an important class of compounds. They are often used in cosmetics and pigments, and utilized as potentially biodegradable agrochemicals.[7-9] Polyfunctionalized 4*H*-pyrans also constitute a structural unit of many natural products[10-11] and biologically interesting compounds which possess various pharmacological activities,[12] such as antiallergic[9] and antitumor[13] activities. 4*H*-Pyran derivatives are also potential calcium channel antagonists[14] which are structurally similar to biologically active 1,4-dihydropyridines.

The persistent bacterial infections have been on the rise with more and more antibacterial agents reaching the masses. There has been a focus on fused chromenes,[15] fused pyrans,[16] 2-pyrones,[17] etc. as antibacterial agents. Aytemir et al.[18] reported the antimicrobial activity of 4-oxo-4*H*-pyran derivatives and showed that one of the synthesised compounds is as active as ceftazidime in inhibiting *S. aureus*. Kidwai et al.[19] investigated the 2-amino-4*H*-pyrans derivatives for their antibacterial activity. In view of the reported biological significance of 4*H*-pyrans and our interest in developing newer antibacterial agents, we focused our attention towards them.

3.1.3 Accounted Routes for the Synthesis of Pyran Derivatives

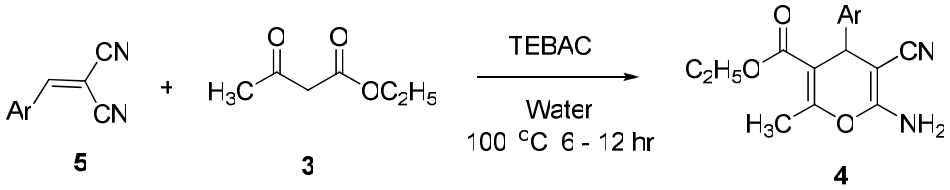
Earlier 2-amino-4*H*-pyrans were synthesised by the cyclisation of arylidenemalononitriles with β-dicarbonyl compounds in presence of base such as piperidine,[20] morpholine, pyridine,[21] triethylamine,[22-23] sodium methoxide, or 1,1,3,3-tetramethylguanidine. Most of these methods also involve use of volatile solvents, require longer reaction time (~12 h) and difficult to recover catalysts. The following are some of the protocols for the synthesis of pyran derivatives.

Seshu Babu et al.[24] reported an efficient synthesis of polyfunctionalized 4*H*-pyrans in one-pot through condensation of an arylaldehyde, malononitrile, and an active methylenic diketo compound using a heterogeneous strong basic Mg/La mixed oxide catalyst (Scheme 3.1). The protocol required heating the reaction mixture at 65 ^{o}C for 1-3 hours in methanol followed by a simple workup. This procedure offers advantages in terms of higher yields with reusability of the catalyst.



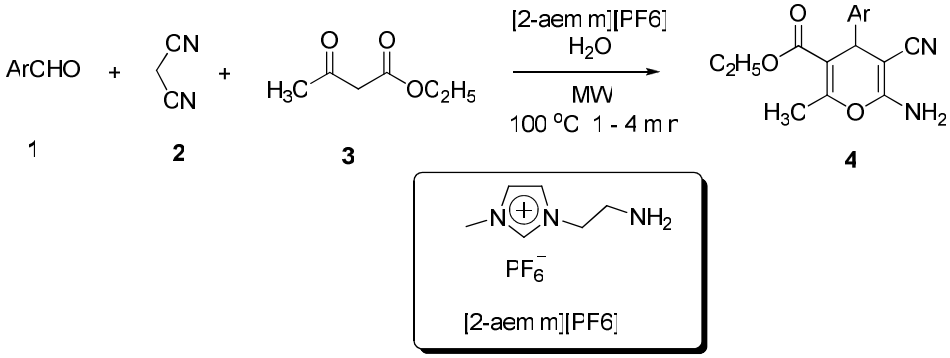
Scheme 3.1 Synthesis of polyfunctionalized 4*H*-pyrans

A clean and simple synthesis of ethyl 6-amino-5-cyano-4-aryl-2-methyl-4*H*-pyran-3-carboxylate derivatives was accomplished[25] in high yields via the reaction of arylmethylidenemalononitriles with ethyl acetoacetate in aqueous media catalysed by triethylbenzylammonium chloride (TEBAC). The reaction required 100 °C for 6-12 hours followed by a simple workup which involves filtration of the solid product. The products were crystallised from water-DMF (Scheme 3.2).



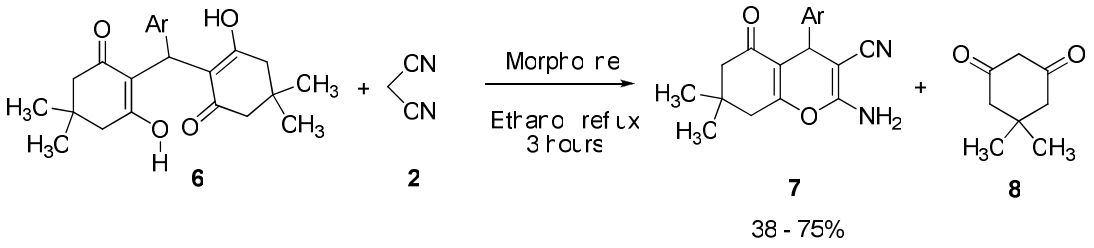
Scheme 3.2 TEBAC catalysed synthesis of 4*H*-pyran derivatives **4**

Peng and Song used a mixture of catalytically active ionic liquid, 1-methyl-3-(2-aminoethyl)imidazolium hexafluophosphate [2-aemimPF6], and water for the synthesis of 4*H*-pyrans **4** using computer-controlled microwave irradiation.[26] A mixture of aromatic aldehydes **1**, malononitrile **2**, ethyl acetoacetate **3**, water and [2-aemim][PF6] was subjected to microwave irradiation (100 °C) for 1-4 min (Scheme 3.3). The workup of the protocol was simple and the yields were good. The reaction medium was reused successfully.



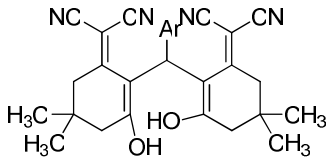
Scheme 3.3 Synthesis of 4*H*-pyrans **4** in ionic liquid

Gheath and Al-Orffi has synthesised different bisdimedones and reacted with malononitrile to obtain different dialkylidenes (Figure 3.1). These reactions were failed to give the desired alkylidenes, instead different 4*H*-pyrans were obtained in moderate to good yield (Scheme 3.4). Moreover in this work a new method of pyran synthesis has been established. The formation of 4*H*-pyrans was construed to the fragmentation of bis dimedone into two parts by the attack of the nucleophilic carbon of malononitrile to a methine carbon followed by attacking of enolic hydroxyl group to cyano group.[27]



6 2 7 8

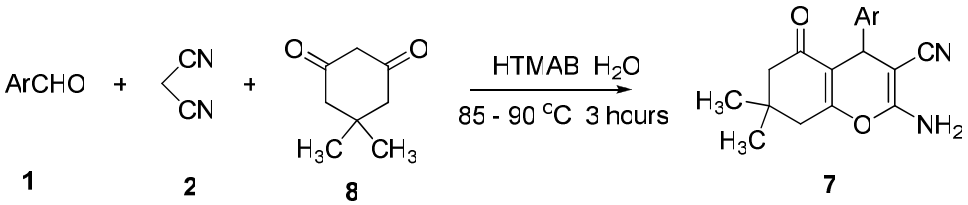
Scheme 3.4 Synthesis of 4*H*-pyrans **7** in presence of morpholine



9

Figure 3.1 Dialkylidene

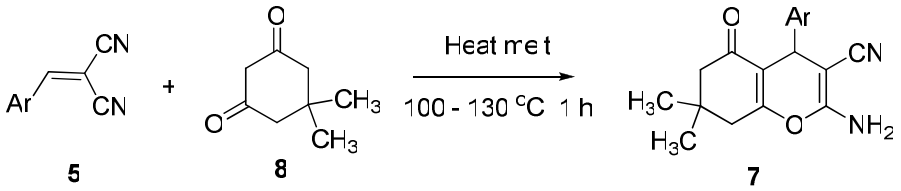
The synthesis of 2-amino-3-cyano-4-aryl-7,7-dimethyl-5-oxo-4*H*-5,6,7,8-tetrahydrobenzo[*b*] pyran derivatives **7** using hexadecyltrimethyl ammonium bromide (HTMAB) as the catalyst (10 mol%) was achieved by Jin and coworkers in good to excellent yields.[28] This method required heating at 80-90 ^{o}C for upto 4 hours (Scheme 3.5). The role of HTMAB as a catalyst in the reaction was attributed to its emulsification property.



1 2 8 7

Scheme 3.5 HTMAB catalysed synthesis of 4*H*-pyran derivatives **7**

Kaupp and coworkers[29] developed a heat-melt technique for the synthesis of **7** in quantitative yields under solvent-free conditions without the use of any auxiliaries. The arylidenemalononitrile **5** and dimedone **8** were taken in stoichometric amounts and heated at 100 – 130 ^{o}C upto 1 hour (Scheme 3.6). The products were directly obtained after cooling and drying. Quantitative Michael addition reaction of **5** with **8** followed by rearrangement and cyclisation gave **7** in three cascades



5 **8** **7**

Scheme 3.6 Synthesis of 4*H*-pyrans **7** with heat-melt technique

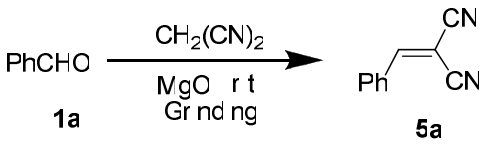
3.2 The Present Study

Herein, the one-pot synthesis of 4*H*-pyran compounds is described using sequential reaction of aromatic aldehyde, malononitrile and β-dicarbonyl compounds in presence of magnesium oxide as a catalyst under solvent-free condition at room temperature. The magnesium oxide was successfully employed, in view of our success in the synthesis of 2-aminochromenes.[30] The interesting biological properties of 2-aminopyrans leveraged the interest in the synthesis of these molecules. The synthesised compounds were screened for *in vitro* antibacterial activity against gram negative and gram positive standard strains of bacteria using Broth Microdilution MIC (Minimum Inhibitory Concentration) method and zone of inhibition assay.[31]

3.3 Results and Discussion

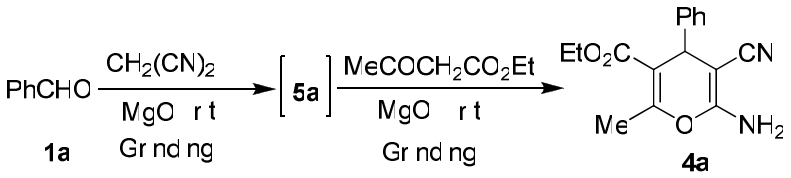
3.3.1 Chemistry

In our attempts to develop a facile one-pot solvent-free protocol, initially we focused on the efficient condensation of benzaldehyde **1a** (1.0 mmol) and malononitrile (1.0 mmol) at room temperature with mechanochemical mixing. This provided only traces of **5a** even with extended grinding. However, continuously grinding the mixture of benzaldehyde **1a** (1 mmol) and malononitrile (1 mmol) at room temperature in presence of MgO (4 mg, 0.1 mmol) turned out to be successful with almost quantitative formation of **5a** within five minutes (Scheme 3.7).



Scheme 3.7 Synthesis of benzylidenemalononitrile **5a** in presence of MgO

Subsequent addition of ethyl acetoacetate (1 mmol) to the reaction mixture with vigorous grinding afforded the 2-amino-4*H*-pyrans **4a** in poor yield. However, addition of another 16 mg (0.4 mmol) of MgO led to the formation of **4a** in good yield along with traces of unreacted benzylidenemalononitrile **5a** and ethyl acetoacetate. Further, addition of a few drops of water and grinding the reaction mixture at room temperature led to rapid (15 min) formation of pure 2-amino-4*H*-pyran **4a** (Scheme 3.8).



Scheme 3.8 One-pot synthesis of 4*H*-pyran **4a** catalysed by MgO

After the completion of reaction, the residue was taken into methanol and filtered. The crude product was obtained by evaporating the filtrate. Recrystallisation of the crude product led to the isolation of crystalline solid **4a** in 77% yield. The IR spectrum of **4a** exhibited bands at 3412, 3334, 2199, 1693 cm^{-1} indicating the presence of NH_2, C≡N and C=O functionalities, respectively. The ^1H NMR spectrum evidenced a characteristic singlet at δ 4.43 due to C-4H. The spectral data and physical properties of **4a** were found to be in agreement with the literature values.[25]

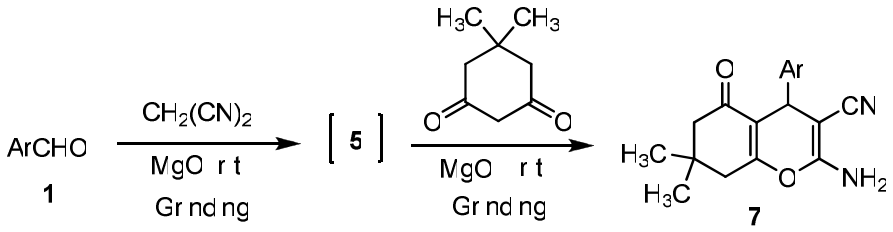
Encouraged by this successful three-component reaction, synthesis of diverse 2-amino-4*H*-pyrans **4b-h** was undertaken. The aromatic aldehydes bearing electron-withdrawing and electron-donating groups were found to be equally effective to produce 2-amino-4*H*-pyrans **4b-h** in very good yields (Table 3.1). The scope of this one-pot reaction was further extended by replacing ethyl acetoacetate with 5,5-dimethyl-cyclohexane-1,3-dione (Scheme 3.9) and various 2-amino-4*H*-pyrans **7a-g** were prepared (Table 3.1).

Table 3.1 Synthesis of 2-amino-4*H*-pyrans **4a-h** and **7a-g**

Compd.[a]	Ar	Yield [%]	M.p. [°C]
4a	C_6H_5	77	195-196
4b	3-ClC_6H_4	94	173-176
4c	4-ClC_6H_4	79	172-174
4d	4-$CH_3C_6H_4$	87	177-179
4e	3-OHC_6H_4	88	164-165
4f	4-$OCH_3C_6H_4$	89	142-144
4g	4-$NO_2C_6H_4$	86	180-183
4h	3-$NO_2C_6H_4$	92	182-183
7a	C_6H_5	75	226-228
7b	4-$OCH_3C_6H_4$	86	197-199
7c	3-$NO_2C_6H_4$	83	201-205
7d	4-$NO_2C_6H_4$	78	175-176
7e	4-ClC_6H_4	86	202-203
7f	4-$CH_3C_6H_4$	73	209-211
7g	3-OHC_6H_4	75	224-226

[a]Compounds were characterized by their spectral data (IR and ^{1}H NMR). [b]Yields refer to pure isolated products.

The catalyst was recycled by a simple workup. After the completion of reaction, MgO was removed by filtration, washed with methanol and dried at the pump. The recovered catalyst was reused for second and third consecutive cycles without any significant loss in catalytic activity (77%, 77% and 76%, respectively, for the three consecutive cycles in the synthesis of **4a**).

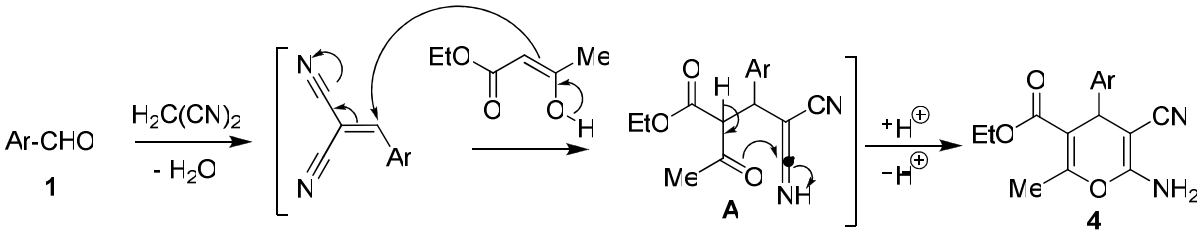


Scheme 3.9 One-pot synthesis of 2-amino-4*H*-pyrans **7** catalysed by MgO

3.3.2 Plausible Mechanism

Mechanistically, the initial condensation of aromatic aldehyde with malononitrile in presence of MgO leads to the formation of arylidenemalononitrile **5** with the loss of a water molecule.[32] The nucleophilic addition of the enolizable ethyl acetoacetate to

arylidenemalononitrile **5** followed by intramolecular cyclisation of the resulting species **A** produce the 2-amino-4*H*-pyrans **4** (Scheme 3.10). Similar reaction mechanism applies for the synthesis of 2-amino-4*H*-pyrans **7a-g**.



Scheme 3.10 Proposed mechanistic path in the synthesis of 4*H*-pyrans **4**

3.3.3 Antibacterial Activity

The synthesised compounds were screened for their antibacterial activity against three bacterial strains, namely *Escherichia coli* (MTCC 41), *Staphylococcus aureus* (MTCC 1144) and *Pseudomonas putida* (MTCC 1072). The non-pathogenic strain *Pseudomonas putida* is ampicillin resistant and is closely related to the pathogenic strain *Pseudomonas aeruginosa.* The antimicrobial activity assay (MIC and the zone of inhibition) was performed for the compounds at 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0 and 128.0 μg/mL concentrations. The MIC assay is to test the sensitivity of microorganisms to an antimicrobial agent. A set of tubes with multiple concentrations of compounds was prepared in growth medium (LB broth). The tubes were then inoculated with the microorganisms, incubated for 12-16 h, and examined for growth of bacteria. Broth tubes that appear turbid are indicative of bacterial growth while tubes that remain clear indicate no growth. Growth seems to diminish as the concentration of some compounds increase, and eventually appeared to reduce at higher concentrations.

The zone of inhibition assay is to find the extent of sensitivity of microorganisms to the organic compound being tested. This antimicrobial activity assay was performed for the compounds at different concentrations. The bacterial isolate was inoculated uniformly on to the surface of an agar plate. A filter disk impregnated with a known amount of compound was applied to the surface of the plate and the compound was allowed to diffuse into the adjacent medium. A bacterial lawn appeared on the plate after incubation for 16 h. The antimicrobial activity of the compound was recorded as the size of zone inhibition. The size of the zone obtained at a particular concentration is directly

proportional to the sensitivity of the organism to the salt and thus the zone of inhibition in the disk diffusion test is inversely related to the MIC.

3.3.4 Structure-Activity Relationship (SAR)

The results of antibacterial studies are given in Table 3.2. Among the synthesised **4a-h** and **7a-g**, the compounds **4a, 4b**, **4f, 7b, 7c, 7d, 7e** and **7g** were showing complete inhibition at 128 μg/mL or less. The rest of the compounds showed incomplete inhibition.

Among the 2-amino-4*H*-pyrans (**4a-h**), the compound **4a** completely inhibited *E. coli* at 64 μg/mL but it could inhibit *S. aureus* and *P. putida* at 128 μg/mL. Introducing a methoxy group at *para* position of the C-4 aryl ring (**4f**) made it relatively ineffective towards *E. coli* but found to be active against other two strains. The compound **4b** with a chloro group at the *meta* position of C-4 aryl ring exhibited activity similar to the compound **4f**. Any other substitution at the positions 3 and 4 of the C-4 aryl ring retarded the efficiency of the resulting compounds.

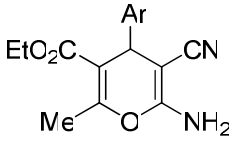
The compound **7a** resulted in decreased activity when compared to **4a** but placing a nitro group at para position of the C-4 aryl ring on 2-amino-4*H*-pyran resulted in a potent compound **7d,** when compared to all other compounds, especially with its counterpart **4g**. Placing a hydroxy group at *meta* position of the C-4 aryl ring in 2-amino-4*H*-pyran resulted in **4e,** which was active towards *P. putida* and the activity is enhanced when positions 5 and 6 of 2-amino-4*H*-pyran were fused to give **7g**. Further, the MIC of **7g** towards *P. putida* is 64 μg/mL whereas ampicillin was inactive upto 256 μg/mL. Though a possible explanation for this cause is unclear, fusing the positions 5 and 6 of the 4*H*-pyran ring resulted in compounds **7b-g** with the activity increased substantially.

3.4 Conclusion

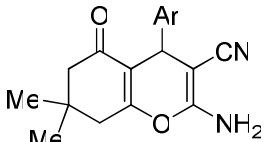
A facile, convenient and environmentally benign one-pot synthesis of 2-amino-4*H*-pyrans is described under solvent-free conditions using magnesium oxide as a recyclable catalyst in good yields. The antibacterial assay of the compounds **4a, 4b**, **4f, 7b, 7c, 7d, 7e** and **7g** showed complete inhibition of bacterial growth at 128 μg/mL or less and the rest of the compounds exhibited incomplete inhibition. The noteworthy compound **7d** proved to be active in terms of overall potency and the compound **7g** showed selective inhibition towards *P. putida*. The compounds with electron-rich substituents on the aryl ring imparted good antibacterial activity. Further modifications can be easily imparted by

altering any of the three components, *viz.* aromatic aldehydes, α-cyano active methylene compounds and enolisable β-diketones.

Table 3.2 MIC (μg/mL) and the zone of inhibition (in mm) values of various 2-amino-4*H*-pyrans (**4a-h**) and (**7a-g**) in gram positive and gram negative bacteria.



4a-h



7a-g

		E. coli			*S. aureus*			*P. putida*		
	Ar	**MIC (μg/mL)**	**Zone of inhibition (mm)**		**MIC (μg/mL)**	**Zone of inhibition (mm)**		**MIC (μg/mL)**	**Zone of inhibition (mm)**	
			128 μg/mL	**64 μg/mL**		**128 μg/mL**	**64 μg/mL**		**128 μg/mL**	**64 μg/mL**
4a	C_6H_5	64	>3	>3	128	>3	<1	128	>3	<1
4b	3-ClC_6H_4	128	>3	<1	128	>3	<1	128	>3	<1
4c	4-ClC_6H_4	>128	<1	<1	>128	<1	<1	>128	<1	<1
4d	4-$CH_3C_6H_4$	>128	<1	<1	>128	<1	<1	>128	<1	<1
4e	3-OHC_6H_4	>128	<1	<1	>128	<1	<1	128	>3	<1
4f	4-$OCH_3C_6H_4$	128	>3	<1	128	>3	<1	128	>3	<1
4g	4-$NO_2C_6H_4$	>128	<1	<1	>128	<1	<1	>128	<1	<1
4h	3-$NO_2C_6H_4$	>128	<1	<1	>128	<1	<1	>128	<1	<1
7a	C_6H_5	>128	>2	<1	>128	<1	<1	>128	>2	>2
7b	4-$OCH_3C_6H_4$	64	>3	>3	>128	>2	>2	128	>3	>2
7c	3-$NO_2C_6H_4$	128	>3	<1	128	>3	>2	128	>3	<1
7d	4-$NO_2C_6H_4$	64	>3	>3	64	>3	>3	128	>3	<1
7e	4-ClC_6H_4	128	>3	>2	128	>3	>2	128	>3	>2
7f	4-$CH_3C_6H_4$	>128	>2	>2	>128	>2	>2	128	>3	>2
7g	3-OHC_6H_4	128	>3	>2	>128	>2	>2	64	>3	>3
Std	Ampicillin	16	>5	>5	16	>5	>5	>256	0	0

3.5 Experimental Details

3.5.1 General

All the laboratory grade reagents were obtained commercially. Melting points were recorded on Buchi530 melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu IRPrestige-21 FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in parts per million (δ) and coupling constants

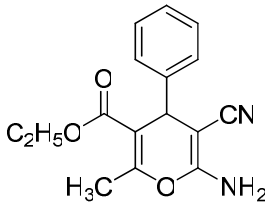
(J) in Hz. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} precoated aluminium sheets.

3.5.2 General Procedure for the Synthesis of 2-Amino-4*H*-pyrans

A mixture of aromatic aldehyde (1.0 mmol), malononitrile (1.0 mmol) and MgO (0.5 mmol) were grinded at room temperature till it formed a solid (10 min). Ethyl acetoacetate (1.0 mmol) or 5,5-dimethyl-cyclohexane-1,3-dione (1.0 mmol) and 2-3 drops of water were added to the mixture and continued grinding till it formed a solid (15 min). On completion of the reaction, as indicated by TLC, the solid was dissolved in methanol and filtered. The solvent was distilled off under vacuum and residue so obtained was recrystallised from methanol.

Analytical data of 2-amino-4*H*-pyrans **4a-h** and **7a-g**:

Ethyl 6-amino-5-cyano-2-methyl-4-phenyl-4*H*-pyran-3-carboxylate (4a)

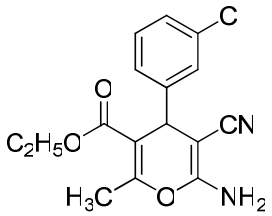


M.p.: 195-196 ^{o}C (Lit. M.p.[33]:194-196 ^{o}C).

IR (KBr, *v* cm^{-1}): 3412 (NH_2), 3334 (NH_2), 2199 (C≡N), 1693 (C=O).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31-7.19 (m, 5H), 4.44 (s, 2H), 4.43 (s, 1H), 4.03 (q, *J* = 7.12 Hz, 2H), 2.38 (s, 3H), 1.09 (t, *J* = 7.12 Hz, 3H).

Ethyl 6-amino-4-(3-chlorophenyl)-5-cyano-2-methyl-4*H*-pyran-3-carboxylate (4b)

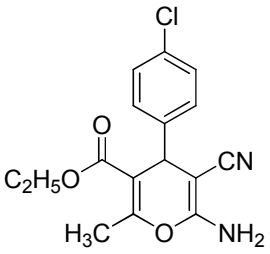


M.p.: 173-176 ^{o}C (Lit. M.p.[25]: 177-178 ^{o}C).

IR (KBr, *v* cm^{-1}): 3400 (NH_2), 3329 (NH_2), 2191 (C≡N), 1693 (C=O), 779 (C-Cl);

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.12 (m, 4H), 4.55 (s, 2H), 4.40 (s, 1H), 4.04 (q, *J* = 7.12 Hz, 2H), 2.35 (s, 3H), 1.09 (t, *J* = 7.12 Hz, 3H).

Ethyl 6-amino-4-(4-chlorophenyl)-5-cyano-2-methyl-*4H*-pyran-3-carboxylate (4c)

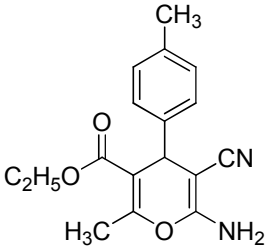


M.p.: 172-174 ^oC (Lit. M.p.[34]: 175-177 ^oC).

IR (KBr, *v* cm^{-1}): 3410 (NH_2), 3333 (NH_2), 2193 (C≡N), 1693 (C=O), 760 (C-Cl).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.25 (d, *J* = 8 Hz, 2H), 7.15 (d, *J* = 8 Hz, 2H), 5.93 (s, 2H), 4.38 (s, 1H), 4.03 (q, *J* = 7.16 Hz, 2H), 2.36 (s, 3H), 1.12 (t, *J* = 7.16 Hz, 3H).

Ethyl 6-amino-5-cyano-2-methyl-4-(4-methylphenyl)-4*H*-pyran-3-carboxylate (4d)

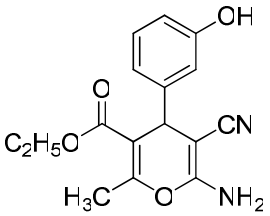


M.p.: 177-179 ^oC.

IR (KBr, *v* cm^{-1}): 3414 (NH_2), 3336 (NH_2), 2202 (C≡N), 1693 (C=O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.07 (s, 4H), 5.80 (s, 2H), 4.35 (s, 1H), 4.02 (q, *J* = 7.12 Hz, 2H), 2.35 (s, 3H), 2.30 (s, 3H), 1.12 (t, *J* = 7.12 Hz, 3H).

Ethyl 6-amino-5-cyano-4-(3-hydroxyphenyl)-2-methyl-4*H*-pyran-3-carboxylate (4e)

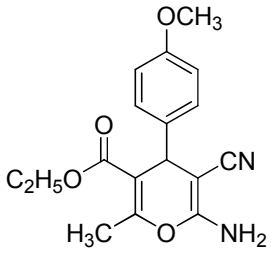


M.p.: 164-165 ^oC.

IR (KBr, *v* cm^{-1}): 3414 (NH_2), 3336 (NH_2), 2202 (C≡N), 1693 (C=O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.85 (s, 1H), 7.07 (t, *J* = 8 Hz, 1H), 6.67-6.64 (m, 3H), 5.74 (s, 2H), 4.31 (s, 1H), 4.03 (q, *J* = 7.12 Hz, 2H), 2.35 (s, 3H), 1.13 (t, *J* = 7.12 Hz, 3H).

Ethyl 6-amino-5-cyano-4-(4-methoxyphenyl)-2-methyl-4*H*-pyran-3-carboxylate (4f)

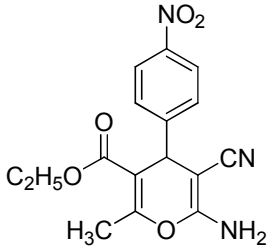


M.p.: 142-144 ^{o}C (Lit. M.p.[34]: 137-139 ^{o}C).

IR (KBr, *v* cm^{-1}): 3405 (NH_2), 3323 (NH_2), 2210 (C≡N), 1693 (C=O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 6.96 (d, *J* = 14.6 Hz, 2H), 6.66 (d, *J* = 14.6 Hz, 2H), 5.53 (s, 2H), 4.21 (s, 1H), 3.88 (q, *J* = 7.16 Hz, 2H), 3.61 (s, 3H), 2.19 (s, 3H), 1.12 (t, *J* = 7.16 Hz, 3H).

Ethyl 6-amino-5-cyano-2-methyl-4-(4-nitrophenyl)-4*H*-pyran-3-carboxylate (4g)

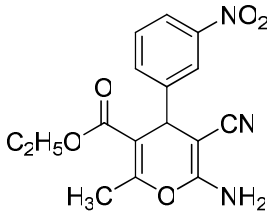


M.p.: 180-183 ^{o}C.

IR (KBr, *v* cm^{-1}): 3408 (NH_2), 3331 (NH_2), 2198 (C≡N), 1693 (C=O), 1520 (N=O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.15 (d, *J* = 4.8 Hz, 2H), 7.39 (d, *J* = 4.8 Hz, 2H), 6.20 (s, 2H), 4.52 (s, 1H), 4.03 (q, *J* = 7.08 Hz, 2H), 2.41 (s, 3H), 1.11 (t, *J* = 7.08 Hz, 3H).

Ethyl 6-amino-5-cyano-2-methyl-4-(3-nitrophenyl) -4*H*-pyran-3-carboxylate (4h)

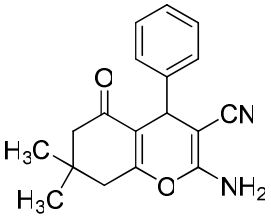


M.p.: 182-183 ^{o}C (Lit. M.p.[35]: 184 ^{o}C).

IR (KBr, *v* cm^{-1}): 3402 (NH_2), 3327 (NH_2), 2191 (C≡N), 1693 (C=O), 1531 (N=O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.10-8.03 (m, 2H), 7.61-7.49 (m, 2H), 5.98 (s, 2H), 4.55 (s, 1H), 4.04 (q, *J* = 7.12 Hz, 2H), 2.41 (s, 3H), 1.12 (t, *J* = 7.12 Hz, 3H).

2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-5-oxo-4-phenyl-4*H*-chromene-3-carbonitrile (7a)

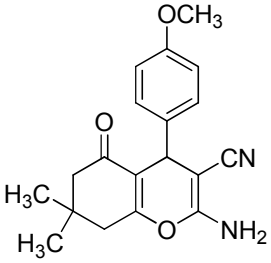


M.p.: 226-228 °C (Lit. M.p.[29]: 233-234 °C).

IR (KBr, *v* cm^{-1}): 3394 (NH_2), 3325 (NH_2), 2199 (C≡N), 1676 (C=O), 1215 (C-O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.27-7.18 (m, 5H), 4.50 (s, 2H), 4.40 (s, 1H), 2.45 (s, 2H), 2.25 (d, J_{AB} = 16 Hz, H-6a), 2.19 (d, J_{AB} = 16 Hz, H-6b), 1.11 (s, 3H), 1.04 (s, 3H).

2-Amino-5,6,7,8-tetrahydro-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (7b)

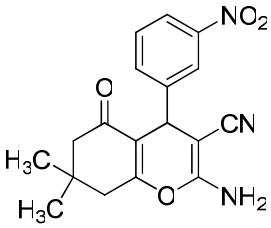


M.p.: 197-199 °C (Lit. M.p.[28]: 199-201 °C).

IR (KBr, *v* cm^{-1}): 3379 (NH_2), 3325 (NH_2), 2195 (C≡N), 1682 (C=O), 1211 (C-O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.14 (d, *J* = 8.68 Hz, 2H), 6.80 (d, *J* = 8.68 Hz, 2H), 5.60 (s, 2H), 4.30 (s, 1H), 3.76 (s, 3H), 2.45 (s, 2H), 2.23 (d, J_{AB} = 16 Hz, H-6a), 2.16 (d, J_{AB} = 16 Hz, H-6b), 1.10 (s, 3H), 1.02 (s, 3H).

2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-4-(3-nitrophenyl)-5-oxo-4*H*-chromene-3-carbonitrile (7c)

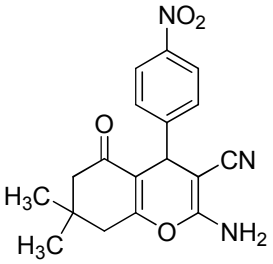


M.p.: 201-205 °C (Lit. M.p.[28]: 208-211 °C).

IR (KBr, *v* cm^{-1}): 3437 (NH_2), 3333 (NH_2), 2187 (C≡N), 1674 (C=O), 1211 (C-O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.06-8.04 (m, 2H), 7.65-7.62 (m, 1H), 7.52-7.48 (m, 1H), 6.30 (s, 2H), 4.47 (s, 1H), 2.52 (s, 2H), 2.26 (d, J_{AB} = 16 Hz, H-6a), 2.17 (d, J_{AB} = 16 Hz, H-6b), 1.12 (s, 3H), 1.03 (s, 3H).

2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-4-(4-nitrophenyl)-5-oxo-4*H*-chromene-3-carbonitrile (7d)

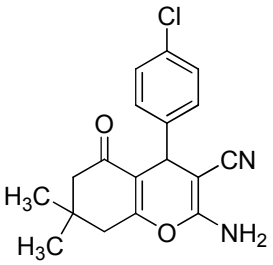


M.p.: 175-176 ^{o}C (Lit. M.p.[29]: 174-176 ^{o}C).

IR (KBr, *v* cm^{-1}): 3518 (NH_2), 3375 (NH_2), 2187 (C≡N), 1682 (C=O), 1215 (C-O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.14 (d, *J* = 8.72 Hz, 2H), 7.43 (d, *J* = 8.72 Hz, 2H), 6.07 (s, 2H), 4.47 (s, 1H), 2.50 (s, 2H), 2.26 (d, J_{AB} = 16 Hz, H-6a), 2.17 (d, J_{AB} = 16 Hz, H-6b), 1.12 (s, 3H), 1.02 (s, 3H).

2-Amino-4-(4-chlorophenyl)-5,6,7,8-tetrahydro-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile (7e)

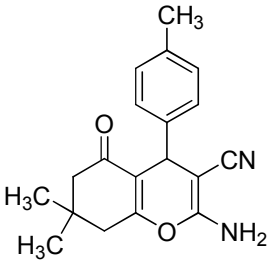


M.p.: 202-203 ^{o}C (Lit. M.p.[29]: 218 ^{o}C).

IR (KBr, *v* cm^{-1}): 3379 (NH_2), 3325 (NH_2), 2187 (C≡N), 1674 (C=O), 1215 (C-O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.24 (d, *J* = 8.52 Hz, 2H), 7.18 (d, *J* = 8.52 Hz, 2H), 5.79 (s, 2H), 4.33 (s, 1H), 2.46 (s, 2H), 2.24 (d, J_{AB} = 16 Hz, H-6a), 2.16 (d, J_{AB} = 16 Hz, H-6b), 1.11 (s, 3H), 1.02 (s, 3H).

2-Amino-5,6,7,8-tetrahydro-7,7-dimethyl-5-oxo-4-(4-methylphenyl)-4*H*-chromene-3-carbonitrile (7f)

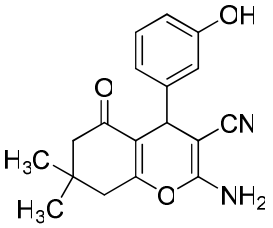


M.p.: 209-211 ^{o}C (Lit. M.p.[28]: 208-210 ^{o}C).

IR (KBr, *v* cm^{-1}): 3398 (NH_2), 3329 (NH_2), 2195 (C≡N), 1672 (C=O), 1211 (C-O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 7.11-7.05 (m, 4H), 5.60 (s, 2H), 4.30 (s, 1H), 2.46 (s, 2H), 2.19 (d, J_{AB} = 16 Hz, H-6a), 2.16 (d, J_{AB} = 16 Hz, H-6b), 1.10 (s, 3H), 1.03 (s, 3H).

2-Amino-5,6,7,8-tetrahydro-4-(3-hydroxyphenyl)-7,7-dimethyl-5-oxo-4*H*-chromene-3-carbonitrile(7g)



M.p.: 224-226 ^{o}C.

IR (KBr, *v* cm^{-1}): 3452 (NH_2), 3414 (NH_2), 2199 (C≡N), 1651 (C=O), 1215 (C-O).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ 8.91 (s, 1H), 7.05 (t, J = 7.76 Hz, 1H), 6.63 (m, 3H), 6.11 (s, 2H), 4.20 (s, 1H), 2.47 (s, 2H), 2.23 (d, J_{AB} = 16 Hz, H-6a), 2.16 (d, J_{AB} = 16 Hz, H-6b), 1.11 (s, 3H), 1.05 (s, 3H).

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CHAPTER - IV

A Facile Synthesis and Src Kinase Inhibitory Activity of 1,2,3-Triazoles

This chapter deals with the synthesis of 1,4-disubstituted-1*H*-1,2,3-triazoles under facile conditions from easily available starting materials and describes the studies on their Src kinase inhibition. The one-pot synthesis was achieved by the reaction of α-bromo ketones/α-tosyloxy ketones, sodium azide and terminal alkynes in the presence of aqueous PEG 400 (1:1, v/v) using the click chemistry approach. The synthesis was carried out with α-bromo ketones and further ameliorated using α-tosyloxy ketones. The reaction proved extremely facile and diversely substituted compounds were synthesised. The protocol was advantageous in terms of short reaction time, simple workup and requires benign and reusable reaction media. The compounds were evaluated for Src kinase inhibitory activity. Structure-activity relationship analysis demonstrated that insertion of phenyl and *p*-tolyl at position 4 and less bulkier aromatic group at position 1 contribute critically to the modest Src inhibition activity (IC_{50} = 32-43 μM) of 1,2,3-triazoles.

Chapter 4

4.1 Introduction

4.1.1 c-Src Kinase

Protein tyrosine kinases (PTKs) play a critical role in signaling between cells in multi-cellular animals. An important aspect of these signaling mechanisms is the recognition of phosphotyrosine by small protein modules known as SH2 domains (Src homology domains) which were first discovered in the Src proteins. PTKs catalyse the phosphorylation of phenolic group of tyrosine residue in many substrate proteins by the transfer of γ-phosphate group of ATP.[1] The first step in such mechanisms is the activation of cell surface receptors (such as the insulin receptor, or epidermal growth factor (EGF) receptor) by extracellular ligands. The activation of the receptor results in the activation of tyrosine kinases, which generate phosphotyrosine residues inside the cell. These phosphotyrosine residues act as "beacons" which attract signaling proteins that contain SH2 domains to the receptors.[2]

The heterotrimeric G proteins, G_q-, $G_{12/13}$- and $G_{i/o}$-coupled GPCRs are all known to regulate mitogenesis via the transactivation of Src-dependent integrin signalling complexes (Figure 4.1). G_q- and $G_{i/o}$-coupled receptors also utilize Src to activate a variety of MAPK pathways. G_s-, $G_{i/o}$- and G_q-coupled receptors promote proliferation via the activation of the STAT transcription factors, and this has been postulated to be Src-dependent (Figure 4.1; shown in dashed lines). Full STAT activity may require phosphorylation by JAKs and MAPKs. Dashed lines also identify the probable involvement of multiple, unidentified intermediates in the transcriptional regulation of cell cycle proteins.[3]

The non-receptor tyrosine kinases of the Src family, Src, Yes, Lck, Fyn, Lyn, Fgr, Hck, Blk and Yrk, share a great deal of structural homology and are present in cytoplasm.[4-5] Src tyrosine kinase plays a prominent role in regulating cell growth and differentiation. Src has been implicated in development of variety of cancers. Src mutations and/or overexpression have been correlated with tumor growth, metastasis, and angiogenesis.[1-2,6]

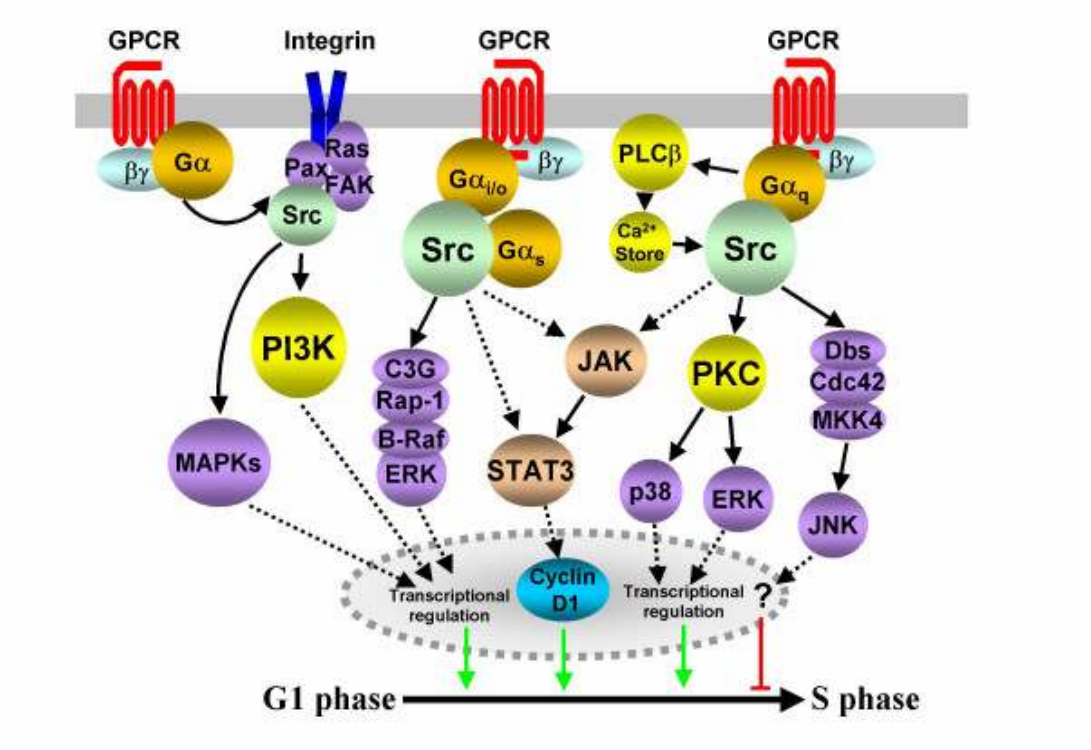


Figure 4.1 Role of Src kinase in signal transduction

The Src kinase has a structure composed of two peptide binding domains, in addition to a catalytic kinase domain. One of the peptide binding domains is the SH2 domain, and the other is an SH3 domain, which recognizes polyproline helices. The N-terminal region is myristylated, so the protein is associated with the cell membrane (Figures 4.2 and 4.3).[7]

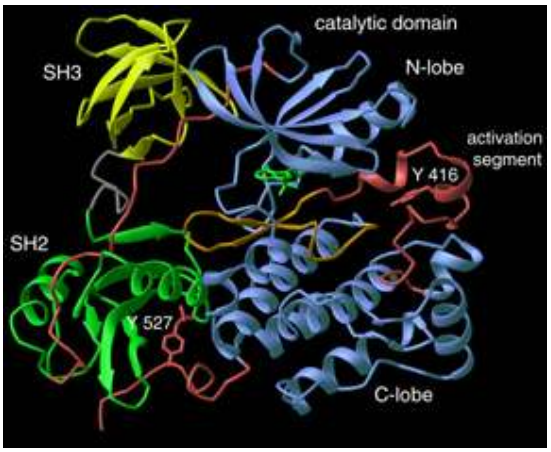


Figure 4.2 Src kinase and its homology domains

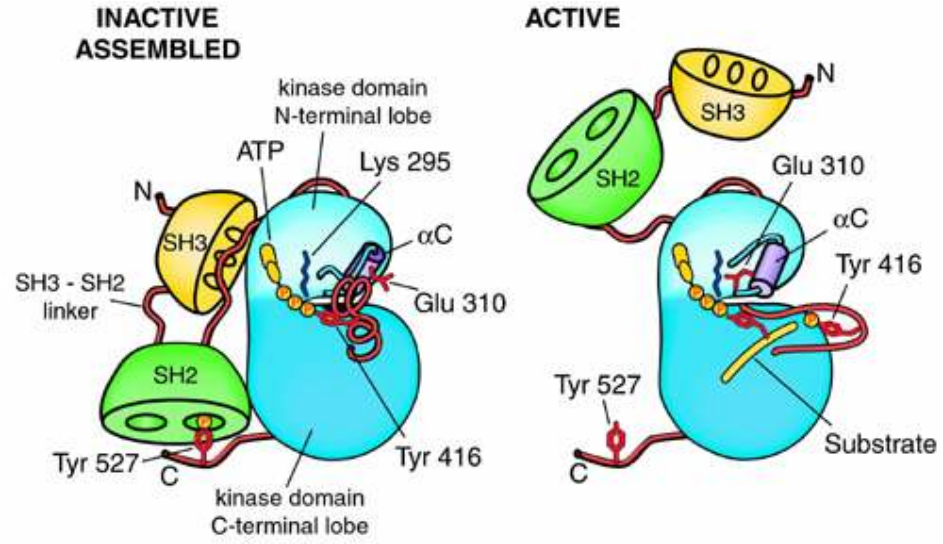


Figure 4.3 Src kinase in its basal and active states

Various structural motifs have been reported to target Src kinase[8-10] such as quinolinecarbonitriles,[11-14] ATP-phosphopeptide conjugates,[15] pyrazolopyrimidines,[16] purines,[16] imidazo[1,5-a] pyrazines,[17] benzotriazines,[18] pyrimidoquinolines,[19] pyridopyrimidinones[20], and quinazolines.[21] Imatinib **1**, a well known marketed PTK inhibitor, is used to treat a number of malignancies like chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GISTs). Dasatinib **2** is another marketed kinase inhibitor that inhibits Src family tyrosine kinases and BCR/ABL and is approved to use after Imatinib treatment. A 3-quinolinecarbonitrile based Src kinase inhibitor, Bosutinib **3**, is undergoing rigorous trials for cancer treatment (Figure 4.4).[22]

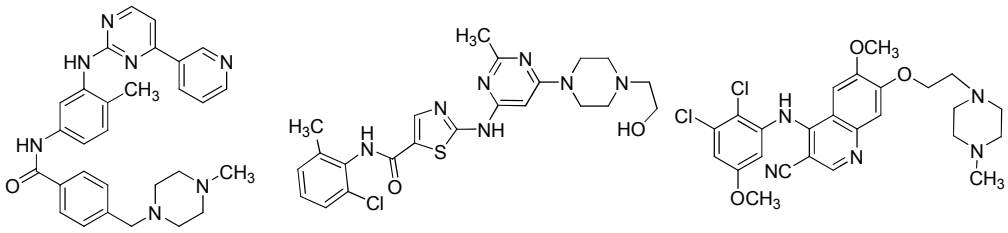


Figure 4.4 Marketed drugs **1-3** that target tyrosine kinase

4.1.2 Polyethylene Glycol in Organic Transformations

Organic reactions in environmentally benign media have attracted significant interest among researchers due to the detrimental impact of toxic and volatile solvents on the environment.[23-26] In the past, without losing reaction efficiency, chemists have

demonstrated the utility of benign alternative solvents such as ionic liquids, water and polyethylene glycol (PEG) as a replacement of toxic and volatile organic solvents.[27]

PEG is used extensively for a variety of purposes, ranging from additives in pharmaceutical industry to various medical purposes.[28-30] PEG is nontoxic and nonvolatile as well as miscible with water.[31] The use of PEG has been reviewed as a green reaction media where its phase-transfer properties and applications of PEG solutions in water have been highlighted. Also recent applications, as a scaffold and solvent for homogeneous Pd-catalysed carbon-carbon couplings such as Suzuki and Heck,[32] as a solvent for the Michael addition of amines to conjugated amines,[33] for catalytic hydrogenations with PtO_2,[34] and as a recyclable medium in asymmetric aldol reactions,[35] have been reported.

PEG has been found to be stable to acid, base, high temperature,[36-38] O_2, H_2O_2 high oxidation systems,[39] and $NaBH_4$ reduction systems,[40] although partial oxidation of the PEG terminal $–CH_2OH$ group to –COOH may occur in systems such as $H_2O_2–Na_2WO_4$.[37] In addition, PEG may be recovered from aqueous solution by extraction with a suitable solvent or by direct distillation of water or solvent.[41]

The water soluble polymer PEG can be considered a cosolvent in water which leads to an apparent decrease of the aqueous solution polarity.[42] The consequent increase in solubility of organic molecules has led to the application of PEG as a solvent and a phase transfer catalyst (PTC) in organic synthesis. PEG, in aqueous solution, acting as a co-solvent, significantly changes many of the properties of water. Solutions of PEG in water may be viewed as monophasic, consisting of two homogeneously mixed components, or as biphasic, having large hydrated polymer molecules separated by regions of free water.

There is a significant effect of PEG on the polarity of the aqueous solutions. The solution polarity of PEG and copolymer solutions are little less than that of pure water but the polarity further decreases with increasing polymer proportion.[43]

4.1.3 Biological Significance of 1,2,3-Triazole Derivatives

The 1,2,3-triazole, a five-member nitrogen-containing heterocycle, is often encountered as a structural unit of compounds possessing diverse biological activities including herbicidal, fungicidal, antibacterial, antiallergic, selective β_3 adrenergic receptor agonism,

antiHIV and anticonvulsant.[44-53] Many 1,2,3-triazoles are also used as dyes, optical brightners, agrochemicals, photographic materials and corrosion inhibitors.[42,46,48,54-61] Stability towards metabolic degradation, and capability of hydrogen-bond formation make 1,2,3-triazoles an attractive connecting unit to discover novel biologically interesting chemical entities.[62-65]

Bock et al. have proven that 1,4-disubstituted 1,2,3-triazoles can serve as transoid amide bond mimics in natural compounds without compromising biological activity.[66] The triazole was introduced into a cyclotetrapeptide and its activity is tested for tyrosinase activity in comparision to its native type. The activity remains unaffected even after replacing amide bond with 1,2,3-triazole (Figure 4.5). This study clearly demonstrates that analogues of an inaccessible natural product are readily available *via* Cu(I)-catalysed alkyne–azide coupling and that these analogues show retention of biological activity when compared with the parent cyclotetrapeptide.

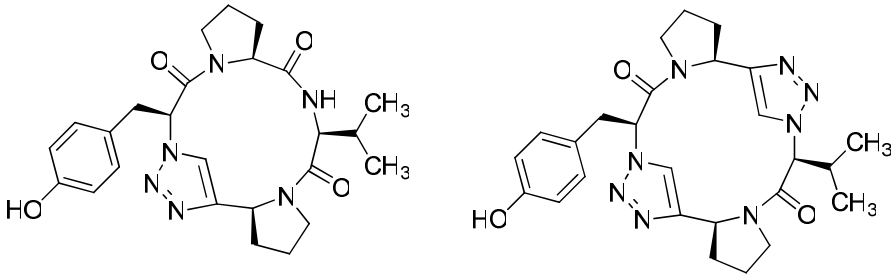


Figure 4.5 Amide bond substituted 1,2,3-triazoles in cyclotetrapeptides **4** and **5**

A series of α-diazo-β-oxoaldehyde compounds were condensed with different amines to yield 4-acyl-1*H*-1,2,3-triazoles.[67] The synthesised compounds were investigated for their inhibition activities against *Mycobacterium tuberculosis* $H_{37}R_v$ and the MIC of one of the compounds, **6**, is as low as 12.5 μg/mL (Figure 4.6).

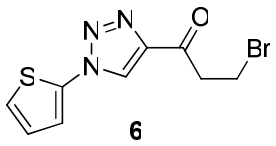


Figure 4.6 1,2,3-Triazole **6** with antitubercular activity

Heras and coworkers synthesised 1,4-disubstituted 1,2,3-triazoles **7** by the 1,3-dipolar cycloaddition of peracetylated glucopyranosyl azides to propargyl halides and tested their cytostatic activity in HeLa cells (Figure 4.7).[68] The compounds **7a** and **7b** exhibited ED_{50} of 3.5 and 2 μg/mL respectively.

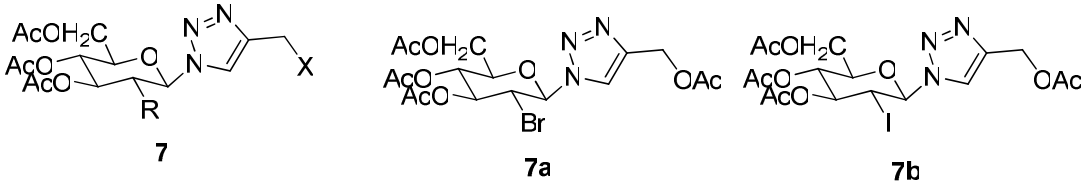


Figure 4.7 Peracetylated glucopyranosyl triazoles as cytostatic compounds

It was demonstrated[69] that the 1,2,3-triazole is an effective replacement for a peptide group in HIV-1 protease inhibitors. This has been illustrated with the combinatorial modification of Amprenavir **8** by azide–alkyne click chemistry followed by enzyme inhibition and structural analysis (Figure 4.8). The HIV-1 protease inhibition is evaluated in wild type and three other mutants. The compounds **9** and **10** exhibited significant activity.

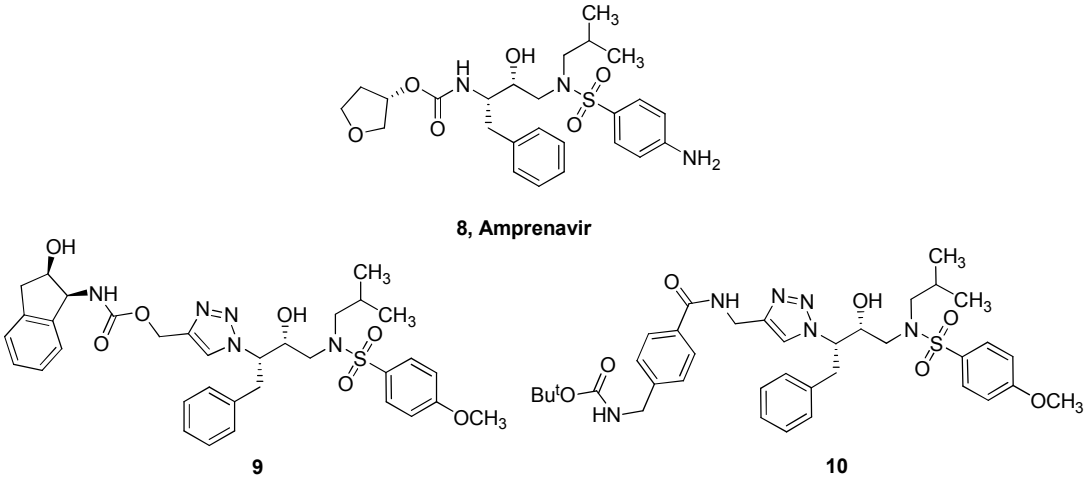
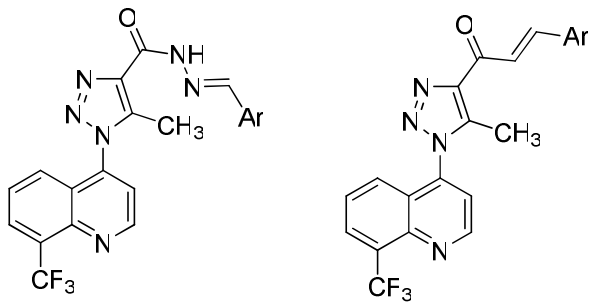


Figure 4.8 Amprenavir **8** and its analogues **9**, **10**

A series of 1,2,3-triazoles (**11** and **12**) were synthesised via 1,3-dipolar cycloaddition in good yields and antimicrobial activity of these derivatives containing quinoline moiety against wide range of bacterial and fungal stains (Figure 4.9) are evaluated. The antimicrobial activity study revealed that all the compounds screened showed good antibacterial activity (MIC: 6 – 25 μg/mL) and moderate antifungal activity (MIC: 6 – 25 μg/mL). Among the newly synthesised derivatives, compounds with 3-methyl thienyl substituent are found to increase antimicrobial activity.



11 **12**

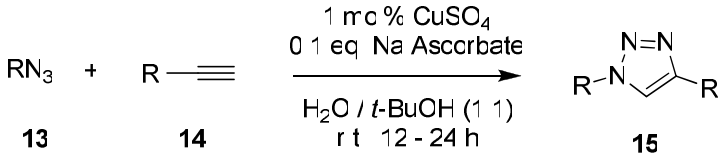
Figure 4.9 1,2,3-Triazoles **11** & **12** with antifungal and antimicrobial activity

4.1.4 Reported Protocols for the Synthesis of 1,2,3-Triazole Derivatives

In the past, various methods were developed for the synthesis of 1,2,3-triazoles. Of the existing procedures, the 1,3-dipolar cycloaddition reaction of azides with alkynes is a very useful organic transformation which has been extensively studied by Huisgen et al.[70-72] Recently Sharpless and co-workers have reported remarkable high yielding syntheses of 1,2,3-triazoles with excellent regioselectivity.[45]

Some of the reported methods for the synthesis of 1,4-disubstituted 1,2,3-triazoles are described below:

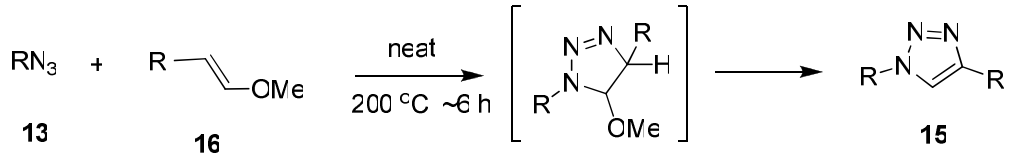
The Cu(I)-catalysed, stepwise cycloaddition of azides **13** to terminal alkynes **14** exhibits broad scope and provides 1,4-disubstituted 1,2,3-triazoles **15** in excellent yields and high regioselectivity (Scheme 4.1).[73]



13 **14** **15**

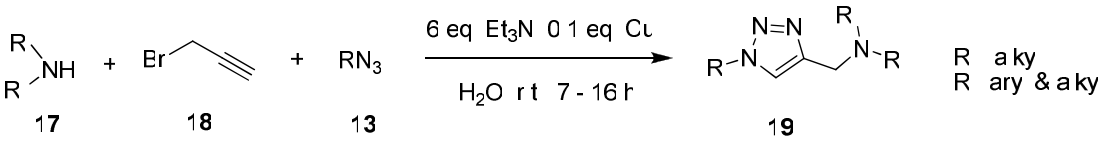
Scheme 4.1 Cu(I)-catalysed cycloaddition of azides and terminal alkynes

1,2,3-Triazoles are prepared in good to modest yields by cycloaddition of alkyl azides **13** onto enol ethers **16** under solvent-free conditions (Scheme 4.2).[74] The reaction can access ring-fused triazoles **15** that are unavailable by azide-alkyne cycloaddition and was easily scalable. The 1,2,3-triazole products bear functionality that may be readily derivatized.



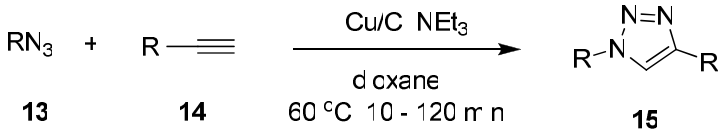
Scheme 4.2 Synthesis of 1,2,3-triazoles **15** under solvent-free conditions

A Cu(I)-catalysed three-component reaction of amines **17**, propargyl halides **18** and azides **13** forms 1-substituted-1*H*-1,2,3-triazol-4-ylmethyl-dialkylamines **19** in water (Scheme 4.3).[75] Synthetic advantages are high atom economy, low environmental impact, wide substrate scope, mild reaction conditions and good yields.



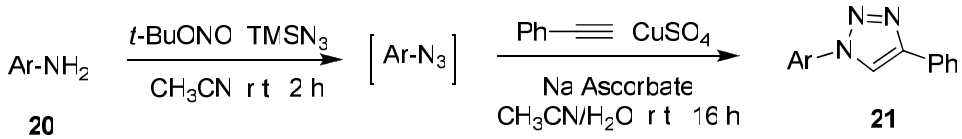
Scheme 4.3 One-pot synthesis of 1,2,3-triazolyl methyl dialkylamines **19**

Highly efficient click chemistry between azides **13** and terminal alkynes **14** can be heterogeneously catalysed by copper nanoparticles mounted within the pores of activated charcoal. Reactions can be accelerated with stoichiometric amounts of Et_3N or by increasing the reaction temperature using microwave irradiation (Scheme 4.4).[76]



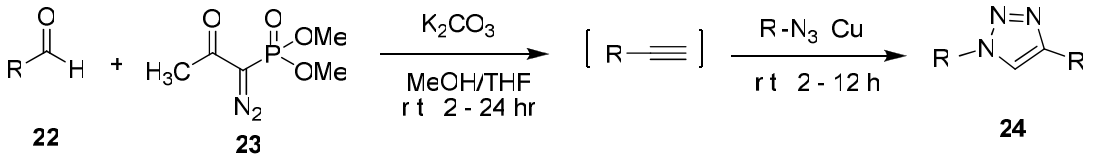
Scheme 4.4 Synthesis of 1,2,3-triazoles **15** catalysed by copper nanoparticles

The synthesis of aromatic azides from the corresponding amines was accomplished under mild conditions with *tert*-butyl nitrite and azidotrimethylsilane (Scheme 4.5). 1,4-Disubstituted 1,2,3-triazoles **21** were obtained in excellent yields from various aromatic amines **20** without the need for isolation of the azide intermediates.[77]



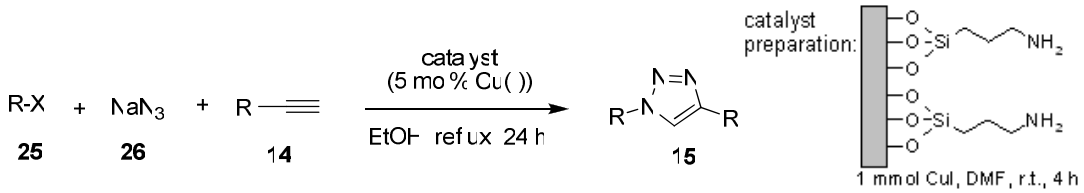
Scheme 4.5 One-pot synthesis of 1,2,3-triazoles **21** from arylamines

A reliable and operationally simple one-pot reaction for a one-carbon homologation of various aldehydes **22** with Bestmann's reagent **23** followed by Cu(I)-catalysed azide-alkyne click chemistry gives 1,4-disubstituted 1,2,3-triazoles **24** in good yields without the need for isolation of the alkyne intermediates (Scheme 4.6).[78]



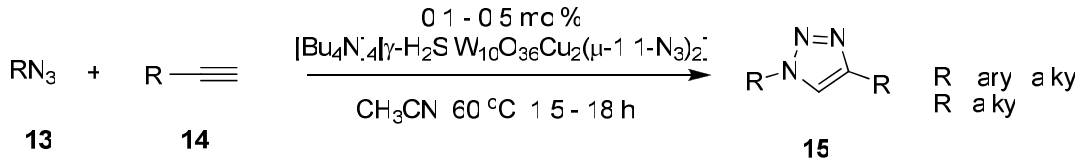
Scheme 4.6 One-pot synthesis of 1,2,3-triazoles **24** from aldehydes **22**

Cu(I) immobilized on 3-aminopropyl-functionalized silica gel catalysed the reaction of terminal alkynes **14** with benzyl- or alkyl halides **25** and sodium azide **26** in ethanol to give 1,4-disubstituted 1,2,3-triazoles **15** in good to excellent yields (Scheme 4.7).[79] Furthermore, the silica-supported copper could be recovered and recycled by simple filtration.



Scheme 4.7 One-pot synthesis of 1,2,3-triazoles **15** from benzyl/alkyl halides **25**

A dicopper-substituted γ-Keggin silicotungstate acts as an efficient precatalyst for the regioselective 1,3-dipolar cycloaddition of organic azides **8** to alkynes **9** (Scheme 4.8). Various substrates were efficiently converted to the corresponding 1,2,3-triazole derivatives **10** in excellent yields without any additives.[80]



Scheme 4.8 Dicopper catalyzed cycloaddition of azides **13** with alkynes **14**

4.2 The Present Study

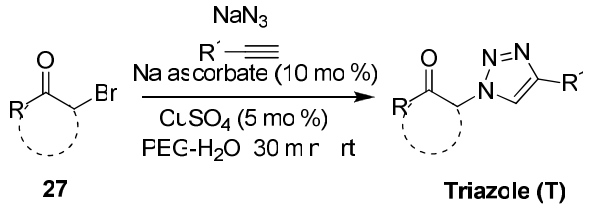
Herein, we describe the synthesis and evaluation of 1,4-disubstituted 1,2,3-triazoles as a novel template for Src kinase inhibition. The rational for choosing these derivatives is discussed in section 4.2.3. The synthesis of 1,4-disubstituted 1,2,3-triazoles was carried

out in one-pot starting from α-halo ketones / α-tosyloxy ketones. The reaction takes place under facile conditions in short time. The simplicity of its workup, tolerability of the protocol to diversely substituted starting materials and recyclability of the reaction media are the noteworthy features.

4.3 Results and Discussion

4.3.1 Synthesis of 1,2,3-Triazoles from α-Bromo Ketones / α-Tosyloxy Ketones

α-Bromo ketones can be easily prepared by the selective α-bromination of the appropriate acetyl compound.[81-85] Isolation and purification of some α-azido ketones from the reaction of α-bromo ketones and sodium azide is difficult due to incomplete conversion.[86-88] Therefore, a method which avoids the isolation and handling of α-azidoketones via *in situ* generation from the reaction of α-bromo ketones with sodium azide is highly attractive and safe. In our efforts to prepare a novel and diverse library of 1,4-disubstituted 1,2,3-triazoles, we report herein a one-pot synthesis of these interesting compounds using Cu(I)-catalysed reaction of α-bromo ketones **27**, terminal acetylenes and sodium azide in aqueous PEG 400 at room temperature (Scheme 4.9).



Scheme 4.9 Synthesis of 1,2,3-triazoles **T**

"Click" azide-alkyne cycloaddition reaction is reported in a wide variety of organic solvents, including THF,[89] *t*-BuOH:H_2O,[90] DMSO:H_2O,[59] MeCN,[91] and 1,4-dioxane:H_2O.[72] Various solvents were screened including environmentally benign solvents (Table 4.1).

Our initial attempts in aqueous medium failed to produce good yields. Encouraged by our recent success with benign aqueous PEG 400, we turned our attention to the PEG 400.[92-93] The reaction of α-bromo ketone, sodium azide, and phenylacetylene in presence of Cu(I) in PEG 400 afforded 1,2,3-triazoles **T** but the reaction took 24 h to complete (Table 4.1**)**.

Table 4.1 Screening of solvents for the synthesis of 1,2,3-triazoles **T**

S. No.	Solvent	Time (h)	Yield (%)
1	*t*-BuOH	24	65
2	*t*-BuOH-H_2O (1:1, v/v)	20	80
3	H_2O	6	15
4	PEG 400	24	80
5	PEG 400-H_2O (1:1, v/v)	0.5	90
6	PEG 400-H_2O (3:1, v/v)	4	75
7	PEG 400-H_2O (1:3, v/v)	10	53
8	Ethanol	5	50
9	Acetonitrile	8	45
10	DMF	17	30

Attributing this sluggish reaction to the high viscosity of the PEG 400, we zeroed in on the aqueous PEG 400 system. After several combinations of PEG 400 and water, it was observed that equal amounts of both the solvents is ideally suited for the efficient preparation of 1,2,3-triazoles. The reaction time with this aqueous PEG 400 system was as low as 30 min when compared to 12-24 h with *t*-BuOH/H_2O (1:1, v/v). We expect this prodigious behaviour is due to the phase transfer catalytic nature of PEG 400.

Under these optimized conditions, reaction of α-bromo acetophenone, sodium azide and phenylacetylene resulted in the exclusive formation of 4-phenyl-1-(2-phenylethan-2-on-1-yl)-1*H*-1,2,3-triazole **T1** in 90% yield at room temperature in 30 minutes. The IR spectrum of **T1** (Table 4.3) displayed a strong band at about 1690 cm^{-1} indicating the presence of a ketonic functionality. The ^1H NMR spectrum of **T1** displayed a distinct singlet at δ = 8.19 for the triazolyl C_5-H proton (Figure 4.10). The ^{13}C NMR spectrum of **T1** showed a peak at δ = 195.03 corresponding to C=O carbon (Figure 4.11). The mass spectrum of this compound showed the molecular ion peak at *m/z* = 264.3 $(M+H)^+$, which is in agreement with the calculated value (Figure 4.12).

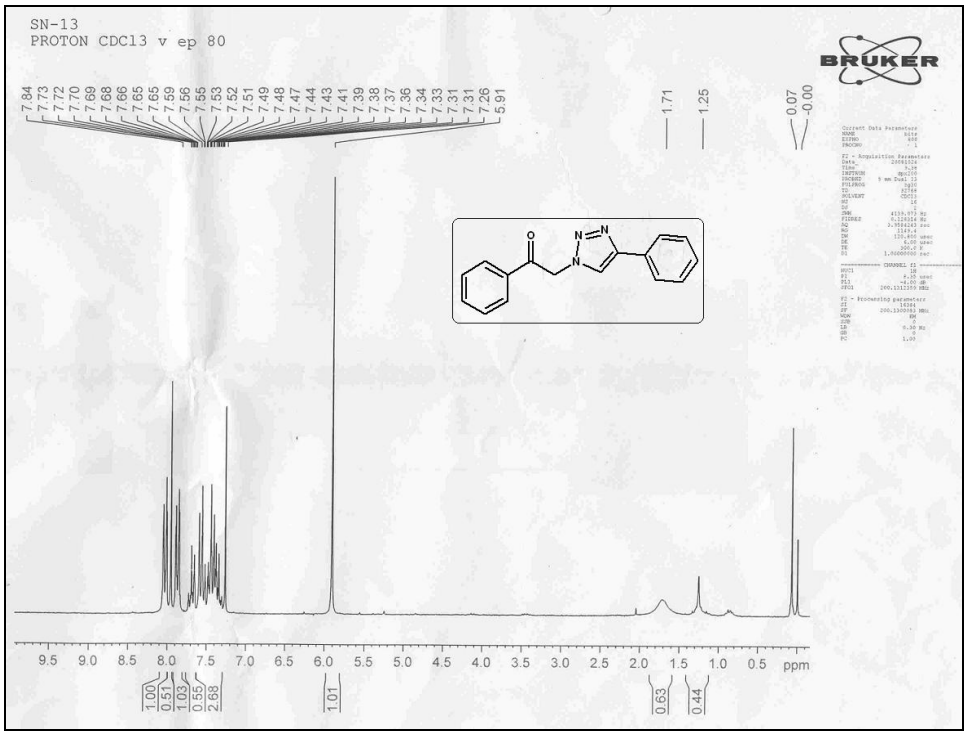


Figure 4.10 ^{1}H NMR of compound **T1**

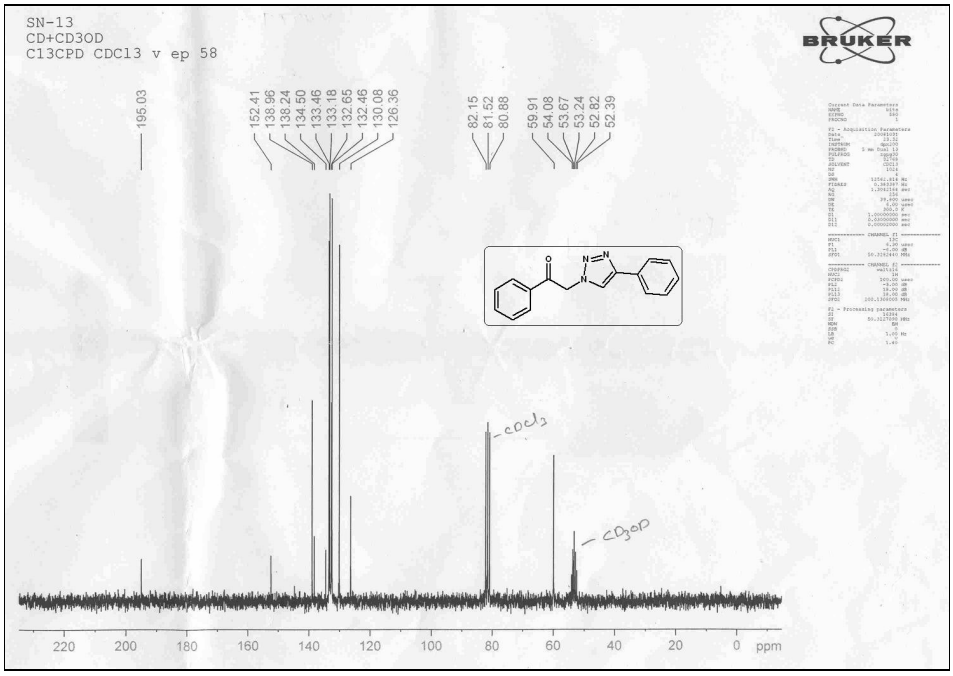


Figure 4.11 ^{13}C NMR of compound **T1**

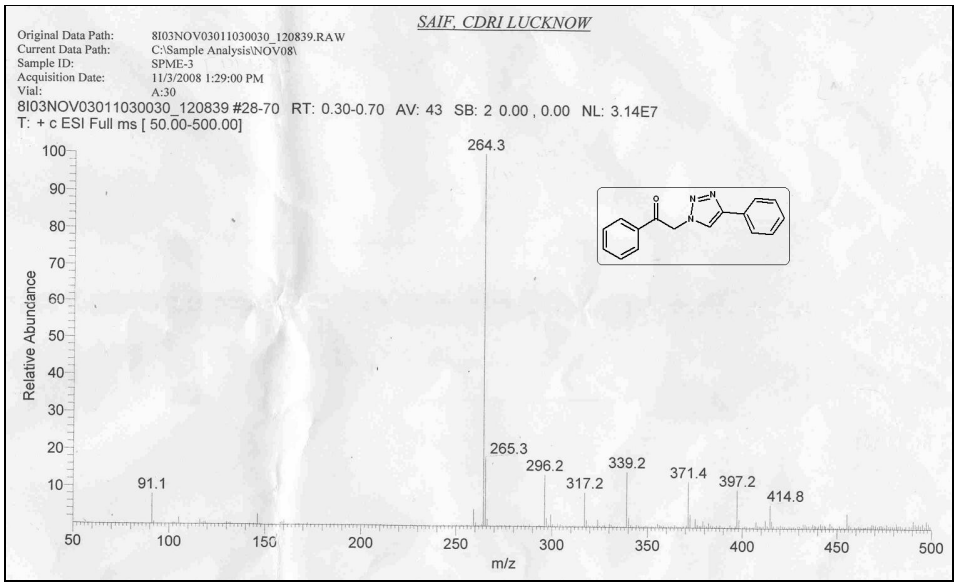


Figure 4.12 Mass spectrum of compound **T1**

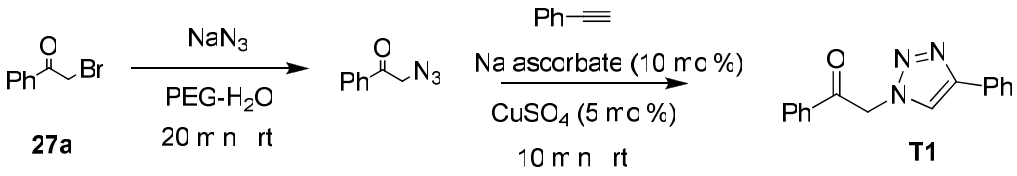
Encouraged by the success with initial efforts, we decided to extend the scope of this one-pot protocol by choosing various α–bromo compounds and terminal acetylenes. All the α–bromoketones, α–bromoacetonitrile, α–bromo-*N,N*-dimethylacetamide and terminal acetylenes reacted smoothly to afford diverse 1,4-disubstituted-1,2,3-triazoles **T2-24** in good yields (Table 4.3 & 4.4). Regioselective synthesis of 1,4-disubstituted 1,2,3-triazole analogues with diverse functionalities further demonstrates the usefulness of this protocol. After completion of the reaction, the products were isolated by simple filtration or percolating the crude product through a bed of silica gel. All the products were characterized by IR, NMR and MS spectral data.

To check the reusability of the catalyst and aqueous PEG 400 system, after the removal of product **T1**, the solution obtained was recycled for successive reactions. Almost similar results were obtained in the second and third successive recycles in terms of product yield and reaction time (Table 4.2).

Table 4.2 Reuse of reaction media in the synthesis of 1,2,3-triazole **T1**

Cycle	Solvent	Time (min)	Yield (%)
1	PEG 400-Water (1:1, v/v)	30	90
2	PEG 400-Water (1:1, v/v)	30	88
3	PEG 400-Water (1:1, v/v)	30	90

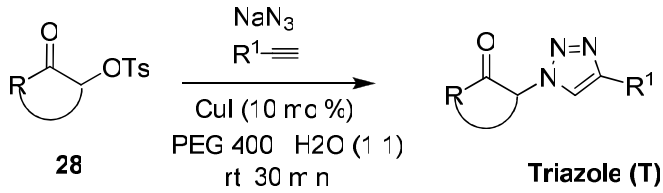
α-Azido ketones are often unstable to heat and light; therefore, *in situ* formation of these compounds is advantageous to handle them safely. Initial formation of the α-azido ketone intermediate was unambiguously confirmed by reacting equimolar quantities of α-bromo ketone and sodium azide in aqueous PEG 400. After the formation of α-azido ketone, as indicated by TLC and IR (2100 cm^{-1}), phenylacetylene was added to the reaction mixture. The products **T1** of this reaction and the one-pot condensation reaction were found to be identical (Scheme 4.10). Higher solubility of reactants in aqueous PEG 400 and formation of PEG 400-sodium cation complex may be responsible for the faster reaction.[31,94-96]



Scheme 4.10 Stepwise formation of 1,2,3-triazoles **T1**

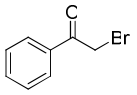
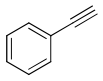
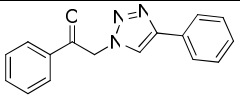
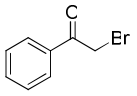
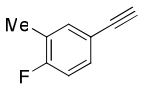
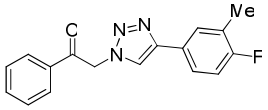
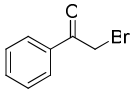
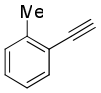
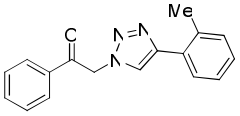
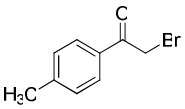
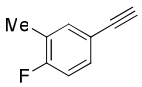
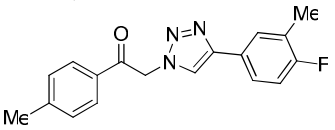
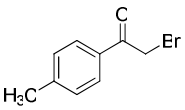
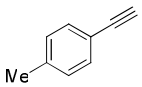
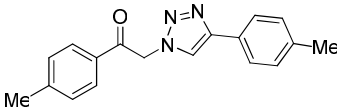
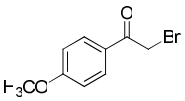
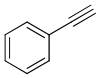
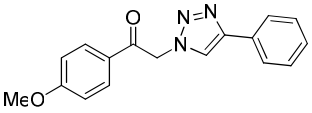
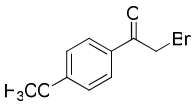
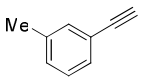
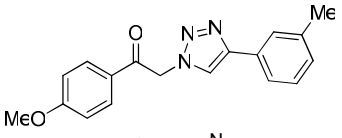
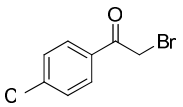
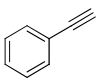
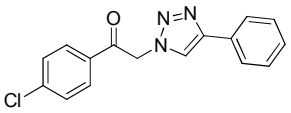
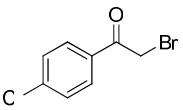
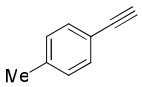
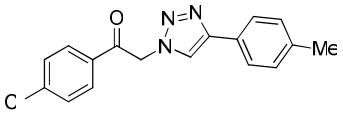
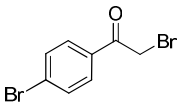
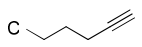
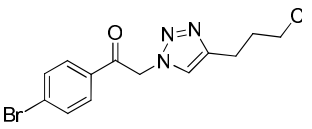
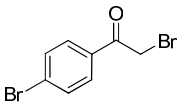
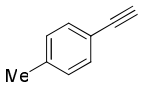
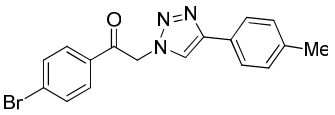
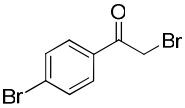
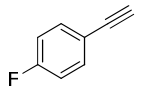
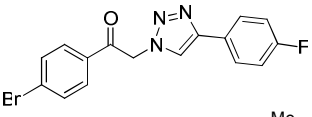
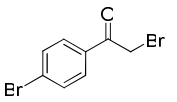
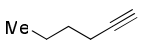
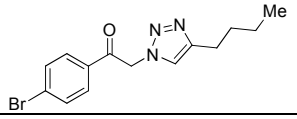
α-Tosyloxy ketones **28**, ideal substitutes for the lachrymatory α-bromo ketones, are easily accessible starting materials for a variety of organic transformations and in the construction of diverse heterocyclic compounds. This useful intermediate can be easily prepared from enolizable ketones using Koser's reagent, [hydroxy(tosyloxy)]iodobenzene.[97]

Keeping in view the various advantages of α-tosyloxy ketones, synthesis of 1,4-disubstituted-1,2,3-triazoles **T** was also attempted with α-tosyloxy ketones. The conditions were also further improvised by replacing copper sulphate and sodium ascorbate with copper iodide. Finally a one-pot, three-component condensation of α-tosyloxy ketones **28**, sodium azide, and terminal alkynes was achieved using the Cu(I)-catalysed "Click" azide-alkyne cycloaddition reaction in aqueous PEG 400 (Scheme 4.11).



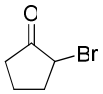
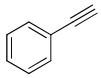
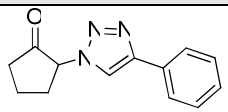
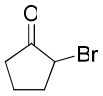
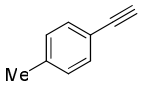
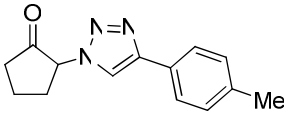
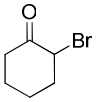
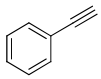
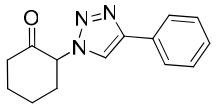
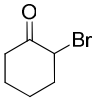
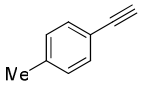
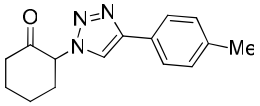
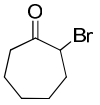
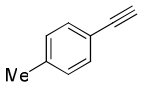
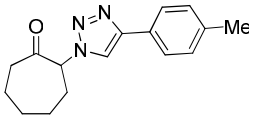
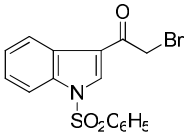
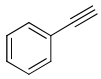
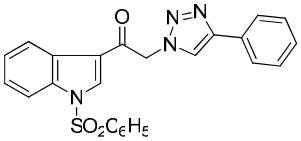
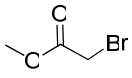
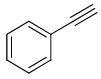
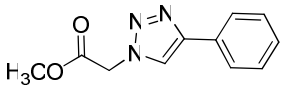
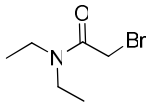
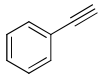
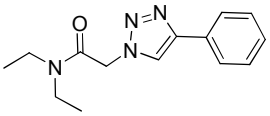
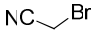
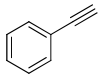
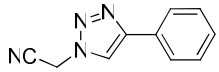
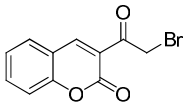
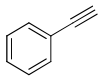
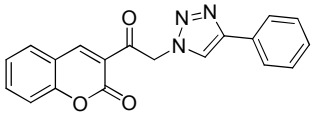
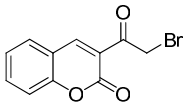
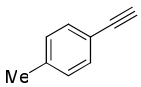
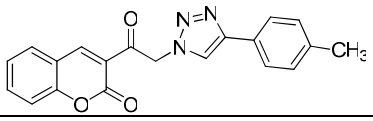
Scheme 4.11 Regioselective synthesis of 1,2,3-triazoles **T**

Table 4.3 Sequential synthesis of 1,2,3-triazoles **T** from α-bromo arylketones **27**

S.No.	α-Bromo ketone		Alkyne	Product		Yield (%)[a]
1.		**(27a)**			**(T1)**	90
2.		**(27a)**			**(T2)**	86
3.		**(27a)**			**(T3)**	85
4.		**(27b)**			**(T4)**	88
5.		**(27b)**			**(T5)**	90
6.		**(27c)**			**(T6)**	86
7.		**(27c)**			**(T7)**	84
8.		**(27d)**			**(T8)**	87
9.		**(27d)**			**(T9)**	85
10.		**(27e)**			**(T10)**	75
11.		**(27e)**			**(T11)**	65
12.		**(27e)**			**(T12)**	83
13.		**(27e)**			**(T13)**	76

[a]Yields refer to pure and isolated products

Table 4.4 Synthesis of 1,2,3-triazoles **T** from α-bromo ketones **27**

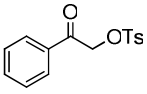
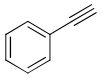
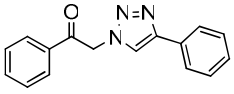
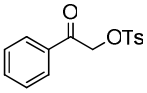
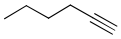
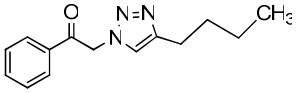
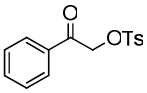
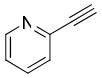
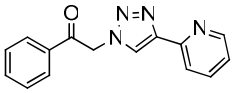
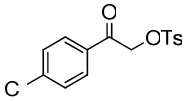
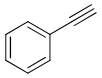
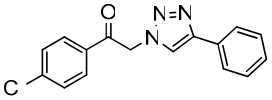
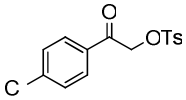
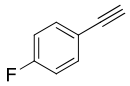
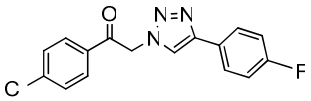
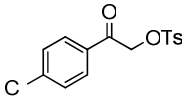
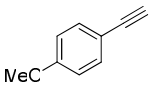
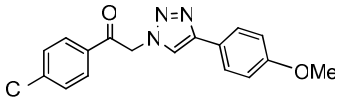
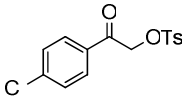
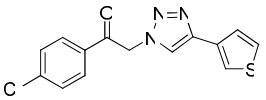
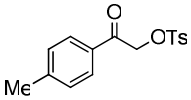
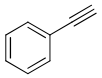
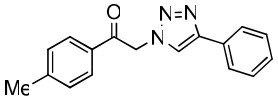
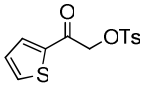
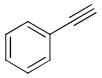
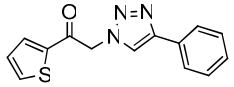
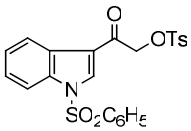
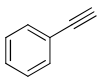
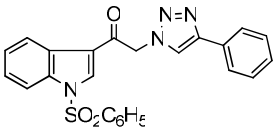
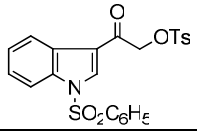
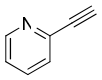
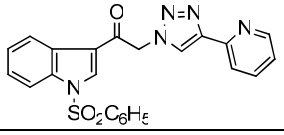
S.No.	α-bromo ketone		Alkyne	Product		Yield (%)[a]
1.		**(27f)**			**(T14)**	86
2.		**(27f)**			**(T15)**	81
3.		**(27g)**			**(T16)**	83
4.		**(27g)**			**(T17)**	85
5.		**(27h)**			**(T18)**	81
6.		**(27i)**			**(T19)**	80
7.		**(27j)**			**(T20)**	77
8.		**(27k)**			**(T21)**	78
9.		**(27l)**			**(T22)**	79
10.		**(27m)**			**(T23)**	68
11.		**(27m)**			**(T24)**	62

[a]Yields refer to pure and isolated products

One-pot reaction of α-tosyloxy acetophenone, sodium azide and phenylacetylene in presence of Cu(I)-catalysed in aqueous PEG 400 at room temperature afforded pure 4-phenyl-1-(2-phenyl ethan-2-on-1-yl)-1*H*-1,2,3-triazole **T1** in 82% yield. The IR spectra of **T1** exhibited a strong band at about 1695 cm^{-1} indicating the presence of ketonic functionality. Its ^1H NMR displayed a characteristic singlet at δ 8.19 for triazolyl C_5-H. High resolution mass spectrum of this compound showed the molecular ion peak at *m/z* 263.2415 which is in agreement with the calculated value, *m/z* 263.2939.

Under optimized conditions, synthesis of a variety of 1,4-disubstituted-1,2,3-triazoles **T** was undertaken using various α-tosyloxy ketones **28** and terminal alkynes (Table 4.5 & 4.6). Aromatic, heterocyclic and aliphatic terminal alkynes underwent three-component condensation smoothly to afford a wide range of 1,4-disubstituted-1,2,3-triazoles. The rich variety in the α-tosyloxy ketones also demonstrates the functional group tolerance of the reaction.

Table 4.5 Synthesis of 1,4-disubstituted-1,2,3-triazoles **T** from α-tosyloxy ketones **28**

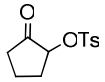
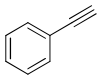
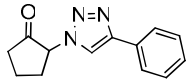
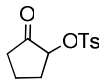
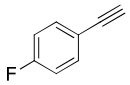
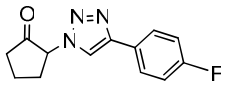
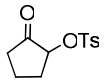
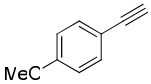
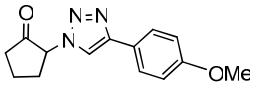
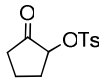
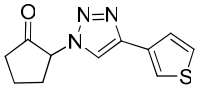
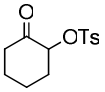
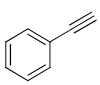
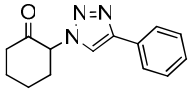
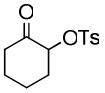
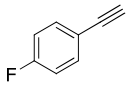
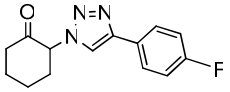
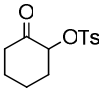
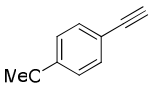
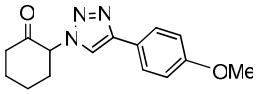
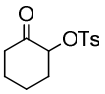
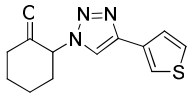
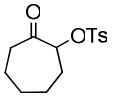
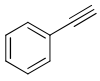
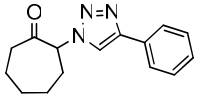
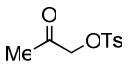
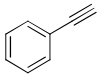
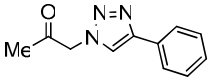
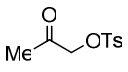
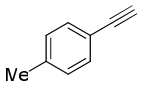
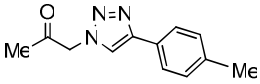
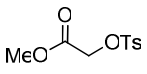
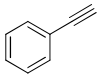
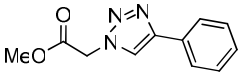
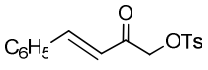
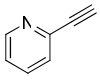
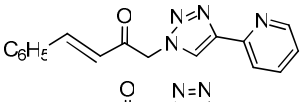
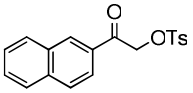
S.No.	α-Tosyloxy ketone		Alkyne	Product		Yield (%)[a]
1.		**(28a)**			**(T1)**	82
2.		**(28a)**			**(T25)**	82
3.		**(28a)**			**(T26)**	78
4.		**(28b)**			**(T8)**	79
5.		**(28b)**			**(T27)**	68
6.		**(28b)**			**(T28)**	70
7.		**(28b)**			**(T29)**	71
8.		**(28c)**			**(T30)**	81
9.		**(28d)**			**(T31)**	80
10.		**(28e)**			**(T19)**	85
11.		**(28e)**			**(T32)**	83

[a]Yields refer to pure and isolated products

The α-tosylate ester reacted smoothly to produce 1,4-disubstituted 1,2,3-triazole with 10 mol% of Cu(I) (Entry 12, Table 4.6). Also, tosylate of α,β-unsaturated ketone afforded

1,4-disubstituted 1,2,3-triazole in good yield (Entry 13, Table 4.6). The moderate to good yields of the products show the viability of the reaction at higher scales. All the products **T** were isolated by simple filtration after dilution with water. Analytically pure products were obtained by percolating the crude products through a bed of silica gel.

Table 4.6 Synthesis of 1,2,3-triazoles **T** from α-tosyloxy ketones **28**

S.No.	α-Tosyloxy ketone		Alkyne	Product		Yield (%)[a]
1.		**(28f)**			**(T14)**	81
2.		**(28f)**			**(T33)**	74
3.		**(28f)**			**(T34)**	82
4.		**(28f)**			**(T35)**	78
5.		**(28g)**			**(T16)**	83
6.		**(28g)**			**(T36)**	75
7.		**(28g)**			**(T37)**	80
8.		**(28g)**			**(T38)**	77
9.		**(28h)**			**(T39)**	77
10.		**(28i)**			**(T40)**	90
11.		**(28i)**			**(T41)**	85
12.		**(28j)**			**(T20)**	74
13.		**(28k)**			**(T42)**	76
14.		**(28l)**			**(T43)**	79
15.		**(28m)**			**(T44)**	75

[a]Yields refer to pure and isolated products

4.3.2 Plausible Mechanism

The step-wise mechanism[98-100] involves the initial formation of the α-azido ketone from the α-tosyloxy ketone with sodium azide. The Cu(I) quickly forms an acetylide with the terminal alkyne which in turn forms adduct **A** with α-azido ketone. Subsequent, intramolecular cyclization of **A** produces another cyclic adduct **B** which rearranges to copper-containing 1,2,3-triazole **C.** Finally protonation of **C** leads to 1,2,3-triazole **T** and regeneration of the catalyst (Figure 4.13).

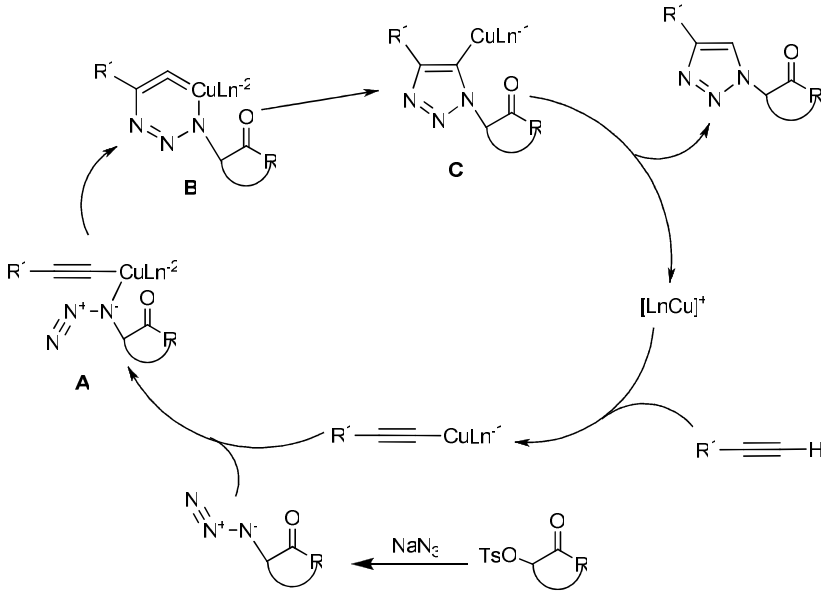


Figure 4.13 Reaction pathway for the formation of 1,2,3-triazoles **T**

4.3.3 Src Kinase Inhibitory Activity of the Synthesised 1,2,3-Triazoles

X-ray studies of phenylpyrazolopyrimidine inhibitors in Hck kinase-PP1 and Lck kinase-PP2[101-102] complexes have revealed a deep, hydrophobic binding pocket near the ATP binding site of Src family kinases for the aryl moiety of the pyrazolopyrimidine template. It was reported earlier that the hydrophobic interaction of the phenyl group with hydrophobic pocket is essential for the binding of 3-phenylpyrazolopyrimidines **29** (Figure 4.14) to the ATP binding site.[15] The pyrazolopyrimidine core resemble the purine core of ATP itself and bind in the nucleotide binding site in the position normally occupied by the adenine base. Any substituent attached to N^1 of pyrazole occupies a mostly hydrophobic cavity in PP1. Most of this hydrophobic cavity remains unfilled. This cavity, in part, formed from side chains of helix αC and helix αD.

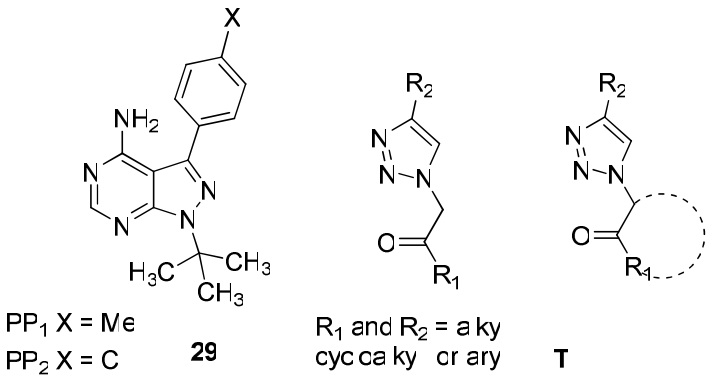


Figure 4.14 3-Phenylpyrazolopyrimidines **29** and 1,4-disubstituted 1,2,3-triazoles **T**.

Herein, the evaluation of 1,4-disubstituted 1,2,3-triazoles **T** as a novel template for Src kinase inhibition is discussed. The 1,2,3-triazoles are important heterocycles that are reported to possess several biological properties. The 1,2,3-triazole based compounds have been previously reported to inhibit p38 MAP kinase and PfPK7 protein kinase.[103-104]

It was hypothesized that substitution at N_1 position of 1,2,3-triazoles with hydrophobic residues may occupy and interact with the hydrophobic binding pocket of Src ATP binding site similar to that of 3-phenylpyrazolopyrimidines. The hydrophobic interactions of the hydrophobic groups with several amino acids in the hydrophobic pocket may contribute to the enhancement of potency.

The synthesised triazoles **T** are categorised into two classes. The first class of compounds (Table 4.7) includes 1,2,3-triazoles where R is a hydrophobic residue, such as phenyl, substituted phenyl, coumarinyl, 2-thienyl, or other nonaromatic substituents (i.e., CH_3, OCH_3, $N(C_2H_5)$). In compounds in class 2 (Table 4.8), R is a cyclopentanone-2-yl, cyclohexanone-2-yl, or cycloheptanone-2-yl substitution. Substitutions at position 4 (R^1) were phenyl, substituted phenyl, short alkyl, or a heteroaromatic (i.e., 2-pyridyl, 3-thienyl). The diversity of hydrophobic substitutions at R and R^1 positions allowed to explore the structure-activity relationship analysis of 1,4-disubstituted 1,2,3-triazoles **T**.

4.3.4 Structure-Activity Relationship

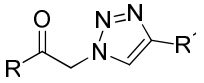
An array of 37 diversely substituted 1,2,3-triazoles were evaluated against Src kinase. The results of Src kinase inhibitory activity of compounds in classes I and II are shown in Tables 4.7 and 4.8, respectively.

In general, most of the compounds in class I with R as non-aromatic alkyl groups (Me, *N*-ethyl, OMe, **T20-21, T40-41**) exhibited weak Src kinase inhibition with IC_{50} values more than 100 μM or minimal inhibitory activity at highest concentration tested (375 μM). Furthermore, compounds with large aromatic groups such as styryl (**T42**), 3-coumarinyl (e.g., **T23**, **T24**) or aromatic groups with a bulky substitution (4-ClC_6H_4**,** 4-BrC_6H_4) in compounds **T8-13, T27-29** showed weak Src inhibitory potency. Attempts to improve the activity by introducing an aliphatic substituent at R^1 (**T13**, **T10**) also resulted in poor inhibition, suggesting that the size of aromatic moiety at R position is critical, and a bulky moiety at this position must be avoided. In contrast, the introduction of less bulkier unsubstituted phenyl and thienyl groups at position 1 in compounds **T3** (IC_{50} = 41.6 μM) and **T31** (IC_{50} = 32.5 μM) in class 1 significantly improved the Src inhibitory activities.

The presence of an electron-donating methyl group in R and R^1 phenyl ring in **T5** (IC_{50} = 49.8 μM) did not result in improved inhibition when compared with **T3**. The introduction of phenyl (**T1**), 4-F-3-$CH_3C_6H_3$ (**T2**), 2-pyridyl (**T26, T42**), and n-butyl (**T25**) as R^1 group drastically decreased the Src inhibitory activity versus **T3**. Introduction of electronegative fluorine also did not improve the activity in other compounds (**T4**, **T27**, and **T12**). These data indicate that the nature of R^1 group contributes significantly to the overall activity.

In order to explore the effect of nonaromatic cyclic functional groups at R position in Src inhibitory activity, a series of analogs having different cyclic ketones and bearing nonaromatic groups at R position were prepared and evaluated (Table 4.8). Compounds **T16** and **T17** with N_1 2-cyclohexanone and C4 phenyl/tolyl groups exhibited modest Src kinase inhibition with IC_{50} values of 43.2 and 33.9 μM, respectively. Introduction of 4-fluorophenyl, 4-methoxyphenyl and 3-thienyl substituents at C4 position of 1,2,3-triazole also led to the compounds (**T33-35, T36-38**) with poor activity. Other compounds in class 2 showed diminished activity versus **T16** and **T17**, confirming the importance of R^1 groups in overall activity.

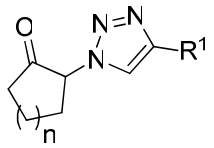
Table 4.7 The Src kinase inhibitory activity of 1,2,3-triazoles **T** (class I).



Compds	R	R^1	IC_{50} (μM)[a]
T1	C_6H_5	C_6H_5	>100.0
T3	C_6H_5	4-$CH_3C_6H_4$	41.6
T2	C_6H_5	4-F-3-$CH_3C_6H_3$	81.0
T26	C_6H_5	2-pyridyl	NA[b]
T42	Styryl	2-pyridyl	>100.0
T25	C_6H_5	*n*-butyl	NA
T5	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	49.8
T4	4-$CH_3C_6H_4$	4-F-3-$CH_3C_6H_3$	82.3
T6	4-$OCH_3C_6H_4$	C_6H_5	>100.0
T7	4-$OCH_3C_6H_4$	3-$CH_3C_6H_4$	72.8
T8	4-ClC_6H_4	C_6H_5	139.0
T9	4-ClC_6H_4	4-$CH_3C_6H_4$	108.7
T27	4-ClC_6H_4	4-FC_6H_4	NA
T28	4-ClC_6H_4	4-$OCH_3C_6H_4$	NA
T29	4-ClC_6H_4	3-thienyl	>150.0
T11	4-BrC_6H_4	4-$CH_3C_6H_4$	NA
T12	4-BrC_6H_4	4-FC_6H_4	NA
T13	4-BrC_6H_4	*n*-butyl	NA
T10	4-BrC_6H_4	1-Cl-butan-4-yl	>100.0
T23	coumarin-3-yl	C_6H_5	89.5
T24	coumarin-3-yl	4- $CH_3C_6H_4$	>150.0
T31	2-thienyl	C_6H_5	32.5
T40	CH_3	C_6H_5	>150.0
T41	CH_3	4-$CH_3C_6H_4$	>150.0
T20	OCH_3	C_6H_5	>100
T21	N(C_2H_5)	C_6H_5	>150.0
Staurosporine	_	_	0.3
PP2	_	_	2.8

[a]The concentration of the compound that inhibited enzyme activity by 50%,; [b]less than 10% enzyme inhibitory activity was observed up to the concentration of 75 μM. NA: No activity

Table 4.8 The Src kinase inhibitory activity of 1,2,3-triazoles **T** (class II).



Compds	n	R^1	IC_{50} (μM)[a]
T14	1	C_6H_5	105.5
T15	1	4-$CH_3C_6H_4$	62.1
T35	1	3-thienyl	NA[b]
T34	1	4-$OCH_3C_6H_4$	NA
T33	1	4-FC_6H_4	NA
T16	2	C_6H_5	43.2
T17	2	4-$CH_3C_6H_4$	33.9
T38	2	3-thienyl	NA
T36	2	4-FC_6H_4	NA
T37	2	4-$OCH_3C_6H_4$	NA
T18	3	4-$CH_3C_6H_4$	66.1

[a]The concentration of the compound that inhibited enzyme activity by 50%,; [b]less than 10% enzyme inhibitory activity was observed up to the concentration of 75 μM. NA: No activity

4.3.5 Molecular Modeling

Molecular modeling was utilized to examine how the structures would fit within the ATP-binding site of the enzyme (Figure 4.15). The modeling studies indicated that tolyl groups in **T3** and **T17** occupy the hydrophobic binding pocket similar to tolyl group of PP1 with slightly different orientations (Figure 4.15). The substitution at N1 position of triazole occupied mostly the hydrophobic cavity of Src ATP binding site similar to that of *t*-butyl group of PP1. The compounds demonstrated only modest inhibitory potency possibly because of mostly hydrophobic interactions. The 4-amino group of PP1 and PP2 is hydrogen bonded to the side chain of Thr338 as well as the carbonyl of Glu339 that contributes significantly to their potency as Src kinase inhibitors.

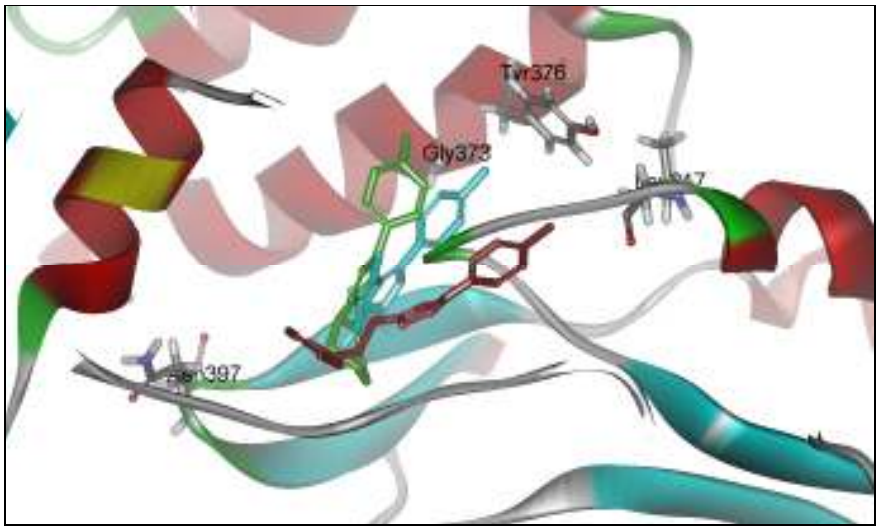


Figure 4.15 Comparison of structural complexes of Src kinase with different 1,2,3-triazoles (**T3**, red; **T17**, green) and PP1 (blue) based on molecular modeling. The compounds are rendered in stick styles. They are the lowest energy conformers predicted for the compounds.

4.4 Conclusions

In conclusion, we have developed a simple, benign and one-pot regioselective synthesis of biologically significant 1,4-disubstituted-1*H*-1,2,3-triazoles in good yields under catalytic conditions. The protocol is applicable to a wide range of α-tosyloxy/α-bromo ketones and acetylenic compounds, and allows the assembly of a diverse set of 1,4-disubstituted-1*H*-1,2,3-triazoles. The α-bromo ketones reacted as efficiently as α-tosyloxy ketones. The use of Cu(I) as a reusable catalyst in aqueous PEG makes this method facile, cost effective, and ecofriendly. Compounds **T3**, **T5**, **T31**, **T16**, and **T17** exhibited modest Src kinase inhibitory activity among the synthesised 1,2,3-triazoles with IC_{50} values in the range of 32-43 μM. Comparison of moderately active compounds indicate that the insertion of C_6H_5- and 4-$CH_3C_6H_4$- at R^1 position in both groups with appropriate less bulkier group at R position in class I is well tolerated for the modest Src inhibition activity of 1,2,3-triazoles. The structure-activity relationship data provide insights for further optimization of this scaffold and/or use in fragment-based discovery of Src kinase inhibitors.

4.5 Experimental Details

All the reagents were purchased from Aldrich and Spectrochem chemicals. The reactions were monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under

UV light (254 nm). Column chromatography, if performed, was using 100-200 mesh silica gel and appropriate mixture of hexane-ethyl acetate for elution. The solvents were evaporated on Buchi rotary evaporator. Melting points (M.p.) were determined with electrothermal capillary melting point apparatus. ^{1}H & ^{13}C NMR spectra were recorded on a Bruker Advance II (400 MHz) spectrometer. The coupling constant (*J*) values are in Hz. High resolution mass spectral data were obtained using electronspray ionization from a Quadruple-Time-of-flight (Q-Tof II) mass spectrometer (Micromass Manchester, U. K.). LC–MS analysis was carried out on an ABI 2000 Q-Trap mass spectrometer fitted with electro-spray ionization (ESI).

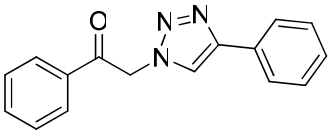
4.5.1 General Procedure for the Synthesis of 1,2,3-Triazoles

From α-bromo ketones: To a solution of α-bromo ketones (1.0 mmol), sodium azide (1.2 mmol) and terminal acetylene (1.0 mmol) in aqueous PEG 400 (2 mL) was added sodium ascorbate (19.8 mg, 10 mol%) and 1 M copper sulfate (50 μL, 5 mol%) solution. The reaction mixture was allowed to stir at room temperature for 30 min. After the reaction was complete, as indicated by TLC, the solid product was filtered, washed and dried to afford pure 1,4-disubstituted-1*H*-1,2,3-triazoles **T** .

From α-tosyloxy ketones: To a stirred solution of α-tosyloxy ketone (1.0 mmol), sodium azide (1.0 mmol) in aqueous PEG 400 (1 ml, 1:1, v/v), terminal alkyne (1.0 mmol), copper iodide (0.1 mmol) were added and allowed to stir at room temperature for 30 min. The reaction mixture appeared turbid. On completion of the reaction as indicated by the TLC, the reaction mixture was diluted with water and filtered at the pump to collect the product or extracted with ethyl acetate (3 x 2 ml). The organic layer was dried over anhydrous sodium sulfate and distilled using rotary vacuum evaporator to afford pure 1,4-disubstituted-1*H*-1,2,3-triazoles **T**.

Analytical data of the synthesised compounds:

4-Phenyl-1-(2-phenylethan-2-on-1-yl)-1*H*-1,2,3-triazole (T1)[72]



M.p.: 159 - 160 ^{o}C.

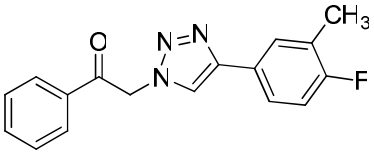
IR (KBr, *v* cm^{-1}): 1695 (CO).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.04-8.00 (m, 2H), 7.95 (s, 1H), 7.89-7.84 (m, 2H), 7.73-7.65 (m, 1H), 7.59-7.31 (m, 5H), 5.91 (s, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 195.0 (CO), 152.4, 139.0, 138.2, 134.5, 133.5, 133.2, 132.7, 132.5, 130.1, 126.4, 59.9.

MS (EI): *m/z* [M^+] calcd for $C_{16}H_{13}N_3O$ 263.2939, found 263.2415.

4-(4-Fluoro-3-methylphenyl)-1-(2-phenylethan-2-on-1-yl)-1*H*-1,2,3-triazole (T2)



M.p.: 156 – 158 ^oC.

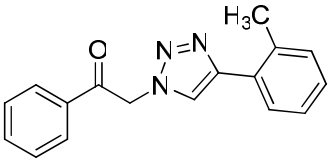
IR (KBr, *v* cm^{-1}): 1693 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.47 (s, 1H), 8.08 (d, *J* = 8.0 Hz, 2H), 7.71-7.57 (m, 5H), 7.22-7.17 (m, 1H), 6.25 (s, 2H), 2.27 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 192.2 (CO), 160.3, 159.1, 145.6, 134.2, 129.0, 128.4, 128.2, 127.0, 124.8, 124.5, 122.9, 115.4, 56.02, 14.2

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{14}FN_3O$: 296.1; found: 296.3.

4-(2-Methylphenyl)-1-(2-phenylethan-2-on-1-yl)-1*H*-1,2,3-triazole (T3)



M.p.: 116 – 119 ^oC.

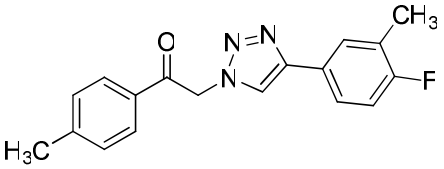
IR (KBr, *v* cm^{-1}): 1712 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.50 (s, 1H), 8.12-8.10 (m, 2H), 7.75-7.60 (m, 5H), 7.37-7.33 (m, 1H), 7.17-7.16 (m, 1H), 6.26 (s, 2H), 2.37 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 192.2 (CO), 146.4, 138.1, 134.3, 134.1, 130.1, 129.0, 128.8, 128.5, 128.2, 125.7, 123.0, 122.3, 56.0, 21.0.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}N_3O$: 278.1; found: 278.3.

4-(4-Fluoro-3-methylphenyl)-1-(2-(4-methylphenyl)ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T4)



M.p.: 155 – 158 ^{o}C.

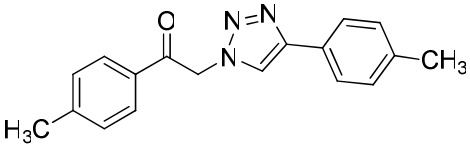
IR (KBr, *v* cm^{-1}): 1681 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.97 (d, *J* = 8.0 Hz, 2H), 7.90 (s, 1H), 7.71-7.69 (m, 1H), 7.62-7.58 (m, 1H), 7.06-7.01 (m, 1H), 6.98 (d, *J* = 8.0 Hz, 2H), 5.82 (s, 2H), 3.89 (s, 3H), 2.31 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 188.2 (CO), 164.1, 160.7, 146.9, 130.1, 128.5, 126.3, 125.9, 124.9, 124.3, 120.8, 114.9, 113.9, 55.1, 54.7, 14.1.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{16}FN_3O$ $(M+H)^+$: 310.1; found: 310.3.

1-(2-(4-Methylphenyl)ethan-2-on-1-yl)-4-(4-methylphenyl)-1*H*-1,2,3-triazole (T5)



M.p.: 132 - 135 ^{o}C.

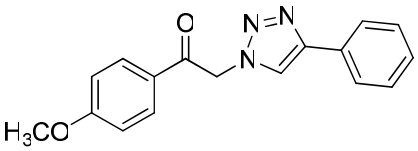
IR (KBr, *v* cm^{-1}): 1691 (CO).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.99 (d, *J* = 8.9 Hz, 2H), 7.90 (s, 1H), 7.74 (d, *J* = 8.1 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 6.99 (d, *J* = 8.9 Hz, 2H), 5.81 (s, 2H), 3.89 (s, 3H), 2.38 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.7, 164.6, 148.2, 138.0, 130.6, 129.5, 127.8, 127.0, 125.7, 121.1, 114.4, 113.7, 55.6, 21.2.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{17}N_3O$: 292.1; found: 292.3.

1-(2-(4-Methoxyphenyl)ethan-2-on-1-yl)-4-phenyl-1*H*-1,2,3-triazole (T6)



M.p.: 148 – 152 ^{o}C.

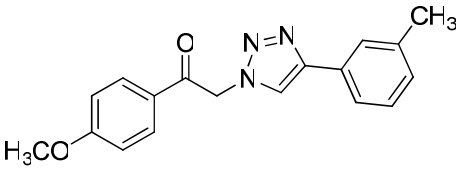
IR (KBr, *v* cm^{-1}): 1697 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.53 (s, 1H), 8.09 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.48-7.45 (m, 2H), 7.36-7.34 (m, 2H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.20 (s, 2H), 3.88 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6): δ = 193.5, 169.0, 152.0, 135.2, 134.9, 133.2, 132.4, 131.5, 130.0, 126.5, 118.7, 60.0, 59.8.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}N_3O_2$: 294.1; found: 294.3.

1-(2-(4-methoxyphenyl)ethan-2-on-1-yl)-4-(3-methylphenyl)-1*H*-1,2,3-triazole (T7)



M.p.: 140 – 142 ^oC.

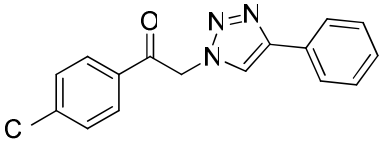
IR (KBr, *v* cm^{-1}): 1695 (CO).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.00-7.98 (m, 3H), 7.74 -7.66 (m, 2H), 7.33-7.28 (m, 1H), 7.15 (d, *J* = 7.5 Hz, 2H), 6.98 (d, *J* = 8.2 Hz, 2H), 5.83 (s, 2H), 3.88 (s, 3H), 2.39 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 190.3 (CO), 163.9, 146.3, 138.1, 130.7, 130.6, 128.8, 128.5, 126.9, 125.7, 123.0, 122.3, 114.2, 69.7, 55.6, 21.0

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{17}N_3O_2$: 308.1399; found: 308.3.

1-(2-(4-Chlorophenyl)ethan-2-on-1-yl)-4-phenyl-1*H*-1,2,3-triazole (T8)



M.p.: 175 – 178 ^oC.

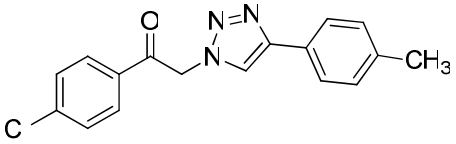
IR (KBr, *v* cm^{-1}): 1685 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.53 (s, 1H), 8.11 (d, *J* = 8.0 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.48-7.45 (m, 2H), 7.37-7.35 (m, 1H), 6.27 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 191.4 (CO), 146.3, 139.2, 132.8, 130.7, 130.1, 129.1, 128.9, 127.9, 125.1, 123.0, 56.0.

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{13}ClN_3O$: 298.0747; found: 297.9593.

1-(2-(4-Chlorophenyl)ethan-2-on-1-yl)-4-(4-methylphenyl)-1*H*-1,2,3-triazole (T9)



M.p.: 179 – 181 ^{o}C.

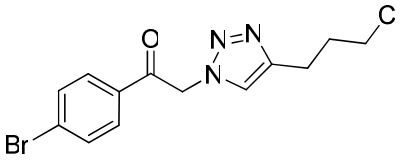
IR (KBr, *v* cm^{-1}): 1697 (CO).

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ = 8.46 (s, 1H), 8.08 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.17 (s, 2H), 2.33 (s, 3H).

^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ = 190.4 (CO), 163.9, 146.3, 137.1, 130.6, 129.5, 128.0, 126.9, 125.0, 122.6, 114.2, 55.6, 20.8

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{14}ClN_3O$: 312.0; found: 312.3.

1-(2-(4-Bromophenyl)ethan-2-on-1-yl)-4-(3-chloropropyl)-1*H*-1,2,3-triazole (T10)



M.p.: 147 – 149 ^{o}C.

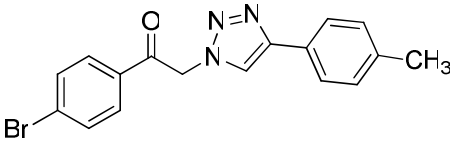
IR (KBr, *v* cm^{-1}): 1693 (CO).

^{1}H NMR (300 MHz, DMSO-*d*$_6$) : 7.86 (d, *J* = 8.49 Hz, 2H), 7.68 (d, *J* = 8.49 Hz, 2H), 7.51 (s, 1H), 5.79 (s, 2H), 3.65-3.58 (m, 2H), 2.96-2.91 (m, 2H), 2.24-2.15 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): 189.7 (CO), 146.8, 132.7, 132.6, 130.0, 129.6, 123.0, 55.3, 44.2, 31.8, 22.7.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{13}H_{13}BrClN_3O$: 344.0; found: 344.3.

1-(2-(4-Bromophenyl)ethan-2-on-1-yl)-4-(4-methylphenyl)-1*H*-1,2,3-triazole (T11):



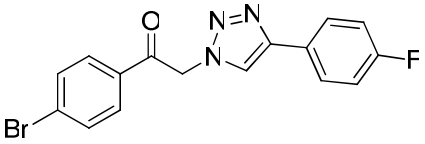
M.p.: 202-206 ^{o}C .

IR (KBr, *v* cm^{-1}): 1703 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90-7.89 (m, 3H), 7.75 (d, *J* = 8.40 Hz, 2H), 7.70 (d, *J* = 8.80 Hz, 2H), 7.24 (d, *J* = 8.00 Hz, 2H), 5.84 (s, 2H), 2.39 (s, 3H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}BrN_3O$: 356.0398; found: 357.9048

1-(2-(4-Bromophenyl)ethan-2-on-1-yl)-4-(4-fluorophenyl)-1*H*-1,2,3-triazole (T12):



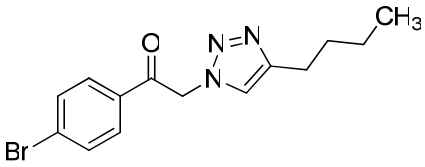
M.p.: 182-185 ^{o}C.

IR (KBr, *v* cm^{-1}): 1695 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90-7.88 (m, 3H), 7.85-7.81 (m, 2H), 7.71 (d, *J* = 8.80 Hz, 2H), 7.13 (t, *J* = 8.80 Hz, 2H), 5.85 (s, 2H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{12}BrFN_3O$: 360.0148; found: 359.8784

1-(2-(4-Bromophenyl)ethan-2-on-1-yl)-4-butyl-1*H*-1,2,3-triazole (T13):



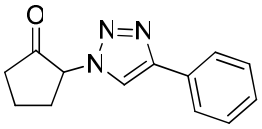
M.p.: 168-171 ^{o}C.

IR (KBr, *v* cm^{-1}): 1693 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, *J* = 8.40 Hz, 2H), 7.69 (d, *J* = 8.80 Hz, 2H), 7.46 (s, 1H), 5.77 (s, 2H), 2.77 (t, *J* = 7.60 Hz, 2H), 1.70 (pentet, *J* = 8.00 Hz, 2H), 1.41 (sextet, *J* = 7.40 Hz, 2H), 0.94 (t, *J* = 7.40 Hz, 3H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{17}BrN_3O$: 322.0555; found: 321.9362

1-(Cyclopentan-1-on-2-yl)-4-phenyl-1*H*-1,2,3-triazole (T14)



M.p.: 80 – 83 ^{o}C.

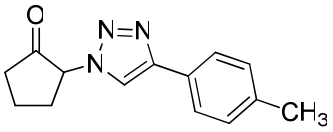
IR (KBr, *v* cm^{-1}): 1732 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.89 (s, 1H), 7.84 (d, *J* = 7.48 Hz, 2H), 7.40–7.44 (m, 2H), 7.35–7.31 (m, 1H), 4.96 (dd, *J* = 11.46 Hz and 8.44 Hz, 1H), 2.86–2.79 (m, 1H), 2.65–2.42 (m, 3H), 2.36–2.29 (m, 1H), 2.12–2.01 (m, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 211.2 (CO), 146.3, 130.4, 130.3, 129.2, 128.7, 127.5, 65.2, 35.4, 29.46, 17.87

HRMS(ESI): *m/z* $[M]^+$ calcd for $C_{13}H_{13}N_3O$: 227.1059; found: 227.1572.

1-(Cyclopentan-1-on-2-yl)-4-(4-methylphenyl)-1*H*-1,2,3-triazole (T15)



M.p.: 104 – 108 ^{o}C.

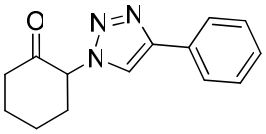
IR (KBr, *v* cm^{-1}): 1753 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.89 (s, 1H), 7.75 (2 H, d, *J* = 8.0 Hz), 7.26 (2 H, d, *J* = 8.0 Hz), 4.96 (dd, *J* = 11.46 Hz and 8.44 Hz, 1H), 2.86–2.79 (m, 1H), 2.65–2.42 (m, 3H), 2.36–2.29 (m, 4H), 2.12–2.01 (m, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 211.2 (CO), 146.3, 137.2, 129.5, 127.8, 125.0, 120.8, 65.2, 35.4, 29.46, 20.79, 17.87

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{15}N_3O$: 242.1; found: 242.3.

1-(Cyclohexan-1-on-2-yl))-4-phenyl-1*H*-1,2,3-triazole (T16)



M.p.: 86 – 87 ^{o}C.

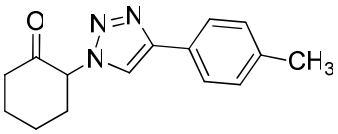
IR (KBr, *v* cm^{-1}): 1715 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.56 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 2H), 7.47-7.44 (m, 2H), 7.35-7.32 (m, 1H), 5.76-5.71 (m, 1H), 2.75-2.67 (m, 1H), 2.45-2.32 (m, 3H), 2.11-2.09 (m, 1H), 1.96-1.92 (m, 2H), 1.76-1.68 (m, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 204.1 (CO), 145.9, 130.8, 128.9, 127.8, 125.0, 121.3, 66.8, 40.4, 33.4, 26.4, 23.6.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{16}N_3O$: 242.1293; found: 242.0473.

1-(Cyclohexan-1-on-2-yl))-4-(4-methylphenyl)-1*H*-1,2,3-triazole (T17)



M.p.: 97 – 99 ^{o}C.

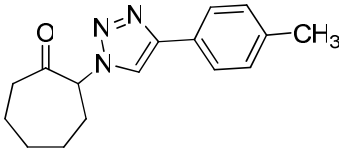
IR (KBr, *v* cm^{-1}): 1725 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.56 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 5.76-5.71 (m, 1H), 2.75-2.67 (m, 1H), 2.45-2.32 (m, 3H), 2.11-2.09 (m, 1H), 1.96-1.92 (m, 2H), 1.76-1.68 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 204.1 (CO), 146.2, 137.0, 129.4, 128.1, 125.0, 121.0, 66.8, 39.7, 33.1, 26.4, 23.6, 20.8.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{17}N_3O$: 256.1; found: 256.3.

1-(Cycloheptan-1-on-2-yl)-4-(4-methylphenyl)-1*H*-1,2,3-triazole (T18)



M.p.: 132 – 136 °C.

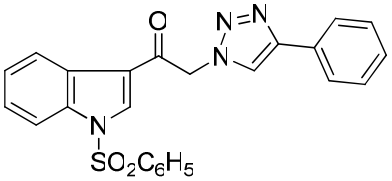
IR (KBr, *v* cm^{-1}): 1710 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 5.84 (dd, *J* = 8.00 Hz and 4.00 Hz, 1H), 2.78-2.74 (m, 1H), 2.54-2.47 (m, 1H), 2.33 (s, 3H), 2.30-2.14 (m, 2H), 1.89-1.78 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 206.4 (CO), 146.0, 137.0, 129.4, 128.1, 125.0, 121.0, 68.1, 41.2, 30.7, 28.6, 26.8, 22.6, 20.8.

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{19}N_3O$: 270.1; found: 270.3.

4-Phenyl-1-(2-[1-(phenylsulfonyl)-1H-indol-3-yl]-ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T19)



M.p.: 158 – 161 °C.

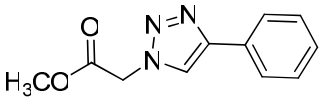
IR (KBr, *v* cm^{-1}): 1685 (CO).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.17 (s, 1H), 8.61 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 2H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 2H), 7.80-7.76 (m, 1H), 7.69-7.65 (m, 2H), 7.49-7.33 (m, 5H), 6.26 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 187.9 (CO), 146.3, 136.3, 135.4, 134.8, 133.9, 130.7, 130.2, 128.9, 127.9, 127.3, 126.8, 126.2, 125.2, 125.1, 123.2, 122.0, 117.5, 113.1, 56.0.

HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{24}H_{19}N_4O_3S$: 443.1178; found: 443.0073.

1-(2-Methoxyethan-2-on-1-yl)-4-phenyl-1*H*-1,2,3-triazole (T20)



M.p.: 79 – 82 °C.

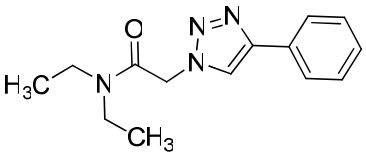
IR (KBr, *v* cm^{-1}): 1759 (CO).

^{1}H NMR (300 MHz, $CDCl_3$): 7.92-7.82 (m, 3H), 7.42-7.27 (m, 3H), 5.21 (s, 2H), 3.80 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): 166.8 (CO), 148.2, 130.4, 128.9, 128.3, 125.8, 121.1, 53.0, 50.8.

MS (EI): m/z $[M+H]^+$ calcd for $C_{11}H_{12}N_3O_2$: 218.1; found: 218.3.

1-(2-(N,N-Diethylamino)ethan-2-on-1-yl)-4-phenyl-1*H*-1,2,3-triazole (T21)



M.p.: 131 – 134 °C.

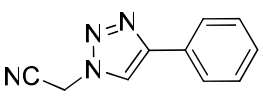
IR (KBr, *v* cm^{-1}): 1651 (CO).

^{1}H NMR (300 MHz, $CDCl_3$): 8.03 (s, 1H), 7.85-7.83 (m, 2H), 7.44-7.39 (m, 2H), 7.39-7.30 (m, 1H), 5.23 (s, 2H), 3.46-3.39 (m, 4H), 1.25 (t, *J* = 7.15 Hz, 3H), 1.15 (t, *J* = 7.10 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): 164.0 (CO), 148.0, 130.7, 128.8, 128.1, 125.8, 121.4, 50.9, 42.0, 41.0, 14.4, 12.8.

MS (EI): m/z $[M+H]^+$ calcd for $C_{14}H_{18}N_4O$: 259.2; found: 259.3.

1-Cyanomethyl-4-phenyl-1*H*-1,2,3-triazole (T22)



M.p.: 95 – 98 °C.

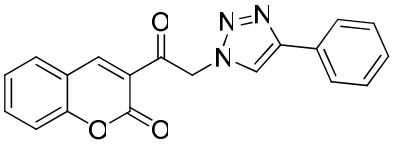
IR (KBr, *v* cm^{-1}): 2286 (CN).

^{1}H NMR (200 MHz, $CDCl_3$): 8.0 (s, 1H), 7.86-7.81 (m, 2H), 7.51-7.38 (m, 2H), 5.40 (s, 2H).

^{13}C NMR (50 MHz, $CDCl_3$): 149.6, 129.9, 129.4, 129.3, 126.3, 120.3, 113.1, 38.0.

MS (EI): m/z $[M+H]^+$ calcd for $C_{10}H_9N_4$: 185.1; found: 185.2.

1-(2-(Coumarin-3-yl)ethan-2-on-1-yl)-4-phenyl-1*H*-1,2,3-triazole (T23)



M.p.: 152 – 154 ^oC.

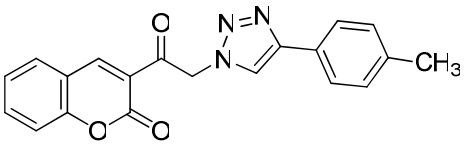
IR (KBr, *v* cm^{-1}): 1730 (CO).

^1H NMR (400 MHz, DMSO-d_6): δ = 8.44 (s, 1H), 8.05 – 8.00 (m, 2H), 7.83 – 7.75 (m, 4H), 7.55 – 7.43 (m, 4H), 6.06 (s, 2H).

^{13}C NMR (100 MHz, DMSO-d_6): δ = 189.5 (CO), 148.7, 148.3, 137.1, 135.3, 135.1, 131.2, 131.1, 129.5, 125.2, 125.1, 125.0, 122.5, 118.0, 116.3, 116.2, 57.6

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{13}N_3O_3$: 332.1; found: 332.3.

1-(2-(Coumarin-3-yl)ethan-2-on-1-yl)-4-(4-methylphenyl)-1*H*-1,2,3-triazole (T24):



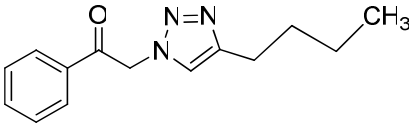
M.p.: 146-148 ^oC.

IR (KBr, *v* cm^{-1}): 1730 (CO).

^1H NMR (400 MHz, $CDCl_3$): δ 8.86 (s, 1H), 7.84 (s, 1H), 7.77-7.72 (m, 4H), 7.45-7.39 (m, 2H), 7.25 (d, *J* = 8.80 Hz, 2H), 6.00 (s, 2H), 2.39 (s, 3H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{16}N_3O_3$: 346.1192; found: 345.9914

4-*n*-Butyl-1-(2-phenyl ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T25):



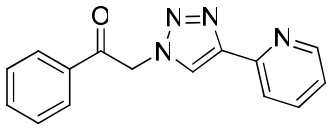
M.p.: 91- 94 ^oC.

IR (KBr, *v* cm^{-1}): 1697 (CO).

^1H NMR ($CDCl_3$): δ = 8.02-7.99 (m, 2H), 7.69-7.65 (m, 1H), 7.56-7.52 (m, 2H), 7.45 (s, 1H), 5.82 (s, 2H), 2.77 (t, *J* = 7.60 Hz, 2H), 1.73-1.66 (m, 2H), 1.46-1.36 (m, 2H), 0.95 (t, *J* = 7.60 Hz, 3H).

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{18}N_3O$ 244.1, found 244.1

4-(2-Pyridyl)-1-(2-phenyl ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T26):



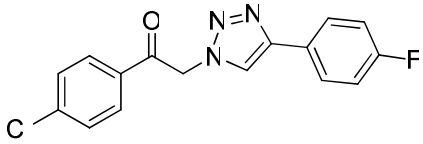
M.p.: 159 – 163 ^{o}C.

IR (KBr, *v* cm^{-1}): 1707 (CO).

^{1}H NMR ($CDCl_3$): δ = 8.64-8.59 (m, 1H), 8.30 (s, 1H), 8.25-8.19 (m, 1H), 8.10-8.01 (m, 2H), 7.87-7.55 (m, 4H), 7.30-7.22 (m, 1H), 5.94 (s, 2H).

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{13}N_4O$ 265.1, found: 265.2

1-(2-(4-Chlorophenyl)ethan-2-on-1-yl)-4-(4-fluorophenyl)-1*H*-1,2,3-triazole (T27):



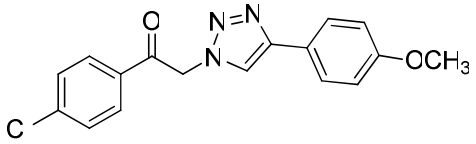
M.p.: 174-177 ^{o}C.

IR (KBr, *v* cm^{-1}): 1693 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J* = 8.80 Hz, 2H), 7.90 (s, 1H), 7.85-7.82 (m, 2H), 7.54 (d, *J* = 8.80 Hz, 2H), 7.13 (t, *J* = 8.80 Hz, 2H), 5.86 (s, 2H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{12}ClFN_3O$: 316.0653; found: 315.9462

1-(2-(4-Chlorophenyl)ethan-2-on-1-yl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (T28):



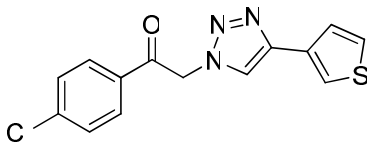
M.p.: 180-184 ^{o}C (dec.)

IR (KBr, *v* cm^{-1}): 1703 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J* = 8.80 Hz, 2H), 7.84 (s, 1H), 7.78 (d, *J* = 8.80 Hz, 2H), 7.53 (d, *J* = 8.80 Hz, 2H), 6.97 (d, *J* = 9.20 Hz, 2H), 5.84 (s, 2H), 3.85 (s, 3H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}ClN_3O_2$: 328.0853; found: 327.9601

1-(2-(4-Chlorophenyl)ethan-2-on-1-yl)-4-(thiophen-3-yl)-1*H*-1,2,3-triazole (T29):



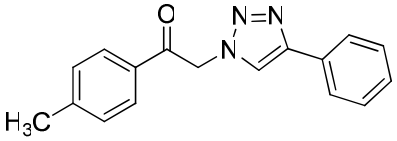
M.p.: 198-201^{o}C.

IR (KBr, *v* cm^{-1}): 1693 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J* = 8.80 Hz, 2H), 7.83 (s, 1H), 7.72 (dd, *J* = 2.80 Hz and 1.20 Hz, 1H), 7.53 (d, *J* = 8.80 Hz, 2H), 7.48 (dd, *J* = 5.20 Hz and 1.20 Hz, 1H), 7.39 (dd, *J* = 5.00 Hz and 3.20 Hz, 1H), 5.84 (s, 2H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{11}ClN_3OS$: 304.0311; found: 303.9194

1-(2-(4-Methylphenyl)-ethan-2-on-1-yl)-4-phenyl-1*H*-1,2,3-triazole (T30):



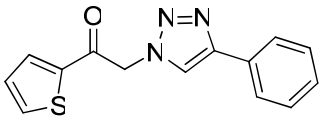
M.p.: 158 – 161 °C.

IR (KBr, *v* cm^{-1}): 1690 (CO).

^{1}H NMR ($CDCl_3$): δ = 7.88-7.85 (m, 3H), 7.79 (d, *J* = 7.32 Hz, 2H), 7.39-7.35 (m, 2H), 7.29-7.27 (m, 3H), 5.81 (s, 2H), 2.38 (s, 3H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{15}N_3O$ 277.3205, found 277.3172.

4-Phenyl-1-(2-(thiophen-2-yl)-ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T31):



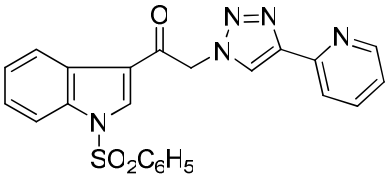
M.p.: 151 °C (dec.)

IR (KBr, *v* cm^{-1}): 1665 (CO).

^{1}H NMR ($CDCl_3$): δ = 7.91 (s, 1H), 7.82-7.79 (m, 3H), 7.74-7.73 (m, 1H), 7.39-7.35 (m, 2H), 7.30-7.26 (m, 1H), 7.17-7.15 (m, 1H), 5.73 (s, 2H).

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{12}N_3OS$ 270.1, found 270.1

4-(2-Pyridyl)-1-(2-[1-(phenylsulfonyl)-1*H*-indol-3-yl].-ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T32):



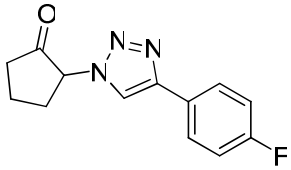
M.p.: 149 – 153 °C.

IR (KBr, *v* cm^{-1}): 1676 (CO).

^{1}H NMR ($CDCl_3$): δ = 8.60 (d, J = 4.28 Hz, 1H), 8.40-8.36 (m, 2H), 8.27 (d, *J* = 7.44 Hz, 1H), 8.21 (d, *J* = 7.96 Hz, 1H), 7.99-7.97 (m, 3H), 7.81 (dt, *J* = 7.72 Hz & *J* = 1.72 Hz, 1H), 7.63-7.59 (m, 1H), 7.52-7.48 (m, 2H), 7.45-7.36 (m, 2H), 7.27-7.24 (m, 1H), 5.79 (s, 2H).

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{18}N_5O_3S$ 444.1, found 444.0.

1-(Cyclopentan-1-on-2-yl)-4-(4-fluorophenyl)-1*H*-1,2,3-triazole (T33):



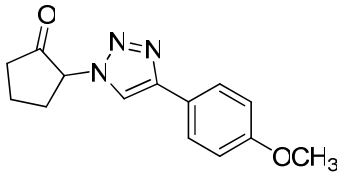
M.p.: 123-125 ^oC.

IR (KBr, *v* cm^{-1}): 1747 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (s, 1H), 7.82-7.79 (m, 2H), 7.14-7.10 (m, 2H), 4.95 (dd, *J* = 11.80 Hz and 8.60 Hz, 1H), 2.87-2.80 (m, 1H), 2.66-2.55 (m, 2H), 2.52-2.42 (m, 1H), 2.38-2.29 (m, 1H), 2.12-2.01 (m, 1H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{13}H_{13}FN_3O$: 246.1043; found: 246.0143

1-(Cyclopentan-1-on-2-yl)-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (T34):



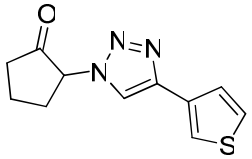
M.p.: 132-134 ^oC.

IR (KBr, *v* cm^{-1}): 1747 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (s, 1H), 7.75 (d, *J* = 8.80 Hz, 2H), 6.95 (d, *J* = 8.80 Hz, 2H), 4.94 (dd, *J* = 11.60 Hz and 8.40 Hz, 1H), 3.84 (s, 3H), 2.83-2.77 (m, 1H), 2.64-2.41 (m, 3H), 2.35-2.28 (m, 1H), 2.11-1.98 (m, 1H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{16}N_3O_2$: 258.1243; found: 258.0299

1-(Cyclopentan-1-on-2-yl)-4-(thiophen-3-yl)-1*H*-1,2,3-triazole (T35):



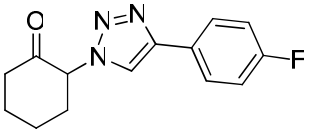
M.p.: 142-144 ^oC.

IR (KBr, *v* cm^{-1}): 1745 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (s, 1H), 7.69 (dd, *J* = 2.80 Hz and 1.20 Hz, 1H), 7.46 (dd, *J* = 4.80 Hz and 1.20 Hz, 1H), 7.38 (dd, *J* = 5.00 Hz and 2.80 Hz, 1H), 4.94 (dd, *J* = 11.60 Hz and 8.40 Hz, 1H), 2.86-2.79 (m, 1H), 2.65-2.54 (m, 2H), 2.52-2.42 (m, 1H), 2.37-2.29 (m, 1H), 2.13-2.00 (m, 1H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{12}N_3OS$: 234.0701; found: 233.9906

1-(Cyclohexan-1-on-2-yl))-4-(4-fluorophenyl)-1*H*-1,2,3-triazole (T36):



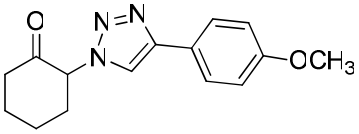
M.p.: 160-162 ^oC.

IR (KBr, *v* cm^{-1}): 1720 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83-7.80 (m, 3H), 7.14-7.09 (m, 2H), 5.45 (dd, *J* = 13.40 Hz and 6.20 Hz, 1H), 2.72-2.52 (m, 3H), 2.29-2.12 (m, 3H), 2.02-1.77 (m, 2H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{14}H_{15}FN_3O$: 260.1199; found: 260.0308

1-(Cyclohexan-1-on-2-yl))-4-(4-methoxyphenyl)-1*H*-1,2,3-triazole (T37):



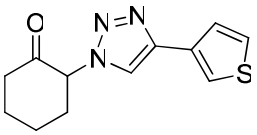
M.p.: 152-155 ^oC

IR (KBr, *v* cm^{-1}): 1720 (CO).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78-7.76 (m, 3H), 6.97-6.94 (m, 2H), 5.44 (dd, *J* = 13.00 Hz and 5.60 Hz, 1H), 3.84 (s, 3H), 2.70-2.51 (m, 3H), 2.27-2.15 (m, 2H), 2.00-1.77 (m, 3H).

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}N_3O_2$: 272.1399; found: 272.0415

1-(Cyclohexan-1-on-2-yl))-4-(thiophen-3-yl)-1*H*-1,2,3-triazole (T38):

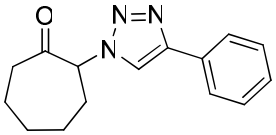


M.p.: 199-201 ^oC.

IR (KBr, *v* cm^{-1}): 1730 (CO).

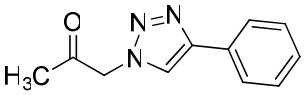
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (s, 1H), 7.70 (dd, *J* = 3.20 Hz and 1.20 Hz, 1H), 7.47 (dd, *J* = 5.00 Hz and 1.40 Hz, 1H), 7.38 (dd, *J* = 4.80 Hz and 2.80 Hz, 1H), 5.45 (dd, *J* = 13.40 Hz and 5.40 Hz, 1H), 2.72-2.52 (m, 3H), 2.29-2.12 (m, 3H), 2.01-1.77 (m, 2H).
HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{12}H_{14}N_3OS$: 248.0858; found: 247.9972

1-(Cycloheptan-1-on-2-yl)-4-phenyl-1*H*-1,2,3-triazole (T39):



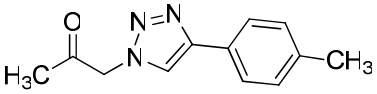
M.p.: 96 – 99 °C.
IR (KBr, *v* cm^{-1}): 1710 (CO).
^{1}H NMR ($CDCl_3$): δ 7.92 (s, 1H), 7.87-7.84 (m, 2H), 7.44-7.41 (m, 2H), 7.35-7.31 (m, 1H), 5.70 (dd, *J* = 11.00 Hz and 3.40 Hz, 1H), 2.82-2.75 (m, 1H), 2.66-2.58 (m, 1H), 2.33-2.27 (m, 1H), 2.16-2.01 (m, 4H), 1.89-1.74 (m, 2H), 1.47-1.39 (m, 1H).
MS (EI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}N_3O$ 256.1, found 256.1.

4-Phenyl-1-(propan-2-on-1-yl)-1*H*-1,2,3-triazole (T40):



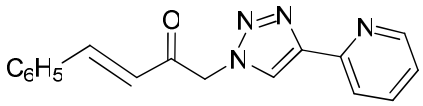
M.p.: 170 °C (dec.)
IR (KBr, *v* cm^{-1}): 1720 (CO).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86-7.84 (m, 3H), 7.45-7.41 (m, 2H), 7.36-7.33 (m, 1H), 5.25 (s, 2H), 2.28 (s, 3H).
HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{12}N_3O$: 202.0980; found: 202.0243

4-(4-Methylphenyl)-1-(propan-2-on-1-yl)-1*H*-1,2,3-triazole (T41):



M.p.: 185 °C (dec.)
IR (KBr, *v* cm^{-1}): 1728 (CO).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (s, 1H), 7.73 (d, *J* = 8.00 Hz, 2H), 7.24 (d, *J* = 8.00 Hz, 2H), 5.24 (s, 2H), 2.38 (s, 3H), 2.27 (s, 3H).
HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{12}H_{14}N_3O$: 216.1137; found: 216.0341

4-Phenyl-1-(2-(2-phenylethene-1-yl)-ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T42):



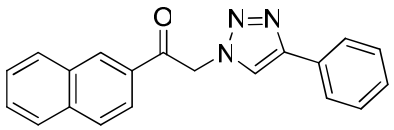
M.p.: 138 - 140 °C.

IR (KBr, *v* cm^{-1}): 1672 (CO).

^{1}H NMR ($CDCl_3$): δ = 7.93 (s, 1H), 7.88-7.86 (m, 2H), 7.79 (d, *J* = 16.4 Hz, 1H), 7.59-7.56 (m, 2H), 7.46-7.40 (m, 5H), 7.37-7.33 (m, 1H), 6.79 (d, *J* = 16.0 Hz, 1H), 5.51 (s, 2H).

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{16}N_3O$ 290.1, found: 290.0.

1-((Naphthalen-3-yl)-ethan-2-on-1-yl)-(4-phenyl)-1*H*-1,2,3-triazole (T43):



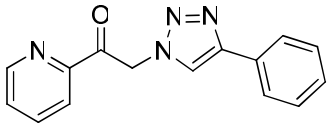
M.p.: 176 – 181 °C

IR (KBr, *v* cm^{-1}): 1703

^{1}H NMR ($CDCl_3$): δ = 8.57 (s, 1H), 8.06-7.87 (m, 7H), 7.70-7.60 (m, 2H), 7.46-7.42 (m, 2H), 7.36-7.33 (m, 1H), 6.04 (s, 2H).

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{16}N_3O$ 314.1, found 314.2.

(4-Phenyl)-1-((pyridin-2-yl)-ethan-2-on-1-yl)-1*H*-1,2,3-triazole (T44):



M.p.: 155 °C (dec.)

IR (KBr, *v* cm^{-1}): 1722.

^{1}H NMR ($CDCl_3$): δ = 8.77-8.75 (m, 1H), 8.13-8.11 (m, 1H), 7.95-7.88 (m, 4H), 7.62-7.59 (m, 1H), 7.47-7.43 (m, 2H), 7.38-7.33 (m, 1H), 6.20 (s, 2H).

MS (EI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{13}N_4O$ 265.1, found 265.1.

4.5.2 Protocol for the c-Src Kinase Screening of 1,2,3-Triazoles T

The effect of synthesised compounds on the activity of c-Src kinase was determined by HTScan Src Kinase Assay Kit, catalogue number 7776 from Cell Signaling Technology; according to manufacturer's protocol. Streptavidin coated plates was purchased from Pierce. In summary, the kinase reaction was started with the incubation of the reaction

cocktail (GST-Src kinase in 1.25 mM DTT) with prediluted compounds (dissolved in 1% DMSO) for 5 min at room temperature. ATP/substrate cocktail was added to the mixture. The biotinylated substrate (catalogue number 1366) contains the residues surrounding tyrosine 160 (Tyr160) of signal transduction protein with a sequence of EGIYDVP. The reaction mixture was incubated for 30 min at room temperature. The kinase reaction was stopped with the addition of EDTA (pH 8.0). The reaction solution was transferred into 96-well streptavidin plates (Pierce, part number 15124), diluted with 75 µl double distilled water, and incubated at room temperature for 60 min. At the end of the incubation, the wells were washed three times with 0.05% Tween-20 in PBS buffer (PBS/T). After that to the each well was added phosphotyrosine antibody (P-Tyr-100) and the wells were incubated for another 60 min. After washing three times with PBS/T, the wells were incubated with secondary anti-mouse IgG antibody, which was HRP-conjugated for next 30 min at room temperature. The wells were washed five times with PBS/T and then were incubated with 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) substrate for 5 min. The reaction was stopped by adding of stop solution to each well and mixed well and read the absorbance at 450 nm using a microplate reader (Molecular devices, spectra Max M2). IC_{50} values of the compounds were calculated using ORIGIN 8.1 (origin lab) software. IC_{50} is the concentration of the compound that inhibited enzyme activity by 50%. All the experiments were carried out triplicate.

4.5.3 Molecular Modeling Method

Simulations were performed with the Accelrys Discovery Studio 2.5 modeling package, with the CHARMm-based force field. Model of PP1 bound to Src was constructed based on the X-ray crystal structures of PPA bound to Hck (1QCF) and AMP-PNP bound to c-Src (2SRC) templates from RCSB Protein Data Bank. The coordinates and positions of the backbone atoms of PP1 were superimposed on the corresponding atoms in AMP-PNP after which Hck was deleted. For refinement, the PP1-Src complex underwent CHARMm minimization. All default parameters were used in the minimization processon. For the molecular modeling of **3b** and **4h**, after initial minimization, the coordinates and positions of the backbone atoms of tolyl groups and triazole were superimposed on the corresponding atoms in PP1 in complex with c-Src after which PP1 was deleted. The minimization was carried out as described above.

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CHAPTER - V

One-pot Regioselective Synthesis of 1,4-Diaryl-1*H*-1,2,3-triazoles

The work described in this chapter reveals a facile, expeditious and regioselective synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles. Keeping in view the biological importance of 1,4-diaryl-1*H*-1,2,3-triazoles and the dearth in the existing protocols, efforts were made in developing a sustainable and ecofriendly process. This one-pot protocol requires sequential addition of equimolar quantities of diaryliodonium salt, sodium azide and terminal alkynes in aq. PEG 400 (1:1, v/v) with 10 mol% of CuI as catalyst. The simple workup, recyclability of the reaction media and short time are the noteworthy features of this protocol. A variety of readily available diaryliodonium salts were employed with different substituted terminal alkynes. All the yields of the products were in the range of 70 – 85 %.

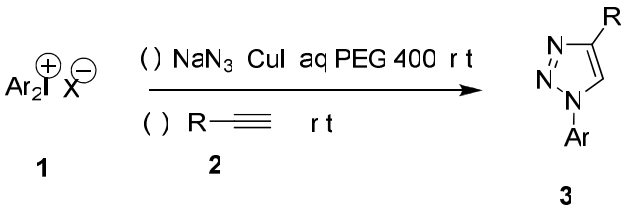
Chapter 5

5.1 Introduction

1,2,3-Triazoles have received significant attention due to their applications in fields such as materials, chemical and biological sciences.[1-3] They are generally stable to moisture and air. They are also reported to possess diverse biological activities including anti-HIV[4], antiallergic[5], antifungal[6-7] and antimicrobial.[8]

With increasing stringent regulatory considerations, the development of environmentally benign, efficient and economical methods is vital and challenging in organic synthesis. From this perspective, multicomponent reactions (MCRs) in benign media (PEG, water, ionic liquids etc.) are particularly worthwhile due to their ecofriendly, atom economic, high convergence, and less laborious process.

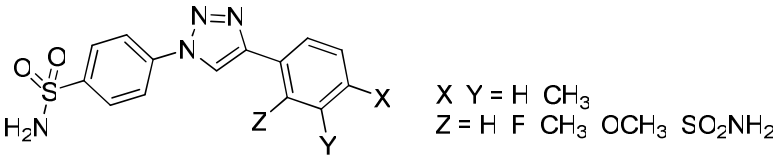
An environmentally benign and sustainable protocol is developed for the one-pot synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles by *in situ* generation of arylazides from the reaction of diaryliodonium salts and sodium azide followed by coupling with terminal acetylenes (Scheme 5.1).



Scheme 5.1 Synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles **3**

5.1.1 Biological Significance of 1,4-Diaryl-1*H*-1,2,3-triazoles

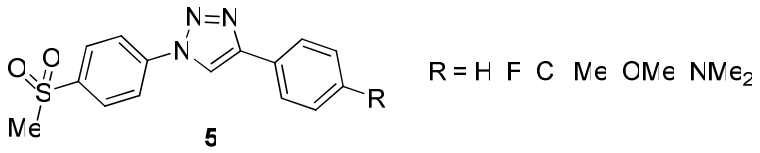
1,4-Diaryl-1*H*-1,2,3-triazoles have exhibited significant activity in various applications. Poulsen and coworkers[9] synthesised a set of 1,4-diaryl-1*H*-1,2,3-triazoles (Figure 5.1) using the click chemistry approach. An inhibition study of the human cytosolic isozymes carbonic anhydrase (CA) I and II, and the mitochondrial isozymes CA VA and VB was carried out with the synthesised 1,2,3-triazoles **4** compounds. Mitochondrial carbonic anhydrases are potential targets for antiobesity therapies, acting to reduce lipogenesis through a novel mechanism of action. These compounds showed effective inhibiton (low nanomolar) of the human mitochondrial carbonic anhydrase isozymes VA and VB (K_i = ~10 nM).



4

Figure 5.1 1,4-Diaryl-1*H*-1,2,3-triazoles **4** as carbonic anhydrase inhibitors

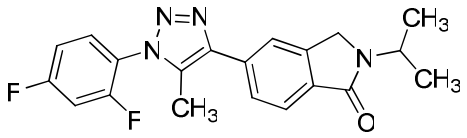
A set of 1,4-diaryl-1*H*-1,2,3-triazoles **5** were demonstrated to exhibit *in vitro* cyclooxygenase-1 (COX-1) and COX-2 inhibition (Figure 5.2).[10] The 1,2,3-triazoles **5** showed selectivity towards COX-2 (IC_{50}: ~ 0.2 μM). The selectivity index of the compound with R = H was 139.



5

Figure 5.2 1,4-Diaryl-1*H*-1,2,3-triazoles **5** as cyclooxygenase inhibitors

The 1,2,3-triazole **6** was reported to exhibit metabotropic glutamate receptor (mGluR) 1 allosteric antagonism (Figure 5.3).[11] Analgesic effects of **6** were seen in the formalin test in rats. The 1,2,3-triazole **6** selectively blocked methamphetamine induced hyperlocomotion and disruption of prepulse inhibition but did not elicit catalepsy or impair motor functions. An efficient synthesis of **6** is also investigated.[12]



6

Figure 5.3 mGluR1 allosteric antagonist **6**

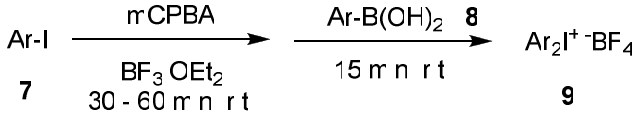
5.1.2 Synthesis of Diaryliodonium Salts

Diaryliodonium salts, an important class of trivalent iodine reagents with two carbon ligands, are well known for their potential applications in photochemistry, organic chemistry and medicinal chemistry.[13-15] The electron-deficient nature and leaving-group ability of diaryliodonium salts make them the best known versatile arylating agents with a variety of nucleophiles. They are applied in the synthesis of a number of heterocycles, arylation of keto compounds, phenylation of nitro compounds, arylation of ethylenic

compounds, synthesis of biphenylenes, generation of benzyne and arylation of heteroatoms (X, N, O).[16-21]

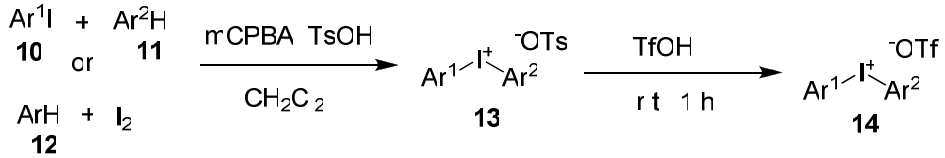
They are non-toxic, non-explosive, readily available and found to be stable towards heat and oxygen and are widely used in variety of organic transformations.[22-25] Very recently several improved methods have been reported for the simple preparation of different diaryliodonium salts from the corresponding arenes in one step.[26-30] A few elegant and effortless protocols for the synthesis of diaryliodonium salts are highlighted below:

Bielawski and coworkers[26] have demonstrated an efficient and rapid one-pot synthesis of symmetric and unsymmetric diaryliodonium tetrafluoroborates **9** from iodoarenes **7** and arylboronic acids **8** (Scheme 5.2). Both electron-deficient and electron-rich salts can be synthesised in a regiospecific manner, and the substitution pattern can easily be varied. An *in situ* anion exchange with triflic acid gives access to the corresponding diaryliodonium triflates.



Scheme 5.2 One-pot synthesis of diaryliodonium tetrafluoroborates **9**

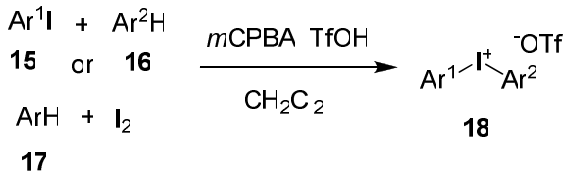
A direct synthesis of symmetric and unsymmetric electron-rich diaryliodonium salts **13** was described with the use of mCPBA and TsOH.[27] An *in situ* anion exchange led to the corresponding triflate salts **14**. The symmetric electron-rich iodonium salts were obtained from arenes **12** and molecular iodine whereas unsymmetric salts were obtained from arylhalides **10** and arenes **11** (Scheme 5.3).



Scheme 5.3 One-pot synthesis of electron-rich diaryliodonium salts **14**

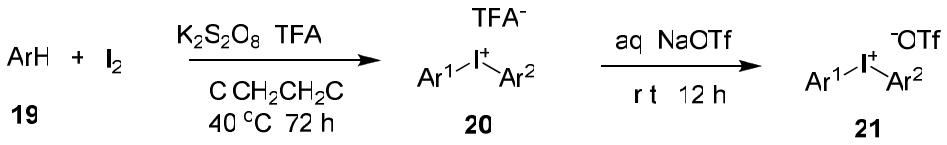
A facile, direct synthesis of diaryliodonium triflates **18** from the corresponding aryl iodide **15** and arene **16** has been described (Scheme 5.4).[31] The method is fast, high yielding, operationally simple and has a large substrate scope. A few electron-rich salts were synthesised from aryliodide and the corresponding arene, and electron-deficient salts were formed by the reaction of a substituted aryliodide with benzene. This protocol

was extended to the synthesis of limited iodonium salts directly from iodine and arenes **17** circumventing the need for aryliodides.



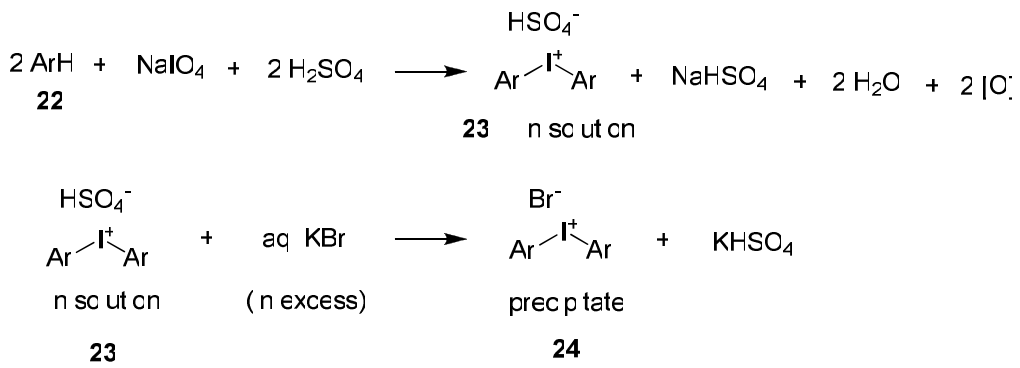
Scheme 5.4 One-pot synthesis of diaryliodonium triflates **18**

A simple method for the preparation of diaryliodonium triflates was described by Hossain and coworkers.[28,32] This new method gave the required salts in good yields by the reaction of arenes **20** with elemental iodine, potassium peroxodisulfate ($K_2S_2O_8$), trifluoroacetic acid (TFA), and 1,2-dichloroethane at 40 °C, followed by treatment with NaOTf (Scheme 5.5). This protocol fails in case of electron rich substrates like anisole, mesitylene and *p*-xylene.



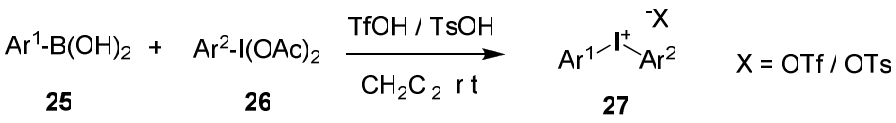
Scheme 5.5 One-pot synthesis of diaryliodonium triflates **21**.

A simple synthesis of symmetrical diaryliodonium bromides **24** was described by the reaction of arenes **22**, sodium metaperiodate and sulfuric acid followed by treatment with aqueous KBr (Scheme 5.6).[33] Several crude symmetrical diaryliodonium bromides **24** were readily metathesized to give the respective pure diaryliodonium tetrafluoroborates. The reaction requires heating at 50 – 55 ^{o}C for 3 hours followed by precipitation with aqueous KBr at room temperature for 1 h. This protocol applies fairly well for electron-deficient arenes.



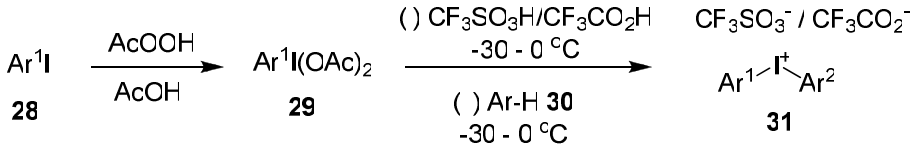
Scheme 5.6 Synthesis of diaryliodonium bromides **24**

A mild and simple method for the preparation of aryl(phenyl)iodonium triflates **27** and heteroaryl(phenyl)iodonium tosylates **27** was demonstrated[34] from the appropriate arylboronic acids **25** (Scheme 5.7). This eliminated the need for toxic and acid sensitive reagents as precursors for the preparation of diaryliodonium salts.



Scheme 5.7 Regioselective synthesis of diaryliodonium salts **27**.

Shah and coworkers[30] described a generalized synthesis of unsymmetrical functionalized diaryliodonium salts **31** through the direct reaction of bis(acetoxy)iodoarenes **29** with arenes **30** in a trifluoromethanesulfonic or trifluoroacetic acid medium (Scheme 5.8). This protocol is applicable only for electron-rich arenes and aryliodides **28**.



Scheme 5.8 Synthesis of unsymmetric diaryliodonium salts **31**.

5.1.3 Reported Protocols for the Synthesis of 1,2,3-Triazoles

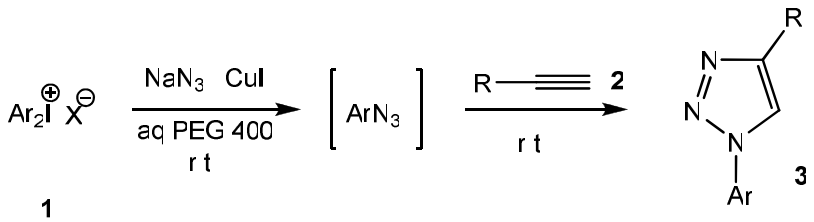
The reported preparations of 1,2,3-triazoles is comprehensively briefed in Chapter 4.1.4. The preparation of 1,2,3-triazoles is typically accomplished via Cu(I)-catalysed 1,3-dipolar cycloaddition between azides and alkynes.[35-38] Alternative methods for their synthesis include the reaction of nitriles and diazo compounds, cycloaddition between nitroethenes and trimethylsilyl azide in the presence of a catalyst, addition of

bromomagnesium acetylides to azides, and reactions of sodium phenylacetylide and a sodium alkoxide with tosyl or mesyl azides.[39-43] Another report describes the synthesis of 1,2,3-triazoles on a solid support.[44] The Cu(I)-catalysed azide–alkyne coupling reaction, the so-called 'click' reaction, was reported by Sharpless and coworkers for the regioselective preparation of 1,4-disubstituted-1,2,3-triazoles in high yields.[45-46]

5.2 The Present Work

Arylazides are very useful starting materials for the click reaction and are generally prepared by treatment of diazonium salts with azide ions.[47-48] Nucleophilic displacement of the diaryliodonium salt by the azide ion can only be achieved if the aromatic ring is sufficiently activated. Most of the existing protocols generally require long reaction times, a large excess of inorganic azide, high boiling polar solvents (DMF or DMSO) and afford low to moderate yields of products. Previously, the synthesis of arylazides was reported starting from arylbromides/iodides or arylamines.[49-51] Some of these protocols require microwave irradiation or heating at reflux, the use of freshly prepared reagents such as *tert*-butyl nitrite, or expensive reagents and ligands, and/or an inert atmosphere. In addition, low molecular weight azides are dangerous to isolate because of their explosive nature.[52-53] Thus, a simple and cost-effective method for the synthesis of 1,2,3-triazoles that avoids the isolation of intermediate arylazides is highly desirable.

The present work describes a new and sequential one-pot synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles which involves *in situ* generation of arylazides via reaction of diaryliodonium salts and sodium azide, followed by coupling with a terminal alkyne (Scheme 5.9).



Scheme 5.9 One-pot synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles **3**

5.3 Results and Discussion

During our initial efforts to prepare phenyl azide *in situ*, the reaction of diphenyliodonium triflate with sodium azide was attempted in water at different

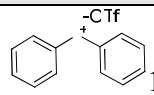
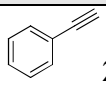
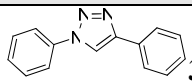
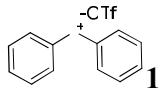
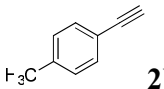
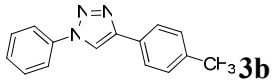
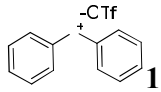
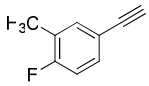
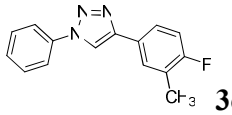
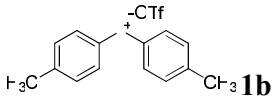
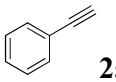
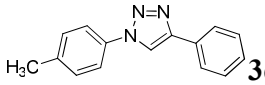
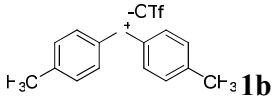
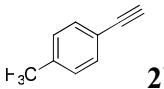
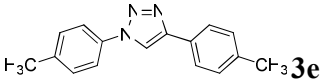
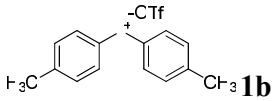
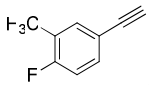
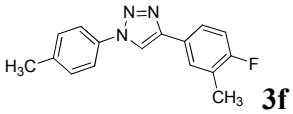
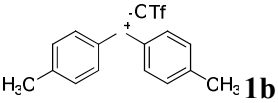
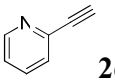
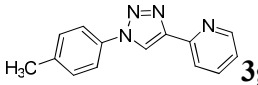
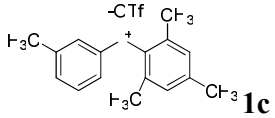
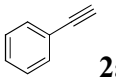
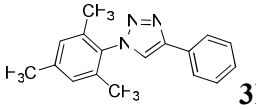
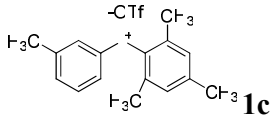
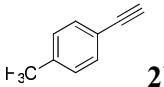
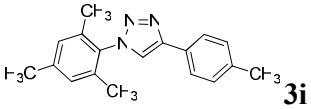
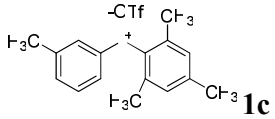
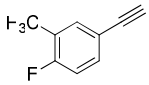
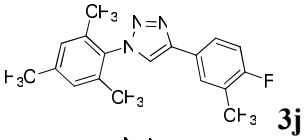
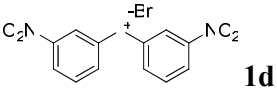
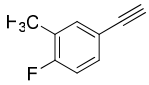
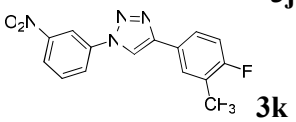
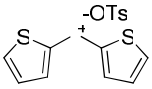
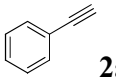
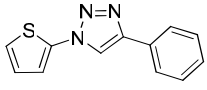
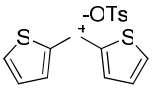
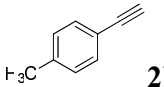
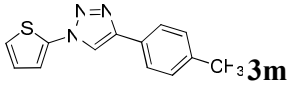
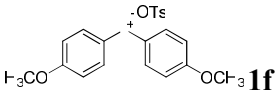
temperatures, however, the desired product was not obtained. Generally, water is added as a reaction media in click reactions, not just for reactivity reasons, but also because water is the best heat-sink for handling the heat output when click reactions are performed. Yet another advantage of water as a reaction solvent is that its presence prevents interference from simple protic functional groups, like alcohols and amides, which are ubiquitous in biologically active organic molecules.

In view of our successful synthesis of 2-aminochromenes in aqueous polyethylene glycol 400 (PEG 400)[54] and the known advantages of this benign medium, we instead explored the use of this polyether–water system as solvent for this reaction.[54-58] The best result for the *in situ* generation of a phenyl azide was obtained using Cu(I) iodide in PEG 400–water (1:1, v/v) solution at room temperature. As a result of the weak nucleophilicity of the counter anions and their high solubility, both diphenyliodonium triflate and diphenyliodonium tosylate salts were suitable and equally reactive starting materials for the *in situ* generation of phenyl azide in good yield.

Following successful conversion of diphenyliodonium triflate into phenyl azide with Cu(I) iodide in PEG 400–water (1:1, v/v) solution, we next developed a new, sequential, one-pot synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles (Scheme 5.9). To our delight, the reaction of diphenyliodonium triflate with sodium azide, followed by a Cu(I)-catalysed 1,3-dipolar cycloaddition with phenylacetylene in aqueous PEG 400, afforded pure 1,4-diphenyl-1*H*-1,2,3-triazole (**3a**) in 85% yield (Table 5.1). The structure of **3a** was confirmed unambiguously by NMR and mass spectral data. The ^{1}H NMR spectrum of **3a** exhibited a characteristic singlet at δ = 8.20 ppm due to the triazolyl C_5-H proton. The high resolution mass spectrum of this compound gave a molecular ion peak at *m/z* = 221.0997 which was in agreement with the calculated value (221.0953).

The scope of this one-pot method was examined using diaryliodonium salts **1a–f** and various terminal alkynes **2a-e** affording the products **3a-n**. In addition to the triflate and tosylate salts, the use of bis(3-nitrophenyl)iodonium bromide (**1d**) gave triazole **3k** in 78% yield. The heteroaryl salt, bis(2-thienyl)iodonium tosylate (**1e**) yielded 1,4-diaryl-1*H*-1,2,3-triazoles **3l** and **3m** in 74% and 71% yields, respectively.

Table 5.1 Sequential synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles **3**

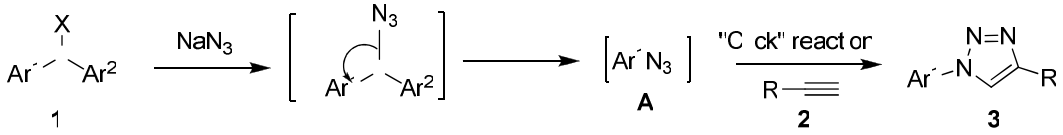
S.No.	Iodonium salt 1	Alkyne 2	Product 3	Yield (%)[a]
14.	 **1a**	 **2a**	 **3a**	85
15.	 **1a**	 **2b**	 **3b**	79
16.	 **1a**	 **2c**	 **3c**	75
17.	 **1b**	 **2a**	 **3d**	82
18.	 **1b**	 **2b**	 **3e**	76
19.	 **1b**	 **2c**	 **3f**	71
20.	 **1b**	 **2d**	 **3g**	77
21.	 **1c**	 **2a**	 **3h**	85
22.	 **1c**	 **2b**	 **3i**	81
23.	 **1c**	 **2c**	 **3j**	78
24.	 **1d**	 **2c**	 **3k**	78
25.	 **1e**	 **2a**	 **3l**	74
26.	 **1e**	 **2b**	 **3m**	71
27.	 **1f**	**2e**	**3n**	85

Notably, the cycloadditions of *in situ* generated arylazides with terminal alkynes were faster in aqueous PEG 400 solution (30 minutes) compared to those reported using *tert*-

butanol–water (12–24 hours).[35] The competing formation of an N–H triazole was observed when the three components were added simultaneously. The use of various diaryliodonium salts **1a-f** and terminal alkynes **2b-e** demonstrates the functional group tolerance of this three-component reaction (Table 5.1). The products were isolated by extraction into diethyl ether. Further, the recyclability of the solvent and catalyst was demonstrated by successive syntheses (three times) of 1,4-diphenyl-1*H*-1,2,3-triazole (**3a**) using recovered aqueous PEG 400, without any appreciable loss in yield (first cycle: 85%; second cycle: 83%; third cycle: 78%).

5.3.1 Plausible Mechanism

Mechanistically, the nucleophilic substitution reaction of diaryliodonium salts with sodium azide proceeds via *in situ* formation of arylazides **A** which then undergo 1,3-dipolar cycloadditions with terminal alkynes (Please refer Scheme 4.12 for the mechanism of azide-alkyne Click reaction) to afford 1,4-diaryl-1*H*-1,2,3-triazoles **3** (Scheme 5.10). Symmetric diaryliodonium salts (**1a,b** and **1d–f**) gave the products by way of nucleophilic attack of the azide ion on either of the aryl rings, whereas unsymmetrical diaryliodonium salt **1c** underwent attack on the more sterically demanding aromatic ring, falling in line with earlier reported studies.[23,59-61]



Scheme 5.10 Mechanistic pathway in the synthesis of 1,2,3-triazoles **3**

5.4 Conclusion

An efficient, safe, one-pot, regioselective synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles in good yields was developed under catalytic conditions, using various diaryliodonium salts and terminal alkynes. Diaryliodonium salts are advantageous over arylhalides in the preparation of 1,2,3-triazoles as aryl halides needs the undesirable conditions such as heating at reflux, the use of freshly prepared reagents such as *tert*-butyl nitrite, or expensive reagents and ligands, and/or an inert atmosphere. A diverse library of substituted 1,2,3-triazoles was synthesised exhibiting the scope of this protocol. The recyclability of the aqueous PEG 400 solvent along with the catalyst is a noteworthy feature.

5.5 Experimental Details

5.5.1 General

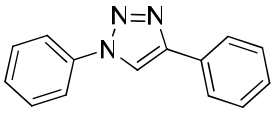
Melting points were obtained using a Ez-melt MPA 120 melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using TMS as the internal standard. Chemical shifts are given in parts per million (δ) and coupling constants (*J*) in Hz. IR spectra were recorded on IR Prestige-21 FTIR spectrophotometer. HRMS data were obtained using electrospray ionization on a Quadrupole–Time-of-flight (Q-Tof II) mass spectrometer (Micromass, Manchester, UK). Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} precoated aluminum sheets.

5.5.2 General Procedure for the One-pot Synthesis of 1,4-Diaryl-1*H*-1,2,3-triazole

A mixture of diaryliodonium triflate (**1a**) (430 mg, 1.0 mmol), NaN_3 (65 mg, 1.0 mmol) and CuI (19 mg, 10 mol%) in PEG 400–H_2O (1 mL, 1:1, v/v) was stirred at r.t. for 30 min. Terminal alkyne (102 mg, 1.0 mmol) was added and the reaction mixture was stirred at r.t. for a further 30 min. On completion of the reaction, as indicated by TLC, the product was extracted into diethyl ether (3 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was washed with hexane (5 mL) and the solid thus obtained was recrystallised from a mixture of ethyl acetate-diethyl ether to afford pure product.

Analytical data of the synthesised 1,2,3-triazoles **3**:

1,4- diphenyl-1*H*-1,2,3-triazole (3a).[62]



Yield: 85%

M.p.: 180–182 °C (Lit. M.p.[17]: 183–184 °C).

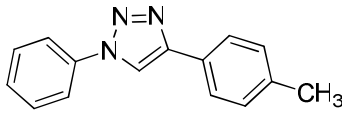
IR (KBr, *v* cm^{-1}): 3121, 3053, 2918, 2853, 1599, 1504, 1229, 1042, 826, 758.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.20 (s, 1H), 7.92 (d, *J* = 7.4 Hz, 2H), 7.80 (d, *J* = 7.7 Hz, 2H), 7.57–7.53 (m, 2H), 7.48–7.45 (m, 3H), 7.40–7.35 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.5, 137.2, 130.3, 129.9, 129.0, 128.9, 128.5, 125.9, 120.6, 117.7.

HRMS (ESI): *m*/*z* $[M]^+$ calcd for $C_{14}H_{11}N_3$: 221.0953; found: 221.0997.

4-(4-Methylphenyl)-1-phenyl-1*H*-1,2,3-triazole (3b)



Yield: 79%

M.p.: 166–169 °C.

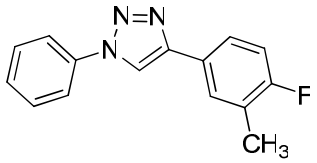
IR (KBr, *v* cm^{-1}): 2912, 2860, 1597, 1495, 1227, 1093, 1042, 814, 756.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (s, 1H), 7.81–7.77 (m, 4H), 7.55–7.52 (m, 2H), 7.44 (t, *J* = 7.4 Hz, 1H), 7.27–7.25 (m, 2H), 2.39 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.5, 138.4, 137.2, 129.8, 129.7, 128.7, 127.5, 125.8, 120.6, 117.3, 21.4.

HRMS (ESI): *m/z* $[M]^+$ calcd for $C_{15}H_{13}N_3$: 235.1109; found: 235.1396.

4-(4-Fluoro-3-methylphenyl)-1-phenyl-1*H*-1,2,3-triazole (3c)



Yield: 75%

M.p.: 144–147 °C.

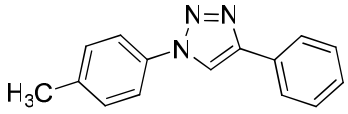
IR (KBr, *v* cm^{-1}): 3121, 2922, 2866, 1599, 1557, 1495, 1225, 1124, 1043, 816, 760, 689.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 (s, 1H), 7.79–7.76 (m, 3H), 7.68–7.65 (m, 1H), 7.57–7.53 (m, 2H), 7.46 (t, *J* = 7.4 Hz, 1H), 7.08 (t, *J* = 9.0 Hz, 1H), 2.34 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.5, 147.8, 137.1, 129.9, 129.1, 128.9, 126.1, 125.6, 124.9, 120.6, 117.4, 115.6, 14.6.

HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{13}FN_3$: 254.1094; found: 254.1090.

1-(4-Methylphenyl)-4-phenyl-1*H*-1,2,3-triazole (3d)



Yield: 82%

M.p.: 166–168 °C.

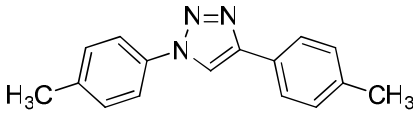
IR (KBr, *v* cm^{-1}): 3125, 2914, 2860, 1520, 1481, 1229, 1045, 816, 692.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.15 (s, 1H), 7.92–7.90 (m, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.47–7.44 (m, 2H), 7.38–7.32 (m, 3H), 2.43 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.3, 139.0, 134.9, 130.4, 130.3, 129.0, 128.4, 125.9, 120.5, 117.7, 21.2.

HRMS (ESI): *m/z* [M^+] calcd for $C_{15}H_{13}N_3$: 235.1109; found: 235.1525.

1,4-Bis(4-methylphenyl)-1*H*-1,2,3-triazole (3e)



Yield: 76%

M.p.: 196–199 °C.

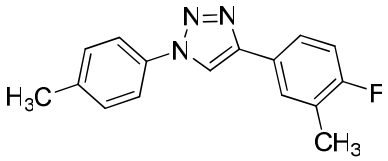
IR (KBr, *v* cm^{-1}): 3105, 3022, 2916, 2860, 1520, 1497, 1229, 1022, 814.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (s, 1H), 7.79 (d, *J* = 8.1 Hz, 2H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 2.42 (s, 3H), 2.39 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.4, 138.9, 138.3, 134.9, 130.3, 129.6, 127.6, 125.8, 120.5, 117.3, 21.4, 21.2.

HRMS (ESI): *m/z* [M+H]$^+$ calcd for $C_{16}H_{16}N_3$: 250.1344; found: 250.1346.

4-(4-Fluoro-3-methylphenyl)-1-(4-methylphenyl)-1*H*-1,2,3-triazole (3f)



Yield: 71%

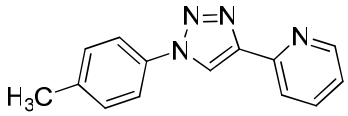
M.p.: 168–171 °C.

IR (KBr, *v* cm^{-1}): 3123, 2951, 2924, 2864, 1557, 1520, 1495, 1223, 1124, 1043, 816.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.09 (s, 1H), 7.76 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.67–7.64 (m, 3H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.07 (t, *J* = 8.9 Hz, 1H), 2.43 (s, 3 H), 2.34 (s, 3H).

HRMS (ESI): *m/z* [M + H]$^+$ calcd for $C_{16}H_{14}FN_3$: 268.1250; found: 268.1245.

2-[1-(4-Methylphenyl)-1*H*-1,2,3-triazol-4-yl]pyridine (3g)



Yield: 77%

M.p.: 128–129 °C.

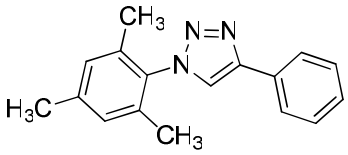
IR (KBr, *v* cm^{-1}): 3107, 3025, 2920, 1595, 1520, 1232, 1036, 989, 814.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.63–8.61 (m, 1H), 8.57 (s, 1H), 8.27–8.24 (m, 1H), 7.84–7.79 (m, 1H), 7.70–7.67 (m, 2H), 7.34 (d, *J* = 8.6 Hz, 2H), 7.28–7.25 (m, 1H), 2.43 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.1, 149.5, 148.8, 139.0, 137.0, 134.7, 130.3, 123.1, 120.5, 120.4, 120.0, 21.1.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{14}H_{13}N_4$: 237.1140; found:237.1142.

1-Mesityl-4-phenyl-1*H*-1,2,3-triazole (3h)



Yield: 85%

M.p.: 113–116 °C.

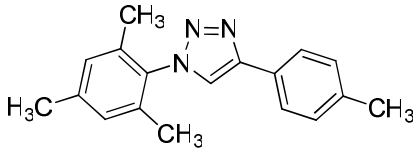
IR (KBr, *v* cm^{-1}): 3136, 2916, 2860, 1607, 1495, 1223, 1034, 856, 768.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.94–7.92 (m, 2H), 7.83 (s, 1H), 7.48–7.44 (m, 2H), 7.38–7.34 (m, 1H), 7.01 (s, 2H), 2.37 (s, 3H), 2.02 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.6, 140.2, 135.2, 133.6, 130.6, 129.1, 129.0, 128.3, 125.8, 121.5, 21.2, 17.4.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{17}H_{17}N_3$: 264.1501; found: 264.1800.

1-Mesityl-4-(4-methylphenyl)-1*H*-1,2,3-triazole (3i)



Yield: 81%

M.p.: 125–128 °C.

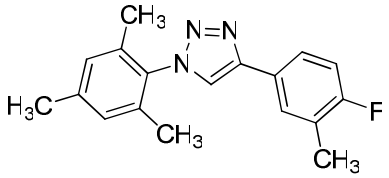
IR (KBr, *v* cm^{-1}): 2920, 2859, 1607, 1487, 1224, 1038, 856, 806.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.81 (d, *J* = 8.1 Hz, 2H), 7.79 (s, 1H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.00 (s, 2H), 2.40 (s, 3H), 2.36 (s, 3H), 2.01 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.7, 140.1, 138.2, 135.2, 133.6, 129.6, 129.1, 127.7, 125.7, 121.2, 21.4, 21.2, 17.4.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{18}H_{20}N_3$: 278.1657; found: 278.1654.

4-(4-Fluoro-3-methylphenyl)-1-mesityl-1*H*-1,2,3-triazole (3j)



Yield: 78%

M.p.: 112–115 °C.

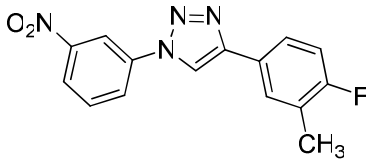
IR (KBr, *v* cm^{-1}): 2922, 2854, 1603, 1487, 1227, 1049, 824.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (dd, *J* = 7.3 and 1.6Hz, 1H), 7.77 (s, 1H), 7.69–7.65 (m, 1H), 7.08 (t, *J* = 9.0 Hz, 1H), 7.01 (s, 2H), 2.36 (s, 3H), 2.35 (s, 3H), 2.01 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.4, 147.0, 140.2, 135.2, 133.5, 129.2, 129.0, 126.4, 125.5, 124.8, 121.2, 115.6, 21.2, 17.4, 14.7.

HRMS (ESI): *m/z* $[M]^+$ calcd for $C_{18}H_{18}FN_3$: 295.1485; found: 295.1831.

4-(4-Fluoro-3-methylphenyl)-1-(3-nitrophenyl)-1*H*-1,2,3-triazole (3k)



Yield: 78%

M.p.: 175–177 °C.

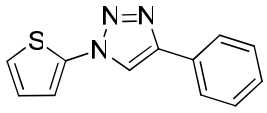
IR (KBr, *v* cm^{-1}): 3098, 1611, 1537, 1504, 1348, 1232, 1045, 804.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (s, 1H), 7.70–7.65 (m, 3H), 7.60–7.56 (m, 1H), 7.47–7.43 (m, 2H), 7.01 (t, *J* = 8.9 Hz, 1H), 2.27 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.6, 148.1, 135.9, 134.7, 130.1, 129.2, 129.1, 125.9, 125.8, 125.6, 124.9, 121.7, 117.2, 115.6, 14.7.

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{15}H_{12}FN_4O_2$: 299.0944; found:299.0943.

4-Phenyl-1-(2-thienyl)-1*H*-1,2,3-triazole (3l)



Yield: 74%

M.p.: 136–139 °C.

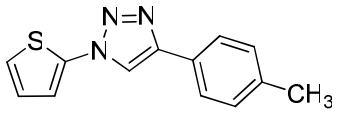
IR (KBr, *v* cm^{-1}): 3098, 2922, 2851, 1556, 1485, 1230, 1034, 822, 763.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.10 (s, 1H), 7.89 (d, *J* = 4.0 Hz, 2H), 7.46 (t, *J* = 7.2 Hz, 2H), 7.39–7.35 (m, 1H), 7.29 (dd, *J* = 3.6, 1.2 Hz, 1H), 7.24 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.07–7.04 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.4, 138.6, 129.9, 128.9, 128.6, 126.3, 125.9, 122.9, 118.9, 118.2.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{10}N_3S$: 228.0595; found: 228.0604.

4-(4-Methylphenyl)-1-(2-thienyl)-1*H*-1,2,3-triazole (3m)



Yield: 71%

M.p.: 167–170 °C.

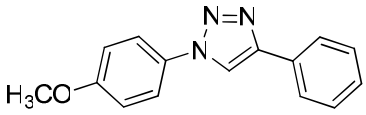
IR (KBr, *v* cm^{-1}): 3109, 2914, 2857, 1558, 1495, 1466, 1221, 1038, 818, 710.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (s, 1H), 7.77 (d, *J* = 8.1 Hz, 2H), 7.28–7.25 (m, 3H), 7.23–7.21 (m, 1H), 7.05–7.03 (m, 1H), 2.39 (s, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.4, 138.6, 138.5, 129.6, 127.0, 126.3, 125.8, 122.8, 118.5, 118.1, 21.4.

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{13}H_{12}N_3S$: 242.0752; found: 242.0758.

1-(4-Methoxyphenyl)-4-phenyl-1*H*-1,2,3-triazole (3n)[62]



Yield: 85%

M.p.: 164–165 °C (Lit. M.p.[17]: 160–161 °C).

IR (KBr, *v* cm^{-1}): 3126, 2841, 1611, 1518, 1252, 1030, 829, 768.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11 (s, 1H), 7.92–7.89 (m, 2H), 7.69 (d, *J* = 9.0 Hz, 2H), 7.47–7.43 (m, 2H), 7.38–7.34 (m, 1H), 7.04 (d, *J* = 9.0 Hz, 2H), 3.88 (s, 3H).

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CHAPTER - VI

An Efficient One-pot Synthesis and Anticonvulsant Activity of 2,5-Disubstituted-1,3,4-thiadiazoles

The current chapter accounts the simple and expeditious synthesis of 2-arylamino-5-aryl-1,3,4-thiadiazoles by the one-pot reaction of equimolar arylaldehydes, hydrazine hydrate and arylisothiocyanates in methanol at 80 ^{o}C followed by oxidative cyclization in presence of ferric ammonium sulphate. The 2-arylamino-5-aryl-1,3,4-thiadiazoles were evaluated for their *in vivo* anticonvulsant activity in subcutaneous picrotoxin induced seizures in swiss albino mice. Some of the 1,3,4-thiadiazoles were moderately active and the structure-activity relationship studies revealed that the presence of bulky substituents on the C-5 aryl ring leads to diminished activity, whereas, bulky substituents on the C-2 arylamino ring elevates the activity.

Chapter 6

6.1 Introduction

1,3,4-Thiadiazoles **1** are five-member ring systems that have instituted their prominence by exhibiting a wide variety of biological activities and as intermediates in several organic preparations.[1-7] The marketed drugs, acetazolamide, methazolamide, sulfamethazole, globucid etc showcase their therapeutic potential. FABT, a most promising thiadiazole anticancer compound in malign tumors of nervous system, inhibits proliferation in tumor cells by decreasing cell division and inhibiting metastasis.[8-9] Thiadiazoles, bioisosters of thiazoles and oxadiazoles, are known to have interesting electro-optical properties[10] and also act as corrosion & oxidation inhibitors,[11] complexation reagents for dyes and metal ions.[12-15]

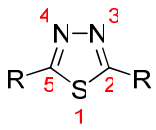


Figure 6.1 1,3,4-Thiadiazole **1**

6.1.1 Definition of Epilepsy

Epilepsy is the tendency to experience sporadic seizures due to an imbalance between the excitatory and inhibitory neurotransmitters in the brain.[16-17] Epilepsy is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. Epilepsy, as defined by WHO is "a chronic disorder characterized by recurrent seizures due to excessive discharge of cerebral neurons".[18] Epilepsy is the second most common chronic neurological condition reported by neurologists[19] affecting 0.4-0.8% of the population world wide.

The main types of epilepsy are partial and generalized seizures. The further classification of two main types of epilepsy is as shown in Table 6.1. Partial seizure means that the electrical storm emerges from only one area of the brain. However, generalized seizure is the one in which the seizures or electrical storms are involving the whole brain at once.

6.1.2 Cause of Epileptic Seizure: GABA as an Endogenous Neurotransmitter

γ-Amino-4-butyric acid (GABA) **2** (Figure 6.2), a non-protein amino acid is the major inhibitory neurotransmitter in the mammalian brain.[20] The inhibitory role of GABA was first elucidated by Elliott.[17]

Table 6.1 Classification of seizures

Partial seizure	a. Simple partial seizures b. Complex partial seizures c. Seizures with secondary generalization
Primary generalized seizure	a. Absence (petit mal) b. Tonic-clonic (grand mal) c. Tonic
Unclassified seizures	a. Neonatal seizures b. Infantile spasms

The overall balance between neuronal excitation and inhibition is maintained by the concentration of GABA in the brain. It is, therefore, described as the brain's natural calming agent.

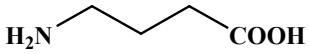


Figure 6.2 γ-Amino-4-butyric acid **2**

GABA receptors are commonly divided into two groups: chloride channel-coupled (ionotropic) $GABA_A$ receptors which belong to the superfamily of ligand-gated ion channels (LGICs) mediate the majority of fast synaptic inhibition in the brain.[20-21] Figure 6.3 is the structural representation of $GABA_A$ receptor.

On the other hand, G-protein-coupled (metabotropic) $GABA_B$ receptors couple to Ca^{2+} and K^+ channels via G-proteins and second messenger systems, activated by baclofen (β-*p*-chlorophenyl-GABA) and resistant to drugs that modulate $GABA_A$ receptors.[20-21] Figure 6.4 represents the $GABA_B$ receptor. The degradation of GABA is by the enzyme GABA aminotransferase (GABA-AT). The reduction of GABAergic neuronal activity plays an important role in a number of neurological disorders, including epilepsy.[22-23] Several animal models of convulsions have been developed to evaluate the anti-seizure

activity. Drugs that increase the content of GABA in brain, exhibit anticonvulsant activity against seizures induced by maximum electroshock (MES), pentylenetetrazole (PTZ) and lithium-pilocarpine (Li-Pilo). The MES is best-validated method for estimation of antiepileptic drugs in generalized tonic-clonic seizures.[24-25]

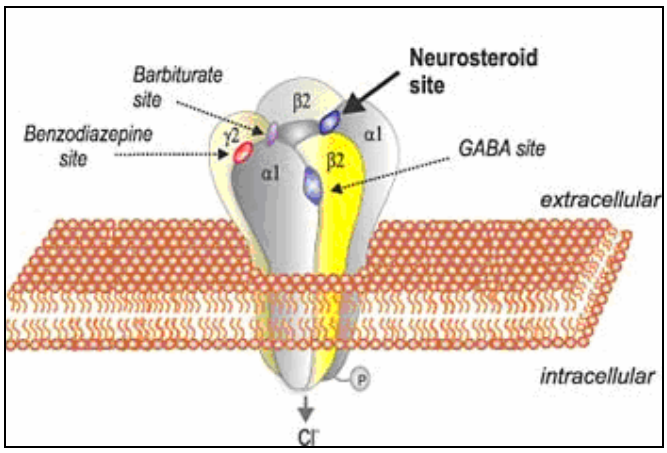


Figure 6.3 Structure of $GABA_A$ receptor

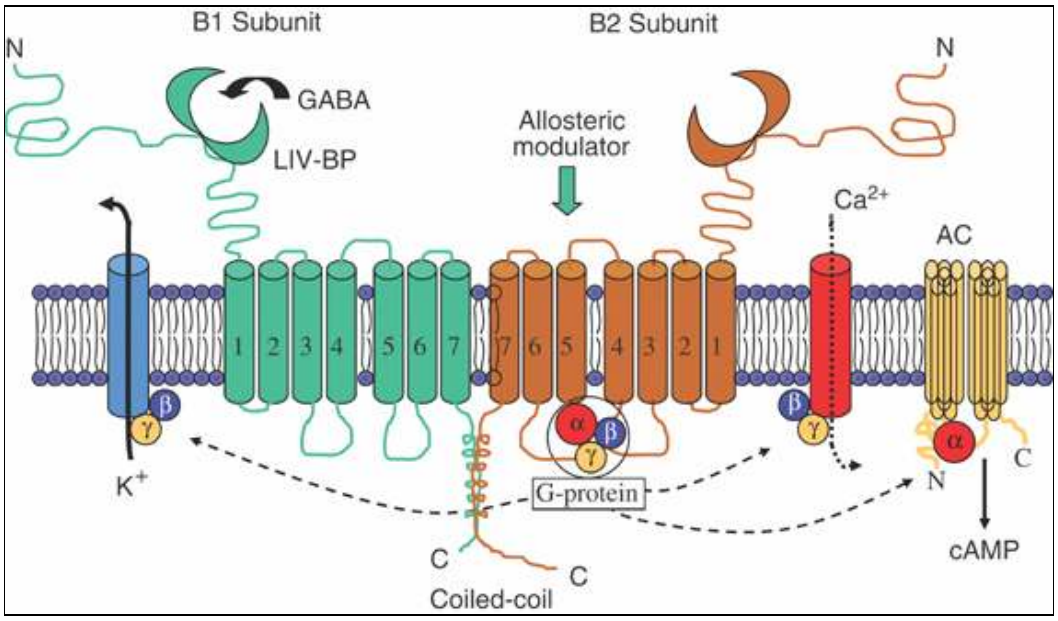


Figure 6.4 Structure of $GABA_B$ receptor

6.1.3 Class of Drugs for Anticonvulsant Activity

In recent years, the field of antiepileptic drug development is quite dynamic, affording many promising research opportunities. Despite progress in understanding the pathogenesis of epilepsy, the cellular basis of human epilepsy still remains a mystery.

Approach to drug therapy is directed towards the control of symptoms that is the suppression of seizures; due to the absence of a specific etiological understanding. Recently, several attempts were made to design a general pharmacophore for the different anticonvulsant classes such as benzodiazepines,[26] barbiturates[27] and triazolines.[28] Unfortunately the various postulated pharmacophore models could not depict a clear picture about the mode of action of these classes of anticonvulsants. Although these drugs have been shown to be effective in reducing seizures in a number of patients, their efficacy does not appear to be superior to that of the drugs developed earlier. Hence, over the past few decades, research aimed at achieving successful delivery of GABA into the CNS has resulted in the discovery of various GABA analogs with improved pharmacological activities. In general, compounds containing 5, 6, or 7-membered heterocyclic ring have anticonvulsant activity. Some of the well known marketed anticonvulsant drugs are Phenytoin (**3**), Phenobarbitone (**4**), Diazepam (**5**), Gabapentin (**6**), Felbamate (**7**) and Vigabatrin (**8**) (Figure 6.5).

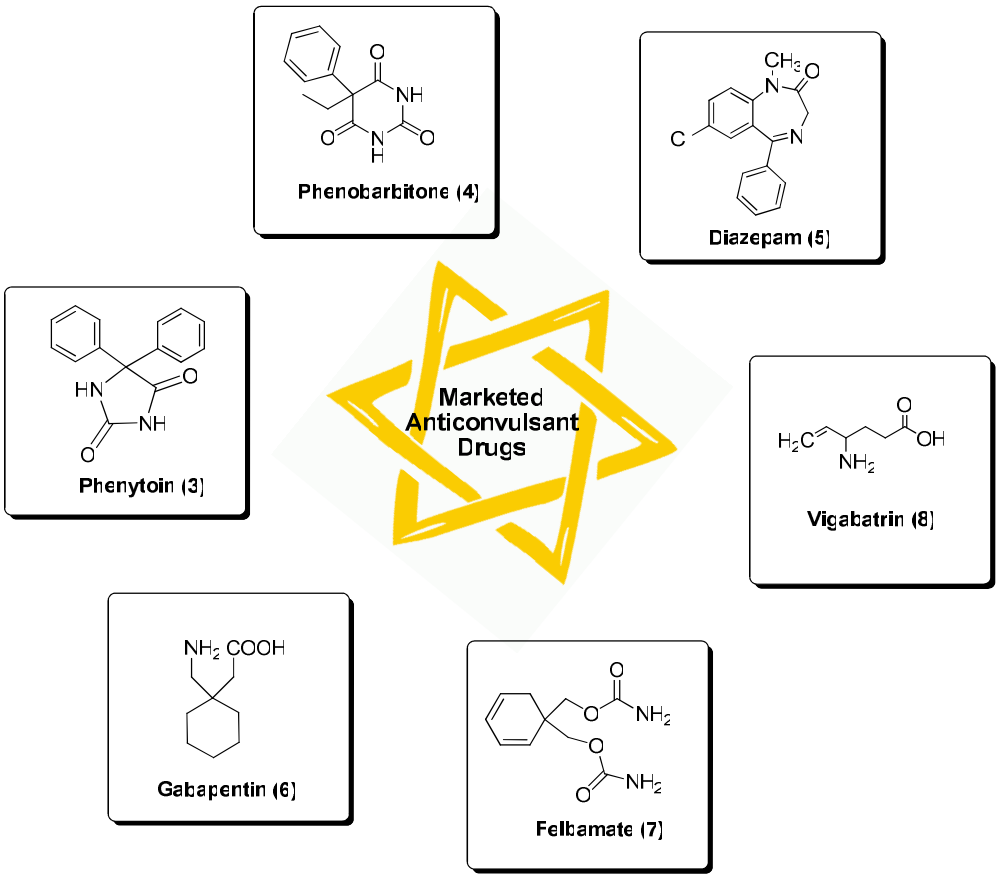


Figure 6.5 Commonly known marketed anticonvulsant drugs **4-8**

Some of the limitations associated with marketed drugs are patient incompliance, continous usage leading to hepatic failure, risk of sudden unexpected death of the epileptic person.[29] Therefore, there is a need for more effective and less toxic antiepileptic drugs. Thus the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry.

6.1.4 Biological Significance of 1,3,4-Thiadiazoles

5/2-Amino-1,3,4-thiadiazoles are interesting pharmacophores and their biological activities are extended over a broad-spectrum. The aromaticity of thiadiazole ring system confers it *in vivo* stability and less toxicity, in general.[30] Thiadiazoles have exhibited potential antiglaucoma,[31] antiinflammatory,[1] antitumor,[7] antiulcer,[32] antibacterial,[33] antiviral,[34] analgesic,[35] antiepileptic,[5] antifungal[33] and radioprotective agent.[36] The 1,3,5-thiadiazole, its isomeric forms and bioisosters are investigated for their antiepileptic action due to their potential to afford a therapeutic drug.

Ahmed and Yusuf[37] prepared a set of benzyl and chlorobenzyl substituted 1,3,4-thiadiazole imines **9** and **10**. The chlorobenzyl substituted compounds **10** were as potent as phenytoin (standard reference drug). The % protection in MES-induced hind limb extension was at 83.3 or 100 for all compounds at a dose of 20 mg/kg. The compounds did not show any neurotoxicity in the Rotarod and ethanol potentiation test.

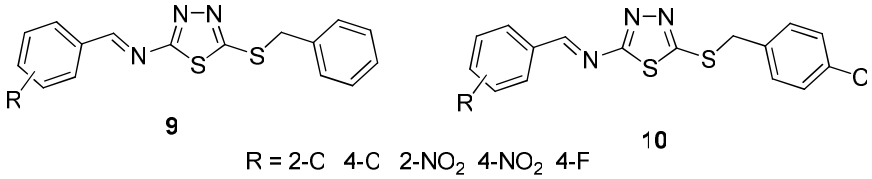


Figure 6.6 1,3,4-Thiadiazole imines **9** and **10**

Rajak and coworkers[5] synthesised a series of 1,3,4-thiadiazoles and studied their anticonvulsant activity with a hypothesized four-site pharmacophore model. Compound **11a** at 100 mg/kg was found to possess significant anticonvulsant activity in MES and scPTZ models employed for anticonvulsant evaluation. Few other compounds also demonstrated a marked anticonvulsant property. The results of the study validated that the pharmacophore model with four binding sites is essential for anticonvulsant activity.

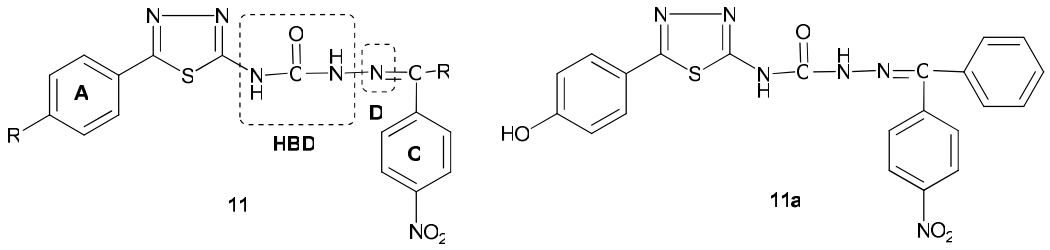


Figure 6.7 Pharmacophoric model of 1,3,5-thiadiazole **11**. Hydrophobic ring system (A), Hydrogen-binding domain (HBD), Distant aryl ring (C), Electron-donor moiety (D).

The 2,5-Disubstituted-1,3,4-thiadiazoles **12** and **13** were reported for their anticonvulsant activity.[33] The degree of protection afforded by these compounds at a dose of 100 mg/kg ip against pentylenetetrazole-induced convulsions in mice ranged from 20 to 90% and the seizure latency varied from 4 to 8 min against 80% protection and 7 min seizure latency exhibited by the reference drug sodium valproate. The compounds with R = ethyl and *m*-fluorophenyl in **12** showed maximum protection of 90% and 70%, respectively (Fig. 6.8).

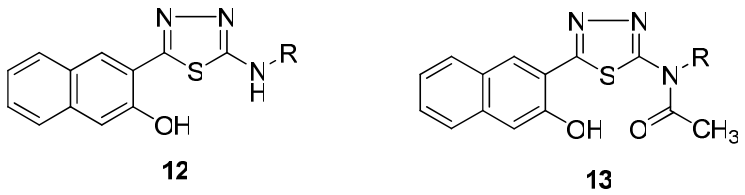


Figure 6.8 1,3,4-Thiadiazoles **12** and **13**

Foroumadi et al.[6] prepared novel 2-amino-1,3,4-thiadiazoles and screened for their anticonvulsant activity by evaluating the ability of these compounds to protect mice against convulsion induced by lethal doses of pentylentetrazole (PTZ) and maximal electroshock (MES). One of the synthesised compounds **14** proved to be most active compound in both MES and PTZ tests with ED_{50} values of 20.11 and 35.33 mg/kg, respectively.

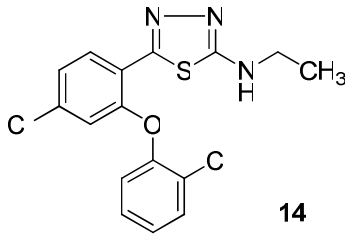
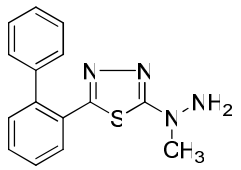


Figure 6.9 Most active 1,3,4-thiadiazole **14**

Researchers at Reckitt and Colman plc have extensively explored 1,3,4-thiadiazoles **15-18** for their anticonvulsant activity.[38] The investigation on the 2-aryl-5-hydrazino-l,3,4-thiadiazoles**15** (Figure 6.11) revealed that the combination of preferred aromatic substituents in the second position coupled with alkyl substitution on the hydrazine moiety leads to a number of potent compounds lacking sedation, ataxia, or lethality. The 5-(2-biphenylyl)-2-(1-methylhydrazino)-l,3,4-thiadiazole **15a** (Figure 6.10) equated favorably with the standard drugs phenytoin, phenobarbital, and carbamazepine.



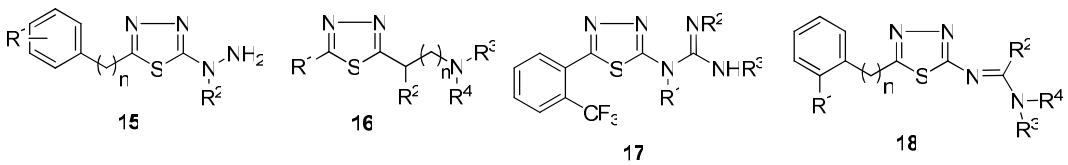
15a

Figure 6.10 Highly potent 1,3,4-thiadiazole **15a**

Further explorations on 5-aminoalkyl-1,3,4-thiadiazoles[39] **16** (Figure 6.11) led to a potent compound, 2-(aminomethyl)-5-(2-biphenylyl)-1,3,4-thiadiazole, which showed the same potency as that of standard drugs. The potency remained unperturbed on alkylation of the side-chain nitrogen atom and potency plunged by aryl substitution or chain lengthening. Replacement of the 2-biphenyl group by phenyl or benzyl also led to inactive compounds.

The investigations on 2-aryl-5-guanidino-l,3,4-thiadiazoles[40] **17** (Figure 6.11) showed that unsubstituted guanidine possesses potent anticonvulsant properties (ED_{50} in MES: ~ 20 mg/kg). Any substitution on the guanidine moiety is detrimental for the activity. Further studies revealed that the unsubstituted guanidine **17** possess considerable degree of sedative activity.

The studies on the anticonvulsant activity of 2-aryl-1,3,4-thiadiazole amidines[41] **18** (Figure 6.11) led to a potent compound, *N*'-(5-(2-(trifluoromethyl)phenyl)-1,3,4-thiadiazol-2-yl)acetimidamide. The compounds **18** exhibited a high level of neurotoxicity and sedation.



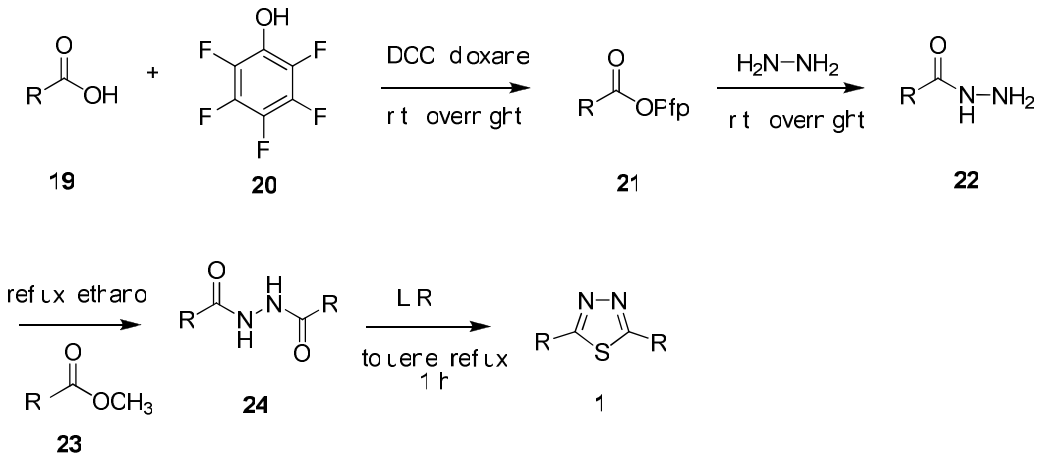
15 16 17 18

Figure 6.11 Potent anticonvulsant 5-hydrazino-l,3,4-thiadiazoles **15**, 5-aminoalkyl-1,3,4-thiadiazoles **16**, 5-guanidino-1,3,4-thiadiazoles **17**, 2-amidino-1,3,4-thiadiazoles **18**.

6.1.5 Synthetic Routes for 2,5-Disubstituted-1,3,4-thiadiazoles

Generally, preparation of 1,3,4-thiadiazoles involve use of diacylhydrazide precursors under different reaction conditions. Symmetrical 2,5-disubstituted-1,3,4-thiadiazoles are prepared by condensation of arylaldehydes, hydrazine and sulfur in ethanol under microwave irradiation.[42] 2-Mercapto-5-substituted-1,3,4-thiadiazoles are prepared by reacting carbon disulfide with hydrazide under basic conditions followed by cyclization under acidic conditions.[43] The general routes for their preparation involve synthesis of acyl thiohydrazides and cyclization under acidic conditions, thionation of acyl hydrazides followed by cyclization under acidic conditions or cyclization of thiosemicarbazones. Following are some of the general protocols for the synthesis of 1,3,4-thiadiazoles in brief.

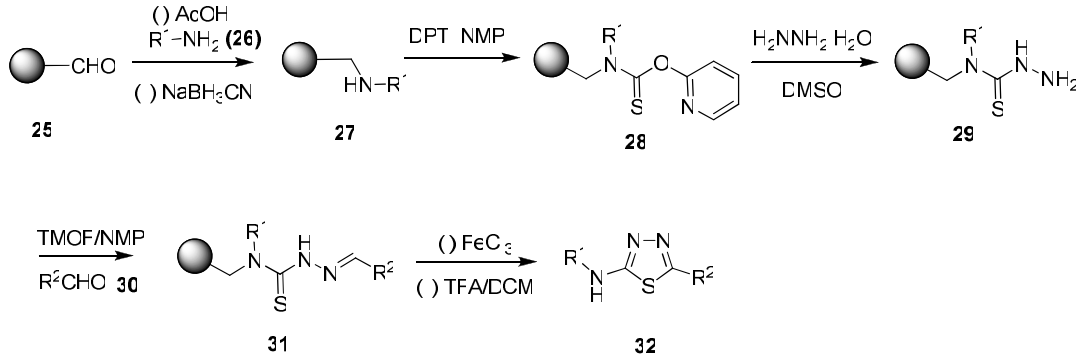
Gierczk and Zalas[44] reported the synthesis of 2,5-disubstituted-1,3,4-thiadiazoles **1** over four steps (Scheme 6.1). The preparation of *N,N′*-diaroylhydrazines **24** was adopted from an earlier report.[45] The preparation of *N,N′*-diaroylhydrazines **24** involves reaction of arylhydrazides **22** and arylesters **23**. The arylhydrazides **22** were prepared using pentafluorophenyl (Pfp) ester **21**, which in turn was prepared by the activation of aryl carboxylic acids **19**. The synthesis of 1,3,4-thiadiazole **1** was achieved in high yields by refluxing *N,N′*-diaroylhydrazines **24** with Lawesson's Reagent (L. R.) in toluene for 1 h.



Scheme 6.1 Synthesis of 2,5-disubstituted-1,3,4-thiadiazoles **1**

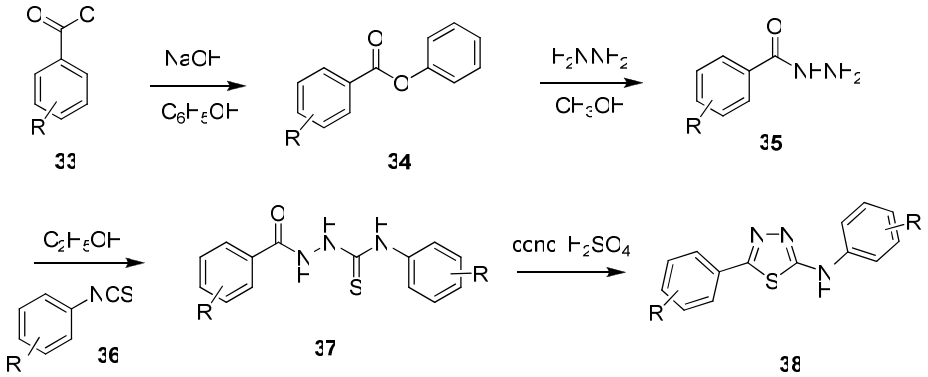
A robust protocol for the solid phase synthesis of 5-alkyl/aryl-2-alkylamino-1,3,4-thiadiazoles **31** was described based on a common resin bound thiosemicarbazide (Scheme 6.2).[46] The protocol has been verified by the preparation of a library with diverse substitutions that gave 1,3,4-thiadiazoles in good to excellent yields and purity. 2-(3,5-

Dimethoxy-4-formylphenoxy)ethoxymethyl polystyrene **25** was treated with a range of primary amines **26** under standard reductive amination conditions to yield the respective resin bound benzyl amine derivates **27**. The transformation of a resin bound primary amine to an isothiocyanate **28** was achieved upon treatment with di-(2-pyridyl)-thionocarbonate (DPT). The isothiocyanate **28** was subsequently reacted with hydrazine to give a thiosemicarbazide **29**. Treatment of **29** with aldehydes **30** in a mixture of trimethyl orthoformate (TMOF) and NMP yielded the thiosemicarbazone **31**. Cyclization of **31** was achieved by treating the resin with a solution of iron(III) chloride in DCM/MeOH. Finally, cleavage of resin with TFA yielded substituted 2-amino-5-substituted-1,3,4-thiadiazoles **32**.



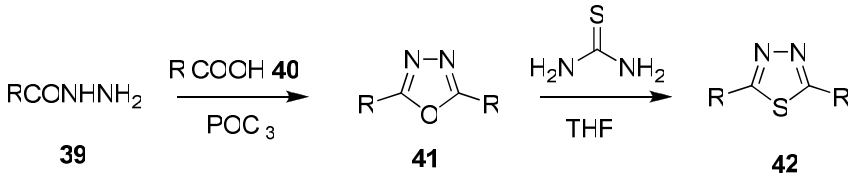
Scheme 6.2 Solid phase synthesis of 2,5-disubstituted-1,3,4-thiadiazoles **32**

Oruc and coworkers[47] described the synthesis of 1,3,4-thiadiazoles **38** over four steps starting from acyl halides **33** (Scheme 6.3). The acyl halides **32** were reacted with phenol under basic conditions to generate esters **34** which were then reacted with hydrazine to yield hydrazides **35**. Reaction of isothiocyanates **36** with **35** afforded acyl thiosemicarbazides **37** which were further cyclized to thiadiazoles **38** in presence of concentrated sulphuric acid.



Scheme 6.3 Synthesis of 2-arylamino-1,3,4-thiadiazoles **38**

The 1,3,4-thiadiazoles **42** were achieved in good yields from the reaction of 1,3,4-oxadiazoles **41** with thiourea (Scheme 6.4).[48] 1,3,4-oxadiazoles **42** were prepared from acid hydrazides **39** on treatment with different carboxylic acids **40** in the presence of phosphorus oxychloride.



39 41 42

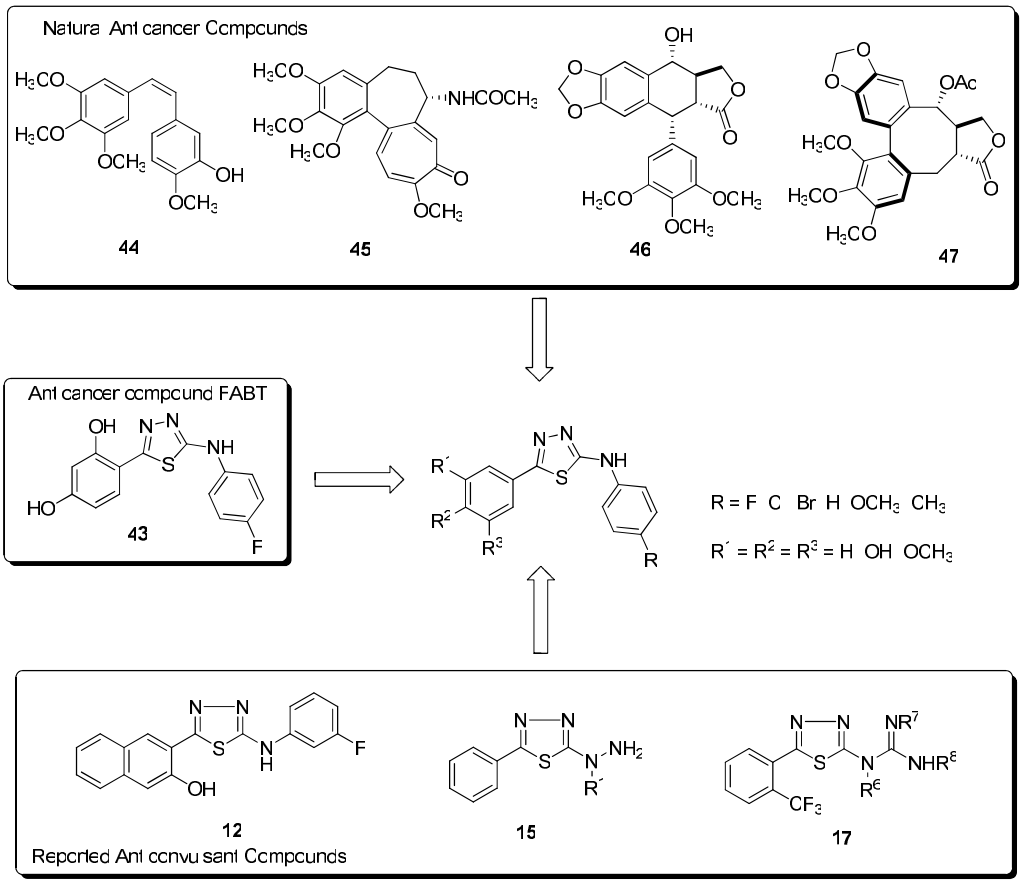
Scheme 6.4 Synthesis of 1,3,4-thiadiazoles **42** from 1,3,4-oxadiazoles **41**

6.2 The Present Work

The 2-amino-1,3,4-thiadiazoles are explored for variety of biological activities. They have exhibited a very good anticonvulsant activity. A careful examination of the anticonvulsant compounds **12**, **15** and **17** show the importance of C5 aryl ring and C2 amine substitution in the 1,3,4-thiadiazole heterocycle. Many other reports have described the antiepileptic activity with aryl substitution at C5 postion and free/substituted amines (alkyl, guanidyl, alkylthio, amidiniyl) at the C2 position. The derivatives with aryl substitution at fifth position and arylamine substitution at the second position are not well explored.

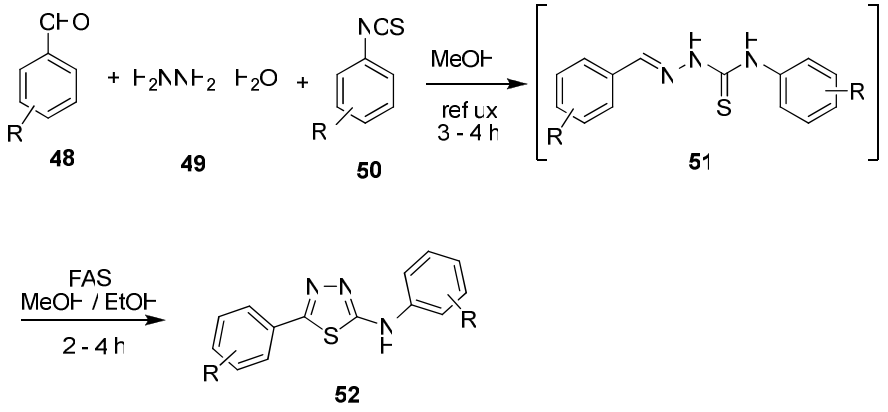
Further, the 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) **43** exhibited most promising anticancer effects.[8] It was shown that the major structural feature of several known natural antimitotic agents (Combretastatin A-4 (**44**), Colchicine (**45**), Podophyllotoxin (**46**), Steganacin (**47**) in Scheme 6.5), which bind at the Colchicine site on tubulin, is a trimethoxy aryl unit.[49-50] The incorporation of crucial structural features of reported anticonvulsant (5-aryl and 2-arylamino) and anticancer (trimethoxyphenyl and hydroxy,methoxyphenyl moieties) into 1,3,4-thiadiazoles may lead to a potent compound with dual activities making the approach similar to combination therapy but combined in one drug (Scheme 6.5).

Several methods are reported for the synthesis of 1,3,4-thiadiazoles.[42,44,48,51-53] Most of the protocols are time consuming, laborious and require at least 3-4 steps from easily available starting materials.



Scheme 6.5 Rational for the design of 1,3,4-thiadiazoles

We have developed a one-pot synthesis of 1,3,4-thiadiazoles **52** which involves the refluxing of arylaldehyde, hydrazine hydrate and arylisothiocyanate followed by oxidative cyclization with ferric ammonium sulphate [$NH_4Fe(SO_4)_2\ 12H_2O$] (Scheme 6.6).

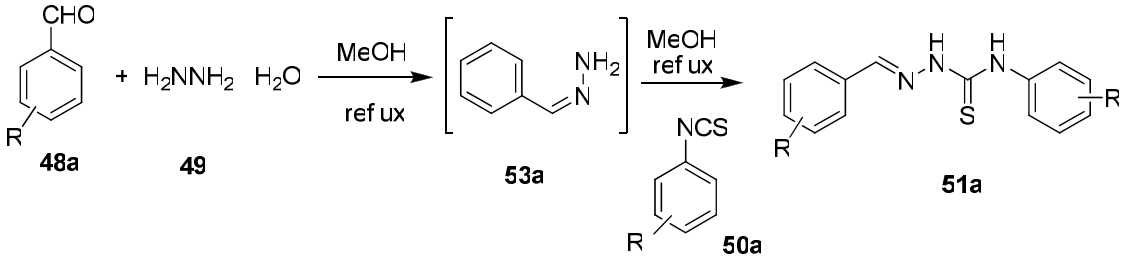


Scheme 6.6 One-pot synthesis of 1,3,4-thiadiazoles **52**

6.3 Results and Discussion

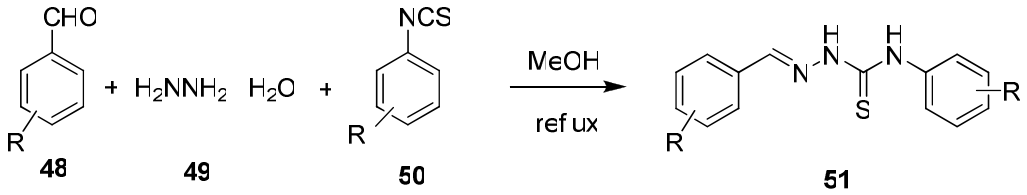
6.3.1 Chemistry

Our initial efforts to prepare 1,3,4-thiadiazoles **52** involve one-pot sequential reaction of Vanillin, hydrazine hydrate and phenyl isothiocyanate in methanol. The reaction of equimolar quantities of Vanillin **48a** and hydrazine **49** in methanol under reflux conditions yielded the intermediate benzylidene hydrazine **53a**. Phenyl isothiocyanate **50a** was added to the reaction pot and refluxed to afford thiosemicarbazone **51a**.



Scheme 6.7 Sequential synthesis of thiosemicarbazone **51a**

With successful formation of thiosemicarbazone **51a**, next we tried to prepare by simultaneous addition of Vanillin, hydrazine hydrate and phenyl isothiocyanate in methanol. To our delight we found that the thiosemicarbazone **51a** was obtained in good yields (90%). It was isolated by simple filtration and subjected to oxidative cyclization.



Scheme 6.8 One-pot synthesis of thiosemicarbazone **51**

Taking a cue from literature reports, the cyclization of **51a** was attempted in refluxing ethanol using different reagents (Table 6.2). Of the screened reagents, the ferric ammonium sulfate, $NH_4Fe(SO_4)_2.12H_2O$ (FAS) smoothly yielded the desired product in good yields.

The overall one-pot protocol was established by the reaction of equimolar quantities of Vanillin, hydrazine and phenyl isothiocyanate in excess methanol under reflux for 4 h. Upon completion of the reaction, as indicated by TLC, the solid product was allowed to

settle down. The excess solvent was decanted and added 3 equivalents of finely ground FAS to the reaction pot and heated at 80 ^{o}C for 5 h. The reaction mixture is then filtered at the pump and washed with excess hot solvent. The solvent is concentrated on rotary evaporator to obtain the crude product which was recrystallised from ethanol. The formation of the thiadiazole **52a** was confirmed by its ^{1}H NMR & mass spectral data (Figure 6.11 and 6.12).

Table 6.2 Conditions investigated for the conversion of thiosemicarbazone **51a** to **52a**

S. No.	Reagent	Solvent	Temp. (^{o}C)	Time (h)	Yield (%)
1	$FeCl_3$ (10% soln.	EtOH	reflux	4	10
2	$FeCl_3$ (4 mmol)	H_2O	90	overnight	0
3	FAS (1 mmol)	EtOH	reflux	5	40
4	FAS (3 mmol)	EtOH	reflux	5	80
5	FAS (6 mmol)	EtOH	reflux	5	75
6	$ZnCl_2$ (excess)	EtOH	reflux	24	0
7	AcOH (excess)	-	reflux	24	0
8	IBD (1 mmol)	CH_2Cl_2	0 to r.t.	4	0
9	HTIB (1 mmol)	CH_2Cl_2	0 to r.t.	4	0
10	$HgCl_2$ (1.1 mmol)	MeCN	r.t.	10	0

The optimized protocol was extended to other arylisothiocyanates and arylaldehydes. The reaction of vanillin with substituted arylisothiocyanates afforded products **52b-e** in good yields (75 – 85%). All arylisothiocyanates were almost equally reactive to generate the 1,3,4-thiadiazoles **52**. Strongly deactivated substrate, 3,4,5-trimethoxybenzaldehyde, also reacted as efficiently as vanillin affording products **52f-j** with good yields (70 – 80%).

Employing 3-cyclopentyloxy-4-methoxybenzaldehye with phenyl isothiocyanate resulted in product **52k** with relatively low yields. The reason for this was ascertained to be the cleavage of cyclopentyl group of the aldehyde in presence of FAS. It was confirmed with a trial reaction of isovanillin with phenylisothiocyanate under same conditions.

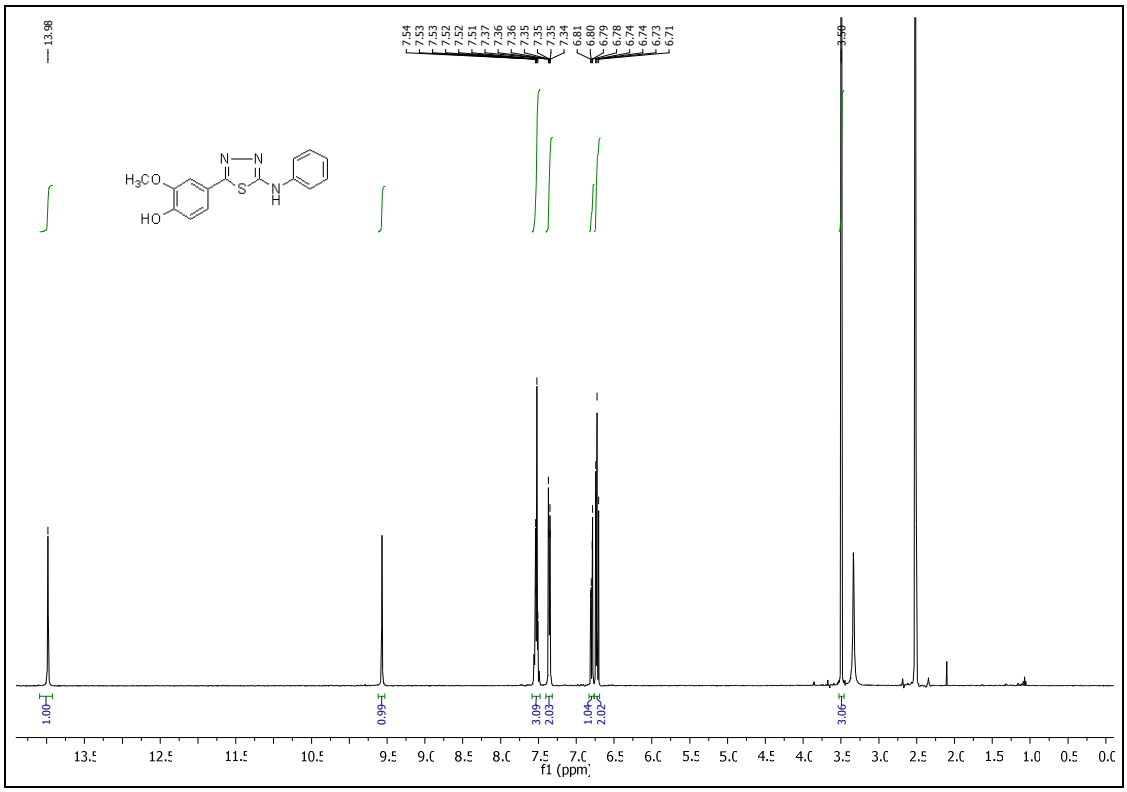


Figure 6.12 ^{1}H NMR spectrum of compound **52a**

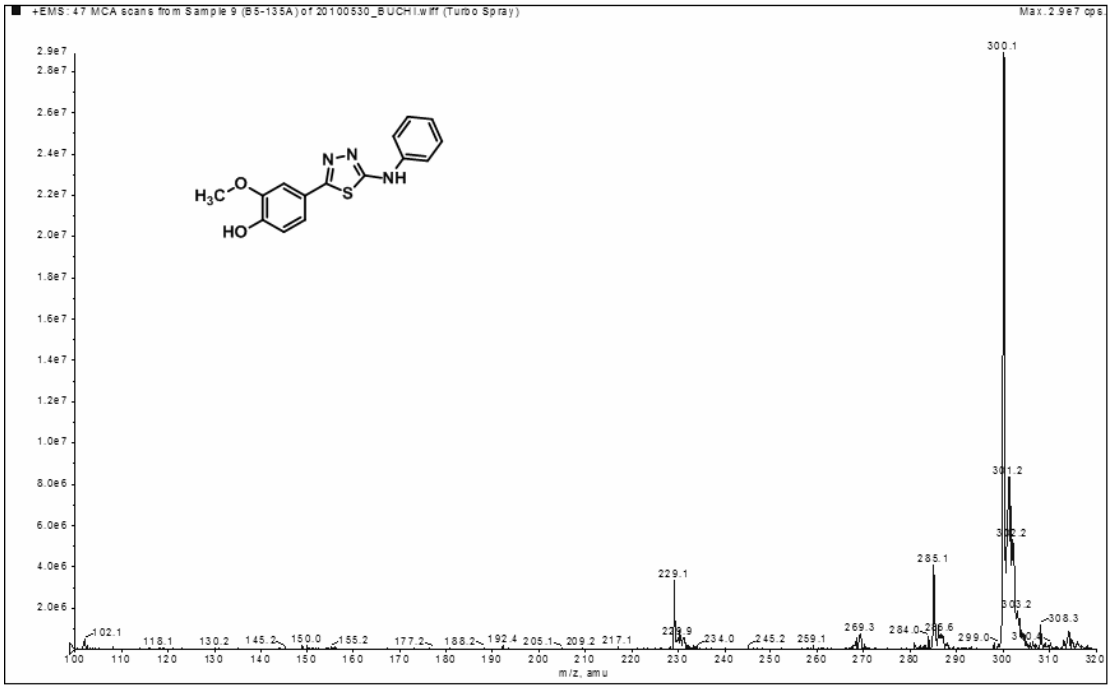
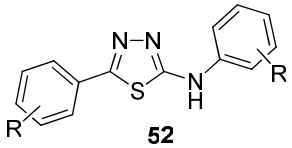


Figure 6.13 Mass spectrum of compound **52a**

Table 6.3 Synthesised 2-arylamino-5-aryl-1,3,4-thiadiazoles **52**

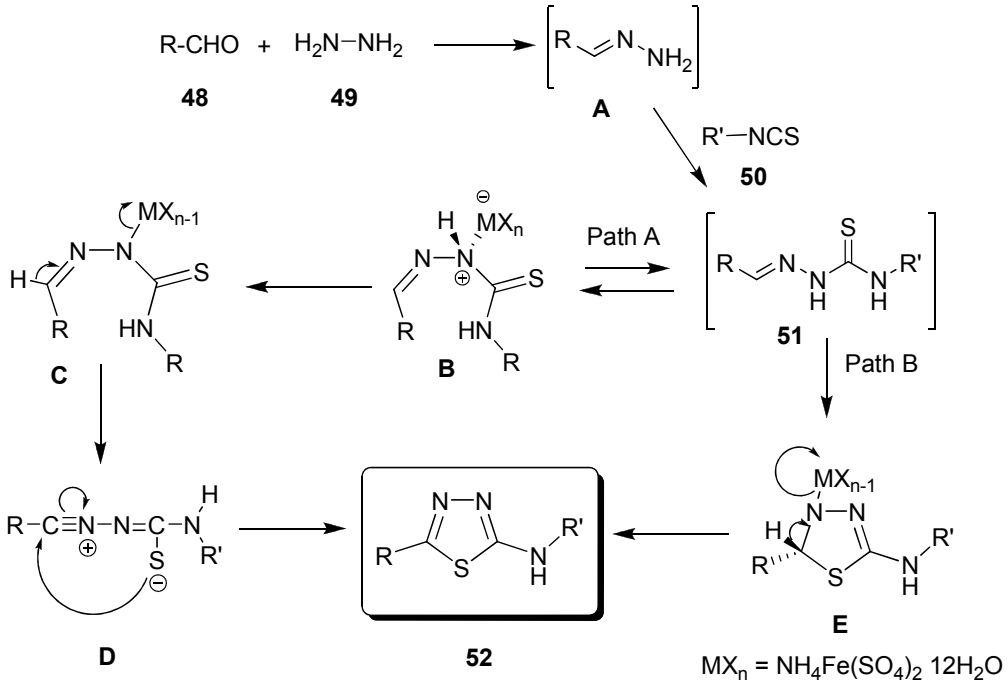


52

Compd.	R	R'	Yield (%)
52a	4-OH-3-OCH_3	H	85
52b	4-OH-3-OCH_3	4-CH_3	82
52c	4-OH-3-OCH_3	4-Cl	78
52d	4-OH-3-OCH_3	4-NO_2	76
52e	4-OH-3-OCH_3	4-OCH_3	80
52f	3,4,5-$(OCH_3)_3$	H	78
52g	3,4,5-$(OCH_3)_3$	4-CH_3	75
52h	3,4,5-$(OCH_3)_3$	4-Cl	74
52i	3,4,5-$(OCH_3)_3$	4-NO_2	72
52j	3,4,5-$(OCH_3)_3$	4-OCH_3	81
52k	3-OC_5H_9-4-OCH_3	H	55

6.3.2 Plausible Mechanism

A plausible mechanism would be the initial formation of the hydrazone **A** from the reaction of aldehyde **48** and hydrazine **49** which further reacts with isothiocyanate **50** to generate the thiosemicarbazone **51**. Probably, the reaction of **51** with FAS may involve two paths (Path A and Path B) as shown in Scheme 6.9. Probably the early stage of the reaction involves the reversible electrophilic attack of the hard acid iron (III) on the hardest basic site among N-2 and N-4 of the open chain structure **B** or on N-4 of the cyclic structure **E**. If there is no substituent at N-2 then the reaction may proceed or through a variety of pathways (**Path A**) to the nitrilimine intermediate **D** that can undergo a 1,5-electrocyclization or to a 5 *endo* dig process when the open chain structure is predominant or through **Path B** when thiosemicarbazone is in the cyclic structure.



Scheme 6.9 Plausible mechanism for the formation of 1,3,4-thiadiazoles **52**

6.3.3 Structure-Activity Relationship (SAR)

The 2-arylamino-5-aryl-1,3,4-thiadiazoles **52a-k** were evaluated for their anticonvulsant potential on swiss albino mice using subcutaneous picrotoxin induced seizure model in comparison with standard drug diazepam (Please refer section 6.6.2 for experimental details). A tentative comparison of the structure of 1,3,4-thiadiazoles **52** and their activity proves helpful to understand the structure-activity relationship (SAR).

Most of the compounds exhibited moderate anticonvulsant activity. The compounds with 4-OH-3-$OCH_3C_6H_3$ substitution at the R position and C_6H_5 (**52a**) at R' exhibited moderate activity with the mean onset of 16.8 min at 50 mg/kg dose. The mean number of seizures was 15.7, which was more than the number of seizures at 100 mg/kg (11.4). The compounds with 4-OH-3-$OCH_3C_6H_3$ substitution at the R position and 4-$CH_3C_6H_4$ (**52b**) and 4-$NO_2C_6H_4$ (**52d**) at R^1 exhibited significant anticonvulsant activity at 100 mg/kg and 50 mg/kg doses with the onset of seizures at greater than 27 min and 20 min, respectively. The number and duration of seizures was more than compound **52a**. The 4-ClC_6H_5 substitution at R^1 proved beneficial in both R substitutions, 3,4,5-$(OCH_3)_3C_6H_2$ **52h** and 4-OH-3-$OCH_3C_6H_3$ **52c**. The onset of seizures was more than 20 min at 100 mg/kg dose but the number of seizures was relatively high in compound **52h** with no mortality.

Table 6.4 Anticonvulsant activity of 1,3,4-thiadiazoles **52a-k** in scPIC induced seizures.

S.No.	Compd.	Dose (mg/kg)	Mean Onset of Seizure (min)	Mean No. of Seizures	Mean Duration of Seizure	Mortality
1.	**52a**	100	18.8 (1.4)	11.4 (2.2)	1.9 (0.6)	1 (0.2)
		50	16.8 (1.3)	15.7 (2.6)	3.0 (1.1)	1 (0.2)
2.	**52b**	100	29.0 (3.4)	12.6 (1.2)	3.5 (0.5)	1 (0.2)
		50	26.5 (2.5)	16.2 (1.4)	4.4 (1.2)	1 (0.2)
3.	**52c**	100	20.4 (4.1)	05.4 (2.8)	1.5 (0.4)	0
		50	16.8 (3.7)	09.5 (4.4)	2.9 (0.4)	1 (0.2)
4.	**52d**	100	27.4 (1.7)	12.5 (1.4)	3.5 (0.6)	0
		50	21.6 (2.4)	14.4 (1.8)	5.6 (0.8)	0
5.	**52e**	100	25.6 (3.5)	10.8 (3.9)	2.2 (0.7)	1 (0.2)
		50	15.4 (2.0)	17.2 (4.2)	4.3 (1.4)	1 (0.2)
6.	**52f**	100	17.2 (2.2)	16.0 (5.7)	6.2 (7.2)	0
		50	14.9 (2.7)	29.0 (2.0)	8.2 (3.8)	0
7.	**52g**	100	14.2 (2.2)	10.0 (3.2)	3.9 (0.6)	0
		50	13.1 (4.6)	15.0 (5.6)	4.2 (1.5)	0
8.	**52h**	100	23.7 (7.6)	18.0 (9.1)	4.1 (1.7)	0
		50	17.5 (6.1)	19.0 (6.2)	6.7 (1.8)	0
9.	**52i**	100	21.9 (1.8)	12.0 (4.9)	2.5 (0.5)	0
		50	18.6 (1.5)	11.0 (5.9)	4.5 (2.0)	0
10.	**52j**	100	20.6 (3.6)	13.0 (7.2)	4.0 (1.2)	0
		50	16.7 (3.2)	18.0 (2.3)	7.6 (2.4)	0
11.	**52k**	100	18.4 (1.4)	19.2 (2.5)	6.6 (0.9)	0.8 (0.2)
		50	14.6 (2.4)	24.3 (3.7)	8.3 (1.6)	1 (0.2)
12.	**Control**	-	13.6 (0.6)	25.4 (3.1)	7.5 (1.1)	4 (0.2)
13.	**Diazepam**	1	25.6 (1.5)	06.0 (0.3)	1.9 (0.6)	0

The values in the parantheses indicate the Standard Error of the Mean (SEM).

The compounds **52a**, **52f** and **52k** with unsubstituted phenyl at R' showed weak activity with the mean number of seizures greater than 16 and mean onset at less than 18 min for 100 mg/kg dose indicating that substitution on the phenyl ring at R' is mandatory. The 4-$NO_2C_6H_4$ at R' and 3,4,5-$(OCH_3)_3C_6H_2$ at R led to **52i** with delayed onset (21.9 min at 100 mg/kg) but

lasted with more number of seizures in contrast to **52d**. The compound **52g** with R' as a 4-$CH_3C_6H_4$ substituent and 3,4,5-$(OCH_3)_3C_6H_2$ as R proved least active with much early onset (14.2 min at 100 mg/kg dose) and more number of seizures (10.0) in distinguishing it from **52b**.

The activity data obtained asserts that bulky substitutions on the phenyl ring at R position and an unsubstituted phenyl at R' proves detrimental for the activity. The activity may be further ameliorated with a more bulky and multiple substitutions on the R' phenyl ring. The moderate activity of 1,3,4-thiadiazoles **52a-k** in scPIC model suggests that their mechanism of action is probably by mediation of $GABA_A$ receptors.

6.4 Conclusions

The one-pot reaction of arylaldehyde, hydrazine and arylisothiocyante led to produce thiosemicarbazone which upon oxidative cyclization with FAS resulted in facile synthesis of 1,3,4-thiadiazoles **52a-k** in good yields. The advantages of the protocol include simple reaction workup, easy availability of starting materials and purification by recrystallisation. The anticonvulsant evaluation of 1,3,4-thiadiazoles **52a-k** resulted in moderate activity. The structure-activity relationship studies revealed that bulky substitutions on the C-5 aryl ring leads to decreased activity and unsubstituted arylamino ring at C-2 also diminishes the activity. The activity can be further improved by the structural variation in the C-5 aryl and C-2 arylamino rings.

6.5 Experimental Details

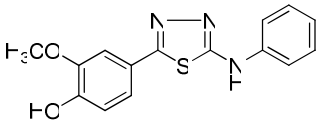
All the reagents were purchased from Aldrich and Spectrochem chemicals. The reaction was monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualized by fluorescence quenching under UV light (254 nm). Column chromatography, if performed, was using 100-200 mesh silica gel and appropriate mixture of hexane-ethyl acetate for elution. The solvents were evaporated on Buchi rotary evaporator. Melting points (M.p.) were determined with electrothermal capillary melting point apparatus. ^1H & ^{13}C NMR spectra were recorded on a Bruker Avance II (400 MHz) spectrometer. The coupling constant (*J*) values are in Hz. High resolution mass spectra data were obtained using electrospray ionization from a Quadruple-Time-of-flight (Q-Tof II) mass spectrometer (Micromass Manchester, U. K.). LC–MS analysis was carried out on an ABI 2000 Q-Trap mass spectrometer fitted with electrospray ionization (ESI).

6.5.1 General Procedure for the One-pot Synthesis of 1,3,4-Thiadiazoles

A mixture of arylaldehyde, hydrazine and arylisothiocyanate was refluxed in excess methanol for 6 h. Upon completion of the reaction, as indicated by TLC, the reaction mixture was allowed to cool to room temperature and the solid product was allowed to settle down. The excess solvent was decanted and added 3 equivalents of finely ground FAS to the reaction pot and heated at 80 ^{o}C for further 5 h. The reaction mixture was then filtered at the pump and washed with excess hot solvent. The filtrate was concentrated on rotary evaporator to obtain the crude 1,3,4-thiadiazole **52** which was recrystallised from ethanol.

Analytical data of the synthesised 1,3,4-thiadiazoles **52**:

2-(3-Methoxy-4-hydroxyphenyl)-5-(phenylamino)-1,3,4-thiadiazole (52a)



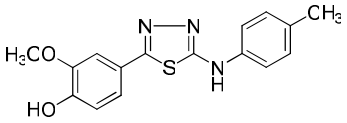
Yield: 85%

M.p.: 246-247 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.98 (s, 1H), 9.57 (s, 1H), 7.58 – 7.48 (m, 3H), 7.40 – 7.32 (m, 2H), 6.80 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.73 (dd, *J* = 9.8, 5.1 Hz, 2H), 3.50 (s, 3H).

MS(ESI): *m/z* $(M+H)^+$ calcd. for $C_{15}H_{13}N_3O_2S$: 300.1; found: 300.1

2-(3-Methoxy-4-hydroxyphenyl)-5-(4-methylphenylamino)-1,3,4-thiadiazole (52b)



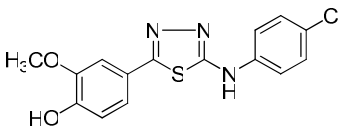
Yield: 82%

M.p.: 248-249 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.57 (s, 1H), 9.61 (s, 1H), 7.27 (d, *J* = 35.9 Hz, 4H), 6.75 (d, *J* = 19.5 Hz, 3H), 3.53 (s, 3H), 2.38 (s, 3H).

MS (ESI): *m/z* $[M + H]^+$ calcd for $C_{16}H_{15}N_3O_2S$: 314.1; found: 314.2

2-(3-Methoxy-4-hydroxyphenyl)-5-(4-chlorophenylamino)-1,3,4-thiadiazole (52c)



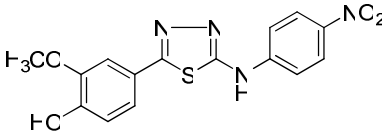
Yield: 78%

M.p.: 268-270 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 14.03 (s, 1H), 9.60 (s, 1H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.41 (d, *J* = 8.6 Hz, 2H), 6.81 (s, 1H), 6.79 – 6.72 (m, 2H), 3.57 (s, 3H).

MS(ESI): *m/z* $(M+H)^+$ calcd. for $C_{15}H_{12}ClN_3O_2S$: 334.0; found: 334.0

2-(3-Methoxy-4-hydroxyphenyl)-5-(4-nitrophenylamino)-1,3,4-thiadiazole (52d)



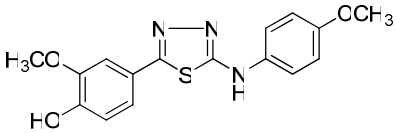
Yield: 76%

M.p.: 280-284 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 14.14 (s, 1H), 9.62 (s, 1H), 8.42 – 8.33 (m, 2H), 7.74 – 7.66 (m, 2H), 6.86 (s, 1H), 6.73 (s, 2H), 3.57 (s, 3H).

MS(ESI): *m/z* $(M+H)^+$ calcd. for $C_{15}H_{12}N_4O_4S$: 345.0; found: 345.0

2-(3-Methoxy-4-hydroxyphenyl)-5-(4-methoxyphenylamino)-1,3,4-thiadiazole (52e)



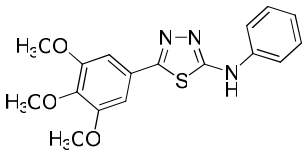
Yield: 80%

M.p.: 228-230 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.93 (s, 1H), 9.56 (s, 1H), 7.29 – 7.24 (m, 2H), 7.09 – 7.02 (m, 2H), 6.83 – 6.77 (m, 2H), 6.72 (d, *J* = 8.1 Hz, 1H), 3.82 (s, 4H), 3.55 (s, 3H).

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{16}H_{15}N_3O_3S$: 330.1; found: 330.2

2-(3,4,5-Trimethoxyphenyl)-5-(phenylamino)-1,3,4-thiadiazole (52f)



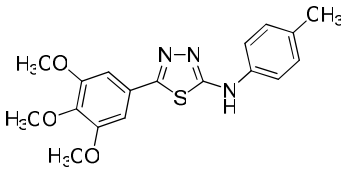
Yield: 78%

M.p.: 218-220 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.66 (s, 1H), 7.57-7.55 (m, 2H), 7.41 (m, 2H), 7.17 (s, 1H) 7.05 (s, 2H), 3.92(s, 6H), 3.86 (s, 3H).

MS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{17}H_{17}N_3O_3S$: 344.1069; found: 344.2816

2-(3,4,5-Trimethoxyphenyl)-5-(4-methylphenylamino)-1,3,4-thiadiazole (52g)



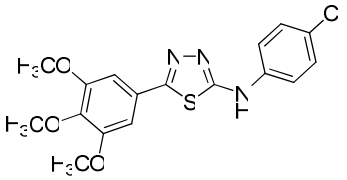
Yield: 75%

M.p.: 225-227 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.98 (s, 1H), 7.69-7.66 (m, 1H), 7.432 (d, *J* = 7.60 Hz, 1H), 7.22 (d, *J* = 7.60 Hz, 2H), 7.04 (s, 2H), 3.92 (s, 6H), 3.78 (s, 3H), 2.3 (m, 3H).

MS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{19}N_3O_3S$: 358.1225; found: 358.5128

2-(3,4,5-Trimethoxyphenyl)-5-(4-chlorophenylamino)-1,3,4-thiadiazole (52h)



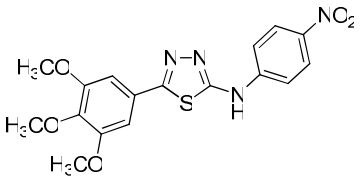
Yield: 74%

M.p.: 240-243 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 9.80 (s, 1H), 7.58 (d, *J* = 8.80 Hz, 1H), 7.51 (s, 2H), 7.35-7.32 (m, 1H) 7.15 (s, 1H), 7.06 (s, 1H) 3.92 (s, 6H), 3.88 (s, 3H).

MS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{17}H_{16}ClN_3O_3S$: 378.0679; found: 378.3997

2-(3,4,5-Trimethoxyphenyl)-5-(4-nitrophenylamino)-1,3,4-thiadiazole (52i)



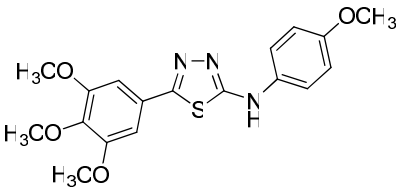
Yield: 72%

M.p.: 224-228 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.37 (d, *J* = 8.00 Hz, 1H), 8.16 (d, *J* = 8.00 Hz, 2H), 7.83 (d, *J* = 8.10 Hz, 2H), 7.65 (d, *J* = 8.10 Hz, 1H), 6.53 (s, 1H), 3.92 (s, 3H), 3.76 (s, 3H), 3.61 (s, 3H).

MS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{17}H_{16}N_4O_5S$: 389.0920; found: 389.4628

2-(3,4,5-Trimethoxyphenyl)-5-(4-methoxyphenylamino)-1,3,4-thiadiazole (52j)



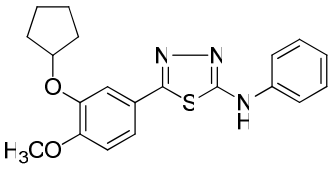
Yield: 81%

M.p.: 191-193 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.64 (s, 1H), 7.48 (d, *J* = 8.80 Hz, 2H), 7.03 (s, 2H), 6.92 (d, *J* = 8.80 Hz, 2H), 3.91(s, 6H), 3.85 (s, 3H), 3.81 (s, 3H)

MS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{19}N_3O_4S$: 374.1175; found: 374.5354

2-(3-Cyclopentyloxy-4-methoxyphenyl)-5-(phenylamino)-1,3,4-thiadiazole (52k)



Yield: 55%

M.p.: 223-225 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.8 (s, 1H), 7.53-7.47 (m, 4H), 7.36-7.31 (m, 2H), 6.98 (d, *J* = 8 Hz, 1H) 6.78 (d, *J* = 8 Hz, 1H), 4.35-4.34 (m, 1H), 3.81 (s, 3H), 1.70-1.50 (m, 8H).

MS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{22}N_3O_2S$: 368.1433; found: 368.7004

6.5.2 Pharmacology- *in vivo* Anticonvulsant Screening

The neuropharmacological studies were conducted on Swiss albino mice (20-30g). The animals were housed six mice per cage at constant temperature under a 12 h light/dark cycle, with food and water *ad libitum.* All experiments and procedures described herein were reviewed and approved by the Institutional Animal Ethics Committee. The various neuropharmacological tests performed were as follows:

***In vivo* screening protocol**

Test solutions of all compounds were prepared in 0.5% w/v methyl cellulose (scPIC tests) and 30% v/v PEG 400, and the animals were dosed 30 min prior to testing. All the compounds were administered intraperitoneally in a volume of 0.01 mL/g body weight of mice at doses 50, or 100 mg/kg (n = 4-6).

Subcutaneous picrotoxin-induced seizure threshold test (scPIC):

The test compounds were evaluated for their ability to antagonize scPIC-induced convulsions in mice after i.p. administration. After 30 min of drug administration a convulsive dose of picrotoxin (3.15 mg/kg) was injected subcutaneously in a volume of 0.01 mL/g body weight into each of the mice. The mice were placed in isolated cages and observed for the next 45 min for the presence or absence of threshold convulsions. Absence of a threshold convulsion was taken as the end point, which indicates that the test substance has the ability to elevate the picrotoxin seizure threshold.

6.6 References

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CHAPTER - VII

A One-pot Expeditious Synthesis of 5-(2′-Indolyl)thiazoles

The work described in this chapter reveals a facile and expeditious one-pot synthesis of 5-(2′-indolyl)thiazoles, analogues oxazolyl indole alkaloids. The reaction of thioamides with 3-tosyloxypentane-2,4-dione led to *in situ* formation of 5-acetylthiazole which upon treatment with arylhydrazines in polyphosphoric acid results in exclusive formation of 5-(2′-indolyl)thiazoles. A library of 5-(2′-indolyl)thiazoles was synthesised by using various thioamides and arylhydrazines in moderate yields. The protocol is appreciable in terms of its exploratory power, use of readily available starting materials and simple experimentation.

Chapter 7

7.1 Introduction

Indole alkaloids from plants and marine origin have been widely documented for their chemistry and biology. Many of these alkaloids have received attention due to their structural novelty and biological significance. An "azole" is a class of five-membered nitrogen containing heterocycles ring with atleast one other non carbon atom such as nitrogen, sulfur or oxygen. The 5-(2′-Indolyl)thiazoles **2** possess an indole ring at C-5 position of thiazole ring as in 5-(3′-indolyl)azoles **1** but connected at the second position of indole unlike third position in natural derivatives (Figure 7.1).

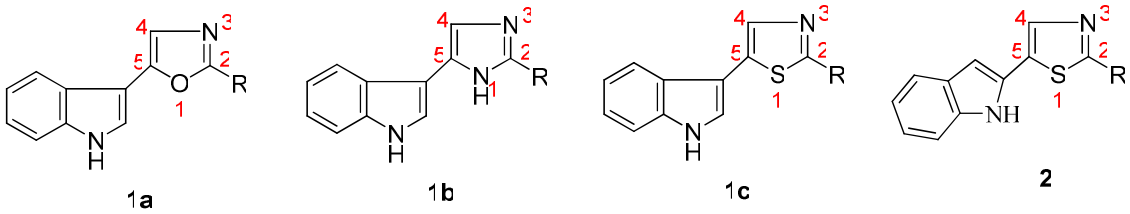


Figure 7.1 5-(3′-Indolyl)azoles **1** and 5-(2′-Indolyl)thiazoles **2**

Thiazole derivatives are the one of the revered compounds with many applications and biological activities attributed to them. Indolyl thiazoles and its biosteres are found in many natural products. Camalexin **3**, which was produced as phytoalexins in the leaves of *Camelina sativa* (Cruciferae) in response to infection by the fungus *Alternaria brassicae*, was elucidated to be (3′-indolyl)-2-thiazoles.[1-2] The naturally occurring BE 10988 (**4**), an inhibitor of topoisomerase, was shown to be a thiazole-substituted indolequinone (Figure 7.2).[3] Several syntheses of analogues of these two natural products were introduced, from a Grignard reaction of substituted indoles and 2-bromothiazole,[4-5] with a Hantzsch reaction as the key step,[6-8] or by photocyclisation of substituted (indol-1-yl)thioureas.[9] The thiazole and benzothiazole derivatives of indole have been found to exhibit antimicrobial[4-5] and cytotoxic activities.[6-8]

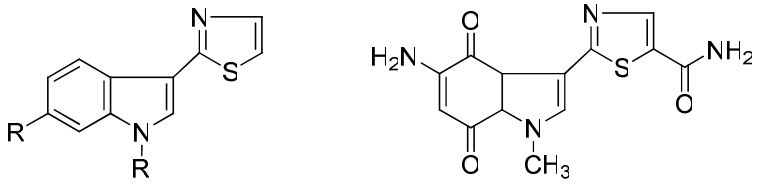


Figure 7.2 Naturally occurring indolyl heterocycles (**3** and **4**)

Biologically active alkaloids, 3-substituted indoles structurally represent a major class of marine invertebrates. These alkaloids include compounds either with the true indole nucleus or those derived from it such as dihydroindole, pseudoindoxyl, and oxoindole. The first indole alkaloid, 3-indolyl-imidazol-4-one (**5**) (Figure 7.3) with an imidazolinone ring in the third position was isolated from the tunicate *dendrodoa grossularia*.[10]

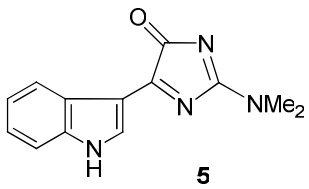


Figure 7.3 Structure of first indole alkaloid **5**

7.1.1 Natural Products: Indolylazole Scaffold

Many natural products,[11-12] synthetic biologically active compounds[13] and molecular sensors[14] contain oxazole heterocycle. The Pimprinine family[15] comprises of 2,5-disubstituted oxazole moiety at third position of the indole ring as shown in figure 7.6. This family includes Pimprinine (**6**), 2-methyl-5-(3′-indolyl)oxazole isolated from *Streptomyces pimprina,*[16] the homologous 2-ethyl-5-(3′-indolyl)oxazole (**7**), Pimprinethine isolated from *Streptomyces cinnamoneus*,[17] *n*-propyl and *n*-butyl analogues WS-30581 A (**8**) and B (**9**), respectively were isolated from *Streptoverticillium waksmanii*[18]*,* 2-benzyl analogue, Pimprinaphine (**10**) isolated from *Streptoverticillium olivoreticuli.*[19] Exploration of cancer cell growth inhibitory constituents of *Pseudomonas syringae* pv. *Coronafaciens* led to the isolation of 2-isobutyl-5-(3′-indolyl)oxazole, (Labradorin 1) (**11**) and 2-*n*-pentyl-5-(3′-indolyl)oxazole (Labradorin 2) (**12**). Camalexin (**3**), an 2-(3′-indolyl)thiazole analogue is the characteristic phytoalexin of *Arabidopsis thaliana* which is induced by a great variety of plant pathogens (Figure 7.4).[20]

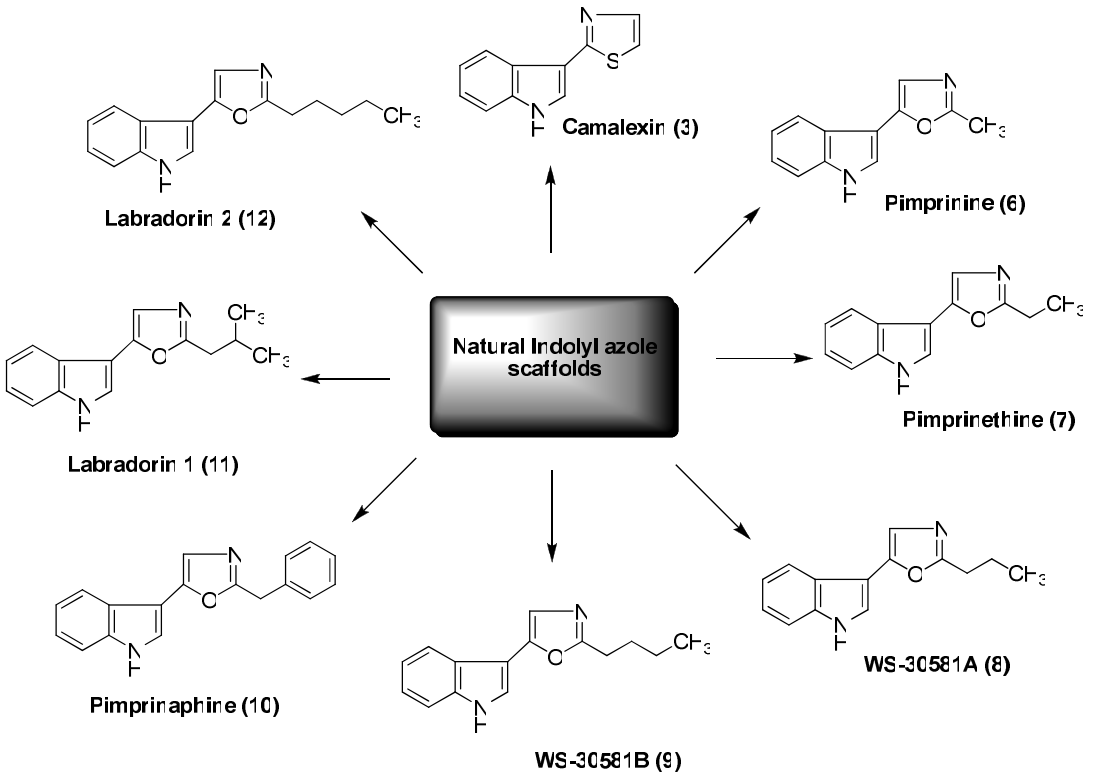


Figure 7.4 Naturally occurring simple 5-(3′-indolyl)azoles

Examples of some complex oxazolyl indole alkaloids (Figure 7.5) include Martefragin A (**13**), an indolyl-peptide substituted on an oxazole moiety which was isolated from red algae, *Martensia fragillis.* It shows an inhibitory activity against lipid peroxidation.[15] The Almazole family is found mostly in red seaweed and only Almazole C (**14**) shows antibacterial activity against gram-negative bacteria. Almazole A-D have a 2,5-disubstituted oxazole ring inserted between indole and peptide *N,N*-dimethyl-L-phenyl alaninamide moieties.[21-22]

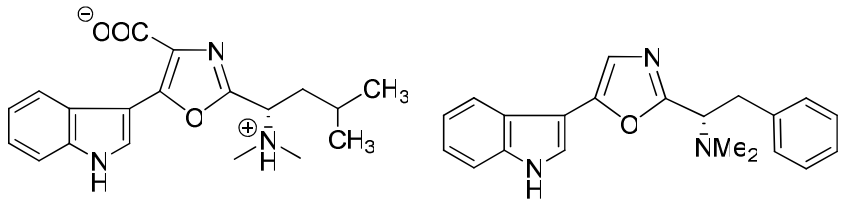


Figure 7.5 Complex oxazolyl indole alkaloids **13** and **14**

The bis(indolyl)alkaloids possess a heterocyclic ring at third position of indole for example an imidazole ring, in anti-inflammatory alkaloid Nortopsentin A-D (**15**)[23-24]

isolated from marine sponge *Topsentina genitrix*,[25-26] or piperazine in Dragmacidin (**16**)[27-28] (Figure 7.6) or pyrimidine ring in Merdians A-E (**18**)[29-30] or an oxazole ring in Martefragin (**13**).[31] Nortopsentin **15** and Topsentin (**17**) represent a class of deep-sea sponge metabolites and exhibit potent biological activities such as antitumor,[32] antiviral,[33] and antiinflammatory.[34]

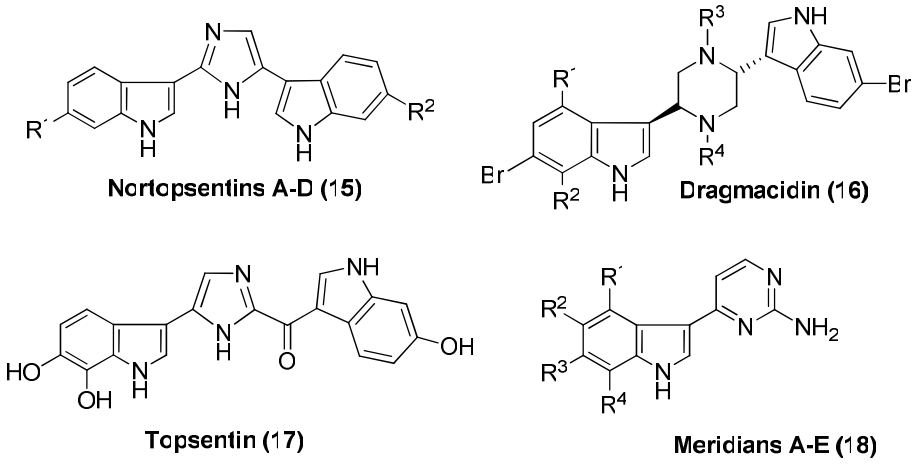


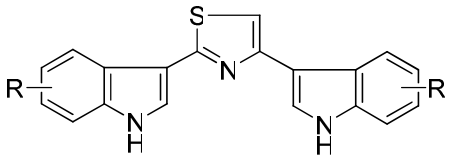
Figure 7.6 Bis(indolyl) alkaloids **15-17** and Meridians **18**

7.1.2 Biological Significance of Indolylazoles

Pimprinine (**6**) was first isolated by Bhate *et al*.[16] and chemically synthesised by Joshi *et al*.[35] It exhibited antiepileptic[36] and monoamine oxidase inhibitory activities.[37] Recently, the strain *Streptomyces* CDRIL-312 produced Pimprinine extracellularly[38] showed promising anticonvulsant activity in both minimum and maximum electric seizure threshold test in mice. Its anticonvulsant activity was very much comparable to that of phenyl hydantion sodium. WS-30581 A (**8**) and WS-30581 B (**9**)**,** were reported to have potent inhibitory effects on platelet aggregation.[19,39] Labradorin 1 (**11**) afforded GI_{50} values of 9.8 and 6.2 µg/mL against the human cancer cell lines NCI-H460 (lung-NSC) and BXPC-3, respectively. However, Labradorin 2 (**12**) afforded GI_{50} value of 9.6 µg/mL against both the human cancer cell lines NCI-H460 (lung-NSC) and BXPC-3.[40]

Martefragin A (**13**) is a strong inhibitor of lipid peroxidation than α-tocopherol. Diazonamide A, a halogenated cyclic peptide isolated from the colonial ascidian *Diazona chinensis* exhibits *in vitro* cytotoxicity against human tumour cell lines HCT-116 human colon carcinoma and B-16 murine melanoma cancer cell lines $IC_{50} < 15$ ng/mL.

Novel indolylthiazoles **19–28,** analogues (Figure 7.7) of Nortopsentins were synthesised and evaluated for cytotoxicity in the NCI's *in vitro* disease-oriented antitumor screen against approximately a panel of 60 human tumor cell lines derived from leukemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. All compounds **19–28** exhibited cytotoxic activities against a variety of human cancer cell lines. The compound **19** selectively exhibited *in vitro* cytotoxicities against leukemia and ovarian cancer cell lines, affording GI_{50} of 3.27 μM in K562, 5.31 μM in Molt-4 and 8.14 μM in IGROV1 assay. In the other human tumor cell line assay, the GI_{50} of compound **19** exceeded 100 μM. To test the possibility that substitutes in the indole ring might result in a potency increase, most of the 2,4- bis(3-substituted-indolyl)thiazoles showed broad effects on tumor cell lines from the leukemia, colon cancer, CNS cancer and breast cancer panels while unsubstituted counterpart **19** brought out highly selective activity against leukemia cell lines and IGROV1 ovarian cancer cell lines. The position of bromine in the indole ring plays an important role in cytotoxicity. Dibrominated compound **26** effectively inhibited MCF 4 of breast cancer, affording the GI_{50} of 0.888 μM.[41]



19 R = R = H
20 R = H R = 6-Br
21 R = 6-Br R = H
22 R = R = 6-Br
23 R = H R = 5-Br

24 R = R = 5-Br
25 R = 6-Br R = 6-OMe
26 R = 6-Br R = 5-Br
27 R = 6-OMe R = 6-Br
28 R = 5-Br R = H

Figure 7.7 Indolylthiazoles **19-28**

The indolylthiazole **29** with a pyridyl substituent has inhibited the cancer cell lines promiscuously. The GI_{50} values against leukemia HL-60, CNS cancer SF-295 and renal cancer RXF 393 are 1.96, 2.57 and 2.31 μM, respectively (Figure 7.8).[41]

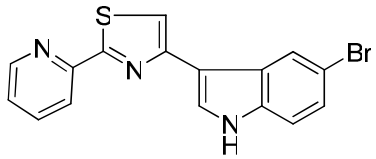


Figure 7.8 An antitumor indolylthiazole **29**

A team of researchers from Italy have synthesised a set of bis(indolyl) derivatives and evaluated their antitumor activity. The bis(indolyl)thiophene **30** proved valuable with potent antitumor activity across a variety of cancer lines (Figure 7.9) and selectivity towards leukemia cell line CCRF-CEM with a GI_{50} of 0.34 μM.[42]

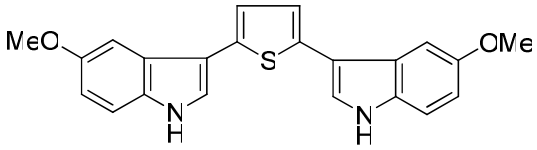


Figure 7.9 Bis(indolyl)thiophene **30**

Our research group prepared a series of 4-(3′-indolyl)oxazoles and studied their cytotoxicity against various cancer cell lines.[43] Of the synthesised 4-(3′-indolyl)oxazoles, compounds **31** and **32** were found to be cytotoxic against cancer cell lines (Figure 7.10). In addition, compound **32** was highly selective against MCF7 and PaCa2 cell lines with IC_{50} values 14.1 and 26 μM, respectively. The structure–activity relationship study showed that *N*-benzylation of indole nitrogen and *p*-fluorophenyl at C-2 position of oxazole ring are important for the activity and selectivity of 4-(3′-indolyl)oxazoles.

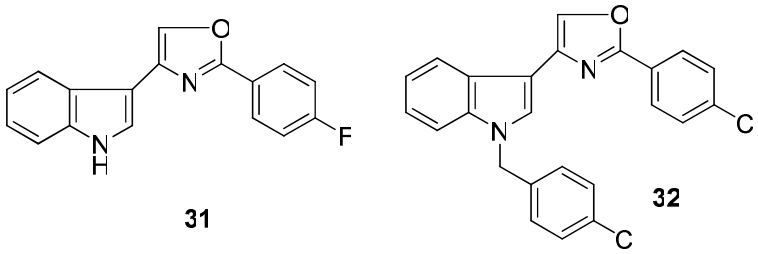


Figure 7.10 Potent and selective anticancer 4-(3′-indolyl)oxazoles **31** and **32**

A team of researchers at Warner-Lambert Company synthesised substituted thiazoles and oxazoles that modulate PPAR activity.[44] They have screened the compounds against HepG2 cells which were transfected with hPPARα, hPPARβ and mPPARγ chimeric receptors. Of a wide array of compounds synthesised and screened for activity, most of them showed good scope of therapeutic applicability for both PPARα and PPARβ. The compound **33** showed an EC_{50} of 572 nM in PPARα and 1.7 nM in PPARβ. Surprisingly, the isosteric indolylthiazole **34** showed no activity (Figure 7.11).

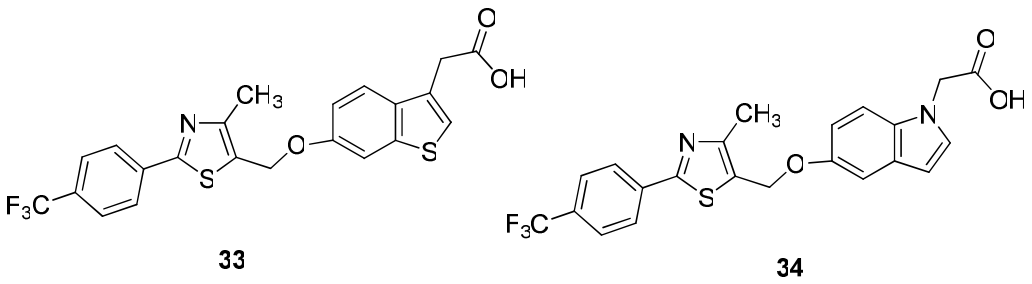


Figure 7.11 Thiazole derivatives **33** and **34** with PPAR activity

Pfizer Inc., US reported 5-heteroyl indole derivatives for treating migraine and other disorders.[45] The synthesis of a plethora of compounds with diverse substitutions was described. The indolyl thiazoles **35** were also indicated as therapeutic probables for migraine (Figure 7.12).

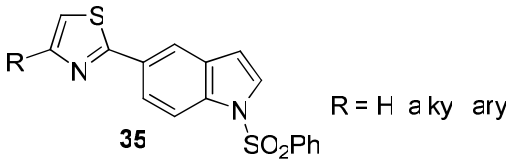


Figure 7.12 Indolylthiazoles **35** for CNS disorders

Masaaki and coworkers[46] at Fujisawa pharmaceutical company have developed thiazolylbenzofuran derivatives **36** that possess leukotriene and SRS-A antagonist or inhibitor activity (Figure 7.13). The synthesis of these particular derivatives was carried out by the reaction of substituted thioamides with 2-substituted benzofuran derivatives.

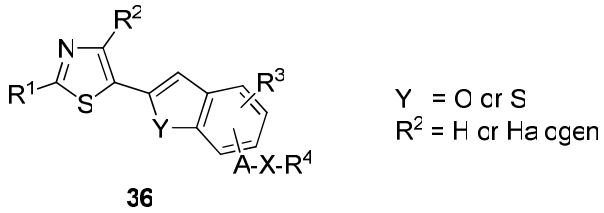


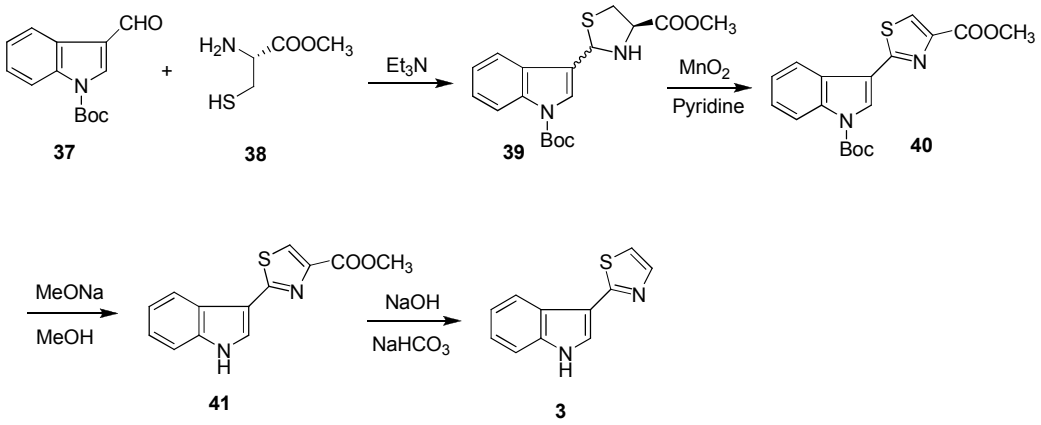
Figure 7.13 The SRS-A inhibitor thiazolylbenzofurans **36**

7.1.3 Synthetic Routes for Indolylazoles

In general, majority of azoles were prepared from precursors such as α-halo ketones,[47] α-diazoketones[48-49] or α-azido ketones[50] in a multi-step synthetic efforts. Synthesis of 5-(3′-indolyl)oxazoles is based on the cyclodehydration of acylaminoketone using harsh reagents such as H_2SO_4, PCl_5, P_2O_5, $SOCl_2$, Ac_2O and Ph_3P.[51] These natural products are also known to be synthesised via classical Robinson-Gabriel method,

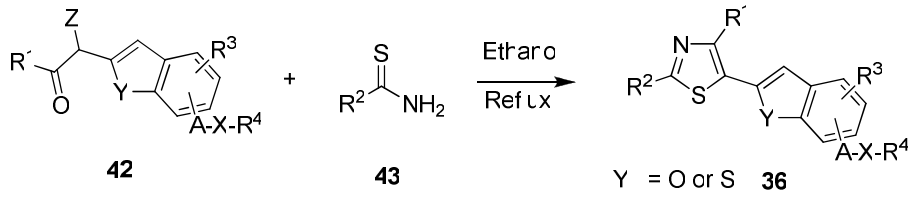
wherein 3-aminoacetylindole is acylated and the resulting ketoamide is cyclodehydrated to indolyloxazole.[52] The cyclisation reagents such as Burgess reagent $POCl_3$/pyridine or Wipf hexachloroethane/triphenylphosphine have also been employed to access the oxazole ring system.[52] Some general routes for the synthesis of indolylazoles are given below:

Dzurilla and coworkers[53] described a new method for the synthesis of Camalexin **3** based on the reaction of 1-(*tert*-butoxycarbonyl)indole-3-carboxaldehyde **37** with methyl L-cysteinate hydrochloride **38**, followed by oxidation to give **40** and decarboxylation to afford **3** (Scheme 7.1).



Scheme 7.1 Synthesis of Camalexin **3**

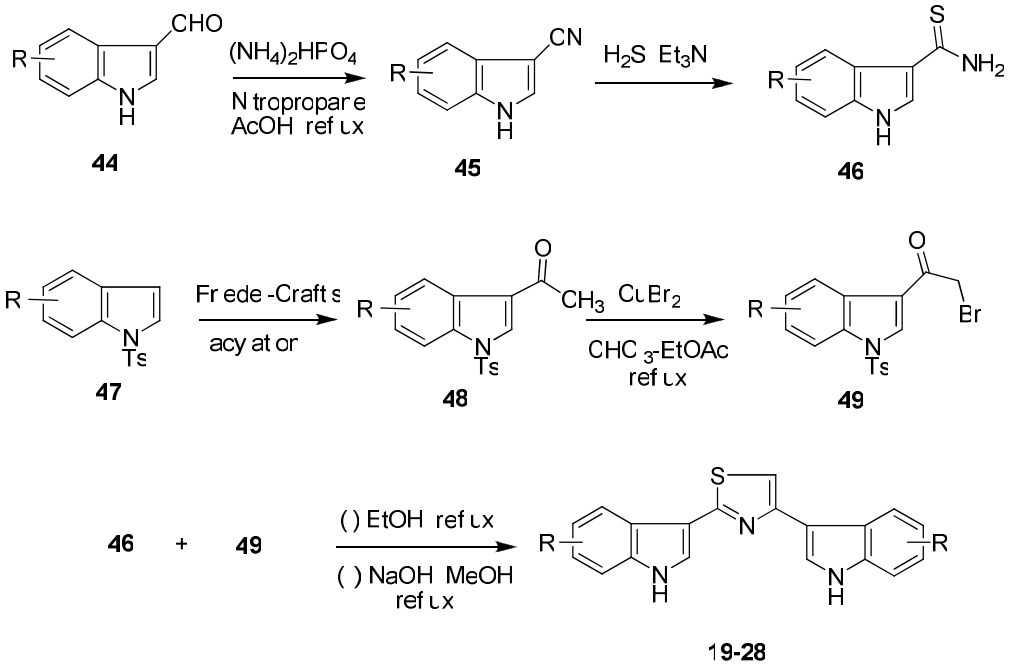
The synthesis of benzofuran/benzothiophene thiazole derivatives **36** was described by Masaaki et al.[54] The thioamide **43** was reacted with 2-substituted benzofuran / benzothiophene derivatives **42** in methanol under reflux conditions (Scheme 7.2). The leaving group Z was described as an acid residue which could be a tosylate or any other relevant moiety.



Scheme 7.2 Synthesis of thiazolylbenzofurans **36**.

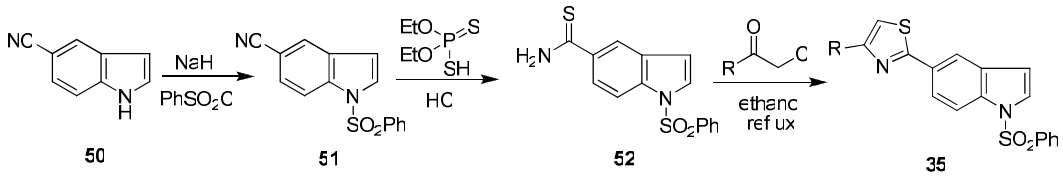
Jiang et al.[41] described a convergent procedure for the synthesis of bis(indolyl)thiazoles. The final step required the reaction of indol-3-thioamide **46** with α-bromo ketone **49** in

ethanol under refluxing conditions followed by deprotection of indoles nitrogen affording the products **19-28** in good yields (Scheme 7.3).



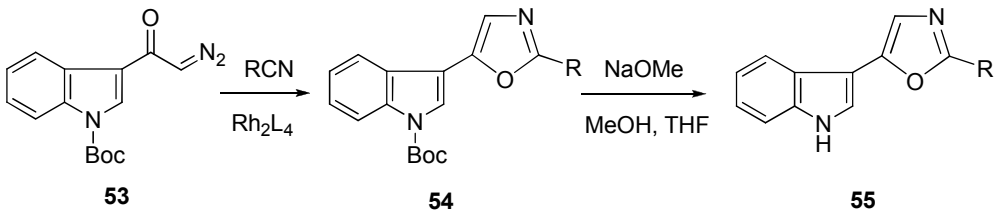
Scheme 7.3 Synthesis of bis(indolyl)thiazoles **19-28**

Nowakowski et al.[45] prepared indolylthiazoles **35** in a linear synthesis starting from 5-cyanoindole **50**. The *N*-protected 5-cyanoindole **51** prepared from **50**, was reacted with diethyldithiophosphate and gaseous HCl to afford *N*-protected indole-5-thioamide **52**. The indolylthiazoles **35** were obtained by reacting indole-5-thioamide **52** with α-chloro ketones in ethanol under reflux conditions (Scheme 7.4).



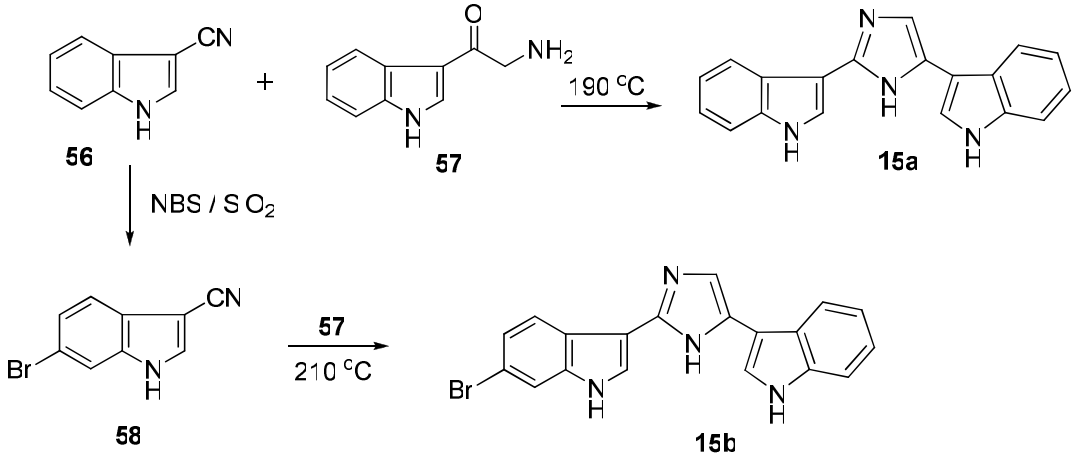
Scheme 7.4 Synthesis of indolylthiazoles **35**

Rhodium (II) catalyzed reaction of *N*-Boc-3-diazoacetylindole **53** with the corresponding nitrile led to Boc-protected oxazolyl indole alkaloids **54**. Subsequent deprotection of **54** led to 5-(3′-indolyl)oxazoles **55** in good yields (Scheme 7.5).[49]



Scheme 7.5 Synthesis of oxazolyl indole alkaloids **55**

A concise synthesis of two bis(indole)alkaloids Nortopsentin D (**15a)** and Nortopsentin B (**15b**) was described from α-aminoketone **57** as a key intermediate. Condensation of **57** with 3-cyanoindole **56** produced Nortopsentin D (**15a)** (Scheme 7.6). Bromination of **56** using N-bromosuccinimide (NBS) afforded bromocyanoindole **58** which on condensation with **57** produced Nortopsentin B (**15b**).[55]



Scheme 7.6 Synthesis of nortopsentins B (**15a**) and Nortopsentin D (**15b**)

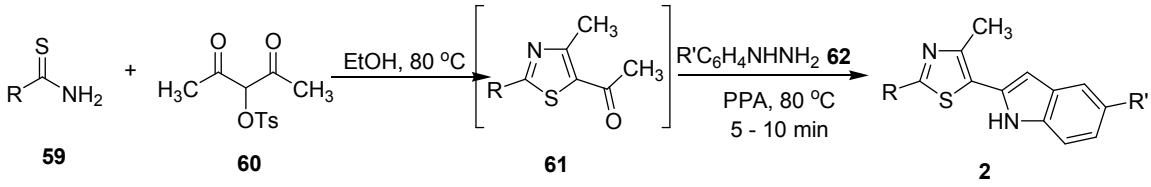
7.2 The Present Study

Bioisosterism is a strategy of medicinal chemistry for the rational design of new drugs, applied with a lead compound for molecular modification.[56] The correct use of this strategy allocates the identification of new classes of lead compound with attractive pharmacotherapeutic activity, simplifying the synthetic route and consequently maximizing the chances for success in discovering medications. In drug design, the purpose of exchanging one bioisostere for another is to enhance the desired biological or physical properties of a compound without making significant changes in chemical structure. There are innumerous reasons like the necessity to improve pharmacological activity, gain selectivity for a determined receptor or enzymatic isoform subtype with simultaneous reduction of certain adverse effects.

Instigated by the role of indolylthiazoles and its bioisoters in variety of therapeutic activities and the paucity of one-pot protocols for their preparation, we have contrived the synthesis of 5-(2'-indolyl)-2-substituted thiazoles involving reaction of substituted thioamides, 3-tosyloxypentane-2,4-dione and arylhydrazines. The thiazole heterocycle in **2** is connected to the second position of indole ring unlike the third position in naturally occurring indole alkaloids that may lead to potent anticancer agent.

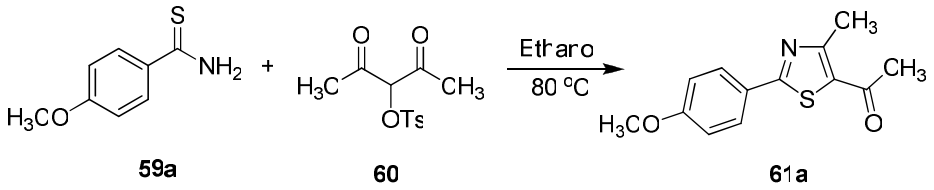
7.3 Results and Discussion

The one-pot synthesis of 5-(2′-indolyl)thiazoles **2** was carried out by *in situ* generating 5-acetylthiazole **61** from the reaction of thioamide **59** with 3-tosyloxypentane-2,4-dione **60** in ethanol and subsequent treatment of **61** with arylhydrazines **62** in PPA (Scheme 7.7).



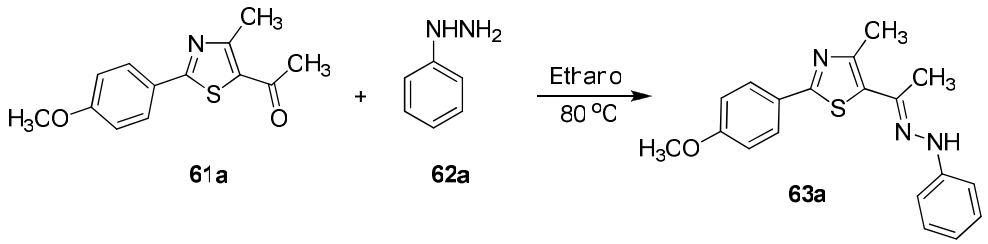
Scheme 7.7 One-pot synthesis of 5-(2′-indolyl)thiazoles **2**

The preparation of indolylthiazoles **2** was initiated with a trial reaction of 4-methoxybenzothioamide **59a** with 3-tosyloxypentane-2,4-dione **60** in ethanol. The reaction mixture was refluxed for 4 hours in ethanol without any catalyst to afford 5-acetylthiazole **61a** in 90% yield.



Scheme 7.8 Synthesis of 5-acetylthiazole **61a**

Upon confirming the intermediacy of the 5-acetylthiazole **61a** by comparing its melting point with literature report,[57] it was further reacted with equimolar quantity of phenylhydrazine (**62a**) in ethanol at 80 °C, to produce hydrazone **63a**.



Scheme 7.9 Synthesis of thiazolylhydrazone **63a**

The solid hydrazone **63a** thus obtained was subjected to Fischer indole cyclisation under different acidic conditions as mentioned in the Table 7.1. In most of the cases either reaction failed to initiate or led to a complex mixture with traces of desired product.

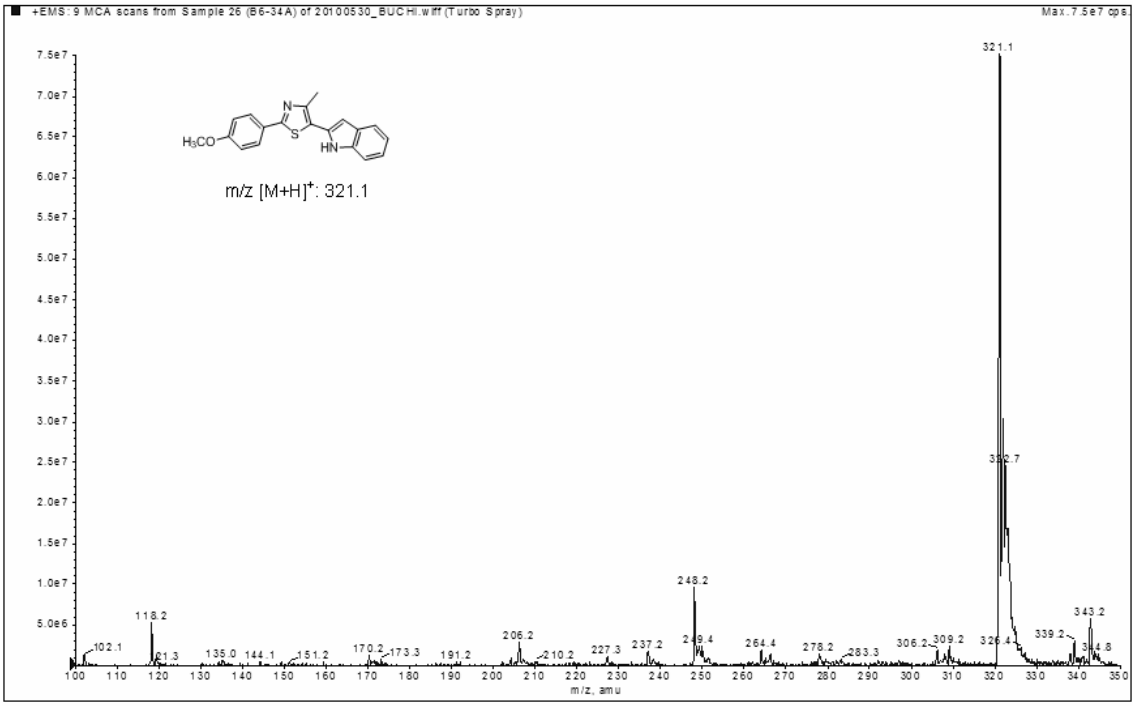
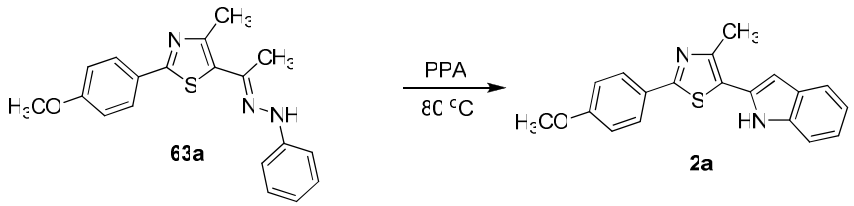


Figure 7.14 Mass spectrum of 5-(2′-indolyl)thiazole **2a**

Finally, the hydrazone **63a** was heated in polyphosphoric acid (PPA) at 80 ^{o}C for 15 min to obtain the desired 5-(2′-indolyl)thiazole **2a** in 30% yield (Scheme 7.10). The structure of **2a** was confirmed by its ^{1}H NMR and Mass spectral data (Figures 7.14 & 7.15). The characteristic singlet of indolyl C3-H at δ 6.68 in ^{1}H NMR confirmed the formation of indole ring and the C4 methyl of thiazole exhibited a singlet at δ 2.67. The mass spectrum of **2a** confirmed the molecular ion peak $[M+H]^{+}$ at *m/z* 321.1 which is in agreement with the calculated value.



Scheme 7.10 Fischer indole cyclisation of thiazolylhydrazone **63**

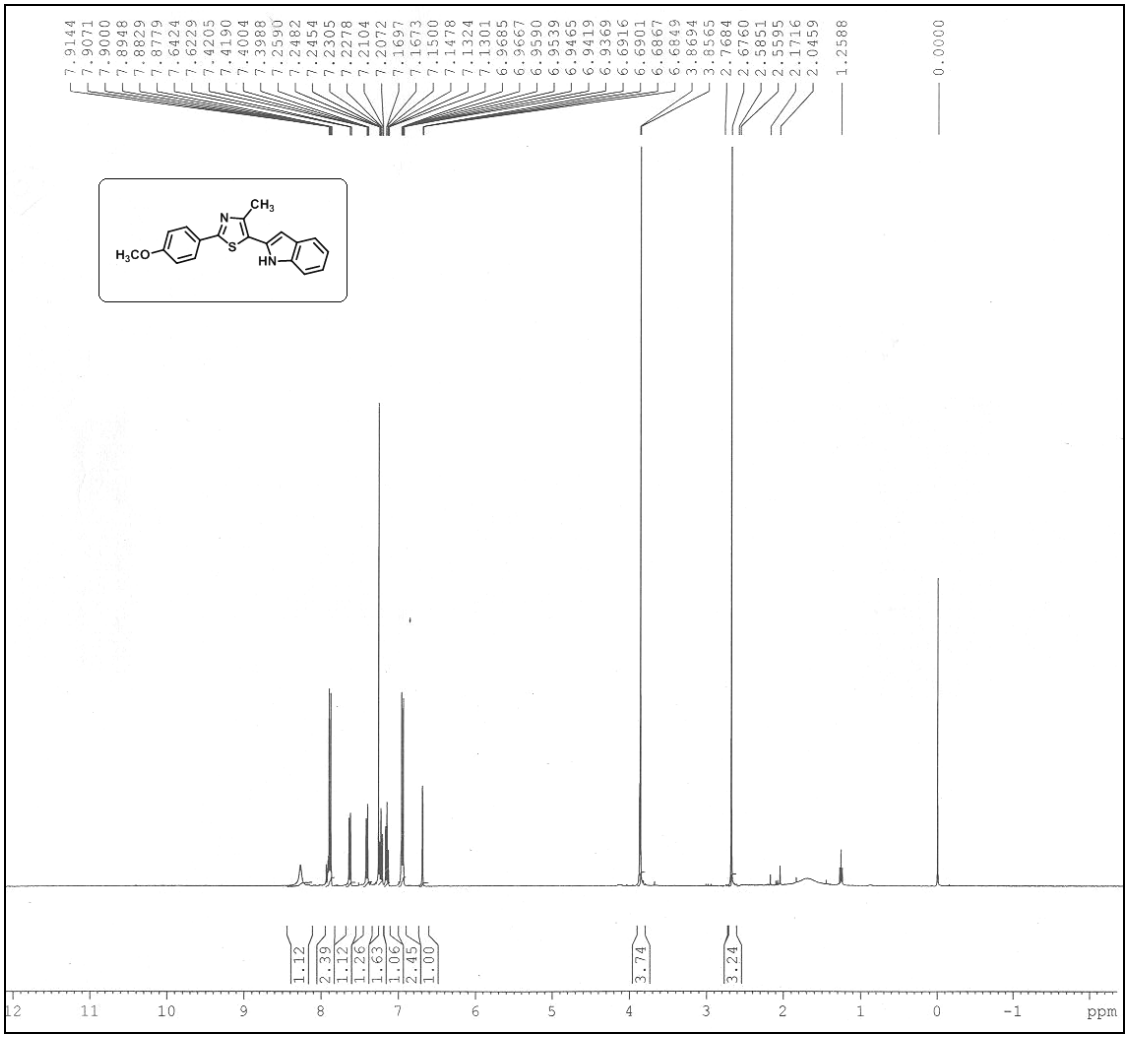


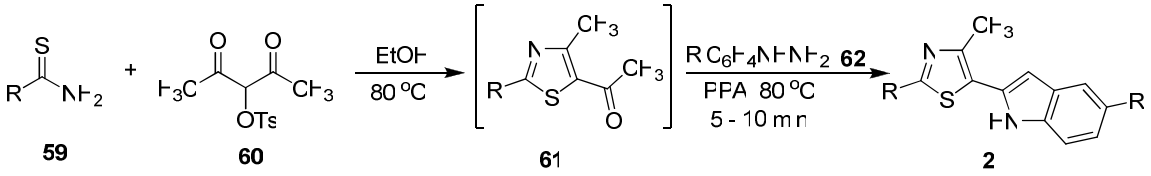
Figure 7.15 ^{1}H NMR spectrum of 5-(2′-indolyl)thiazole **2a**

The reaction was further simplified by the direct reaction of phenylhydrazine **62a** with 5-acetylthiazole **61a** in presence of PPA. Encouraged by the outcome, a one-pot trial was attempted by reacting the equimolar quantities of 4-methoxybenzothioamide **59a** with 3-tosyloxypentane-2,4-dione **60** in ethanol at 80 ^{o}C. Upon completion of the reaction, ethanol was removed and the residue was treated with phenylhydrazine **62a** and 2 drops of PPA to afford indolylthiazole **2a**.

Table 7.1 Fischer-indole cyclisation of hydrazone **63**

S.No.	Reagent	Condition	Solvent	Yield (%)
1	HCl	Reflux	Ethanol	-
2	H_3PO_4 (6M)	Heating (80 ^oC)	-	-
3	H_3PO_4 (18M)	Heating (80 ^oC)	-	-
4	TsOH	Microwave	Neat	-
5	TsOH	Reflux	Ethanol	-
6	TsOH	Reflux	Acetonitrile	-
7	TsOH	Grinding	Neat	-
8	Conc. H_2SO_4	Heating (80 ^oC)	-	-
9	PPA	Heating (r.t.)	-	-
10	PPA	Heating (80 ^oC)	-	30
11	PPA	Reflux (110 ^oC)	Toluene	-
12	$ZnCl_2$	Grinding	Neat	-
13	$ZnCl_2$	Microwave	Neat	-
14	$ZnCl_2$	Reflux	Ethanol	-
15	AcOH	Heating (116 ^oC)	-	-
16	PTA	Reflux (80 ^oC)	Ethanol	-
17	PTA	Grinding	-	-
18	HCOOH	Heating (80 ^oC)	-	-

Further, indolylthiazole **2a** was rapidly obtained in better yield when three equivalents of phenylhydrazine **62a** was allowed to react with 5-acetylthiazole **61a**. In all the cases Fischer indole cyclisation required about 5 min to produce indolylthiazole **2**.

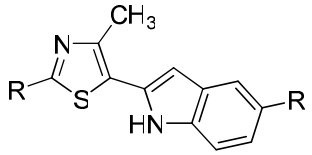


Scheme 7.11 One-pot synthesis of indolylthiazoles **2**

The developed protocol was extended to prepare various 5-(2′-indolyl)thiazoles **2b-k** using thioamides **59** and arylhydrazines **60** in moderate yields. The 4-methoxythiobenzamide, 3,4,5-trimethoxythiobenzamide and 4-chlorothiobenzamide exhibited almost similar reactivity towards 3-tosyloxypentane-2,4-dione. However,

thioacetamide and indolyl thioamide were relatively more reactive affording the products in relatively higher yields.

Table 7.2 Preparation of 5-(2′-indolyl)thiazoles **2**



2

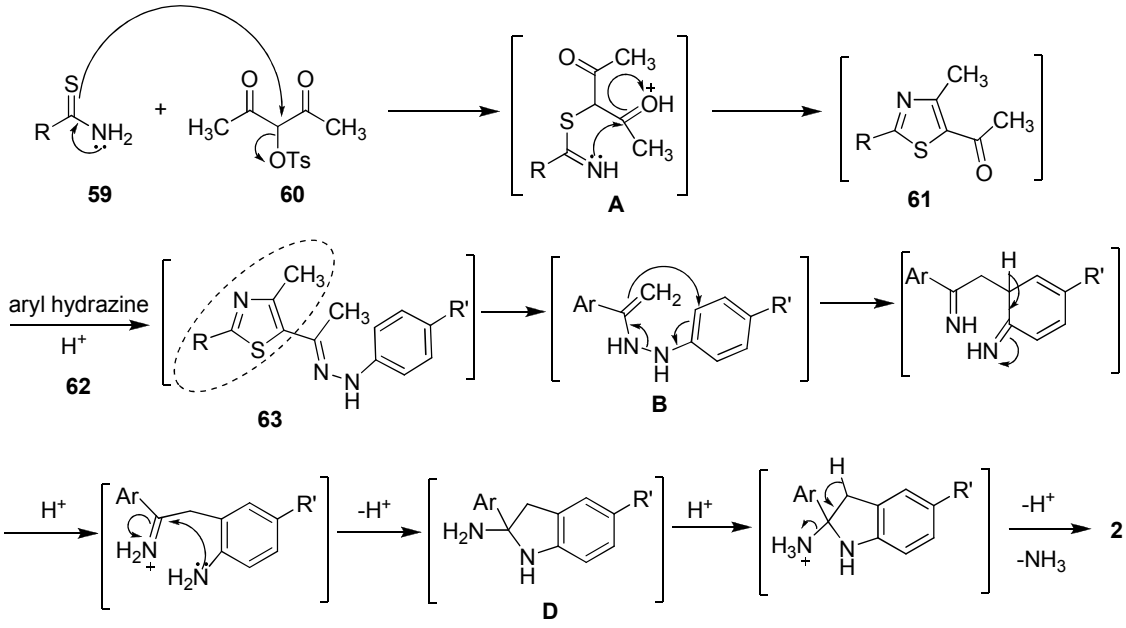
Compd.	R	R′	Yield (%)
2a	4-OMeC_6H_4	H	45
2b	4-OMeC_6H_4	Cl	48
2c	4-ClC_6H_4	H	55
2d	H	Cl	62
2e	Me	H	65
2f	Indol-3-yl	H	60
2g	3,4,5-$(OMe)_3C_6H_2$	H	55
2h	3,4,5-$(OMe)_3C_6H_2$	Cl	52
2i	3,4,5-$(OMe)_3C_6H_2$	F	55
2j	3,4,5-$(OMe)_3C_6H_2$	Br	58
2k	3,4,5-$(OMe)_3C_6H_2$	OMe	50

The thioacetamide and phenylhydrazine reacted to afford **2e** in 65% yield, whereas reaction involving indole-3-thioamide and phenylhydrazine afforded **2f** in 60% yield. The yields of the compounds **2g - 2k** were in the range of 50–60% demonstrating that substituents have little effect on the final products. The reaction of 4-methoxythiobenzamide with phenylhydrazine and 4-chlorophenylhydrazine yielded **2a** and **2b** in 45 and 48% yields, respectively. Arylhydrazines **62** bearing electronegative atom (4-Cl, 4-Br and 4-F) were equally reactive with 5-acetylthiazole **61** whereas arylhydrazines **62** bearing electron-donating group (4-OMe) was relatively more reactive to undergo Fischer indole cyclisation. The yields of the 5-(2′-indolyl)thiazoles **2** were in the range of 45 – 65%.

7.3.1 Plausible Mechanism

Formation of the 5-(2′-indolyl)thiazoles **2** involves the nucleophilic displacement of tosyloxy group of **60** by thioamide **59** to generate species **A** which undergoes cyclisation

with loss of water molecule to afford acetylthiazole **61**. The reaction of **61** with arylhydrazines **62** results in hydrazones **63** which undergoes a [3,3]-sigmatropic rearrangement as depicted in Scheme 7.12 to produce 5-(2′-indolyl)thiazoles **2**.



Scheme 7.12 Plausible mechanistic pathway for the formation of indolylthiazoles **2**

7.4 Conclusions

The synthesis of prominent 5-(2′-indolyl)thiazoles was achieved in one-pot using a sequential reaction of thioamides, 3-tosyloxypentane-2,4-dione and arylhydrazines. All the 5-(2′-indolyl)thiazoles were obtained in moderate yields. Overall, the protocol is appreciable in terms of the high exploratory power, short duration and ease of availability of the starting materials. The generality of this protocol is demonstrated by preparing a diverse library of 5-(2′-indolyl)thiazoles from various thioamides and arylhydrazines. The similitude of the 5-(2′-indolyl)thiazoles to naturally occuring indole-based alkaloids envisage their biological importance.

7.5 Experimental Details

All the reagents were purchased from aldrich and spectrochem chemicals. The reaction was monitored by thin layer chromatography, which was performed on Merck precoated plates (silica gel. 60 F_{254}, 0.25 mm) and was visualised by fluorescence quenching under UV light (254 nm). Column chromatography, if performed, was using 100-200 mesh silica gel and appropriate mixture of hexane-ethyl acetate for elution. The solvents were

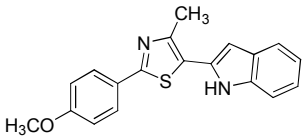
evaporated on Buchi rotary evaporator. Melting points (m.p.) were determined with electrothermal capillary melting point apparatus. ^{1}H spectra were recorded on a Bruker Advance II (400 MHz) spectrometer. The coupling constant (*J*) values are in Hz. LC–MS analysis was carried out on an ABI 2000 Q-Trap mass spectrometer fitted with electrospray ionization (ESI).

7.5.1 General Procedure for the One-pot Synthesis of 5-(2′-Indolyl)thiazoles 2

A mixture of thioamide (2.0 mmol) and 3-tosyloxypentane-2,4-dione (2.0 mmol) was heated at 80 ^{o}C in ethanol for 7 hours. Upon consumption of the thioamide, as indicated by TLC, the solvent was evaporated on rotary evaporator. Substituted phenylhydrazine (6 mmol) and 2 drops of PPA were added to the reaction mixture and heated at 80 ^{o}C for 5–10 min. The reaction mixture is then diluted with water and the desired indolylthiazole was extracted with ethyl acetate. The combined organic phase was washed, dried and evaporated to afford the crude 5-(2′-Indolyl)thiazole which was purified by passing through silica gel column using ethyl acetate-hexane as eluent.

Analytical data of the synthesised 5-(2′-indolyl)thiazoles:

5-(1*H*-Indol-2-yl)-2-(4-methoxyphenyl)-4-methylthiazole (2a)



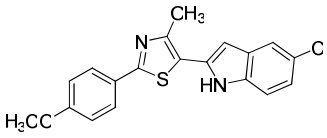
Yield: 45%

M.p.: 182-185 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (s, br, 1H), 7.91 – 7.78 (m, 2H), 7.63 (d, *J* = 7.80 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.23 (dt, *J* = 7.08 and 1.12 Hz, 1H), 7.15 (dt, *J* = 7.88 and 0.92 Hz, 1H), 6.97 – 6.94 (m, 2H), 6.69-6.68 (m, 1H), 3.86 (s, 3H), 2.67 (s, 3H).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{19}H_{16}N_2OS$: 321.1; found: 321.1

5-(5-Chloro-1*H*-indol-2-yl)-2-(4-methoxyphenyl)-4-methylthiazole (2b)



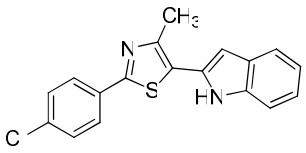
Yield: 48%

M.p.: 197-198 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.05 (s, 1H), 7.86 (d, *J* = 8.76 Hz, 2H), 7.51 (s, 1H), 7.37 (d, *J* = 8.52 Hz, 1H), 7.07 (dd, *J* = 8.48 & 1.84 Hz, 1H), 6.97 (d, *J* = 8.80 Hz, 2H), 6.56 (s, 1H), 3.86 (s, 3H), 2.65 (s, 3H).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{19}H_{15}ClN_2OS$: 355.1; found: 355.1

2-(4-Chlorophenyl)-5-(1*H*-indol-2-yl)-4-methylthiazole (2c)



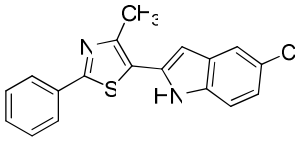
Yield: 55%

M.p.: 172-175 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (s, br, 1H), 7.98 – 7.92 (m, 2H), 7.64 (d, *J* = 7.84 Hz, 1H), 7.46 – 7.42 (m, 3H), 7.17 (dt, *J* = 7.08 and 1.12 Hz, 1H), 7.15 (dt, *J* = 8.04 and 1.04 Hz, 1H), 6.73 (s, 1H), 2.74 (s, 3H).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{13}ClN_2S$: 325.1; found: 325.2

5-(5-Chloro-1*H*-indol-2-yl)-4-methyl-2-phenylthiazole (2d)



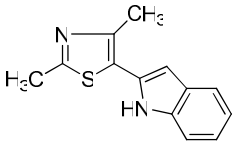
Yield: 62%

M.p.: 154-156 °C.

^{1}H NMR (400 MHz, DMSO-d_6): 11.08 (s, 1H),7.73-7.70 (m, 2H), 7.58-7.56 (m, 1H), 7.43-7.51 (m, 3H), 7.38-7.36 (m, 1H), 7.09-7.06 (m, 1H), 6.58 (s, 1H), 2.65 (s, 3H).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{13}ClN_2S$: 325.1; found: 325.2

5-(1*H*-Indol-2-yl)-2,4-dimethylthiazole (2e)



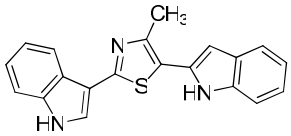
Yield: 65%

M.p.: 238 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (s, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.42 (dd, *J* = 8.1, 0.8 Hz, 1H), 7.17 – 7.11 (m, 1H), 7.04 (ddd, *J* = 7.9, 7.1, 1.0 Hz, 1H), 6.65 – 6.62 (m, 1H), 2.70 (s, 3H), 2.53 (s, 3H).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{13}H_{12}N_2S$: 229.1; found: 229.2

5-(1*H*-Indol-2-yl)-2-(1*H*-indol-3-yl)-4-methylthiazole (2f)



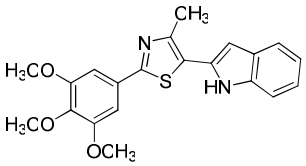
Yield: 60%

M.p.: 146-149 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (s, 1H), 8.33 (s, 1H), 8.01 (s, 1H), 7.92 (s, 1H), 7.55 (s, 1H), 7.45 – 7.38 (m, 4H), 7.24 – 7.21 (m, 3H), 2.17 (s, 3H).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{15}N_3S$: 330.1; found: 330.2

5-(1*H*-Indol-2-yl)-4-methyl-2-(3,4,5-trimethoxyphenyl)thiazole (2g)



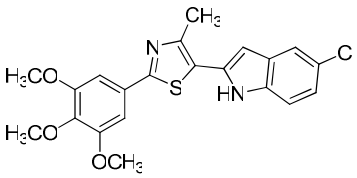
Yield: 55%

M.p.: 126-129 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.25 (s, 1H), 7.65 (d, *J* = 8.02 Hz, 1H), 7.38 (dd, *J* = 8.1, 0.8 Hz, 1H), 7.17 – 7.13 (m, 1H), 7.05-7.03 (m, 1H), 6.95 (s, 2H), 6.64 (s, 1H), 4.02 (s, 6H), 3.93 (s, 3H), 2.80 (s, 3H).

HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{21}H_{20}N_2O_3S$: 381.1; found: 381.1

5-(5-Chloro-1*H*-indol-2-yl)-4-methyl-2-(3,4,5-trimethoxyphenyl)thiazole (2h)



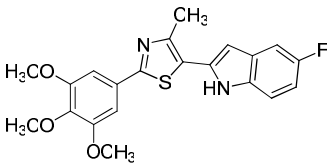
Yield: 52%

M.p.: 126-127 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.25 (s, 1H), 7.51 (s, 1H), 7.37 (d, J = 8.52 Hz, 1H), 7.12 (s, 2H), 7.07 (dd, J = 8.48 & 1.84 Hz, 1H), 6.56 (s, 1H), 4.00 (s, 6H), 3.95 (s, 3H), 2.84 (s, 3H).

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{19}ClN_2O_3S$: 415.1; found: 415.1

5-(5-Fluoro-1*H*-indol-2-yl)-4-methyl-2-(3,4,5-trimethoxyphenyl)thiazole (2i)



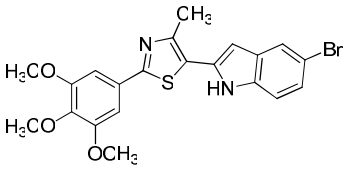
Yield: 55%

M.p.: 128-129 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (s, 1H), 7.62-7.61 (m, 1H), 7.48-7.51 (m, 1H), 6.90 (s, 2H), 7.11-7.09 (m, 1H), 6.59 (s, 1H), 4.02 (s, 6H), 3.97 (s, 3H), 2.87 (s, 3H).

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{19}FN_2O_3S$: 399.1; found: 399.1

5-(5-Bromo-1*H*-indol-2-yl)-4-methyl-2-(3,4,5-trimethoxyphenyl)thiazole (2j)



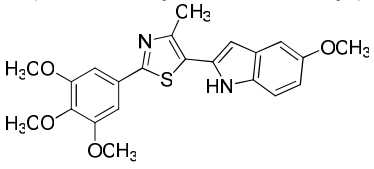
Yield: 58%

M.p.: 128-131 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.39 (s, 1H), 7.98-7.97 (m, 1H), 7.66-7.64 (m, 1H), 7.05 (s, 2H), 7.43-7.41 (m, 1H), 6.76 (s, 1H), 3.97 (s, 6H), 3.93 (s, 3H), 2.82 (s, 3H).

HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{19}BrN_2O_3S$: 458.0; found: 458.1

5-(5-Methoxy-1*H*-indol-2-yl)-4-methyl-2-(3,4,5-trimethoxyphenyl)thiazole (2k)



Yield: 50%

M.p.: 129-132 °C.

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.34 (s, 1H), 7.57-7.56 (m, 1H), 7.38-7.36 (m, 1H), 7.01 (s, 2H), 6.90-6.94 (m, 1H), 6.72 (s, 1H), 3.96 (s, 6H), 3.92 (s, 3H), 3.79 (s, 3H), 2.68 (s, 3H).

HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{22}H_{22}N_2O_4S$: 411.1; found: 411.2

7.6 References

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CHAPTER - VIII

CONCLUSIONS

Chapter 8

8.1 General Conclusions

MCRs have seen a great upsurge in the last decade due to the recognition of their potential in leveraging drug discovery. In the first half of the 20th century few MCRs were known to exist and their importance was feebly realized. It was after the discovery of an isocyanide based MCR by Ugi that truly changed the face of multicomponent chemistry. Following decades have seen a number of Ugi-MCR variants. More and more molecules of the heterocycle pool were explored with much less efforts using MCRs. New principles, classifications and strategies have evolved gradually setting the line for constant growth. The syntheses of drugs like Crixivan were achieved easily while the multi-step synthesis failed to give the products in large scale. The capacity of MCRs to generate enormous libraries, almost to the tune of 10^4, established their vitality in the current synthetic organic chemistry and drug discovery. MCRs could successfully meet the requirements of high throughput screening.

An important challenge in this growing field of MCR chemistry still has to be solved by finding conditions to create many stereoselective MCR products although a little has been achieved in this direction. Contemporary organic synthesis is faced with the challenge of developing simple methods for the rapid construction of complex, biologically potent compounds. The numbers of components that can go into one-pot are increasing with time. So far, eight component reactions are successfully reported kindling the hope for complete one-pot protocols in the near future with minimal waste generation. The high atom economic MCRs are highly preferable over linear multi-step synthesis.

The MCRs have interesting applications in drug discovery and in combating the environmental toxic waste remediation by dwindled wastes. The current thesis reflects the synthesis of various biologically interesting heterocycles such as 2-aminochromenes, 4*H*-pyrans, 1,2,3-triazoles, 1,3,4-thiadiazoles and indolylthiazoles under eco-friendly conditions. Heterogeneous catalysts and benign reaction solvents were employed successfully for the synthesis of biologically interesting heterocycles utilizing variety of reactions such as nucleophilic substitution, cycloaddition, condensation, and oxidative cyclisation. The synthesised heterocycles were evaluated for their biological activity.

8.2 Specific Conclusions

The first chapter gives a comprehensive overview of MCRs and vividly describes their importance in drug discovery and total synthesis of natural products. The application of MCRs in drug discovery is an extremely important tool in the arsenal of an organic chemist. It was shown how pharmacophores can be generated in MCRs leading to potential therapeutic agents. The strategies for innovating novel MCRs were described and the description on exploratory power gives an idea how the substitutions on the reacting components effects the size of the library.

The second chapter emphasizes the synthesis of biologically important 2-aminochromenes utilizing the eco-friendly heterogeneous catalyst, MgO. The nanosized MgO was successfully synthesised and employed in the one-pot reaction of aryl aldehyde, malononitrile and α-naphthol. The reactions were successfully carried out in aq. PEG at room temperature in short time affording high yields. The attractive features of this protocol are: simple experimentation procedure, use of benign reaction solvents, cost effectiveness, the recyclability of catalysts, and its adaptability for the synthesis of a diverse set of 2-aminochromenes.

The third chapter describes the solvent-free synthesis of 4*H*-pyrans from one-pot condensation of arylaldehyde, malononitrile and ethylacetoacetate/dimedone using a benign catalyst, magnesium oxide. The notable advantages of the protocol involve a simplified purification process, short reaction time, one-pot reaction and solvent-free conditions, use of readily available enolisable ketones and excellent yield of products. The antibacterial assay of some of the compounds showed complete inhibition of bacterial growth at 128 μg/mL or less and the rest of the compounds exhibited incomplete inhibition. The compounds with electron-rich substituents on the aryl ring of aldehyde imparted good antibacterial activity.

The fourth chapter of the thesis deals with the synthesis of an array of 1,2,3-triazole derivatives under Click conditions using α-halo ketones / α-tosyloxy ketones, sodium azide and terminal alkynes and studies on their Src kinase inhibitory activity. The protocol was applied to a wide range of α-tosyloxy/α-haloketones and acetylenic compounds which allowed to generate a diverse set of 1,4-disubstituted-1*H*-1,2,3-triazoles. The use of Cu(I) as a reusable catalyst in aqueous PEG makes this method facile, cost effective, and ecofriendly. The 1,2,3-triazoles exhibited moderate Src kinase

inhibitory activity. The structure-activity relationship studies revealed that the insertion of C_6H_5- and 4-$CH_3C_6H_4$- at first position with appropriate less bulkier group at fourth position of 1,2,3-triazoles was well tolerated for the modest Src inhibition activity of 1,2,3-triazoles. Molecular modeling studies provided further information for modification of 1,2,3-triazoles to generate a lead compound.

The chapter five describes the facile, efficient and regioselective one-pot synthesis of 1,4-diaryl-1*H*-1,2,3-triazoles in aqueous PEG. The multicomponent reaction of diaryliodonium salts, sodium azide and terminal alkynes catalysed by Cu(I) rapidly afforded the 1,2,3-triazoles in good yields. The studies on unsymmetrical diaryliodonium salts revealed that the attacking nucleophile prefers the sterically demanding ring or the ring with electron withdrawing substituents. This interesting three-component reaction also encompasses the recyclability aspect of the reaction media.

The sixth chapter reportes the synthesis and *in vivo* anticonvulsant evaluation of 2-aminoaryl-5-aryl-1,3,4-thiadiazoles. The one-pot synthesis was achieved by the reaction of arylaldehyde, hydrazine hydrate and arylisothiocyantes affording the 1,3,4-thiadiazoles in good yields. The anticonvulsant activity of the 1,3,4-thiadiazoles was moderate in comparison to standard drug, diazepam. The structure-activity relationship studies of the synthesised 1,3,4-thiadiazoles revealed that bulky substitutions on the phenyl ring at position-5 led to decreased activity and unsubstituted arylamino ring at position-2 is detrimental for the activity.

The chapter seven describes a novel one-pot synthesis of various indolylthiazoles from readily available thioamides, 3-tosyloxypentane-2,4-dione and arylhydrazines. All the indolylthiazoles were obtained in moderate yields, and the overall protocol was appreciable in terms of the high exploratory power, short duration and use of simple and radily available starting materials. Substituents on the arylthioamides and arylhydrazines had little effect on the course of reaction. The similitude of the 5-(2'-indolyl)thiazoles to naturally occurring 5-(3'-indolyl)thiazoles and other reported molecules envisages their biological importance such as anticancer activity.

8.3 Future Scope of the Research Work

The advent of multicomponent reactions has significantly changed the face of drug discovery. The study on the existing MCRs helps to understand and develop novel multicomponent reactions. There has not been any explicit focus on the stereoselective MCRs. Understanding the cascade of reactions in the one-pot multi-step synthesis helps to establish new routes. Further, the formation of new bonds and points of diversity per step can be increased.

The literature report of an efficient one-pot reaction of up to eight components was developed by the union of MCRs with the formation of nine new bonds and eleven points of diversity. The complexity and diversity of the products can be increased by developing more multiple-multicomponent reactions. Heterogenous catalysts are largely underutilized in MCRs which can be seen as one of the impeding factors for their harness.

The MCRs developed in the current thesis attempted to focus on the highlighted lacunae. Attempts were made to develop reactions under catalytic conditions, both heterogeneous and homogenous, using benign solvents for their efficient transformation. The modular and moderately complex heterocycles prepared in this thesis involve a maximum of three components reaction may be further explored to generate biologically interesting molecules.

The synthesis of 2-aminochromenes was achieved with aromatic aldehydes, malononitrile and α-naphthol. These reactions can be further explored with aliphatic aldehydes, α-cyanoactive methylene compounds and activated phenols which will further improve the scope and applicability of the protocol. The similar improvements can be applied to 4*H*-pyrans to further enlarge its chemical space. These molecules can be further derivatised to generate tacrine like analogs which have a great therapeutic potential.

The scope of the synthesised 1,2,3-triazoles is far and wide. They were evaluated for Src kinase inhibition and have exhibited modest activity which can be further improved by structural modifications. The synthesised 1,3,4-thiadiazoles have the dual potential for anticonvulsant and anticancer activity which might prove useful in conditions that require both activities. These molecules can be evaluated for their anticancer and anticonvulsant activities.

The biologically important 5-(2'-indolyl)thiazoles that were described in chapter 7 have the great potential to generate a lead molecule due to their similitude to naturally occurring indole-based heterocycles envisages many vital biological activities. The scope of the reaction can be further improved with modifications that can be imparted by employing various thioamides, hydrazines and isothiocyanates.

Appendices

List of Publications *[A-1]*

1. Dalip Kumar; V. Buchi Reddy; Anil Kumar; Deendayal Mandal; Rakesh Tiwari; Keykavous Parang "Click chemistry inspired one-pot synthesis of novel 1,2,3-triazoles and their Src kinase inhibitory activity" ***Bioorganic & Medicinal Chemistry Letters*** **(under revision)**

2. Dalip Kumar, V. Buchi Reddy "An Efficient One-Pot Regioselective Synthesis of 1,4-Diaryl-1,2,3-triazoles Using Click Chemistry" ***Synthesis,*** **2010,** 1687-1691.

3. Dalip Kumar; V. Buchi Reddy; Shaswat Sharad; Urvashi Dube; Suman Kapur "A facile one-pot green synthesis and antibacterial activity of 2-amino-4H-pyrans and 2-amino-5-oxo-5,6,7,8-tetrahydro-4H-chromenes" ***European Journal of Medicinal Chemistry*****, 2009,** 44, 3805–3809.

4. Dalip Kumar, V. Buchi Reddy, Rajender S. Varma, A facile and regioselective synthesis of 1,4-disubstituted 1,2,3-triazoles using click chemistry, **Tetrahedron Letters, 2009**, 50, 2065-2068.

5. Dalip Kumar; Gautam Patel; V. Buchi Reddy "Greener and Expeditious Synthesis of 1,4-Disubstituted 1,2,3-Triazoles from Terminal Acetylenes and in situ Generated α-Azido Ketones" ***Synlett*****, 2009**, 399-402.

6. Dalip Kumar; V. Buchi Reddy; Braj G. Mishra; R.K. Rana; Mallikarjuna N. Nadagouda; Rajender S. Varma "Nanosized magnesium oxide as catalyst for the rapid and green synthesis of substituted 2-amino-2-chromenes" ***Tetrahedron***, **2007**, 63, 3093-3097.

List of papers presented in conferences *[A-2]*

1. "A Facile One-Pot Synthesis of 1,2,3-Triazoles and their Src Kinase Inhibitory Activity" at **National Conference on Green and Sustainable Chemistry-2010** organized by Chemistry Group at Birla Institute of Technology and Science during February 19-21, 2010. **(Poster)**

2. "A One-Pot Facile and Expeditious Synthesis of 1,4-Diaryl-1,2,3-triazoles" at **14th International Conference on Chemical Biology for Discovery: Perspectives and Challenges (ISCBC-2010)** organized by the Indian Society of Chemists & Biologists at Central Drug Research Institute, Lucknow, India during January 15-18, 2010. **(Poster. Won the best poster award)**

3. "A Facile One-Pot Greener Synthesis and Antibacterial Activity of 2-Amino-4H-pyran Derivatives" at **The 4th International Conference on Multi-Component Reactions and Related Chemistry (MCR 2009)** at The Urals State Technical University – UPI, Ekaterinburg, Russia during May 24-28, 2009. **(Poster)**

4. "A Facile and Greener Synthesis of 1,4-Disubstituted 1,2,3-triazoles using Click Approach" at the **National Symposium on Green Chemistry: Applications in Science & Engineering (NSGC-2009)** organized by School of Chemistry & Biochemistry, Thapar University at Thapar University, Patiala, Punjab during February 5-6, 2009. **(Oral presentation)**

5. "A Facile and Regioselective Synthesis of 1,4-Disubstituted 1,2,3-Triazoles using Click Chemistry" at the **International Conference on the Interface of Chemistry-Biology in Biomedical Research** organized at Birla Institute of Technology and Science, Pilani during February 22-24, 2008. **(Poster)**

6. "Submicron size magnesium oxide catalysed one pot synthesis of substituted 2-amino-2-chromenes in aqueous media" at the **National Symposium on Challenges in Drug Discovery Research: Networking Opportunities between Academia and Industries** organized at Birla Institute of Technology and Science, Pilani during April 7-8, 2006. **(Poster)**

BRIEF BIOGRAPHY OF THE CANDIDATE *[A-3]*

Buchi Reddy Vaddula has pursued his Bachelor and Master of Pharmacy from Birla Institute of Technology and Science (BITS), Pilani, India in 2003 and 2005 respectively. He took a vertical transfer to M.Pharm. after B.Pharm. program. During his B.Pharm., he did a six-month stint at the R&D Division of Hetero Drugs Limited., Hyderabad. He was also involved in several on-campus lab oriented and study oriented research projects. In 2005, he registered for his Ph.D. at BITS, Pilani under the supervision of Dr. Dalip Kumar. During his doctoral work, he received Junior Research Fellowship (JRF) & Senior Research Fellowship (SRF) from Defence Research & Development Organization (DRDO) and Institute Fellowship from BITS, Pilani. He has published research articles in well renowned international journals and presented papers in conferences/symposium.

Dr. Dalip Kumar is an Assistant Professor & Group leader of Chemistry Group, Birla Institute of Technology and Science, Pilani. He received his Ph.D. degree from Kurukshetra University, Kurukshetra, Haryana in 1997. For his doctoral degree, he worked with Professor Shiv P. Singh in the research area of heterocyclic chemistry. After his doctorate, he worked as a post-doctoral fellow (1997-1999) with Prof. Rajendra S. Verma at Sam Houston State University, TX, USA. He was also associated with Prof. S. M. Kerwin as a postdoctoral fellow (1999-2000), College of Pharmacy, University of Texas at Austin, TX, USA. He joined BITS, Pilani as a lecturer in 2000-2002. Later, in 2002-2004 he moved to University of Maryland, College Park, MD, USA as a research Associate. He rejoined BITS, Pilani as an Assistant Professor, Chemistry Group in 2004 and since then he is continuing with the same. At present, he is also the group leader of chemistry group, BITS, Pilani. He has been involved in research for the last 13 years and in teaching for 8 years. As a result of his research accomplishment, he has around 60 publications in peer reviewed international journals. Currently Dr. Dalip Kumar is guiding five Ph. D. students. Additionally, to his credit he also has one US patent and presently handling two projects from DST and UGC.