Synthesis of Novel Heterocycles and their Pharmacological activity

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Antibiotics are one of our most important weapons to cover bacterial infections and improve human life . Infectious diseases are the leaders among the challenging drug targets because of the multi-drug resist antimicrobial pathogens and continuous rise in the emerging infections from known and unknown sources. Tyrosine kinases are orally active, small molecules that have a favorable safety profile and can be easily combined with other forms of chemotherapy or radiation therapy. The combination of two pharmacophores into a single molecule is an effective and commonly used direction in modern medicinal chemistry for the exploration of novel and highly active compounds. A variety of safe and effective antiinflammatory agents are available, including aspirin and other nonsteroidal anti-inflammatories, with many more drugs under development. The pharmacophoric structural features of the selective COX-2 inhibitors possess a central heterocyclic five member ring system bearing two vicinal aryl moieties, such as pyrazole (celecoxib), 2(5H) furanone (rofecoxib), and isoxazole (valdecoxib).

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Novel Heterocycles and its Activity

Mane , Pavase

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LAXMIKANT SURESH PAVASE

GENERAL REMARKS

1) All the raw materials were obtained commercially and used without further purification.

2) The Melting points of all the synthesized compounds were taken on Veego melting point apparatus model VMP-D and are uncorrected.

3) The progress of the reactions were monitored by Thin Layer Chromatography (TLC), on silica gel-precoated aluminium sheets (Type 60, F254, Merck, Germany) and the spots were detected by exposure to UV lamp at λ 254 nm for 20-30 seconds.

4) IR spectra were recorded on MIRacle10 shimadzu IR Affinity-1 instrument, and values were reported cm^{-1}.

5) ^1H NMR spectra were recorded using $CDCl_3$ and DMSO-d_6 as solvent with tetramethylsilane as an internal standard on Varian 400-MHz instruments and the chemical shift (δ) are reported in parts per million with coupling constants (J) values are reported in Hertz (Hz).

6) Elecronspray ionization-mass spectra were recorded on LC-MS/MS Waters (Aquity) TQ detector instrument.

7) Elemental analysis is reported from Vario Micro Elementar instrument.

LIST OF ABBRIVIATIONS

%	percent
±	Plus or minus
λ	Lambda
°C	Degree Celsius
µg/Kg	Microgram per kilogram
µg/L	Micrograms per litre
gm	grams
cm	Centimetre
hr	hour
mp	melting point
NMR	Nuclear Magnetic Resonance
J	Coupling constant
δ	Chemical shift
Hz	Hertz
s	singlet
d	doublet
t	triplet
m	multiplate
DMSO	Dimethylsulfoxide
DMF	N,N-Dimethylformamide
THF	Tetrahydrofuran
MeOH	Methanol
EtOH	Ethanol
NaH	Sodium hydride
Na_2SO_4	Sodium Sulfate
HCl	Hydrochloric Acid
$POCl_3$	Phosphorous oxychloride
$LiOH.H_2O$	Lithium hydroxide monohydrate
EDC.HCl	1-Ethyl-3-(3-dimethylaminoprppyl)carbodimide.HCl
HOBt	N-Hydroxybenzotriazole
Et_3N	Triethylamine
$NaCNBH_3$	Sodium cynoborohydride
$KHCO_3$	Potassium bicarbonate
NaOH	Sodium hydroxide
$SnCl_2$	tin (II) chloride
$NaNO_2$	sodium nitrate
TLC	Thin Layer Chromatography
TKI	Tyrosine kinase inhibitors
NSAID	Non steroidal anti-inflammatory drugs
IC_{50}	Half maximal inhibitory concentration
MIC	Minimum Inhibitory Concentration
SI	Selectivity index
COX	Cyclooxygenase
ANOVA	Analysis of Variance
SD	Standard deviation
SE	Standard error
AUC	Area under curve
IPGTT	Intraperitonial Glucose Tolerance Test

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Introduction to Therapeutic agents

1. Heterocycles in Medicinal Chemistry

Majority of the organic compounds are characterized by the fact that some atoms in their molecules are joined in rings containing at least one atom of an element other than carbon. The cyclic part of heterocyclic indicates that at least one ring structure is present in such a compound, while the prefix hetero refers to the noncarbon atoms, or heteroatoms, in the ring. In their general structure, heterocyclic compounds resemble cyclic organic compounds that incorporate only carbon atoms in the rings but the presence of the heteroatoms gives heterocyclic compounds physical and chemical properties that are often quite distinct from those of their all-carbon-ring analogs.

The most common heterocycles are those having five or six-membered rings and containing heteroatoms of nitrogen (N), oxygen (O), or sulfur (S). The best known of the simple heterocyclic compounds are pyridine, pyrrole, furan, and thiophene. A molecule of pyridine contains a ring of six atoms-five carbon atoms and one nitrogen atom. Pyrrole, furan, and thiophene molecules each contain five-membered rings, composed of four atoms of carbon and one atom of nitrogen, oxygen, or sulfur, respectively.

History of Heterocyclic Chemistry

The history of heterocyclic chemistry began in the 1800s, in step with the development of organic chemistry. Some noteworthy developments

1818: Brugnatelli isolates alloxan from uric acid.

1832: Dobereiner produces furfural (a furan) by treating starch with sulfuric acid

1834: Runge obtains pyrrole ("fiery oil") by dry distillation of bones

1906: Friedlander synthesizes indigo dye, allowing synthetic chemistry to displace a large agricultural industry

1936: Treibs isolates chlorophyl derivatives from crude oil, explaining the biological origin of petroleum.

1951: Chargaff's rules are described, highlighting the role of heterocyclic compounds (purines and pyrimidines) in the genetic code.

Heterocycles are common structural units in marketed drugs and in medicinal chemistry targets in the drug discovery process. Over 80% of top small molecule drugs by US retail sales in 2010 contain at least one heterocyclic fragment in their structures [1]. In fact, heterocyclic moieties are present in the structures of all top 10 brand name small molecule

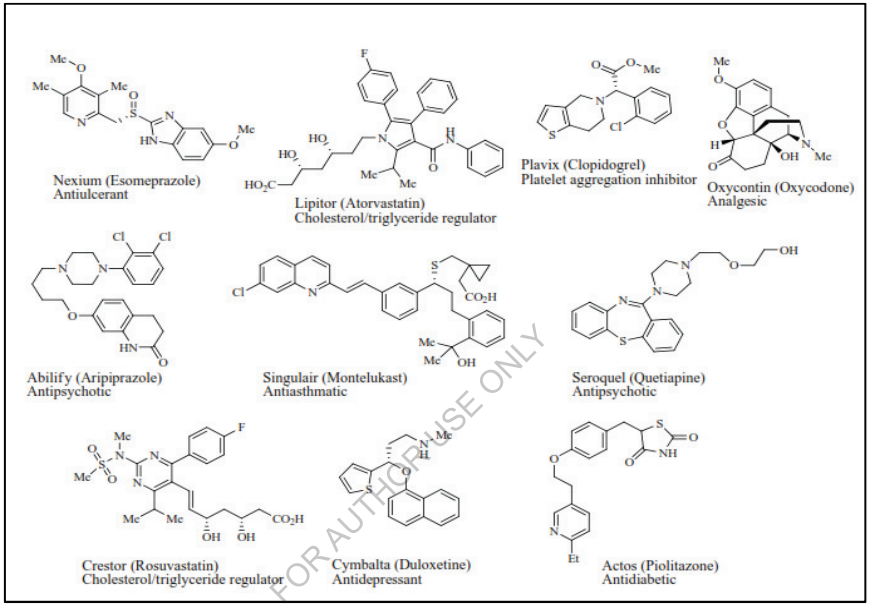

Fig.1: Brand names of small molecule drugs with heterocycles

drugs (**Fig. 1**). The one reason behind such high prevalence of oxygen, sulfur, and especially nitrogen-containing rings in drug molecules is obvious. The research process that leads to identification of an effective therapeutic treatment is largely based on mimicking nature by "fooling" it in a very subtle way. Because heterocycles are the core elements of a wide range of natural products such as nucleic acids, amino acids, carbohydrates, vitamins, and alkaloids, medicinal chemistry efforts often evolve around simulating such structural motifs. However, heterocycles play a much bigger role in the modern repertoire of medicinal chemists. Some of the drug properties that can be modulated by a strategic inclusion of heterocyclic moiety into the molecule include: 1) *potency and selectivity through bioisosteric replacements*, 2) *lipophilicity*, 3) *polarity*, and 4) *aqueous solubility*. Heterocycles are routinely used as bioisosteres for a variety of functional groups in drug candidates. While it is understood that particular bioisosteric replacement would not yield the desired pharmacological effect for

every SAR development, it is nonetheless common belief that heterocycles with a variety of shapes and electronic and physicochemical properties provide fertile grounds for optimization of drug candidates. The pharmacological benefits of employing heterocycles for better potency and specificity can in many cases be explained by their ability to participate in hydrogen bonding with the target protein, where the heterocycle can play the role of either H-acceptor as in heteroaromatic compounds or H-donor as in saturated N-heterocycles.

Hydrogen bonding (HB) is relevant not only for pharmacological properties, but also for physicochemical and transport properties of drug molecules. In their review, Laurence *et al.* [2] have convincingly explained the importance of HB basicity, i.e., HB acceptor ability, in drug molecule design. The strongest HB acceptors among the heterocycles are *N*-methylimidazole, *N*-Methylpyrazole, and pyridine, while the weakest HB acceptors include furan and thiophene. Steric and electronic effects influence the HB of heterocycles. It is also important to realize that HB basicity does not always correlate with proton basicity. Pyridine is much more basic than pyridazine; however, their HB acceptor properties are comparable.

Lipophilicity (estimated most of the time by ClogP, the calculated logarithm of the 1-octanol–waterpartition coefficient) is a key molecular descriptor influencing a variety of properties of drugs [3]. High lipophilicity is often associated with poor metabolic stability and toxicity: ClogP < 5 is part of the rule-of-five guidelines for druglikeness [4], and ClogP < 3 is part of the multiparameter optimization principle for successful CNS drugs [5]. With current average ClogP for marketed drugs at about 2.7, it is imperative to resist the temptation to make large and highly lipophilic compounds [6]. Heterocyclic fragments may drive the ClogP of the compound lower compared to its all-carbon counterparts, and therefore incorporation of heterocycles into the molecule can beadvantageous in that regard. For example, pyridine with ClogP 0.65, oxazole with ClogP -0.18, and others can be considered as candidates for replacing the benzene ring (ClogP 2.12) if reduced lipophilicity is desired. It should be mentioned that reduced lipophilicity does not always produce optimized compounds; for example, cellmembrane permeability can sometimes be negatively affected by such exercise. Other undesired effects may also occur. Thus, formation of reactive metabolites by oxidative bioactivation of thiazole (ClogP 0.49), another poorly lipophilic heterocycle, was uncovered during the development of nonpeptide trombopoeitin receptor agonists [7]. Heterocycles can be also used to increase lipophilicity when it is beneficial. For example, replacement of the carboxylic group by the 10 times more lipophilic tetrazole

fragment was a key strategy in the development of PTB1B inhibitors with improved cell permeability [8].

Polarity of molecule, described as polar surface area (PSA) or topological polar surface area (TPSA), is another molecular descriptor determining drug-likeness. For example, TPSA = 40–90 $A^{\circ 2}$ is considered to be an optimal range for CNS drugs [5]. Heterocycles along with heteroatom-bearing functional groups provide needed flexibility in modulating TPSA. While in most cases heterocycles are used to increase TPSA of less polar compounds, they can also decrease the polarity when used as replacement for more polar groups such as carboxylic acid.

Aqueous solubility is an important drug property determining its oral bioavailability. A number of aromatic and non-aromatic heterocycles exhibit significantly better aqueous solubility than their all-carbon analogs, which is explained by the possibility of hydrogen bonding. There are, however, significant solubility differences between heterocycles, e.g., isoxazole is soluble in water while thiophene has very poor solubility. Some differences are attributed to the hydrogen bond formation in the crystal structure, which leads to limited solubility. Thus, benzimidazole is less soluble in aqueous media than its very close analog benzoxazole.

In summary, heterocycles play a central role in the design of therapeutic molecules. They are utilized to optimize potency and selectivity through bioisosterism and pharmacokinetic and toxicological properties by offering wide opportunities to adjust lipophilicity, polarity, and solubility of the target molecules. It should also be noted that sometimes the desirable result of the heterocycle incorporation into the molecule comes at the expense of negative changes of other parameters of the drug. Recognition of the most important properties for the particular target and their careful manipulation to achieve optimal compromises between potency, selectivity, pharmacokinetic properties, and toxicity is the hardest part of the medicinal chemists' job.

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Section A

1.1. Tyrosine Kinase Inhibitors -New Class of Anticancer Agents

1.1.1. Cancer Research

Cancer is known medically as a malignant neoplasm. And is a broad group of various diseases all involving unregulated cell growth. In cancer cell divide uncontrollably forming malignant tumors and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or blood stream. There are over 200 different known cancers that afflict humans. Cancer can be detected in a number of ways including presence of certain signs and symptoms, screening test or medical imaging. Once a possible cancer is detected it is diagnosed by microscopic examination of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery. In 2007 cancer cause about 13% of all human deaths worldwide. Rates are rising as more people live to an old age and mass lifestyle changes occur in the developing world.

There are many different kinds of cancer. Cancer can develop in almost any organ or tissue, such as the lung, colon, breast, skin, bones, or nerve tissue. There are many causes of cancer, including: Benzene and other chemicals, Drinking excess alcohol, Environmental toxins, such as certain poisonous mushrooms and a type of poison that can grow on peanut plants (aflatoxins), Excessive sunlight exposure, Genetic problems, Obesity, Radiation, Viruses. However, the cause of many cancers remains unknown.

Cancer disease has major health issue on mankind around the globe. The relative rate of death by cancer is very high even in the developed countries. Surprisingly, among all malignant cancer, breast cancer is leading second position. Alcohol-related cirrhosis, and nonalcoholic fatty liver disease allied with obesity, is a major cause of liver cancer cases in US and another western countries.

Rapid and uncontrolled cell growth leads to malignant tumours and occupy the nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or blood stream. There are over 200 different known cancers that afflict humans. Cancer can develop in almost any organ or tissue, such as the lung, colon, breast, skin, bones, or nerve tissue. Cancer can be detected in a number of ways including presence of certain signs and symptoms, screening test or medical imaging. Once a possible cancer is detected, it is diagnosed by microscopic examination of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery.

Cancer is a fundamentally a disease of failure of regulation of tissue growth. In order of a normal cell to transform into a cancer cell, the gene which regulates cell growth and differentiation must be altered. The affected genes are divided into two broad categories. Oncogenes are genes which promote cell growth and reproduction. Tumor suppressor genes are genes which inhibit cell division and survival. Malignant transformation can occur through the formation of novel Oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in manygenes are required to transform a normal cell into a cancer cell. Genetic changes can occur at different levels and by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic DNA.

1.1.2. Tyrosine-kinase Inhibitor (TKI)

A tyrosine-kinase inhibitor (TKI) is a pharmaceutical drug that inhibits tyrosine kinases, enzymes responsible for the activation of signal transduction cascades (through phosphorylation of various proteins). TKIs are typically used as anti-cancer drugs. They are also called tyrphostins, the short name for "tyrosine phosphorylation inhibitor", originally coined in a 1988 publication, which was the first description of compounds inhibiting the catalytic activity of the epidermal growth factor receptor (EGFR). Tyrosine kinases are important mediators of the signaling cascade, determining key roles in diverse biological processes like growth, differentiation, metabolism and apoptosis in response to external and internal stimuli. Recent advances have implicated the role of tyrosine kinases in the pathophysiology of cancer. Though their activity is tightly regulated in normal cells, they may acquire transforming functions due to mutation(s), over expression and autocrine paracrine stimulation, leading to malignancy. Constitutive oncogenic activation in cancer cells can be blocked by selective tyrosine kinase inhibitors and thus considered as a promising approach for innovative genome based therapeutics. The modes of oncogenic activation and the different approaches for tyrosine kinase inhibition, like small molecule inhibitors, monoclonal antibodies, heat shock proteins, immunoconjugates, and antisense peptide drugs are reviewed in light of the important molecules. As angiogenesis is a major event in cancer growth and proliferation, tyrosine kinase inhibitors as a target for anti-angiogenesis can be aptly applied as a new mode of cancer therapy. The review concludes with a discussion on the application of modern techniques and knowledge of the kinome as means to gear up the tyrosine kinase drug discovery process.

1.1.3. Tyrosine kinase signalling pathways

Multicellular organisms live in a complex milieu where signaling pathways contribute to critical links, for their existence. Tyrosine kinases are important mediators of this signal transduction process, leading to cell proliferation, differentiation, migration, metabolism and programmed cell death. Tyrosine kinases are a family of enzymes, which catalyzes phosphorylation of select tyrosine residues in target proteins, using ATP (**Fig.2)**. This covalent post-translational modification is a pivotal component of normal cellular communication and maintenance of homeostasis [1, 2]. Tyrosine kinases are implicated in several steps of neoplastic development and progression. Tyrosine kinase signaling pathways normally prevent deregulated proliferation or contribute to sensitivity towards apoptotic stimuli. These signaling pathways are often genetically or epigenetically altered in cancer cells to impart a selection advantage to the cancer cells.

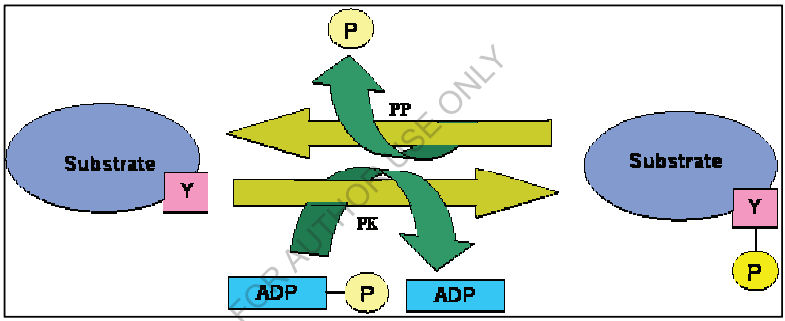

Fig.2: Schematic representation of the mode of action of tyrosine kinase
(*PK represents protein kinase and *PP stands for protein phosphatase)

Thus, it is no wonder that aberrant enhanced signaling emanating from tyrosine kinase endows these enzymes a dominating oncoprotein status, resulting in the malfunctioning of signaling network [3]. The discovery that SRC oncogene having a transforming non receptor tyrosine kinase activity [4], and the finding of EGFR, the first receptor tyrosine kinase paved the way to the understanding of the role and significance of tyrosine kinase in cancer [5]. With the deciphering of the Human Genome Project more than 90 tyrosine kinases have been found out. The more science entangles the intricacies of cellular signaling the more we find the involvement of tyrosine kinase in cellular signaling circuits that are implicated in cancer development. Tyrosine kinases represent a major portion of all oncoprotein that play a transforming role in a plethora of cancers.

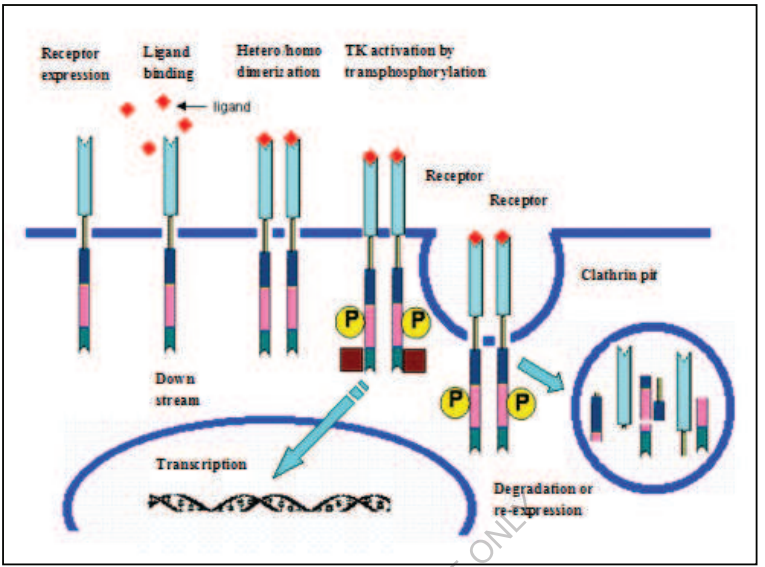

Fig.3: Mechanism of action of tyrosine kinase

1. Receptor expression at membrane claveola **2.** Ligand binding **3.** Hetero/homodimerization leading to tyrosine kinase activation and tyrosine transphosphorylation **4.** Signal transduction **5.** Receptor internalization **6.** Receptor degradation or re-expression.

Hence the identification and development of therapeutic agents for disease states that are linked to abnormal activation of tyrosine kinases due to enhanced expression, mutation or autocrine stimulation leading to abnormal downstream oncogenic signaling have taken a centre stage as a potent target for cancer therapy (**Fig.3**) [6,7].

Tyrosine kinases are primarily classified as receptor tyrosine kinase (RTK) e.g. EGFR, PDGFR, FGFR and the IR and non-receptor tyrosine kinase (NRTK) e.g. SRC, ABL, FAK and Janus kinase. The receptor tyrosine kinases are not only cell surface transmembrane receptors, but are also enzymes having kinase activity. The structural organization of the receptor tyrosine kinase exhibits a multidomain extracellular ligand for conveying ligand specificity, a single pass transmembrane hydrophobic helix and a cytoplasmic portion containing a tyrosine kinase domain. The kinase domain has regulatory sequence both on the N and C terminal end [2,8] .

NRTK are cytoplasmic proteins, exhibiting considerable structural variability. The NRTK have a kinase domain and often possess several additional signaling or protein-protein interacting domains such as SH2, SH3 and the PH domain [9]. The tyrosine kinase domain

spans approximately 300 residues and consists of an N terminal lobe comprising of a 5 stranded β sheet and one α helix, while the C terminal domain is a large cytoplasmic domain that is mainly α helical. ATP binds in the cleft in between the two lobes and the tyrosine containing sequence of the protein substrate interacts with the residues of the C terminal lobe. RTK are activated by ligand binding to the extracellular domain followed by dimerization of receptors, facilitating trans-phosphorylation in the cytoplasmic domain whereas the activation mechanism of NRTK is more complex, involving heterologous protein-protein interaction to enable transphosphorylation [10].

Tyrosine kinase forms a significant share of all oncoproteins thus they take centre stage as possible targets for cancer therapy. Hence low molecular weight tyrosine phosphorylation inhibitors (tyrphostins) have been proposed to be prospective anti-proliferating agents. By late 1980s it was proved that low molecular weight EGFR inhibitors could block EGF dependent cell proliferation [11]. Within a short time reports concerning effective tyrosine kinase inhibitors was the key promising development in anticancer development. With further research in the following years it was quite clear that neglected kinase targets against the biases have come into vogue [12].

1.1.4. Marketed Tyrosine kinase Inhibitors

Most of the tyrosine kinase inhibitors generated since have been ATP mimics. Interestingly many tyrphostins that possess one aromatic ring are substrate mimics [13] . These substrate mimics can be interestingly converted into ATP mimics once the nitrogen of the characteristic benzene malonitrile is incorporated into the second ring. Compounds that are found to be ATP mimics possess at least two aromatic rings. The ATP binding site though evolutionarily conserved can be selectively targeted by taking advantage of the minor difference in the kinase domain. The minor difference leads to changes in hydrogen bonding and hydrophobic interactions resulting in differences of affinity [14]. Of all the tyrosine kinase inhibitors (**Fig.4)** the most successful are Gleevec, Iressa and Tarceva. The novel anticancer drug Gleevec/ Glivec/ Imatinib mesylate (Novartis STI571) is a success for CML and c-kit positive metastatic GIST.

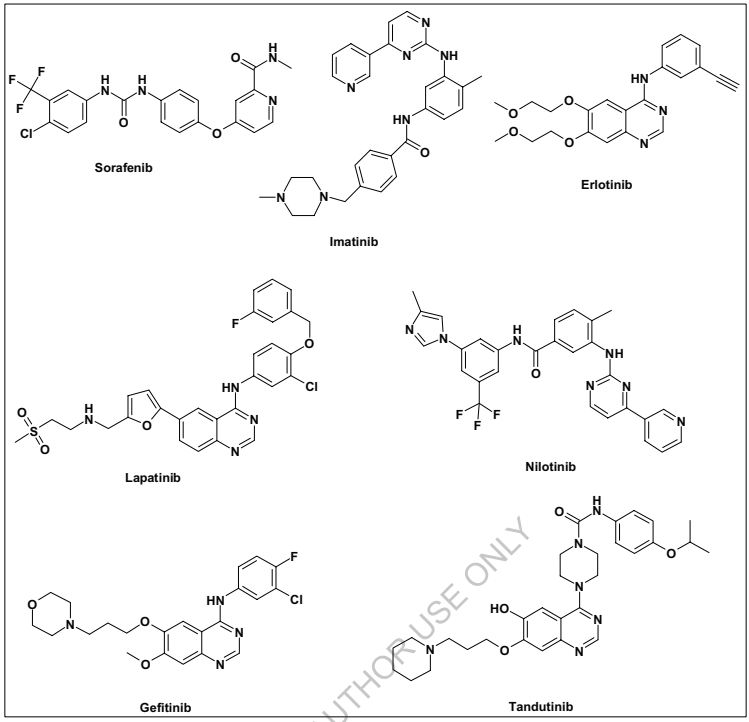

Fig.4: Examples of anticancer Tyrosine Kinase Inhibitors

Gleevec selectively and effectively inhibits the kinase activity of BCR-ABL fusion protein, which is responsible for the constitutive proliferative signaling. It also inhibits TEL-ABL and TEL-PDGFR fusion proteins. STI571 remains bound to the ATP binding cleft of the unphospholrylated (activation loop) Abl, thus establishing extensive contacts with residues lining the cleft and with peptide segments just outside the cleft. A large change in conformation of the nucleotide binding domain is accompanied with the binding of the drug. The binding of STI571 prevents ATP to access the ATP binding cleft and thus inhibits subsequent tyrosine phosphorylation of the substrate [12, 15]. Iressa is a selective inhibitor of EGF receptor tyrosine kinase in non small cell lung cancer and squamous cell carcinoma [16].

The role of tyrosine kinase in the control of cellular growth and differentiation is central to all organisms and has been found to participate in human neoplastic diseases. Tyrosine kinase inhibitors and their potential in clinical application are well documented by dramatic examples like, Gleevec, Iressa and Herceptin. Several tyrosine kinase inhibitors are

undergoing human trials and several are in the pipeline of drug discovery. The activities of these drugs are restricted to cancers with alterations in kinase targets, hence broad application of this treatment strategy are challenging. The quick selection of epidemiologically relevant, durable tyrosine kinase targets coupled to efficient lead finding and optimization needs more intervention in the area of high throughput cancer genome based molecular therapeutics. All these concerted effort may pave the silver lining to tailor made personalised cancer therapeutics.

1.1.5. Synthetic strategies for novel Tyrosine kinase Inhibitors

In our research project to develop newer anticancer molecules, we have adopted two different strategies using two different pharmacophores. First we used thieno [2, 3-d] pyrimidine nucleus to designed and synthesized newer amide and hydrazide molecules of target. Secondly, we designed the novel amide and hydrazide molecules combining the two pharmacophores such as thiazolidine-2,4-dione (2,4-TZD) and sulphonamides.

1.1.5.1. Design & synthesis of novel thieno [2, 3-d] pyrimidine

Tyrosine kinases are orally active, small molecules that have a favorable safety profile and can be easily combined with other forms of chemotherapy or radiation therapy. Several tyrosine kinase inhibitors (TKIs) such as *gefitinib* [17] **(Fig.4)** *erlotinib* [18] (**Fig.4)** and *lapatinib* [19] **(Fig.4)** *have* gained market approval worldwide. TKIs are thus an important new class of targeted therapy that interfere with specific cell signaling pathways and thus allow target specific therapy for selected malignancies. Pyrimidine and its fused ring system is present in Cytosine, adenine, guanine and thiamine, which form a part of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), vitamins and co-enzymes and other purines. A pyrimidine nucleus fused with another heterocycles is act as a core moiety in the discovery of bioactive molecules [20]. Hydrazide and their hetero-cyclic products show evidence of diverse biological activities including antibacterial [24], antifungicidal, analgesic, antituberculosis [21], anticancer [22], anti-inflammatory properties [23]. In addition the thieno [2, 3-d] pyrimidine core, which is evaluated as a bioisostere of the quinazoline core, was used in the mechanism- based design and synthesis of new antitumor agents [25]. A number of thieno [2, 3-d] pyrimidine derivatives with different substituent at C-2 and C-4 positions were found to exert potential antitumor activity [26-27]. It is observed from the literature that C-4 position must contain a hetero atom such as primary nitrogen [Gefitinib

(*Iressa*)] **(Fig.4)** or secondary nitrogen containing side chains on the quinazoline moiety [Tandutinib (*MLN518*)] **(Fig.4)** to have antitumor potency. From literature study it was observed that fusion of five, six or seven member cycloalkyl lipophilic moieties conserved the antitumor activity [26], [28-31] as exhibited by the 4-sustituted 5, 6, 7, 8-tetrahydrobenzo [4, 5] thieno [2, 3-d] pyrimidine. We have prepared a series of new thieno [2, 3-d] pyrimidines by introducing a five member cycloalkyl ring containing secondary nitrogen (L-proline) at C-4 position and Synthesized pharmacophoric moieties of interest such as amides and hydrazides that proved to contribute to antitumor activities.

We design the molecules after modifying the C-4 substitution by secondary nitrogen containing amino acid L- proline and used the substituted anilines like gefitinib, lapatinib and also substituted piperazine side chains to form terminal amides replacing urea. Hydrazide derivates were also prepared to compare its relationship with amide derivatives. We studied the structural modifications of thieno [2, 3-d] pyrimidine by aiming to obtained the new candidates of remarkable antitumor activities.

1.1.5.2. Design & synthesis of novel thiazolidine-2,4-dione

The combination of two pharmacophores into a single molecule is an effective and commonly used direction in modern medicinal chemistry for the exploration of novel and highly active compounds. The two pharmacophores are different in the case of heterobivalent ligands. Therefore, heterobivalent ligands containing pharmacophores that bind to different molecular targets or to two distinct sites on the same molecular target could be beneficial for the treatment of cancer [32]. The thiazolidine-2,4-dione (2,4-TZD) ring is a well-known scaffold in medicinal chemistry and has been used to develop new potential anticancer agents [33-35], such as the PI3Kα inhibitor GSK1059615 and its GSK2126458 analogues (**Fig.5)** [36]. Research into the antitumor efficacy of molecules which contain the 5-arylidene-thiazolidine-2,4-dione system has received significant attention over the last years [37-39]. A series of 5-acridin-9-ylmethylene-3-benzyl-thiazolidine- 2,4-dione analogues showed a moderate antiproliferative activity (IC_{50}: 4.1-58 μM) against a wide panel of cancer cell lines [40]. The α,β-unsaturated carbonyl system of the 5-benzylidene-thiazolidine-2,4-dione could act as a Michael acceptor, suggesting that the alkylation of the β-position of the reactive enone system by biological nucleophiles may be one mechanism by which antiproliferative activity was exerted *in vitro*.

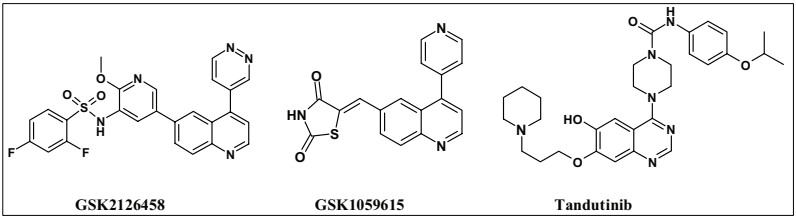

Fig.5. Examples of anticancer sulfonamide, thiazolidinedione and aniline compounds

Thiazolidin-4-one is a versatile scaffold for designing potential bioactive agents. Thiazolidin-4-one derivatives have been reported for broad spectrum of biological activities such as antioxidant [41], anticancer [42-44], anti-inflammatory [40,45], antimicrobial [46-47], anti-HIV [48-49], antiviral [50], anticonvulsant [51-52], and
antihypertensive [53] activities. Sulfonamide plays an essential role in biological activities [54]. Combination of these two mentioned scaffolds in one molecule according seems to be a promising 'hybrid pharmacophores' approach to new anticancer agents.

Since 1,3,4-thiadiazole and thiazolidin-4-one moieties are biologically proven anticancer and antioxidant pharmacophores and substitution in these scaffolds may further enhance their activity, prompted us to undertake this problem. Moreover, the combination of two pharmacophores in a single molecule is a well established hypothesis for synthesis of more active drugs with dual activity. Thus, a series of novel sulphonamide substituted thiazolidine-4-ones were synthesized and evaluated for their antioxidant and anticancer activity. In our earlier anticancer research [55] we have found that L-proline containing amide and hydrazide derivatives showed potential anticancer activities in MCF-7 and HCT-15 cell line when tested *in-vitro*. In continuation to this research here we have combine the two pharmacophores derived from the preclinical candidates of GSK, i.e. aryl sulphonamides and thiazolidinedione. Here we have discussed the structure activity relationship observed after coupling this combination with the L-proline amide and hydrazide analogs.

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Section B

1.2. Cyclooxygenase Inhibitors as anti-inflammatory agents

1.2.1. Inflammatory Research

Inflammation involving the innate and adaptive immune systems is a normal response to infection. However, when allowed to continue unchecked, inflammation may result in autoimmune or auto inflammatory disorders, neurodegenerative disease, or cancer. A variety of safe and effective antiinflammatory agents are available, including aspirin and other nonsteroidal anti-inflammatories, with many more drugs under development. In particular, the new era of anti-inflammatory agents includes ''biologicals'' such as anticytokine therapies and small molecules that block the activity of kinases. Other anti-inflammatories currently in use or under development include statins, histone deacetylase inhibitors, PPAR agonists, and small RNAs. Fast and effective relief of pain and inflammation in the human being is continued to be a major task for the medicinal chemist.

1.2.2. Non-steroidal anti-inflammatory drugs (NSAIDs)

Fast and effective relief of pain and inflammation in the human being is continued to be a major task for the medicinal chemist. Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the alleviation of pain and inflammation associated with a number of pathological conditions [1]. NSAIDs bestow their effect by inhibiting the catalytic activity of cyclooxygenase (COX), which results in a blockage of the formation of prostaglandins (PGs) and thromboxane (TXs) [2-3]. The cyclooxygenase exists as two distinct isoforms (COX-1 and COX-2) [4]. The maintenance of physiological functions such as protection of gastric mucosa, vascular homeostasis and platelet aggregation is governed by the constitutively expressed COX-1 isoform as organization enzyme while the up regulation of the COX-2 is observed in acute and chronic inflammation [5-6]. Thus Inhibition of COX-2 accounts for the anti-inflammatory effects of NSAIDs, whereas interruption of COX-1 leads to gastrointestinal toxicity ranging from ulcers to perforation and bleeding [7]. Time-honoured non-selective NSAIDs such as Indomethacin, Ibuprofen and Aspirin interact with both forms (COX-1 and COX-2); accounting for their anti-

inflammatory activity in addition to their pronounced side effects resulting from the inhibition of gastro protective PGs synthesized through COX-1 pathway [8]. Hence, a number of selective COX-2 inhibitors such as celecoxib I, rofecoxib II and valdecoxib III (coxibs) has been developed and approved for marketing by virtue of their fewer

gastrointestinal side effects compared to traditional NSAIDs (**Fig. 6**). Celecoxib, in the 1, 5-diarylpyrazole class of compound was the first launched selective COX-2 inhibitor, and has excellent selectivity and potent anti-inflammatory activity; having advantage of not associating with increased cardiovascular complications [9] but known to have gastrointestinal side effects [10].

The Pyrazole nucleus exhibit wide range of biological activities which are well known in medicinal literature some investigations to add are; antimicrobial [11-12] antiviral [13] antitumor activities [14] anti-depressant [15]. Latest reports for anti-inflammatory pyrazole includes [8, 16-19]. Among these, 4-functionalized pyrazoles occupy a distinctive position in medicinal chemistry because of current status of their diversified pharmacological profile. The combined updates about medicinal importance and archaeology of research pertaining to the pyrazoles and their anti-inflammatory activity can be studied at ones by referring recent reviews [20-21].

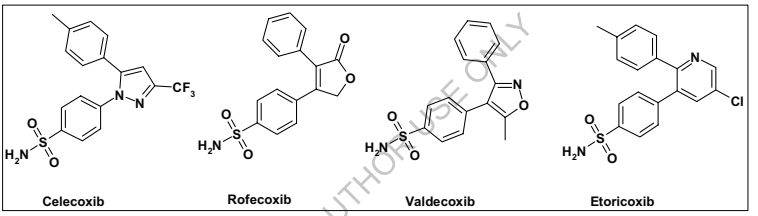
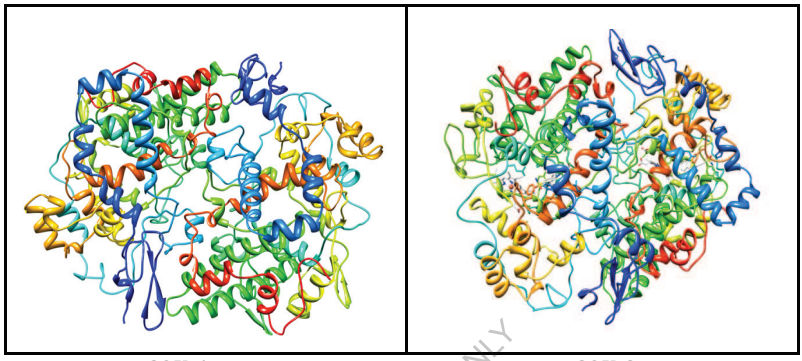


Fig.6: Examples of COX- 2 inhibitors

Reducing pain, inflammation, and fever with salicylate-containing plant extracts can be traced throughout written human history. One hundred and fifty years ago, Felix Hoffman acetylated salicylic acid and created aspirin. Aspirin inhibits the cyclooxygenase (COX) enzymes COX-1 and COX-2, which synthesize inflammatory mediators called prostaglandins and thromboxanes. The ability to block production of prostaglandins and thromboxanes accounts for aspirin being the world's most used therapeutic agent. Second to aspirin are nonsteroidal anti-inflammatory drugs (NSAIDS), which target COX-2 and hence the synthesis of prostaglandins, particularly PGE2. Synthetic forms of natural cortisol (termed glucocorticoids) are also widely used to treat many inflammatory diseases, and despite their side effects, glucocorticoids remain a mainstay for reducing inflammation. Yet, it is still the challenge of the pharmaceutical chemist to develop more effective and less toxic agents to treat the signs and symptoms of acute inflammation as well as the long-term consequences of chronic inflammatory diseases.

1.2.2.1. Cyclooxygenase Inhibitors

It is now known that COX exists as two separate isoforms, COX-1 and COX-2 (**Fig. 7**), which differ markedly in their tissue expression and regulation.10–12 COX-1 is constitutively expressed in most tissues, including the stomach, duodenum, platelets, and kidneys;



COX-1 **COX-2**

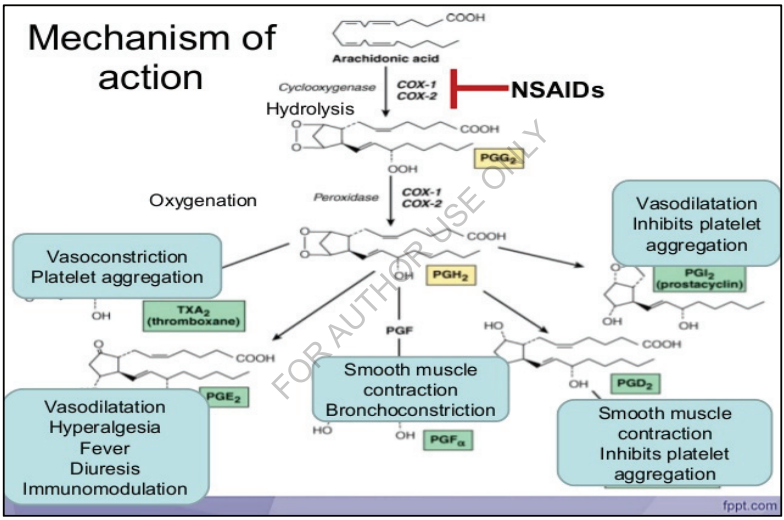
Fig.7: COX-1 and COX-2 structures

COX-1 plays a key part in the production of prostaglandins which regulate important physiological processes such as gastrointestinal cytoprotection (maintaining an effective mucusbicarbonate barrier, submucosal blood flow, quicker and more effective mucosal adaptation to initial tissue damage, and more rapid recovery when such damage occurs). It is also involved in vascular homoeostasis and the maintenance of good renal function and in maintaining normal physiological functions in many other cells. This is an important "housekeeping" role. COX-2, on the other hand, is normally undetectable in most tissues, but it can be induced rapidly, and in large quantities—to 200–300-fold—by cytokines, growth factors, and hormones in the presence of inflammation and other pathological processes.

1.2.3. Mechanism of action for NSAID's

In response to cell injury or receptor activation, phospholipase A2 liberates arachidonic acid from membrane-bound phospholipids in a wide range of tissues (**Fig 8**). The enzyme cox catalyzes the transformation of arachidonic acid to prostaglandin (PG) G2 and subsequently to PGH2. Tissue-specific isomerases convert PGH2 to various prostanoids, which activate receptors and lead to physiological effects. These prostanoids include thromboxane A2 (TxA2) in platelets (platelet activation, vasoconstriction), PGE2 and PGI2 in the gastric mucosa (gastroprotection), PGE2 and PGI2 in the kidney (salt and water excretion), PGE2 in joints (inflammation and

Fig.8: NSAID's and Arachidonic acid pathway



pain), PGI2 in endothelial cells (platelet inhibition and vasodilation) and PGE2 in the central nervous system (pain and fever). The cox-1 isoform of cox is a constitutive enzyme expressed in most tissues and cell types, whereas cox-2 is inducible in inflammation and carcinogenesis. High-dose acetylsalicylic acid and tNSAIDs are nonselective, blocking both isoforms of COX, although the relative degrees of cox-1 and cox-2 inhibition vary substantially among these agents. Their therapeutic efficacy depends on their inhibition of the cox-2-mediated formation of PGE2, which causes inflammation in the joints, and pain and fever in the central nervous system. However, their inhibition of cox-1-mediated PGE2

formation in the gastric mucosa increases the risk of gastrointestinal symptoms, mucosal damage and bleeding. By contrast, the coxibs inhibit only the cox-2-mediated pathways, achieving the desired therapeutic goal of reducing inflammation and pain by blocking PGE2 formation in joints and elsewhere. The coxibs spare cox-1-mediated gastric PGE2 production, preserving its gastroprotective actions. It is now recognized that cox-2 may be expressed constitutively by endothelial cells, and that it is required for the synthesis of vascular protective PGI2 (**Fig 8**). The inhibition of PGI2 by cox-2 selective agents may be an important mechanism of serious adverse cardiovascular events. PGI2 synthesis is also inhibited by high-dose acetylsalicylic acid and tNSAIDs, although possibly not to the same extent as by the cox-2 selective coxibs. Both the coxibs and the tNSAIDs inhibit renal PGE2 and PGI2, resulting in sodium and water retention, as well as elevation of blood pressure. These derangements may contribute to the cardiovascular risks of the coxibs and possibly the tNSAIDs.

1.2.3.1. Gastric damage by NSAID's

NSAIDs may be associated with many gastrointestinal problems, ranging from mild to severe dyspeptic symptoms, the development of gastric or duodenal ulceration, haemorrhage or perforation, and other events which may lead to hospitalisation or death. Endoscopic studies have shown a prevalence rate of 14%–25% of gastric and duodenal ulcers in NSAID users,1 Although endoscopic studies tend to show more gastric than duodenal ulcers associated with NSAID use, patients presenting with gastrointestinal bleeding on NSAIDs may have a similar frequency of gastric and duodenal ulceration. Dyspeptic symptoms occur in up to 60% of patients taking NSAIDs and there is a poor correlation between symptoms and endoscopically proved lesions; up to 50% of endoscopically confirmed ulcers associated with NSAIDs are asymptomatic. NSAIDs interfere with the cyclooxygenase (COX) pathways which lead to the production of prostanoids (prostaglandins, prostacycline, and thromboxane). This interferes with mucosal protection by reducing the effectiveness of the mucus-bicarbonate barrier; gastric acid, and possibly also pepsin, are thus more likely to cause damage. The fact that most NSAIDs are also weak acids may also be a contributory factor.

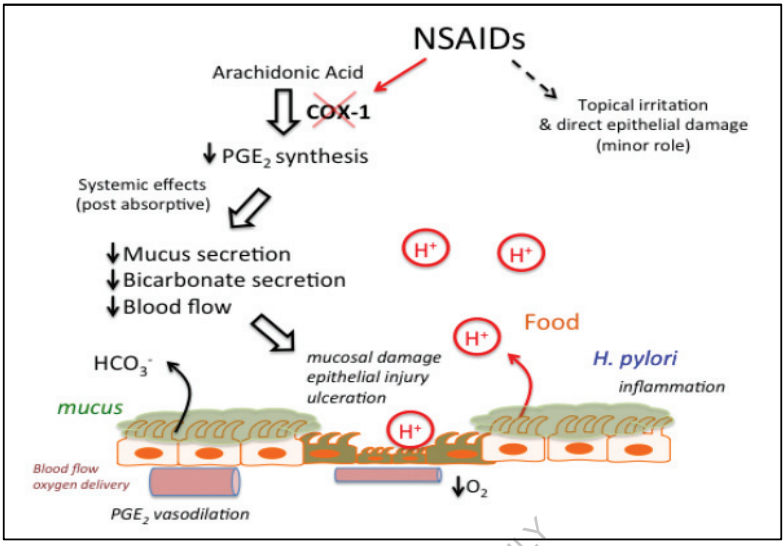


Fig. 9: Pathogenesis of gastric damage by NSAID's

Most conventional NSAIDs are nonselective in their COX inhibition, exerting their anti-inflammatory effects through the inhibition of COX-2, but having adverse effects (such as gastrointestinal mucosal damage and nephrotoxicity) primarily due to inhibition of COX-1(**Fig 9**). Some existing and longstanding NSAIDs, such as etodolac, a known safer NSAID with respect to gastrointestinal damage and which is known to have reduced adverse effects on mucosal prostaglandins, has been subsequently found to have a degree of COX-2 inhibition selectivity.

1.2.4. Synthetic strategies for novel COX-2 Inhibitors

The pharmacophoric structural features of the selective COX-2 inhibitors possess a central heterocyclic five member ring system bearing two vicinal aryl moieties, such as pyrazole (celecoxib), 2(5*H*) furanone (rofecoxib), and isoxazole (valdecoxib). The main part of our research has been devoted to synthetic methods containing the pyrazole nucleus, as a pharmacophoric moiety for potential drugs. Also sulfonamides [22] and hydrazide with their heterocyclic analogs showed evidence of diverse

biological activities including anticancer [23] and anti-inflammatory [24] properties. In particular, the pyrazole nucleus represents a very attractive scaffold to obtain new molecules endowed with anti-inflammatory activities [25-26]. On the basis of these considerations, and

in view of the reported COX-2 inhibitory activities of certain 4-(3-Hydrazinocarbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamidederivatives bearing two aryl moieties at 1- and 5-positions of the pyrazole ring and carrying different substituent on the 5-amino and 3-hydrazinocarbony residue were synthesized. The synthesized compounds have a characteristic molecular pattern and bulk volume to fulfil the pharmacophoric requirements for better recognition at the COX-2 binding active site. The newly synthesized analogues were evaluated for their COX selectivity and their *in-vivo* anti-inflammatory activity.

Though the synthesis of coxibs is engrained in recent years the challenge of exploring the anti-inflammatory activity with promising COX-2 selectivity and safe gastric profile is still in need of investigative accomplishments. In furtherance with our quest associated with synthesis of safe anti-inflammatory agents [25], authors here wish to report an investigation about synthesis, *in-vitro* evaluation of COX selectivity, *in-vivo* anti-inflammatory (AI) activity and evaluation of ulcerogenic liability for two new groups of Hydrazide 1,5- diaryl and amide containing 1,3- diaryl Pyrazoles with promising results.

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Section C

1.3. Antibacterial Agents

1.3.1. Bacterial Research

Antibiotics are one of our most important weapons to cover bacterial infections and improve human life [1]. Bacteria comprise a bulky domain of prokaryotic microorganisms, with a length of few micrometers and are of different shapes like spheres rods and spirals. Usually, bacteria live in association (symbiotic and parasitic) with plants and animals. Large number of bacteria has not yet identified, only about half of the bacterial phyla have species that can be grown in the laboratory. Among all, some bacteria are useful to human life while some are pathogen, emerging a bacterial infection in human body [2]. Bacteria helps in human life such as it is used for making yoghurt, curd, cheese and other fermented foods and also large number of bacteria lives on the skin and in the digestive tract, helping to digestion processes. The human gut contains more than 1000 bacterial species which are beneficial. Other of use bacteria is *Lactobacillus* species that alter the milk sugar into Lactic acid. Also bacteria play an important role in the medicine such as vaccine component and in the production of antibiotics, hormones, antibodies. On the other hand a pathogenic bacterium causes huge level of spoilage leading to the death even in mankind.

1.3.2. Constituents of Bacteria Cell

The bacterial cell differs significantly in structure and function as compared to mammalian cells. The bacterial cytoplasm is separated from the external environment by a cytoplasmic membrane, as shown in **Fig.10**. Following are the functions of the various parts of the bacterial cell.

- **Capsule** - Some species of bacteria have a third protective covering, a capsule made up of polysaccharides (complex carbohydrates). Capsules play a number of roles, but the most important are to keep the bacterium from drying out and to protect it from phagocytosis (engulfing) by larger microorganisms. The capsule is a major virulence factor in the major disease-causing bacteria, such as ***Escherichia coli*** and ***Streptococcus pneumoniae***. Nonencapsulated mutants of these organisms are avirulent, i.e. they don't cause disease.
- **Cell Envelope** - The cell envelope is made up of two to three layers: the interior cytoplasmic membrane, the cell wall, and in some species of bacteria an outer capsule.
- **Cell Wall -** Each bacterium is enclosed by a rigid cell wall composed of peptidoglycan, a protein-sugar (polysaccharide) molecule. The wall gives the cell its shape and surrounds the

cytoplasmic membrane, protecting it from the environment. It also helps to anchor appendages like the pili and flagella, which originate in the cytoplasm membrane and protrude through the wall to the outside. The strength of the wall is responsible for keeping the cell from bursting when there are large differences in osmotic pressure between the cytoplasm and the environment.

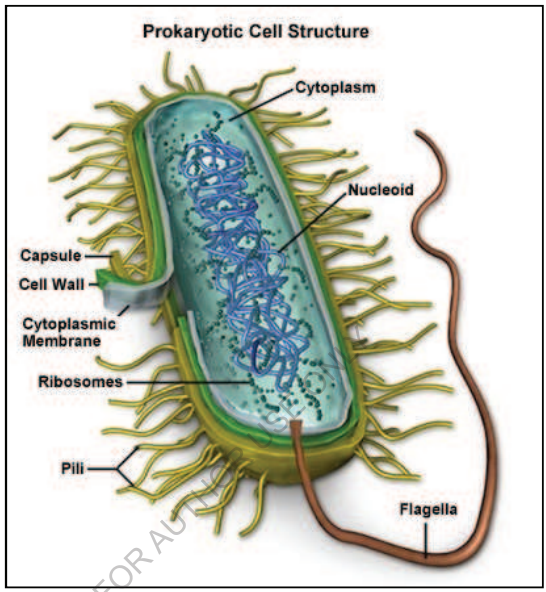

Fig. 10: Bacterial Cell structure

Cell wall composition varies widely amongst bacteria and is one of the most important factors in bacterial species analysis and differentiation. For example, a relatively thick, meshlike structure that makes it possible to distinguish two basic types of bacteria. A technique devised by Danish physician Hans Christian Gram in 1884, uses a staining and washing technique to differentiate between the two forms. When exposed to a gram stain, gram-positive bacteria retain the purple color of the stain because the structure of their cell walls traps the dye. In gram-negative bacteria, the cell wall is thin and releases the dye readily when washed with an alcohol or acetone solution.

- **Cytoplasm** - The cytoplasm, or protoplasm, of bacterial cells is where the functions for cell growth, metabolism, and replication are carried out. It is a gel-like matrix composed of water, enzymes, nutrients, wastes, and gases and contains cell structures such as ribosomes, a chromosome, and plasmids. The cell envelope encases the cytoplasm and all its components.

Unlike the eukaryotic (true) cells, bacteria do not have a membrane enclosed nucleus. The chromosome, a single, continuous strand of DNA, is localized, but not contained, in a region of the cell called the nucleoid. All the other cellular components are scattered throughout the cytoplasm. One of those components, plasmids, are small, extrachromosomal genetic structures carried by many strains of bacteria. Like the chromosome, plasmids are made of a circular piece of DNA. Unlike the chromosome, they are not involved in reproduction. Only the chromosome has the genetic instructions for initiating and carrying out cell division, or binary fission, the primary means of reproduction in bacteria. Plasmids replicate independently of the chromosome and, while not essential for survival, appear to give bacteria a selective advantage.

Plasmids are passed on to other bacteria through two means. For most plasmid types, copies in the cytoplasm are passed on to daughter cells during binary fission. Other types of plasmids, however, form a tubelike structure at the surface called a pilus that passes copies of the plasmid to other bacteria during conjugation, a process by which bacteria exchange genetic information. Plasmids have been shown to be instrumental in the transmission of special properties, such as antibiotic drug resistance, resistance to heavy metals, and virulence factors necessary for infection of animal or plant hosts. The ability to insert specific genes into plasmids have made them extremely useful tools in the fields of molecular biology and genetics, specifically in the area of genetic engineering.

- **Cytoplasmic Membrane** - A layer of phospholipids and proteins, called the cytoplasmic membrane, encloses the interior of the bacterium, regulating the flow of materials in and out of the cell. This is a structural trait bacteria share with all other living cells; a barrier that allows them to selectively interact with their environment. Membranes are highly organized and asymmetric having two sides, each side with a different surface and different functions. Membranes are also dynamic, constantly adapting to different conditions.
- **Flagella** - Flagella (singular, flagellum) are hair like structures that provide a means of locomotion for those bacteria that have them. They can be found at either or both ends of a bacterium or all over its surface. The flagella beat in a propeller-like motion to help the bacterium move toward nutrients; away from toxic chemicals; or, in the case of the photosynthetic cyanobacteria; toward the light.
- **Nucleoid** - The nucleoid is a region of cytoplasm where the chromosomal DNA is located. It is not a membrane bound nucleus, but simply an area of the cytoplasm where the strands of DNA are found. Most bacteria have a single, circular chromosome that is responsible for

replication, although a few species do have two or more. Smaller circular auxiliary DNA strands, called plasmids, are also found in the cytoplasm.

- **Pili** - Many species of bacteria have pili (singular, pilus), small hairlike projections emerging from the outside cell surface. These outgrowths assist the bacteria in attaching to other cells and surfaces, such as teeth, intestines, and rocks. Without pili, many disease-causing bacteria lose their ability to infect because they're unable to attach to host tissue. Specialized pili are used for conjugation, during which two bacteria exchange fragments of plasmid DNA.
- **Ribosomes** - Ribosomes are microscopic "factories" found in all cells, including bacteria. They translate the genetic code from the molecular language of nucleic acid to that of amino acids—the building blocks of proteins. Proteins are the molecules that perform all the functions of cells and living organisms. Bacterial ribosomes are similar to those of eukaryotes, but are smaller and have a slightly different composition and molecular structure. Bacterial ribosomes are never bound to other organelles as they sometimes are (bound to the endoplasmic reticulum) in eukaryotes, but are free-standing structures distributed throughout the cytoplasm. There are sufficient differences between bacterial ribosomes and eukaryotic ribosomes that some antibiotics will inhibit the functioning of bacterial ribosomes, but not a eukaryote's, thus killing bacteria but not the eukaryotic organisms they are infecting.

1.3.2.1. Cell wall of bacteria

The bacterial cell wall is strong to prevent cell lysis, also porous to allow transport across the cell membrane surrounds the bacterial cell. Nearly every genus of bacteria has a cell wall, which is a rigid, carbohydrate-containing structure that surrounds the bacterial cell. The cell wall protects the bacterium from damages [3]. Bacterial cell wall is porous, so small molecules are easily able to pass through the cell wall to the membrane, but large molecules cannot. Hence, the cell wall performs as a coarse filter. The primary function of the cell wall is to maintain the cell shape and prevent cell rupture from osmotic pressure [4].

1.3.2.2. Peptidoglycan

It is a polymer consisting of sugars and amino acids which forms a mesh-like layer outside the plasma membrane of most bacteria, gives the rigidity to bacterial cell.

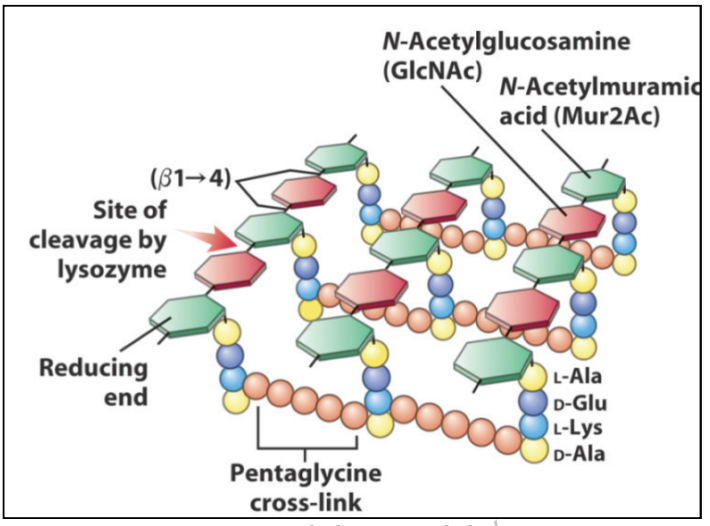

Fig.11: Peptidoglycans cross linkage

The peptidoglycan comprises the alternating residues of β-(1,4) linked *N*-acetyl glucosamine and N-acetylmuramic acid. Attached to the *N*-acetylmuramic acid is chain of three to five amino acids via peptide linkage. The peptide chain can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer. Peptidoglycan (**Fig.11**) serves a structural role in the bacterial cell wall, giving structural strength, as well as neutralizing the osmotic pressure of inner cytoplasm [5-7]. Peptidoglycan helps maintain the structural strength of the cell.

1.3.3. Classification of Bacteria

A Danish Microbiologist, Hans Christian Gram (1853-1938), had developed the Gram stains in order to visualize bacreria more easily under microscope [8]. Based upon the staining pattern pathogenic bacteria have been classified into two main categories viz. Gram (+) and Gram (-ve) by the chemical and physical properties **Fig. 12**. Gram-positive bacteria retain the crystal violet dye, and thus are stained violet, on the other hand Gram-negative bacteria do not; after washing, a counterstain is added that will stain these Gram-negative bacteria a pink colour [9].

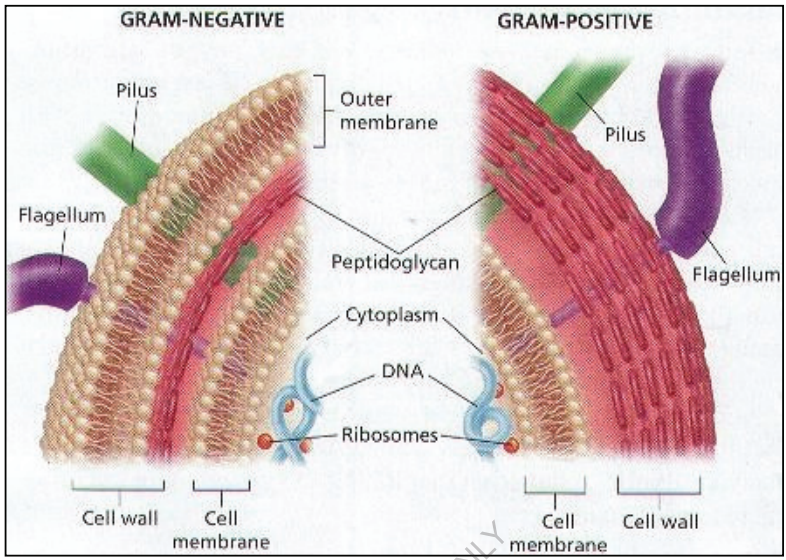

Fig.12: Gram positive and Gram negative bacteria cell wall

1.3.4. Infection caused by bacteria

Bacteria responsible for infections and disease are called as pathogenic bacteria. These disease comes when pathogens enters inside the body and starts to reproduce themselves. Many times pathogenic bacteria produce toxins many times and these chemical are harmful to the body [10]. One of the bacterial diseases with the highest disease burden is tuberculosis, caused by the *Mycobacterium tuberculosis* [11]. As per WHO tuberculosis kills about 2 million people in a year, mostly in sub-Saharan Africa. Pathogenic bacteria give to other globally important diseases like pneumonia, caused by *Streptococcus* and *Pseudomonas*, and food borne illnesses, caused by bacteria such as *Shigella*, *Campylobacter*, and *Salmonella*. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy. Pathogenic bacteria are also the cause of high infant mortality rates in developing countries.

1.3.5. Antibacterial agents

Antibiotics inhibit the growth of bacteria or kill the bacteria. The term antibiotic was used first time by Selman Waksman in 1942. Antibiotics belongs to various category, their categories are depends on how these agent (antibiotics) inhibit the bacterial cell growth [13]. Hence the category/types with their examples are,

1. **Cell wall synthesis inhibitors**

   Marketed drugs like penicillins, cephalosporins, imipenem, meropenem, vencomycin are the cell wall synthesis inhibitors (**Fig.13**).

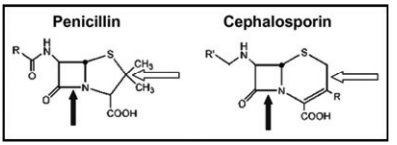

Fig.13: Penicillins & cephalosporins

2. **Bacterial protein synthesis inhibitors.**

   Aminoglycosides, Chloramphenicol, Macrolides, Tetracycline, Linezolide etc are the bacterial protein synthesis inhibitor drugs.

3. **Nucleic acid synthesis inhibitors.**

   Fluoroquinolones, Rifampicin drugs are the nucleic acid synthesis inhibitors.

4. **Folic acid synthesis inhibitors.**

   Sulfonamides, Trimethoprim are bacterial folic acid synthesis inhibitor drugs.

An important quality for an antimicrobial drug is **selective toxicity**, meaning that it selectively kills or inhibits the growth of microbial targets while causing minimal or no harm to the host. Most **antimicrobial drugs** currently in clinical use are antibacterial because the prokaryotic cell provides a greater variety of unique targets or selective toxicity, in comparison to fungi, parasites, and viruses. Examples of antibacterial drugs are reported in **Fig. 14**.

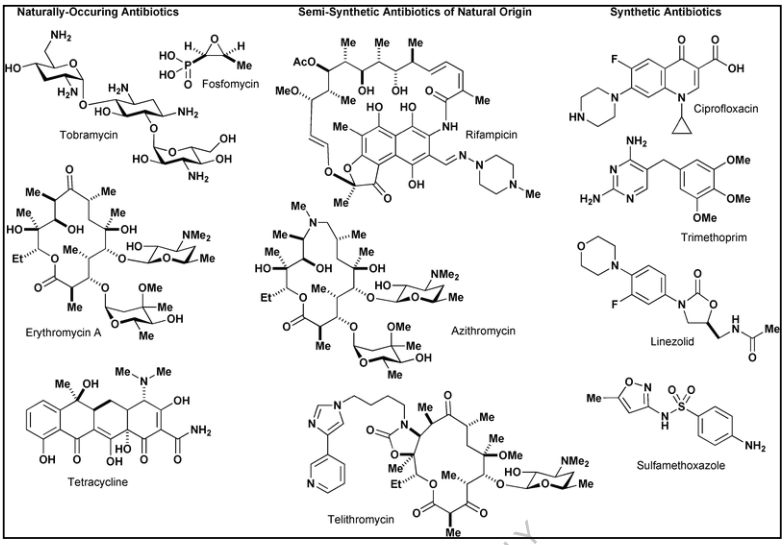

Fig.14: Examples of antibacterial class of drugs

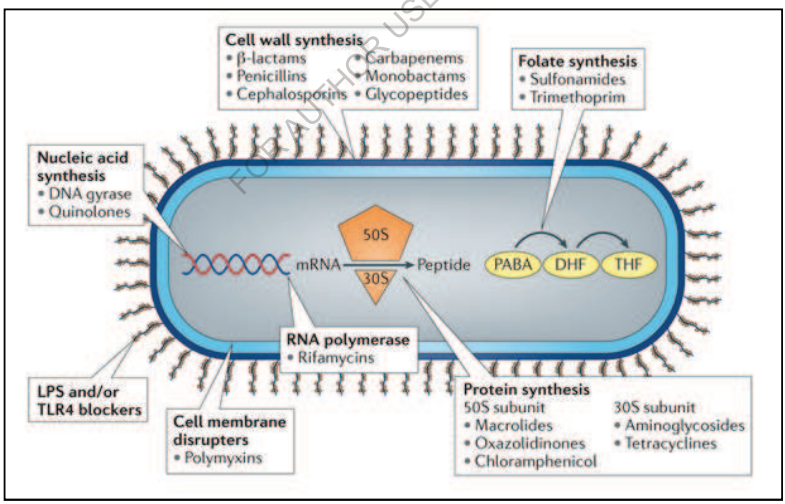

Fig.15: Mechanism of action for antibacterial drugs

Each class of antibacterial drugs has a unique **mode of action** (the way in which a drug affects microbes at the cellular level)and these are summarized in **Fig.15** and **Table 1**.

Table 1: Common Antibacterial Drugs by Mode of Action

Mode of Action	Target	Drug Class
Inhibit cell wall biosynthesis	Penicillin-binding proteins	β-lactams: penicillins, cephalosporins, monobactams, carbapenems
	Peptidoglycan subunits	Glycopeptides
	Peptidoglycan subunit transport	Bacitracin
Inhibit biosynthesis of proteins	30S ribosomal subunit	Aminoglycosides, tetracyclines
	50S ribosomal subunit	Macrolides, lincosamides, chloramphenicol, oxazolidinones
Disrupt membranes	Lipopolysaccharide, inner and outer membranes	Polymyxin B, colistin, daptomycin
Inhibit nucleic acid synthesis	RNA	Rifamycin
	DNA	Fluoroquinolones
Antimetabolites	Folic acid synthesis enzyme	Sulfonamides, trimethoprim
	Mycolic acid synthesis enzyme	Isonicotinic acid hydrazide
Mycobacterial adenosine triphosphate (ATP) synthase inhibitor	Mycobacterial ATP synthase	Diarylquinoline

1.3.6. Bacterial resistance

All types of microbes develop resistance: fungi create antifungal resistance, viruses develop antiviral resistance, protozoa develop antiprotozoal resistance, and bacteria develop antibiotic resistance. Bacterial resistance is the ability of microorganism / bacteria to resist / oppose the effects of antibiotics employed by clinicians [14]. Resistance emerges through by: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from another. Resistance can come very rapidly on account of casual

mutations; or more commonly following gradual buildup over time, and because of misuse of antibiotics or antimicrobials [15]. Resistant microorganisms are complicated to treat, it need of substitute medications or higher doses. Microbial species which are resistant to multiple antimicrobials are called multidrug resistant (MDR). Emergence of MDR resulting in number of death every year. To overcome these troubles specifically design and synthesized these hybrid structures to achieve the biological significance over the existing marketed drugs. The targeted molecules were rationalized and have pharmacophores known for their biological activities.

1.3.7. Synthetic strategies for novel Antibacterial agents

Infectious diseases are the leaders among the challenging drug targets because of the multi-drug resist antimicrobial pathogens and continuous rise in the emerging infections from known and unknown sources. Though there is an availability of a large number of antibiotics and chemotherapeutics for medical use, the emerging resistance drives it for the search of new classes of antimicrobial agents [16, 17]. A potential approach to overcome the problem of antibiotic resistance is to design innovative agents with different modes of action so that no cross resistance with present drugs can occur [18]. Pyrazoles and their variously substituted derivatives exhibit wide range of biological activities and a significant amount of research activity has been directed towards this class. In particular, they are used as antitumor, antimicrobial, antitumor, antihistaminic, antiviral, fungicides, insecticides [19-22]. Some of these compounds have also anti-inflammatory, anesthetic and analgesic properties [23-26]. A typical procedure to synthesis substituted pyrazoles involves base catalyzed aldol condensation followed by the cyclization with active hydrazines [27-30]. It has been observed from the literature that current heterocyclic research showed a significant interest in aryl substituted pyrazoles. [31-33].

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Section D

1.4. Antidiabetic Agents such as Sulfonylureas

1.4.1. Diabetic Research

Diabetes mellitus (**DM**), commonly referred to as **diabetes**, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period [1]. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications [2]. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death [3]. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes [2].

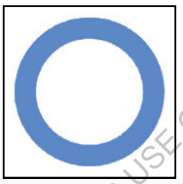


Fig.16: Universal blue circle symbol for diabetes [4].

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced [5]. There are three main types of diabetes mellitus:

- Type 1 DM results from the pancreas's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause is unknown [2].
- Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly [2]. As the disease progresses a lack of insulin may also develop [6]. This form was previously referred to as "non insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The most common cause is excessive body weight and not enough exercise [2].
- Gestational diabetes is the third main form and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels [2].

Type 2 diabetes mellitus (DM) is a disorder characterised by insulin resistance and a progressive decline in pancreatic beta-cell function associated with increasing hyperglycaemia. Defective betacell function occurs early and can be detected in individuals with impaired fasting and/or post-prandial glucose levels (the so-called 'pre-diabetics').

The pathogenesis of type 2 diabetes mellitus is complex and has only been partially clarified. Clearly, the capacity of the pancreas to produce insulin is reduced. On diagnosis, b-cell function is generally reduced to approximately 50% of what is considered normal. In addition to this defect in insulin secretion, there is a reduced sensitivity to the effect of insulin on the target organs (insulin resistance). Insulin resistance is closely related to obesity. Once a state of chronic hyperglycaemia has been reached (diabetes), a number of secondary alterations take place that, although in themselves not the cause, do lead to an additional increase in both insulin resistance and b-cell dysfunction. The term glucose toxicity is used to refer to these secondary defects. **Fig.17** shows the normal relationship between insulin secretion and insulin sensitivity.

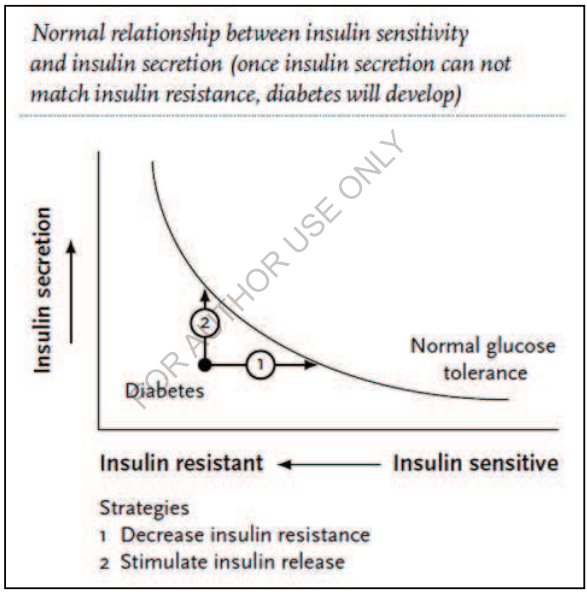


Fig.17: Relationship between insulin sensitivity & secretion

Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight, and avoiding use of tobacco. Control of blood pressure and maintaining proper foot care are important for people with the disease. Type 1 DM must be managed with insulin injections [2]. Type 2 DM may be treated with medications with or without insulin [7]. Insulin and some oral medications can cause low blood sugar [8]. Weight loss surgery in those with obesity is sometimes an effective measure in those with type 2 DM [9]. Gestational diabetes usually resolves after the birth of the baby [10].

As of 2015, an estimated 415 million people had diabetes worldwide [11]. With type 2 DM making up about 90% of the cases [12-13]. This represents 8.3% of the adult population [13]. With equal rates in both women and men [14]. As of 2014, trends suggested the rate would continue to rise [15]. Diabetes at least doubles a person's risk of early death [2]. From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes [7] [11]. The global economic cost of diabetes in 2014 was estimated to be US$612 billion [16]. In the United States, diabetes cost $245 billion in 2012 [17].

Diabetes is chronic disease and the number of people with diabetes has risen from 108 million in 1982 to 422 million in 2014 [18]. Diabetes can be managed by healthy diet, regular physical activity and maintaining a normal body weight. It can be treated through various pathways by controlling blood glucose level [19]. Main disadvantage of these pathways is side effects such as weight gain, hypoglycemia and joint pains. Current treatment for type 2 diabetes is based upon increasing insulin availability, improving sensitivity to insulin, delaying the delivery of insulin and absorption of carbohydrates from gastrointestinal tract or increasing urinary glucose excretion [20].

1.4.2 Types of diabetes mellitus

1.4.2.1. Type 1

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin [21]. It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children

1.4.2.2. Type 2

Type 2 DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion [22]. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 DM is the

most common type of diabetes mellitus. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, high blood sugar can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce the liver's glucose production.

Type 2 DM is primarily due to lifestyle factors and genetics [23]. A number of lifestyle factors are known to be important to the development of type 2 DM, including obesity (defined by a body mass index of greater than 30), lack of physical activity, poor diet, stress, and urbanization [24]. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders [22]. Even those who are not obese often have a high waist–hip ratio [22].

Dietary factors also influence the risk of developing type 2 DM. Consumption of sugar-sweetened drinks in excess is associated with an increased risk [25-26] The type of fats in the diet is also important, with saturated fat and trans fats increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk [23]. Eating lots of white rice also may increase the risk of diabetes [27]. A lack of physical activity is believed to cause 7% of cases [28].

1.4.2.3. Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 DM in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery [29]. However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2 [29]. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases, insulin may be required.

Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital heart and central nervous system abnormalities, and skeletal muscle malformations. Increased levels of insulin in a fetus's blood may inhibit fetal surfactant production and cause respiratory distress syndrome. A high blood bilirubin level may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with

decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.

1.4.3. Drugs for the treatment of Diabetes mellitus

Drugs used in diabetes treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of Insulin, exenatide, liraglutide and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors. Diabetes mellitus type 1 is a disease caused by the lack of insulin. Insulin must be used in Type I, which must be injected. Diabetes mellitus type 2 is a disease of insulin resistance by cells. Type 2 diabetes mellitus is the most common type of diabetes. Treatments include (1) agents that increase the amount of insulin secreted by the pancreas, (2) agents that increase the sensitivity of target organs to insulin, and (3) agents that decrease the rate at which glucose is absorbed from the gastrointestinal tract. Several groups of drugs, mostly given by mouth, are effective in Type II, often in combination. The therapeutic combination in Type II may include insulin, not necessarily because oral agents have failed completely, but in search of a desired combination of effects.

Table 2: Antidiabetic drugs (August 2015)

Class	Generic name (brand name)	Mechanism of action	When to take it	Adverse effects
Sulfonylureas	*Gliclazide* (Diamicron®) *Glimepiride* (Amaryl®) *Glyburide* (Diaβeta®)	Stimulate the pancreas to produce more insulin	Before meals (≤ 30 minutes); Do not take at bedtime	Hypoglycemia (low blood sugar)
Méglitinides	*Natéglinide* (Starlix®) *Répaglinide* (GlucoNorm®)	Stimulate the pancreas to produce more insulin	Before meals (≤ 15 minutes); Do not take at bedtime	Hypoglycemia (low blood sugar)
Biguanides	*Metformine* (Glucophage®)	Reduce the production of glucose by the liver	During meals	Diarrhea, metallic aftertaste, nausea
***Metformine with extended release* (Glumetza®)**			At dinner	
Thiazolidinediones (TZD)	*Pioglitazone* (Actos®) *Rosiglitazone* (Avandia®)	Increase insulin sensitivity of the body cells and reduce the production of glucose by the liver	With or without food, at the same time each day	Swelling due to water retention, weight gain Pioglitazone : inscreased risk of bladder cancer (Health Canada restriction) Rosiglitazone : increased risk of non-fatal heart attack (Health

				Canada restriction)
Alpha-glucosidases inhibitors	*Acarbose* (Glucobay®)	Slow the absorption of carbohydrates (sugar) ingested	With the first mouthful of a meal	Bloating and flatulence (gaz)
Dipeptidyl-peptidase-4 (DPP-4) inhibitors	*Linagliptine* (Trajenta®) *Saxagliptine* (OnglyzaMC) *Sitagliptine* (Januvia®) *Alogliptine* (Nesina®)	Intensify the effect of intestinal hormones (incretines) involved in the control of blood sugar	With or without food, at the same time each day	Pharyngitis, headache
Glucagon-like peptide-1 (GLP-1) agonist	*Exenatide* (Byetta®)	Mimic the effect of certain intestinal hormones (incretines) involved in the control of blood sugar	Injection to take 0 to 60 minutes before breakfast or dinner	Nausea, diarrhea, vomiting
***Liraglutide* (Victoza®)**			Injection to take with or without food, at the same time each day	
Sodium glucose co-transporter 2 inhibitors (SGLT2)	*Canaglifozine* (INVOKANA®)	Help eliminate glucose in the urine	Before the first meal of the day	Genital and urinary infections, more frequent urination
***Dapagliflozine* (Forxiga®)**			Any time of day, with or without food	

The great advantage of injected insulin in Type II is that a well-educated patient can adjust the dose, or even take additional doses, when blood glucose levels measured by the patient, usually with a simple meter, as needed by the measured amount of sugar in the blood. Examples are listed in Table 2.

1.4.3.1. Sulphonylureas

The hypoglycaemic action of sulfonylureas was discovered in 1942 when Marcel Janbon, working in Montpellier, treated a numberof typhoid patients for their infection with the sulphonamide 2254 RP (p-aminobenzenesulfamidoisopropylthiodiazole). There were a number of deaths and a high incidence of side-effects which he correctly interpreted as the result of hypoglycaemia. It was subsequently shown by Auguste Loubatiers that the ability of 2254 RP to lower blood glucose levels resulted from the stimulation of insulin secretion. These pioneering studies led to the widespread use of sulfonylureas in the treatment of non-insulin-dependent diabetes (NIDDM).

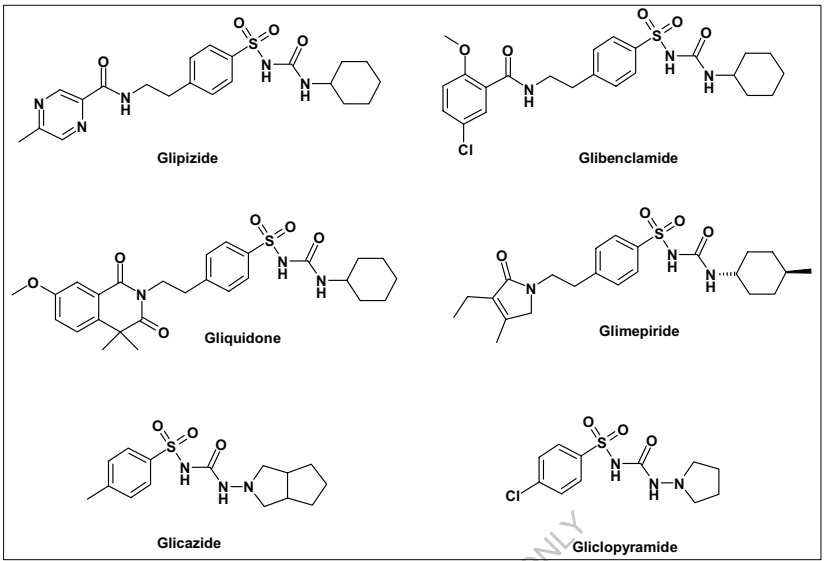


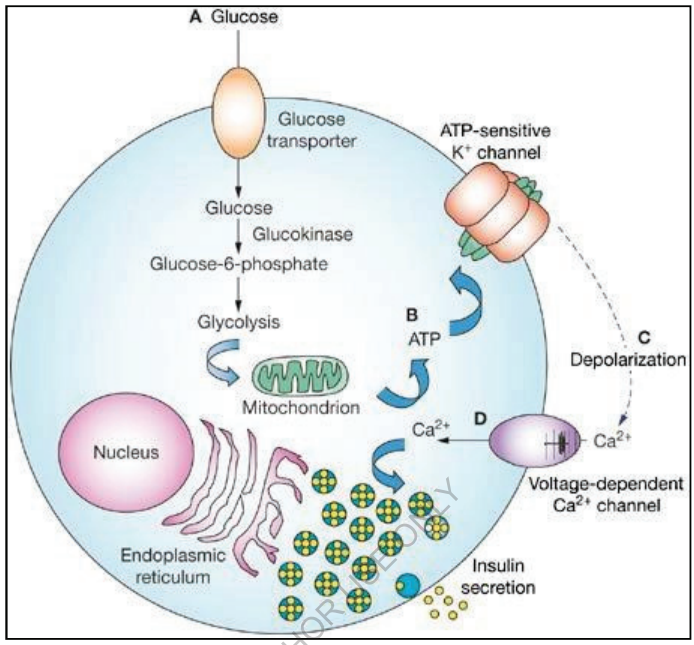
Fig.18: Examples of anti-diabetic sulfonylurea class of drugs

Tolbutamide was introduced in 1956 and was the first of a class of "first generation" sulfonytureas which had clear hypoglycaemic properties and lacked bacteriostatic actions. Subsequently, many thousands of analogues have been synthesised, including the far more potent 'second generation" sulfonylureas such as glibenclamide (**Fig. 18**) [30].

1.4.3.1.1. Mechanism of action for sulfonylurea's

The mechanism of action involves a direct secretory effect on the pancreatic islet beta-cells. Adenosine triphosphate (ATP)-sensitive potassium channels (K+ATP) of the beta-cells play an essential role in the release of insulin and consist of two components: a pore and a regulatory subunit (SUR-1). The sulphonylureas act to enhance the sensitivity of the beta-cell to glucose and, when bound to the transmembrane sulphonylurea receptor (SUR-1), mediate the closing of the potassium-sensitive ATP channels on the cell membrane. Cellular efflux of potassium is reduced and membrane depolarisation takes place.

Fig.19: Mechanism of sulfonulurea's to release insulin in the pancreas



Calcium influx is mediated by the opening of voltage-dependent Ca^{2+}-channels that promote the release of pre-formed insulin granules which lie just adjacent to the plasma membrane (**Fig. 19**) [31].

1.4.3.1.2. Side effects of Sulfonylurea

Sulfonylureas, as opposed to metformin, the thiazolidinediones, exenatide, symlin and other newer treatment agents may induce hypoglycemia as a result of excesses in insulin production and release. This typically occurs if the dose is too high, and the patient is fasting. Some people attempt to change eating habits to prevent this, however it can be counterproductive. Like insulin, sulfonylureas can induce weight gain, mainly as a result of their effect to increase insulin levels and thus utilization of glucose and other metabolic fuels. Other side-effects are: gastrointestinal upset, headache and hypersensitivity reactions [32-33].

1.4.3.2. Thiazolidinone

The TZDs represent a new class of drugs with a new mechanism of action. In Europe, TZDs have been approved for type 2 diabetes mellitus, particularly for overweight patients who are inadequately controlled by diet and exercise alone, for whom metformin is inappropriate because of contraindications or intolerance. TZDs have

also been approved for use in combination therapy. Unlike the situation in the USA, TZDs are not approved, but even contraindicated for use in combination with insulin in Europe. From the day of approval onwards, there has been discussion concerning the exact place of TZDs within the pharmacotherapy of type 2 diabetes mellitus. [34]

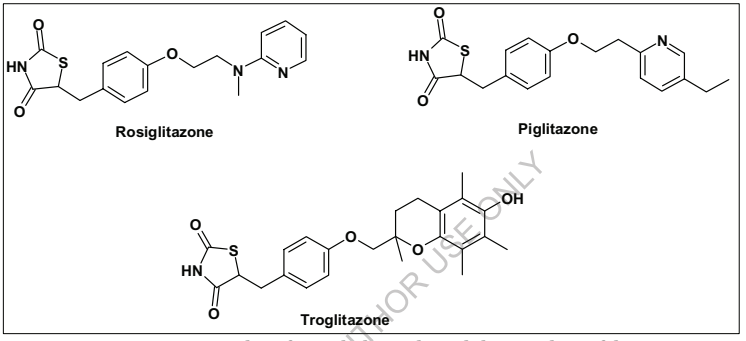


Fig. 20: Examples of anti-diabetic thiazolidinone class of drugs

This class of drug can be used as monotherapy in obese as well as non-obese patients who have failed other conservative measures. TZDs can be used in combination with metformin and sulphonylureas. The use in combination with insulin is prohibited in Europe because of the increased risk of weight gain in the form of adipogenesis and fluid retention. The use of TZDs is contraindicated in acute liver disease owing to the increased risk of hepatotoxicity. Since they decrease hepatic glucose output, the concern exists that they could possibly aggravate hypoglycaemia. With the introduction of this new class of drug in 1997, the world has watched the peroxisome proliferator activated receptor (PPAR)-γ agonists with anticipation. The net effect of these drugs results from stimulation of a nuclear PPAR-γ that regulates the transcription of genes culminating in an increase in insulin sensitivity. Troglitazone, the forerunner drug, was withdrawn in 2000 following reports of fatal hepatotoxicity, and the future of rosiglitazone currently hangs in the balance, owing to a possible increased risk of myocardial infarction and cardiovascular-related deaths.

1.4.3.2.1. Mechanism of action for Thiazolidinones

Thiazolidinediones (TZDs) mediate their function through binding to the PPAR-γ receptor that is expressed predominantly in adipocytes (**Fig. 21**).

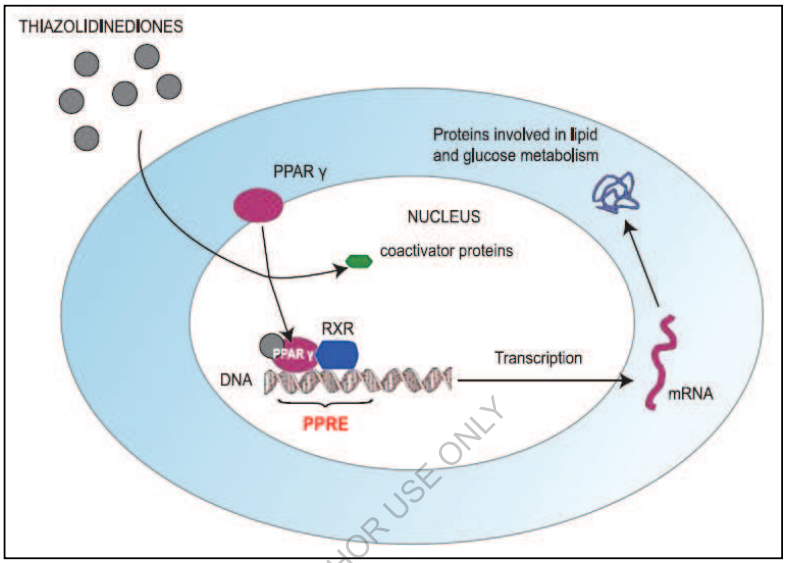


Fig.21: Mechanism of thiazolidinones to release insulin in the pancreas

It is expressed to a lesser extent in muscle and liver tissue. Binding of the PPAR receptor in turn mediates binding to the retinoic-X receptor (RXR-receptor). This heterodimer then binds to a nuclear response element which then switches on gene transcription. Many of the genes that are activated play a central role in carbohydrate and lipid metabolism. TZDs, like metformin, require the presence of insulin to mediate a blood glucose-lowering effect [35-36].

1.4.3.2.2. Side effects of Thiazolidinones

Hypoglycaemia, one of the main side effects of oral blood glucose lowering drugs, does not occur with TZDs, because they do not affect the secretion of insulin. Hypoglycaemia can occur in combination with other drugs, but in that case it is not due to the TZDs. Although the hepatotoxicity of troglitazone has clearly been demonstrated, it has been proven that rosiglitazone and pioglitazone are less associated with hepatotoxicity. In fact a slight improvement in liver enzyme values

usually occurs, probably as a result of the reduction in the amount of liver fat. Two large retrospective analyses showed that the use of pioglitazone or rosiglitazone over a period of one to two years was not associated with an increase in liver failure or hepatitis, in comparison with other oral blood glucose lowering drugs. TZDs are not superior to other oral blood glucose lowering drugs with respect to their glycaemic effect. As such, conventional oral therapy will continue to be important in the treatment of type 2 diabetes mellitus as will combination therapy. TZDs are contraindicated during pregnancy (class Cevidence) based on the observations of growth retardation in animal studies [37]. The challenge of treating type 2 DM grows by the day as the number of patients increase and the adverse effects such as Swelling due to water retention, weight gain Pioglitazone : inscreased risk of bladder cancer (Health Canada restriction) Rosiglitazone : increased risk of non-fatal heart attack (Health Canada restriction). Therefore, a good understanding of the available treatment modalities is of great value. As the pathogenesis of diabetes becomes clearer, exciting new targets for drug therapy will be identified, which provide physicians with more 'fire power' and treatment options in the fight against this disease.

1.4.4. Synthetic strategies for novel Antidiabetic agents

Since sulfonylureas and thiazolidinone moieties are biologically proven antidiabetic pharmacophores and substitution in these scaffolds may further enhance their activity, prompted us to undertake this problem. Moreover, the combination of two pharmacophores in a single molecule is a well established hypothesis for synthesis of more active drugs with dual activity. Thus, a series of novel sulfonylhydrazides and sulfonylureas, substituted with thiazolidine-4-ones were synthesized and evaluated for their hypoglycaemic activity.

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CHAPTER 2

Synthesis and biological studies of novel thieno[2,3-d] pyrimidine derivatives

SECTION A

2.1. Synthesis and anticancer studies of *1-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine-2-carboxylic acid* derivatives

2.1.1. AIM & OBJECTIVES

Tyrosine kinases are orally active, small molecules that have a favourable safety profile and can be easily combined with other forms of chemotherapy or radiation therapy. TKIs are thus an important new class of targeted therapy that interfere with specific cell signalling pathways and thus allow target specific therapy for selected malignancies. A number of thieno [2, 3-d] pyrimidine derivatives with different substituent at C-2 and C-4 positions were found to exert potential antitumor activity [1-2].

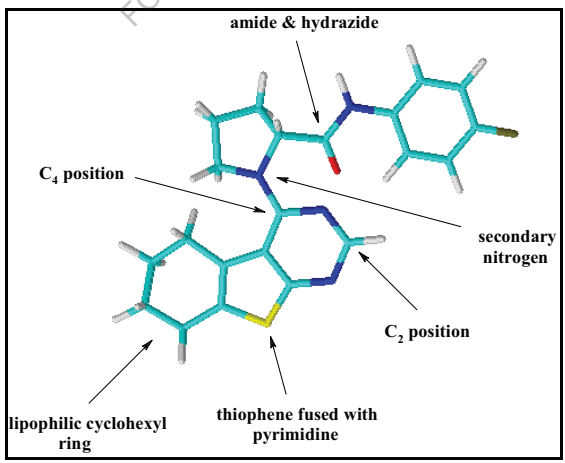


Fig.22: Essential elements of synthesized compounds

We have prepared a series of new thieno [2, 3-d] pyrimidines by introducing a five member cycloalkyl ring containing secondary nitrogen (L- proline) at C-4 position and Synthesized pharmacophoric moieties of interest such as amides and hydrazides that proved to contribute to antitumor activities such as **5** and **13**. Tandutinib (**Fig 4**) consist of C-4 substituted piperazine attached to the quinazoline moiety and formed urea derivative with the propoxy aniline. We design the molecules after modifying the C-4 substitution by secondary nitrogen containing amino acid L- proline and used the substituted anilines like gefitinib, lapatinib and also substituted piperazine side chains to form terminal amides replacing urea. Hydrazide derivates were also prepared to compare its relationship with amide derivatives. We studied the structural modifications of thieno [2, 3-d] pyrimidine by aiming to obtained the new candidates of remarkable antitumor activities.

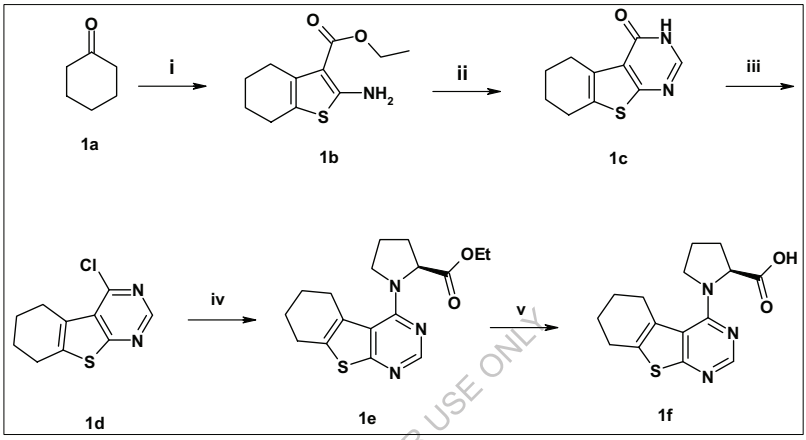
2.1.2. RESULTS AND DISCUSSION

2.1.2.1. Chemistry

The targeted compounds were prepared as outlined in **Scheme 1**. The starting materialEthyl-2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate **1b** was prepared following the method of Gewald [3-4] via the reaction of Cyclohexanone **1a** and sulfur with ethyl cynoacetate in the presence of morpholine. Cyclization of **1b** to **1c** is adopted using the reported reaction condition by refluxing it in formamide [5]. The desired 4-chloro derivative **1d** was obtained via the reaction of **1c** with phosphorous oxychloride in reflux [6]. L-Proline ethyl ester was introduced by replacing Chloro group of thieno [2,3-d] pyrimidine **1d** in Methanol to obtained the Ester intermediate **1e**.Tetrahydrobenzo [4, 5] thieno [2, 3-d] pyrimidine-4yl-pyrrolidine-2-carboxylic acid **1f** was obtained after the alkaline hydrolysis of ester intermediate **1e** by using lithium hydroxide.

Scheme 1

Synthetic route for ***1-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine-2-carboxylic acid*** **(1f)**

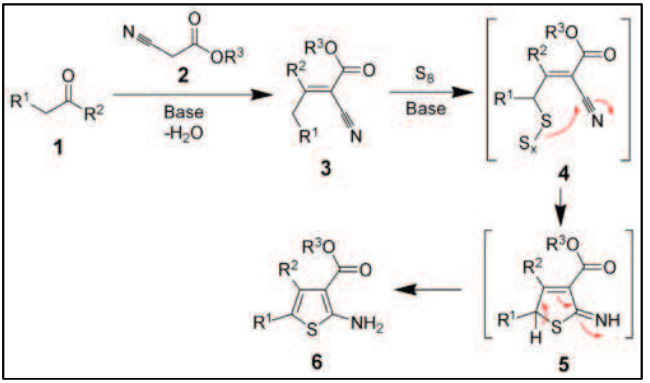


Reagents and conditions**: (i) Ethyl cynoacetate, Sulfur, morpholine, EtOH; (ii) CH_3NO, reflux; (iii) $POCl_3$, reflux (iv) L-proline ethyl ester hydrochloride, Et_3N, MeOH (v) $LiOH.H_2O$, THF: H_2O**

^{1}H NMR spectrum of **1b** revealed the presence of triplet signal at δ 1.33 ppm and quartet signal at δ 4.25 ppm corresponds to ethyl group of ester, and singlet of two protons at δ 5.94 ppm corresponds to amino group. Similarly ^{1}H NMR spectrum of **1c** was identified by the broad singlet at δ 12.31 and singlet at δ 8.00 ppm of the pyrimidin-4(3H)-one. IR spectrum of **1c** showed bands at 3363, 3383 and 1639 cm^{-1} corresponding to the NH and C=O groups, respectively. Chloro intermediate **1d** was assigned by the shifted single signal to 8.72 ppm. Crude ester intermediate **1e** was used directly for hydrolysis reaction, its formation was confirmed by the ESI-MS spectrum showing ES-MS: *m/z* 332.3 $(M+H)^+$ Acid intermediate **1f** shows a characteristic triplet signal at δ 4.80 ppm corresponds to chiral proton of L-proline and pyrimidine proton at δ 8.32 ppm.

Name reactions involved in the synthesis of thienopyrimidine

Gewald Reaction



.

Fig.23: Mechanism of the Gewald reaction

The **Gewald reaction** is an organic reaction involving the condensation of a ketone (or aldehyde when R^2 = H) with a α-cyanoester in the presence of elemental sulfur and base to give a poly-substituted 2-amino-thiophene. [7-8].

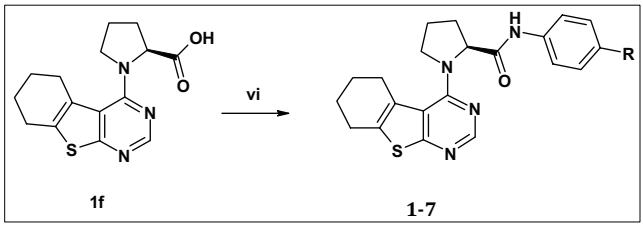
Mechanism of the Gewald Reaction

The reaction mechanism of the Gewald reaction was elucidated 30 years after the reaction was discovered [4]. The first step is a Knoevenagel condensation between the ketone (**1**) and the α-cyanoester (**2**) to produce the stable intermediate **3**. The mechanism of the addition of the elemental sulfur is unknown. It is postulated to proceed through intermediate **4**. Cyclization and tautomerization will produce the desired product (**6**).

We used Cyclohexanone and Ethylcynoacetate in the presence of elemental Sulfur and morpholine to obtained, *Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate* **(1b)**

Scheme 2

Synthetic route for ***1-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-Pyrrolidine-2-carboxylic acid phenylamides*** **(1-7)**

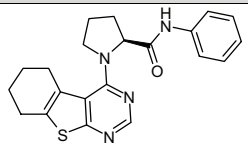
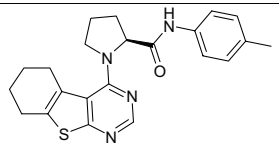
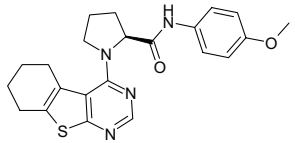


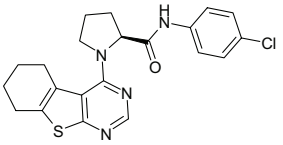
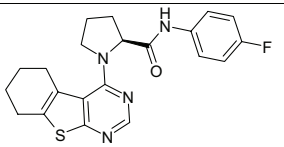
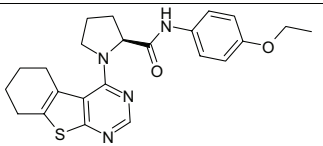
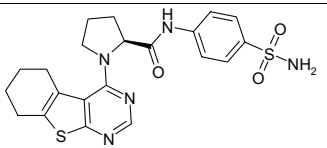
vi) R-Aniline, EDC.HCl, HOBt, DMF, RT

General Procedure

To a solution of 1-(5,6,7,8-tetrehydrobenzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-carboxylic acid **1f** (1g, 0.0033 mol) in DMF (5ml) was added EDC.HCl (0.95g, 0.0050 mol) and R-aniline (0.306g, 0.0033 mol) followed by the HOBt (0.443g, 0.0033 mol). Stirring was continued for 6h. Quenched the reaction mixture with water (50 ml), solid was comes out was filtered, dried and washed with diethyl ether to gave compounds (**1-7)** as a solid product (**Scheme 2**).

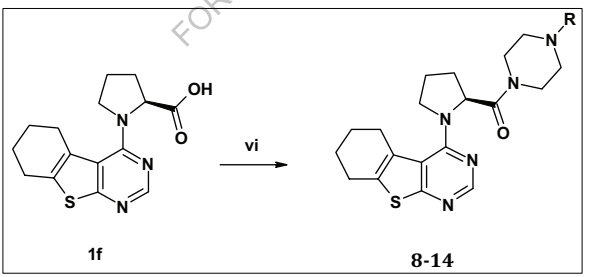
Table 3: Physical & ES-MS (*m/z*) data of synthesized compounds (1-7)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
1		$C_{21}H_{22}N_4OS$	379.3	66	127-129
2		$C_{22}H_{24}N_4O_2S$	393.3	66	136-138
3		$C_{22}H_{24}N_4O_2S$	408.3	67	135-137

4		$C_{21}H_{21}ClN_4OS$	413.4	67	139-141
5		$C_{21}H_{21}FN_4OS$	397.3	65	131-133
6		$C_{23}H_{26}N_4O_2S$	422.3	70	143-145
7		$C_{21}H_{23}N_5O_3S_2$	457.3	63	180-182

Scheme 3

Synthetic route for ***Piperazin-1-yl-[1-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidin-2-yl]-methanone*** **(8-14)**



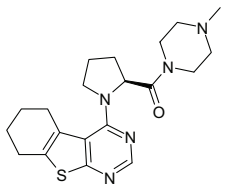
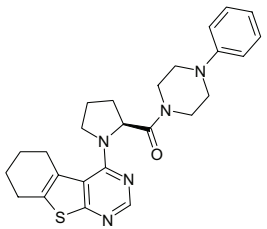
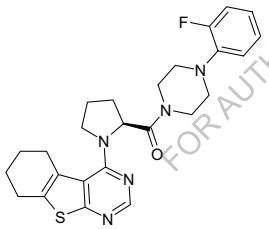
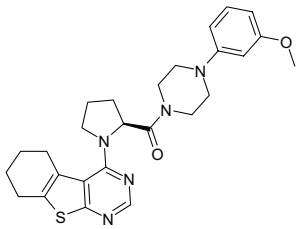
vi) R-Piperazine, EDC.HCl, HOBt, DMF, RT

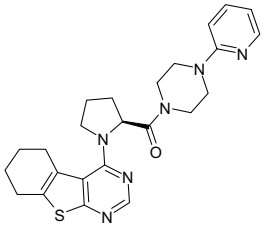
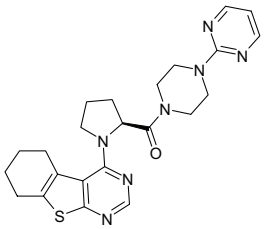
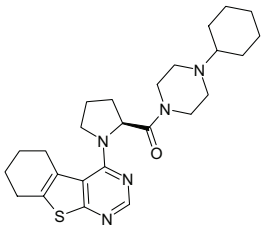
General Procedure

To a solution of 1-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-carboxylic acid **1f** (1 g, 0.0033 mol) in DMF (5 ml) was added EDC.HCl (0.950g, 0.0050 mol) and R- piperazine (0.331g, 0.0033 mol) followed by the HOBt (0.443mg, 0.0033 mol). The reaction mixture was stirred for 6h and was quenched with water (50 ml), solid that

comes out was filtered; dried and washed with diethyl ether to give compounds (**8-14)**; as a solid product. (**Scheme 3**)

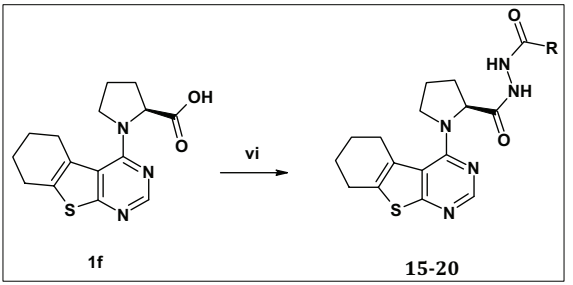
Table 4: Physical & ES-MS (*m/z*) data of synthesized compounds (8-14)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
8		$C_{20}H_{27}N_5OS$	386.1	62	80-82
9		$C_{25}H_{29}N_5OS$	448.4	62	86-88
10		$C_{25}H_{28}FN_5OS$	466.4	62	83-85
11		$C_{26}H_{31}N_5O_2S$	478.2	57	89-91

12		**$C_{24}H_{28}N_6OS$**	448.3	57	90-92
13		**$C_{23}H_{27}N_7OS$**	450.3	87	88-90
14		**$C_{25}H_{35}N_5OS$**	**454.2**	73	102-104

Scheme 4

Synthetic route for ***1-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine -2-carboxylic acid N'-aryl-hydrazide*** **(15-20)**

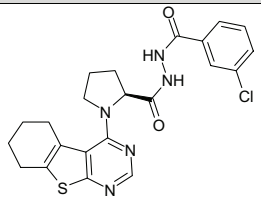
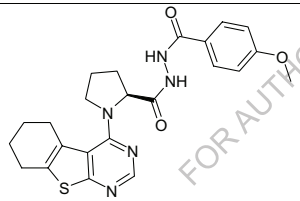
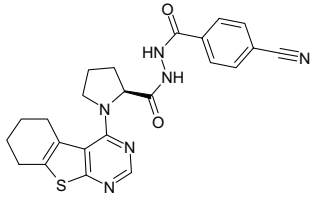
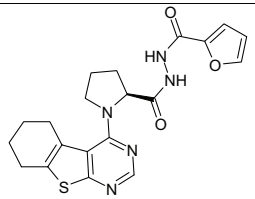


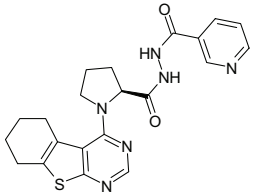
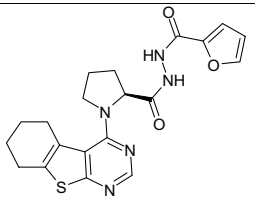
vi) R-Hydrazides, EDC.HCl, HOBt, DMF, RT

General Procedure

To a solution of 1-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d] pyrimidine-4-yl)-pyrolidine-2-carboxylic acid 6 (1g, 0.0033 mmol) in DMF (5 ml) was added EDC.HCl (0.950g, 0.0050 mol) and R-acid hydrazide (0.561 g, 0.0033 mol) followed by the HOBt (0.443g, 0.0033 mol). The reaction mixture was stirred for 6 h and quenched with water (50 ml); the off-white colour solid that came out was filtered, dried, and washed with diethyl ether to gave compounds (**15-20**) as a solid product (**Scheme 4**).

Table 5: Physical & ES-MS (*m/z*) data of synthesized compounds (15-20)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
15		$C_{22}H_{22}ClN_5O_2S$	455.3	87	115-117
16		$C_{23}H_{25}N_5O_3S$	451.3	74	89-91
17		$C_{23}H_{22}N_6O_2S$	446.2	61	117-119
18		$C_{20}H_{21}N_5O_3S$	412.2	84	118-120

19		$C_{21}H_{22}N_6O_2S$	422.2	76	141-143
20		$C_{20}H_{21}N_5O_2S_2$	428.2	85	122-124

2.1.2.2. Evaluation of Anticancer Activity

Secondary amines such as substituted piperazine and primary amines such as substituted anilines were selected to evaluate the structure activity relationship among the novel analogue. Hydrazide derivatives containing aromatic hydrazide were also studied for anticancer activities. A substituted aniline derivative often improves the activity of different pharmacophores and hence plays an important role for various SAR studies. It was also found that these substituted aniline derivatives could be potential anticancer agents [Gefitinib (*Iressa*)] **Fig 4**. With the above facts, here we have designed and prepared seven novel compounds, (**1-7**). The variation was brought across acid core **1f** with five different anilines to get the desired derivatives (**1-7**). In the above series, 4-Fluoro aniline compound **5** has shown remarkable inhibitory activity in MCF-7 cell line (GI_{50}<0.1µmol/L) and good inhibitory activity in HCT-15 cell line (GI_{50}=56.9µmol/L). All the other aniline derivatives showed very poor inhibitory activity in HCT-15 cell line. 4-Chloro aniline **4** revealed inferior activity in MCF-7 cell line than the 4-Fluoro aniline compound **5.** 4-Methoxy aniline **3** found to show better activity than 4-Methyl aniline **2** inMCF-7 cell line, for HCT-15 cell line both are equally inferior. Here, Electron withdrawing group found to improving the potential of inhibiting the anticancer activities in MCF-7 cell line; therefore it was observed that compound **5** and compound **3** are superior to compound **4** and compound **2** respectively. Only compound **4** and compound **5** were showed notable contribution among the compounds **1-5** in Human Colon Cancer Cell Line HCT-15. In conclusion, between the two studied cell lines, compound **5** was found to be potent for MCF-7 cell line.

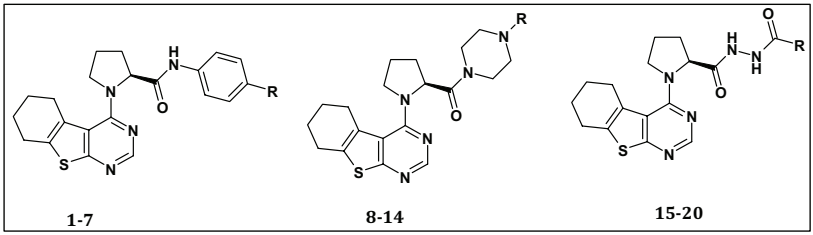
Table 5: Study of compounds on *Human Breast Cancer Cell Line* MCF-7 at 10^{-7} to 10^{-4} molar Drug Concentrations

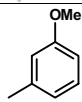
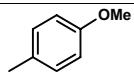
Human Breast Cancer Cell Line MCF-7				
% Control Growth				
Average Molar Drug Concentrations				
Comp. No.	**10^{-7}M (SD)**	**10^{-6}M (SD)**	**10^{-5}M (SD)**	**10^{-4}M (SD)**
1	88.7 (±2.35)	86.4 (±2.35)	80.5 (±4.13)	71.9 (±2.61)
2	92.3 (±6.66)	101.6 (±2.85)	97.8 (±2.92)	41.1 (±2.63)
3	101.1 (±2.46)	101.9 (±1.63)	92.1 (±2.99)	15.9 (±6.74)
4	80.7 (±2.57)	65.7 (±1.26)	49.8 (±2.42)	38.1 (±1.60)
5	**56.4 (±0.7)**	**49.9 (±2.83)**	**41.1 (±3.96)**	**38.2 (±4.63)**
8	90.3 (±3.93)	96.7 (±6.21)	97.0 (±7.91)	69.5 (±2.25)
9	70.8 (±2.70)	55.6 (±1.70)	39.9 (±2.30)	27.8 (±1.45)
10	74.2 (±1.70)	57.2 (±2.20)	38.1 (±2.74)	16.4 (±3.74)
11	85.7 (±2.53)	91.5 (±2.66)	87.9 (±2.74)	31.3 (±2.56)
12	74.1 (±3.09)	57.1 (±3.32)	38.4(±5.08)	16.2 (±3.66)
13	**67.5 (±1.45)**	**44.0 (±2.75)**	**17.0 (±2.02)**	**14.0 (±3.72)**
15	89.8 (±1.26)	94.7 (±3.63)	97.0 (±7.25)	59.1(±3.96)
16	105.5 (±2.55)	111.5 (±2.85)	111.1 (±7.51)	51.3 (±3.03)
17	90.4 (±1.85)	89.2 (±6.92)	97.9 (±8.41)	55.5 (±5.50)
18	111.1 (±2.36)	115.3 (±6.44)	102.1 (±3.08)	25.9 (±6.91)
20	89.1 (±4.10)	90.4 (±6.32)	95.2 (±2.92)	56.0 (±4.77)
ADR	36.1 (±20.42)	23.7(±16.02)	-9.6 (±9.42)	-45.4 (±10.51)

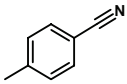
Table 6: Study of compounds on *Human Colon Cancer Cell Line* HCT-15 at 10^{-7} to 10^{-4} molar Drug Concentrations

Human Colon Cancer Cell Line HCT-15				
% Control Growth				
Average Molar Drug Concentrations				
Compound No.	**10^{-7}M (SD)**	**10^{-6}M (SD)**	**10^{-5}M (SD)**	**10^{-4}M (SD)**
1	115.3(±4.33)	114.7(±5.00)	107.6 (±4.85)	75.1 (±1.73)
2	117.7 (±5.99)	118.5 (±1.08)	114.2 (±0.97)	76.5 (±1.42)
3	118.8 (±4.85)	119.3 (±3.60)	111.5 (±6.45)	66.9 (±10.56)
4	111.1 (±2.46)	111.9 (±1.63)	102.1 (±2.99)	25.9 (±6.74)
5	108.2 (±4.95)	111.9 (±4.40)	89.0 (±2.19)	7.6 (±4.23)
8	116.0 (±5.46)	113.8 (±4.51)	108.0(±3.62)	77.3(±0.96)
9	108.1(±12.88)	111.1 (±5.13)	87.2 (±3.04)	24.0 (±1.30)
10	104.8 (±5.70)	109.2 (±4.13)	89.5(±3.06)	5.9 (±1.93)
11	119.1 (±6.86)	114.2 (±7.06)	112.4 (±8.86)	75.0 (±1.51)
12	111.3 (±2.05)	115.7 (±5.89)	102.5 (±4.25)	26.8 (±5.33)
13	99.7 (±3.16)	101.6 (±0.66)	90.8 (±4.02)	18.5 (±6.59)
15	116.3 (±0.90)	112.9 (±1.94)	107.0 (±1.25)	78.6 (±2.11)
16	111.3 (±6.66)	107.2 (±5.15)	100.5 (±1.17)	63.9 (±9.52)
17	119.9 (±5.45)	114.6 (±6.20)	111.7 (±8.87)	74.0 (±3.02)
18	117.5 (±4.13)	118.7 (±3.92)	109.7 (±6.55)	62.5 (±11.01)
20	121.6 (±6.86)	119.2 (±6.90)	109.2 (±9.32)	72.2 (±1.30)
ADR	1.9 (±30.92)	44.2 (±3.91)	-8.1 (±17.78)	-33.2 (±3.46)

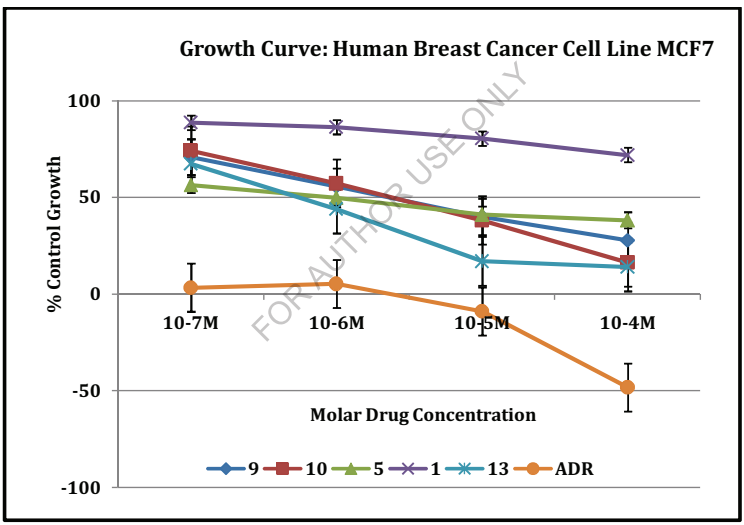
Table 8: GI_{50} of the tested compounds against human tumour cell line



Compounds	R	GI_{50} (µmol/L)	
		Breast MCF-7	Colon HCT-15
1	H	>100	>100
2	$-CH_3$	78.5	>100
3	$-OCH_3$	61.4	>100
4	-Cl	32.2	71.8
5	-F	**< 0.1**	56.9
8	$-CH_3$	>100	>100
9		24.0	67.3
10		20.8	55.2
11		68.7	>100
12		20.3	71.8
13		**< 0.1**	63.5
15		>100	>100
16		88.7	>100

17		>100	>100
18		71.8	>100
20		>100	>100
Reference	***Doxorubicin***	**< 0.1**	**< 0.1**

Secondary nitrogen side chains on the quinazoline moiety [Tandutinib (*MLN518*)] **Fig 4**. exhibits antitumor potency when piperazine was coupled with aniline, to get the terminal urea. In order to enhance the activity here we have modified our synthetic approach and accordingly prepared piperazine 4-substituted amide derivatives **8-14**from same acid core **6**.



Graph 1: % Control Growth Vs Average Molar Drug Concentrations

In our SAR study, 4-pyridinyl piperazine compound **12**, showed intermittent activities in both the cell lines correspond to 4-pyrimidinyl piperazine compound **13** and 4-phenyl piperazine compound **9**. Insertion of the Nitrogen atom in the aromatic ring attached to the piperazine ring increases the activity of compound **9** from (GI_{50}=24.0 µmol/L) to compound **12**(GI_{50}=20.3 µmol/L) for MCF-7 cell line and the trend continues still increase in inhibitory activity reaches to remarkable (GI_{50} < 0.1 µmol/L) value in compound **13**. Alkyl substituted piperazine derivative **8** could not make to contribute towards the inhibitory activity as

compare to aryl substituted piperazine derivatives **9**, **10** and **11**. Between the substituted phenyl piperazine derivatives 4-(2-fluoro phenyl) piperazine compound **10** and 4-(3-methoxy phenyl) piperazine compound **11**, compound **10** is more superior to compound **11**. Electron withdrawing group increases inhibitory activity of **10** and it is also comparable to compound **11** in MCF-7 cell line.

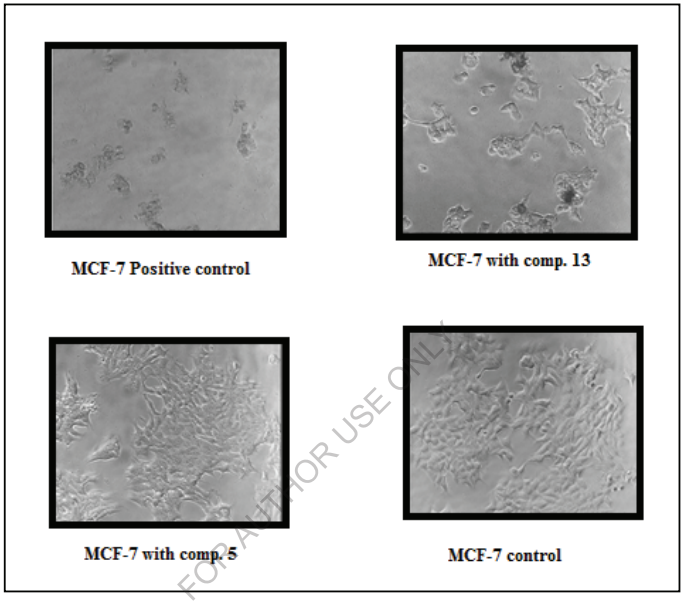


Fig.24: Images of anticancer activities of 5 and 13

For Human Colon Cancer Cell Line HCT-15 activities it was found that compound **10** (GI_{50}= 55.7 µmol/L) is the good inhibitor among the **8-13**. Compound **9** (GI_{50}= 67.3 µmol/L) is inferior to **10**, because the electron withdrawing fluoro group at second position of the phenyl ring was present in **10**. Compound **12** and compound **13** followed the activity trend as they were followed for the MCF-7 cell line. Compound **13** was superior to compound 8e in HCT-15 cell line.

Hydrazide side chains attached to the acid **6** was observed very poor in showing anticancer activities in Human Colon Cancer Cell Line HCT-15. All the synthesized compounds **15-20** was reportedly showed (GI_{50} >100 µmol/L). Compound **16** (GI_{50}= 88.7 µmol/L) and compound **18** (GI_{50} = 71.8 µmol/L) exhibiting the anticancer activities for the human Breast CancerMCF-7 cell line. Compound **16** contains 4-Methoxy benzoic acid hydrazide side

chain and compound **18** was with 2-Furoic acid hydrazide side chain. Compounds **16** and **18** have an oxygen atom present in hydrazide analogue and it might be resemblance to phenolic and ethereal type of oxygen containing Erlotinib (**Fig 4**) to show some activities.

Between the three series of compounds containing substituted anilines **1-7**, substituted piperazine **8-14** and hydrazide side chains **15-20** it was found that secondary substituted piperazine derivatives are exhibiting good inhibitory in both the MCF-7 and HCT-15 cell lines. Primary substituted anilines resulted in improved activities in MCF-7 cell line than HCT-15 cell line and hydrazide compounds were very poor in both the MCF-7 and HCT-15 cell lines.

2.1.3. EXPERIMENTAL

2.1.3.1. Chemistry

***Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate* (1b)**

In to a mixture of Cyclohexanone **1a** (49g, 0.5mol), ethyl 2-cyanoacetate (56g, 0.5mol,) and sulphur (16g, 0.5mol) in 150ml of ethanol was added morpholine (44g, 0.5mol). The mixture was stirred for 8 hr at room temperature. The reaction mixture was diluted with water and the precipitate was collected by filtration and recrystallized from ethanol. **1b** as yellow solid (62g, 55%) mp = 115°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.94 (s, 2H, Ar-N$\underline{H}_2$), 4.25 (q, 2H, J = 7 Hz, -O-C$\underline{H}_2$), 2.69-2.60 (m, 2H, -CH_2-C$\underline{H}_2$-CH_2-), 2.49-2.43 (m, 2H, -CH_2-C$\underline{H}_2$-CH_2-), 1.76-1.66 (m, 4H, -CH_2-C$\underline{H}_2$-CH_2-), 1.33 (t, 3H, J = 7 Hz, -OCH_2-C$\underline{H}_3$); ES-MS: m/z 226.2 $(M+H)^+$.

***5, 6, 7, 8-Tetrahydrobenzo [4, 5] thieno [2, 3-d] pyrimidin-4(3H)-one* (1c)**

The mixture of compound **1b** (35g, 0.16 mol) in 150 ml of formamide was heated at 180°C for 4h and then cooled down to room temperature. The mixture was poured into 200 ml water and filtered. The solid was collected and recrystallized from ethanol. Compound **1c** as yellow solid (25 g, 75%); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.31 (br s, 1H, -CO-N$\underline{H}$-), 8.00 (s, 1H, =C$\underline{H}$-N-), 2.85-2.88 (m, 2H,CH_2-C$\underline{H}_2$-CH_2-), 2.72-2.75 (m, 2H,-CH_2-C$\underline{H}_2$-CH_2-), 1.75-1.82 (m, 4H, -CH_2-C$\underline{H}_2$-CH_2-); ES-MS: m/z 207.2 $(M+H)^+$.

***4-Chloro-5,6,7,8-Tetrahydrobenzo [4,5]thieno[2,3-d]pyrimidine* (1d)**

A suspension of compound **1c** (25g, 0.12 mol) in 150 ml of $POCl_3$ was heated at reflux for 2h. $POCl_3$ was removed at reduced pressure and the residue was poured onto ice and filtered. The solid was washed with water and dried. Compound **1d** as brown solid (23g, 85%); ^{1}H

NMR ($CDCl_3$, 400 MHz):δ 8.72(s,1H, -N=C<u>H</u>-N-), 3.10-3.12 (m, 2H,CH_2-C<u>H_2</u>-CH_2-), 2.88-2.90 (m, 2H,CH_2-C<u>H_2</u>-CH_2-), 1.92-1.95 (m, 4H, -CH_2-C<u>H_2</u>-CH_2-); ES-MS: *m/z* 225.3 $(M+H)^+$.

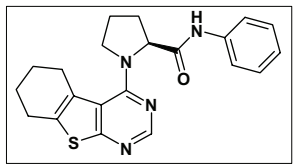
1-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine-2-carboxylicacid ethyl ester (1e)

To a clear solution of compound **1d** (20 g, 0.089 mol) in Methanol 200 ml was added L-Proline ethyl ester hydrochloride (16 g, 0.089 mol) and triethylamine (27 ml, 0.267 mol), stirred the reaction mixture for 3h. Methanol was removed under reduced pressure and residue was taken in EtOAc, washed with water, 1N HCl solution in water and saturated $NaHCO_3$ solution in water. Collected Organic layer was dried over Na_2SO_4 and removed under reduced pressure to yield Ester intermediate **1e** as a yellowish gel (25 g, 84%). This was used for without further purification. ES-MS: *m/z* 332.3 $(M+H)^+$.

1-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine-2-carboxylic acid (1f)

A suspension of ester compound **1e** (25g, 0.075 mol) in 225 ml THF and 25 ml water was added Lithium hydroxide monohydrate (4.2 g, 0.11 mol) at 0°C, and the reaction mixture for 12 h. Distilled out THF under vacuum and to the remaining aqueous residue was added 1N HCl solution in water to adjusted the solution P^H = 4, solid was precipitates out. Filtered the solid and dried Compound **1f** as yellowish solid (16 g, 70%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.23(s, 1H, Pyrimidine-<u>H</u>), 4.80 (t, 1H, *J* = 8 Hz, N-C<u>H</u>-CO), 3.82-3.89 (m, 2H, -N-C<u>H_2</u>-CH_2-), 2.80-2.86 (m, 4H, CH_2-C<u>H_2</u>-C<u>H_2</u>-), 1.75-1.98 (m, 6H, CH_2-C<u>H_2</u>-CH_2-), 1.49-1.50 (m, 2H, CH_2-C<u>H_2</u>-CH_2-); ES-MS: *m/z* 302.2 $(M+H)^-$.

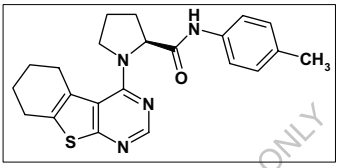
1-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-carboxylic acid phenyl-amide (1)



off white solid (0.82g, Yield= 66%); mp= 127-129°C; ^{1}H NMR(DMSO-d_6, 400 MHz) δ 10.0 (s, 1H, -CO-N<u>H</u>-), 8.21 (s, 1H, Pyrimidine-<u>H</u>), 7.59 (d, *J* = 8 Hz, 2H, Ar-<u>H</u>), 7.27 (t, 2H, *J* =

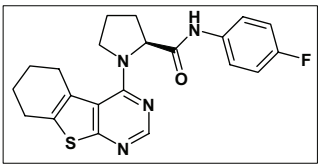
8 Hz, Ar-H),7.0-7.02 (t, 1H, *J* = 7.6 Hz, Ar-H), 4.96 (t, 1H, *J* = 7.6 Hz, N-CH-CO), 3.92-3.96 (m, 1H,-Ar-CH_2-CH_2-), 3.69 (t, 1H, *J* = 8 Hz, -N-CH_2-CH_2-), 2.85-3.01 (m, 4H, -CH_2-CH_2-CH_2-), 2.33-2.38 (m, 1H, -CH_2-CH_2-CH_2-), 1.72-1.98 (m, 6H, -CH_2-CH_2-CH_2-), 1.49-1.51(m, 1H, -CH_2-CH_2-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 22.3, 22.9, 25.3 , 25.4, 28.6, 29.8, 53.8, 62.6, 117.2, 119.3, 119.3, 123.1, 127.8, 128.6, 128.6, 131.8, 139.1, 150.4, 157.5, 167.1, 170.7; ES-MS: *m/z* 379.3(M+H) $^+$; Anal. Calcd. for **$C_{21}H_{22}N_4OS$**; C, 66.64; H, 5.86; N, 14.80;Found: C, 66.61; H, 5.89; N, 14.82.

***1-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-carboxylic acid p-tolylamide* (2)**



brownish solid (0.85g, Yield= 66%); mp= 136-138°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.9 (s, 1H, -CO-NH-), 8.12 (s, 1H, Pyrimidine-H), 7.46 (d, *J* = 9.6 Hz, 2H, Ar-H), 7.08 (d, 2H, *J* = 8.4 Hz, Ar-H), 4.95 (t, 1H, *J* = 7.2 Hz, N-CH-CO), 3.89-3.94 (m, 1H, Ar-CH_2-CH_2-), 3.68 (t, *J* = 7.6 Hz, 1H, -N-CH_2-CH_2-), 2.85-2.97 (m, 4H, -CH_2-CH_2-CH_2-), 2.32-2.35 (m, 1H, -CH_2-CH_2-CH_2-), 2.0 (s, 3H, -CH_3), 1.75-1.98 (m, 6H, -CH_2-CH_2-CH_2-), 1.47-1.51(m,1H, -CH_2-CH_2-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 20.4, 22.3, 22.9 , 25.4, 25.8, 28.6, 29.5, 53.8, 62.5, 117.2, 119.3, 119.3, 127.8, 128.9, 128.9, 131.8, 131.9, 136.6, 150.4, 157.5, 167.1, 170.5; ES-MS: *m/z* 393.3 (M+H) $^+$; Anal.Calcd.for **$C_{22}H_{24}N_4O_2S$**; C, 64.68; H, 5.92; N; 13.71; Found: C, 64.66; H, 5.91; N, 13.74.

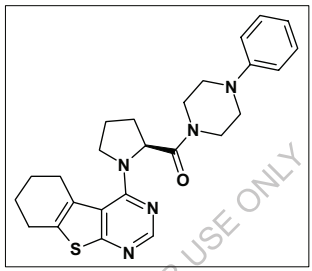
***1-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-arboxylic acid (4-fluoro-phenyl)-amide* (5)**



brownish solid (0.85g, Yield= 65%); mp = 131-133°C, ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.05(s, 1H, -CO-NH-), 8.21 (s, 1H, Pyrimidine-H), 7.59 (d, *J* = 4.8 Hz , 2H, Ar-H), 7.11 (t,

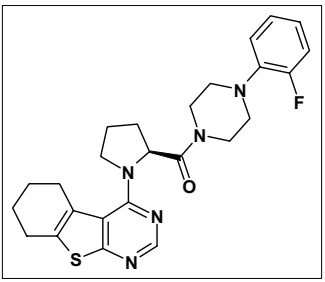
J = 8.4 Hz, 2H, Ar-H), 4.94 (t, 1H, N-CH-CO, *J* = 8.8 Hz), 3.91-3.94 (m, 1H, Ar-CH_2-CH_2), 3.71(t, 1H, *J* = 7.6 Hz, -N-CH_2-CH_2-), 2.85-2.97 (m, 4H, -CH_2-CH_2-CH_2-), 2.32-2.36 (m, 1H, -CH_2-CH_2-CH_2-), 1.74-1.98 (m, 6H, -CH_2-CH_2-CH_2-), 1.50-1.51(m, 1H, -CH_2-CH_2-CH_2-);^{13}C NMR (DMSO-d_6, 100 MHz): δ 22.3, 22.8, 25.3, 25.8, 28.5, 29.8, 53.8, 62.5, 115.0, 115.3, 117.3, 120.9, 121.0, 127.8, 131.8, 135.5, 150.4, 157.5, 159.0, 167.1, 170.6; ES-MS: m/z 397.3 $(M+H)^+$; Anal. Calcd. for **$C_{21}H_{21}FN_4OS$**; C, 63.62; H, 5.34; N, 14.13;Found:C, 63.66; H, 5.37; N, 14.11.

***(4-Phenyl-piperazine-1-yl)-[1-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-yl]-methanone* (7)**



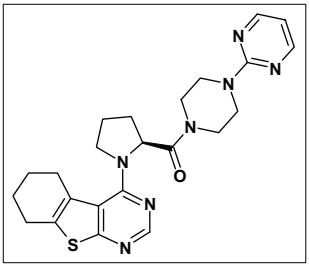
yellowish solid (0.80g, Yield= 62%); mp = 86-88 $^{\circ}$C ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.16 (s, 1H, Pyrimidine-H), 7.23(t, 2H, *J*= 8.4Hz, Ar-H), 6.96 (s, 1H, Ar-H), 6.98 (d, 1H, *J*= 8.4Hz, Ar-H), 6.82 (t, 1H, *J*= 8.4Hz, Ar-H), 5.38(t, 1H, *J*= 8Hz, N-CH-CO), 3.81-3.86 (m, 3H, -CH_2-CH_2-CH_2-), 3.67-3.71(m, 1H, -CH_2-CH_2-CH_2-), 3.60 (br s, 2H, -CH_2-CH_2-CH_2-), 3.28-3.31(m, 2H, -CH_2-CH_2-CH_2-), 3.14-3.17 (m, 2H, -CH_2-CH_2-CH_2-), 3.09 (br s, 1H,-CH_2-CH_2-CH_2-), 2.83-2.88 (m, 3H, -CH_2-CH_2-CH_2-), 2.31-2.32 (m, 1H, -CH_2-CH_2-CH_2-), 1.94-1.99 (m, 3H, -CH_2-CH_2-CH_2-), 1.72-1.99(m, 3H, -CH_2-CH_2-CH_2-), 1.45-1.48 (m,1H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 22.3, 22.8, 25.3 , 25.8, 28.6, 28.6, 41.5, 44.9, 48.5, 48.8, 58.2, 58.2, 115.8, 116.8, 119.3, 127.6, 128.9, 128.9, 131.5, 131.6, 150.4, 150.8, 156.9, 167.1, 170.2; ES-MS: *m/z* 448.4 $(M+H)^+$. Anal. Calcd. for **$C_{25}H_{29}N_5OS$**; C, 67.09; H, 6.53; N, 15.65; Found: C, 67.07; H, 6.57; N,15.67.

***[4-(2-Fluoro-phenyl)-piperazine-1-yl)-[1-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-yl]-methanone* (8)**



yellow solid (1.2g, Yield= 62%), mp = 83-85°C;[1]H NMR (DMSO-d_6, 400 MHz): δ 8.19 (s, 1H, Pyrimidine-H), 7.00-7.18 (m, 4H, Ar-H), 5.37 (t, 1H, *J*= 8Hz, N-CH-CO), 3.82-3.90 (m, 3H), 3.72 (t, 3H, *J*= 6.8Hz, -N-CH$_2$-CH$_2$-), 3.03-3.14 (m, 3H,-CH$_2$-CH$_2$-CH$_2$-), 2.83-2.94 (m, 5H, -CH$_2$-CH$_2$-CH$_2$-). 2.32 (br s, 1H, -CH$_2$-CH$_2$-CH$_2$-), 1.94-1.98 (m, 3H, -CH$_2$-CH$_2$-CH$_2$-), 1.76-1.81(m, 3H, -CH$_2$-CH$_2$-CH$_2$-), 1.47-1.49 (m, 1H,-CH$_2$-CH$_2$-CH$_2$-); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 22.3, 22.8, 25.3 , 25.9, 28.6, 40.2, 41.7, 45.2, 50.2, 50.7, 58.3, 58.3, 115.9, 116.1, 119.6, 119.6, 122.7, 122.8, 124.9, 127.7, 131.8, 150.4, 156.9, 167.1, 170.2; ES-MS: *m/z* 466.4 (M+H)$^+$; Anal. Calcd. for **$C_{25}H_{28}FN_5OS$**; C, 64.49; H, 6.06; N, 15.04; Found: C, 64.51; H, 6.08; N, 15.06.

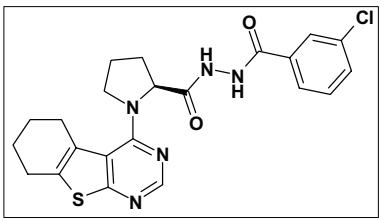
***(4-Pyrimidine-2-yl-piperazine-1-yl)-[1-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrolidine-2-yl]-methanone* (11)**



yellowish solid (1.3g, Yield= 87%); mp= 88-90 °C;[1]H NMR (DMSO-d_6, 400 MHz): δ 8.39 (d, 2H, *J*= 2 Hz, Ar-H), 8.18 (s, 1H, Ar-H), 6.67 (d, 1H, *J*= 4.8 Hz, Ar-H), 5.37 (t, 1H, *J*= 8.4 Hz, N-CH-CO), 3.71-3.91 (m, 8H, CH$_2$-CH$_2$-CH$_2$), 3.54 (br s, 2H,CH$_2$-CH$_2$-CH$_2$), 2.83-2.89 (m, 4H, CH$_2$-CH$_2$-CH$_2$), 2.31-2.35 (m, 1H, CH$_2$-CH$_2$-CH$_2$), 1.91-2.01 (m, 3H, CH$_2$-CH$_2$-CH$_2$), 1.70-1.82 (m, 3H, CH$_2$-CH$_2$-CH$_2$), 1.45-1.49 (m, 1H, CH$_2$-CH$_2$-CH$_2$); ^{13}C NMR

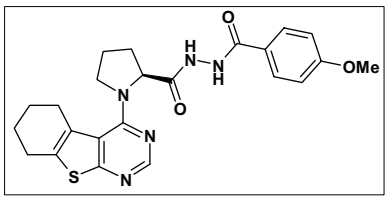
(DMSO-d_6, 100 MHz): δ 22.3, 22.8, 25.3 , 25.9, 28.6, 28.6, 40.2, 41.5, 43.2, 44.7, 53.4, 58.3, 110.4, 116.9, 127.7, 131.5, 150.4, 156.9, 157.9, 157.9, 161.1, 167.1, 170.5; ES-MS: m/z 450.3 $(M+H)^+$; Anal. Calcd. for **$C_{23}H_{27}N_7OS$**; C, 61.45; H, 6.05; N, 21.81; S, 7.13; Found: C, 61.47; H, 6.08; N, 21.83.

3-Chloro-benzoic acid N'-[1-(5,6,7,8,-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrrolidine-2-carbonyl]-hydrazide **(15)**



Off white solid; (1.3g, Yield= 87%); mp = 115-117°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.45 (s, 1H, -NH-NH-), 10.05 (s, 1H, -NH-NH-), 8.26 (s, 1H, Pyrimidine-H), 7.88 (d, 1H, J= 1.6 Hz, Ar-H), 7.81 (d, 1H, J= 8 Hz, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.50-7.54(m, 1H, Ar-H), 4.95 (t, 1H, J= 8 Hz, N-CH-CO), 3.90-3.94 (m, 1H, CH_2-CH_2-CH_2), 3.65-3.69 (m, 1H, CH_2-CH_2-CH_2), 3.11-3.15 (m, 2H, CH_2-CH_2-CH_2), 2.33-2.40 (m, 2H, CH_2-CH_2-CH_2), 1.95-2.02 (m, 4H, CH_2-CH_2-CH_2), 1.74-1.79 (m, 4H, CH_2-CH_2-CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.3, 23.8, 25.3, 25.8, 28.6, 28.6, 52.4, 62.9, 115.9, 116.1, 122.8, 124.9, 127.7, 131.8, 132.5, 134.3, 135.2 151.4, 158.7, 164.3, 167.1, 170.2; ES-MS: m/z 455.3 $(M+H)^+$; Anal. Calcd. for **$C_{22}H_{22}ClN_5O_2S$**: C, 57.95; H, 4.86; N, 15.36; Found: C, 57.93; H, 4.84; N, 15.33.

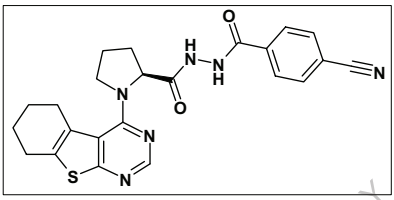
4-Methoxy-benzoicacid N'-[1-(5,6,7,8,-tetrahydro-benzo[4,5]thieno[2,3-]pyrimidine-4-yl)-pyrrolidine-2-carbonyl]-hydrazide **(16)**



(1.1g, Yield= 74 %); mp= 89-91 °C ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.20 (s,1H, -NH-NH-), 9.95 (s, 1H, -NH-NH), 8.14 (s, 1H, Pyrimidine-H), 7.85 (d, 2H, J = 8 Hz, Ar-H), 7.01 (d, 2H, J = 8.4 Hz, Ar-H), 4.95 (t, 1H, J = 8 Hz -N-CH-CO), 3.95 (br s, 1H, CH_2-CH_2-CH_2),

3.80 (s, 3H), 3.77-3.78 (m,1H, CH_2-CH_2-CH_2), 3.06-3.10 (m, 2H,CH_2-CH_2-CH_2), 2.87 (br s, 3H, CH_2-CH_2-CH_2), 2.40 (br s, 1H, CH_2-CH_2-CH_2), 1.77-2.03 (m, 6H, CH_2-CH_2-CH_2) ; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 23.2, 23.8, 25.6, 25.9, 28.3, 28.6, 52.3, 55.9, 62.9, 114.5, 116.1, 123.1, 124.7, 126.8, 127.7, 132.8, 133.5, 151.4, 158.7, 162.2, 164.3, 167.1, 170.2; ES-MS: m/z 451.3 $(M+H)^+$; Anal. Calcd. for **$C_{23}H_{25}N_5O_3S$**: C, 61.18; H, 5.58; N, 15.51; Found: C, 61.15; H, 5.55; N, 15.49.

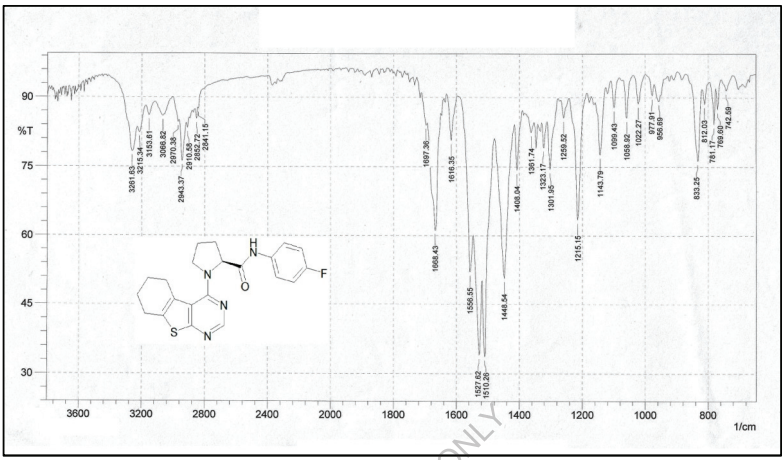
***4-Cyno-benzoic acid N'-[1-(5,6,7,8,-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidine-4-yl)-pyrrolidine-2-carbonyl]-hydrazide* (17)**



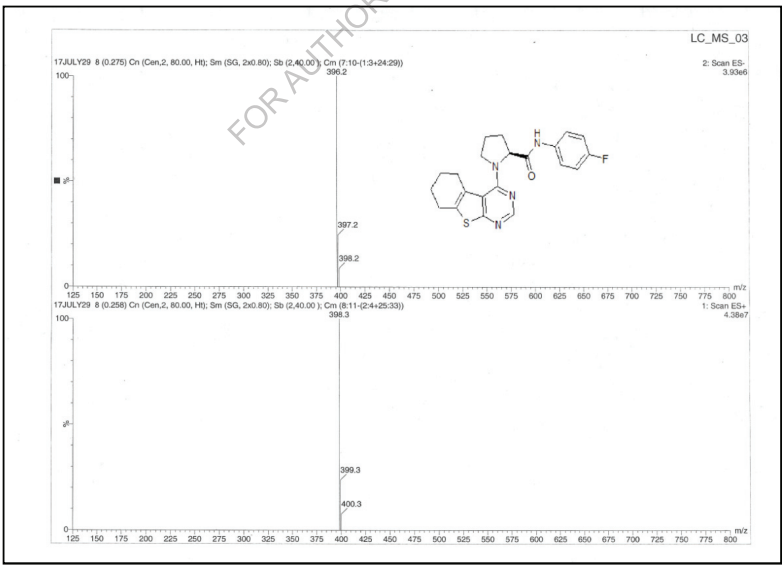
Yellowish solid; (0.9g, Yield= 61 %); mp = 117-119° C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.59 (s, 1H, -NH-NH-), 10.02 (s, 1H, -NH-NH-),8.26 (s, 1H, Ar-H), 7.96-8.08 (m, 4H, Ar-H), 4.95 (t, 1H, J = 8 Hz, N-CH-CO), 3.90-3.96 (m, 1H, CH_2-CH_2-CH_2), 3.67 (t, 1H, J = 8 Hz, CH_2-CH_2- CH_2), 3.11-3.14 (m, 1H, CH_2-CH_2-CH_2),2.85 (br s, 3H, CH_2-CH_2-CH_2), 2.67-2.74 (m, 1H, CH_2-CH_2-CH_2), 2.32-2.40 (m, 1H, CH_2-CH_2-CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 23.3, 24.2, 25.6, 25.9, 29.5, 28.6, 52.3, 62.9, 115.8, 116.1, 123.1, 124.7, 126.8, 127.7, 128.2, 132.8, 133.3, 152.3, 157.8, 162.2, 164.3, 167.1, 170.2; ES-MS: m/z 446.2 $(M+H)^+$; Anal. Calcd. for **$C_{23}H_{22}N_6O_2S$**: C, 61.87; H, 4.97; N, 18.82; Found: C, 61.83; H, 4.94; N, 18.81.

Spectral Data

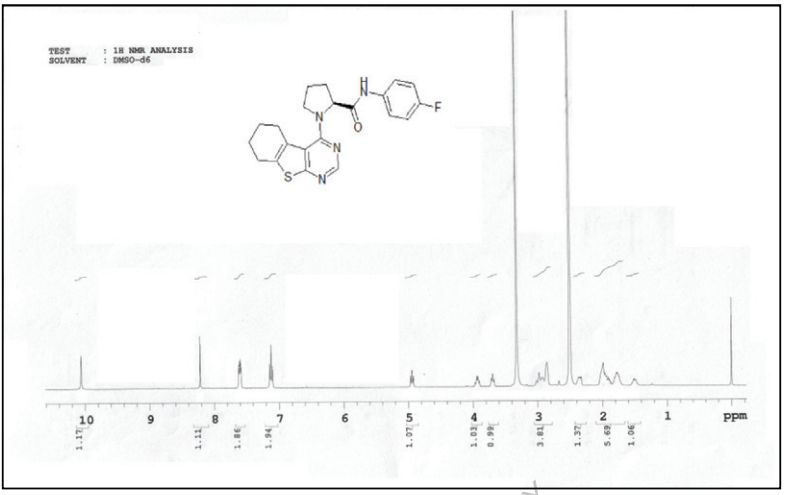
Compound No. 5
IR



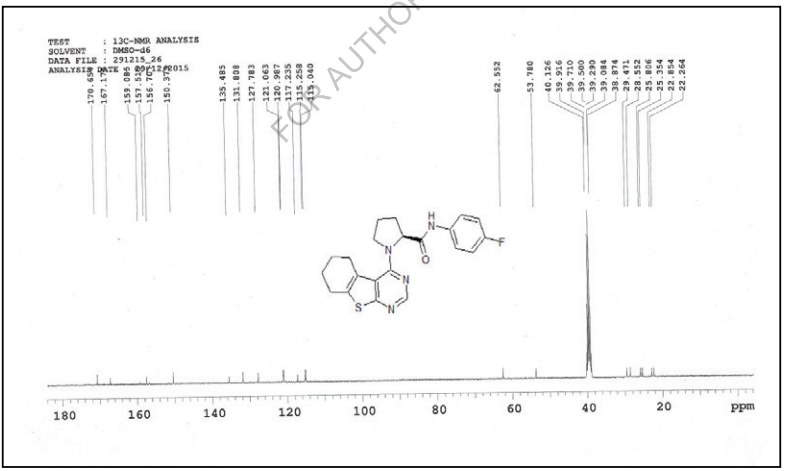
ES-MS



^{1}H NMR (DMSO-d_6, 400 MHz)

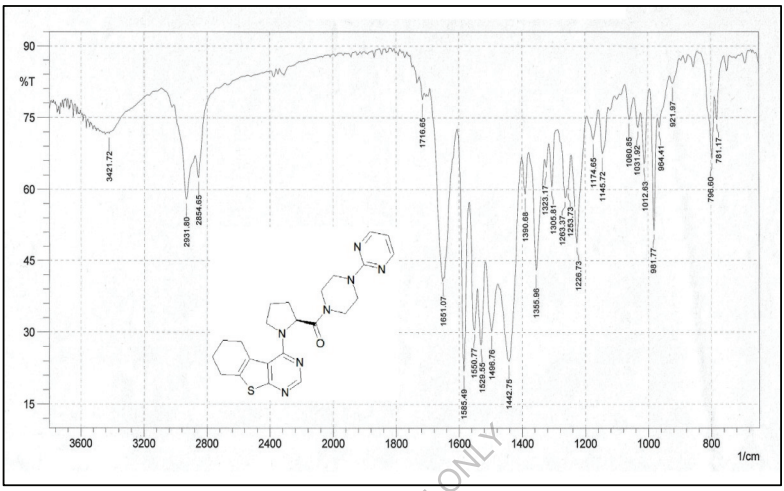


^{13}C NMR (DMSO-d_6, 100 MHz)

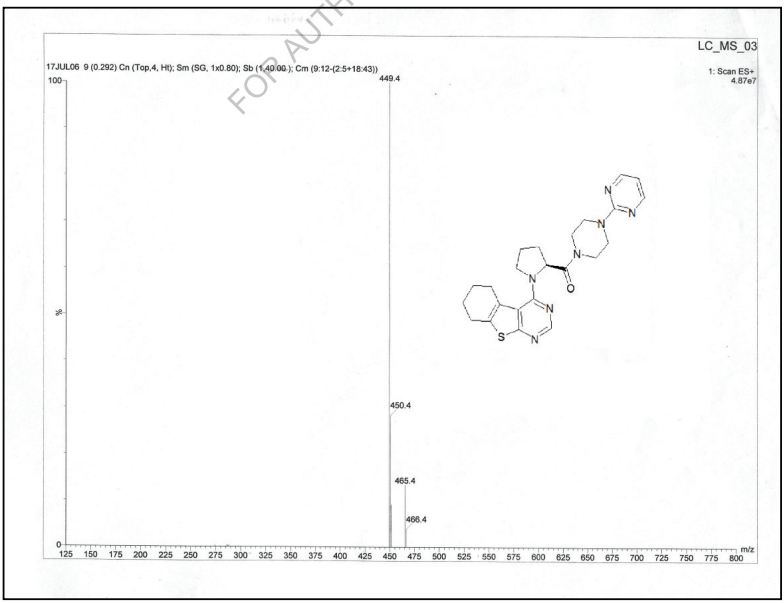


Spectral Data for Compound No. 11

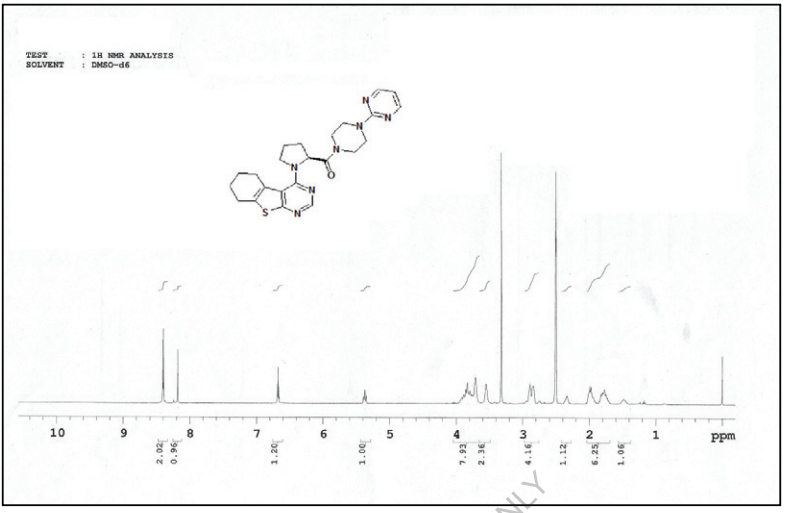
IR



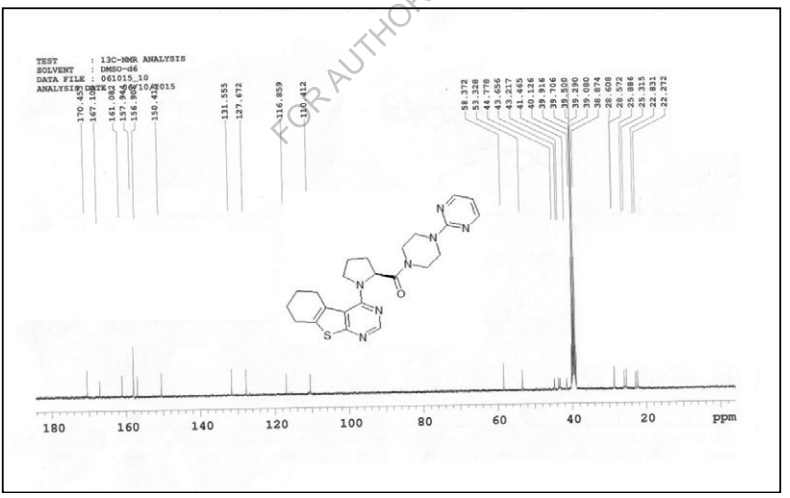
ES-MS



^{1}H NMR (DMSO-d_6, 400 MHz)

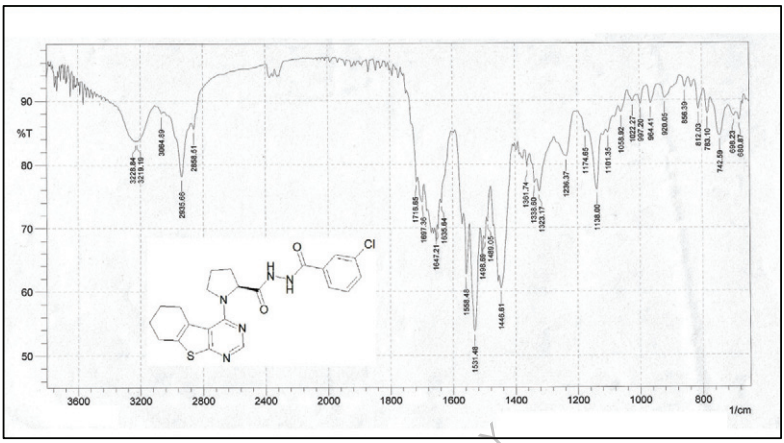


^{13}C NMR (DMSO-d_6, 100 MHz)

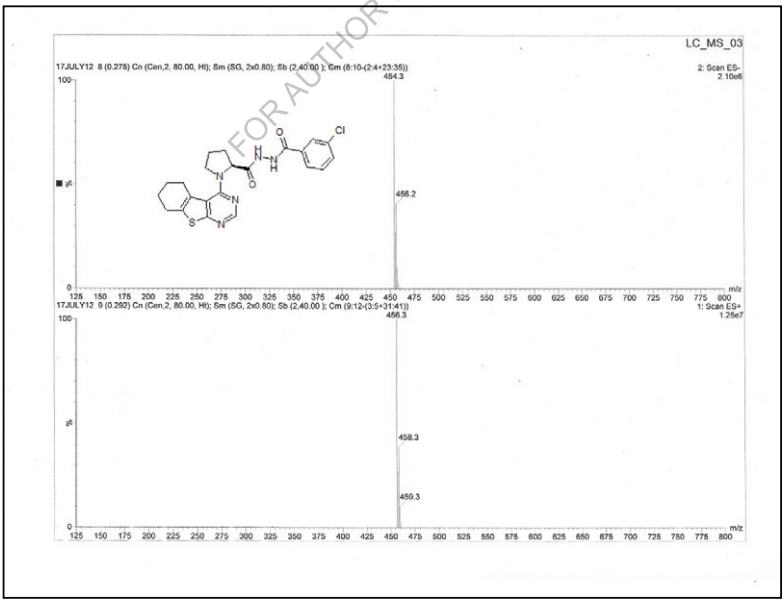


Spectral Data for Compound No. 15

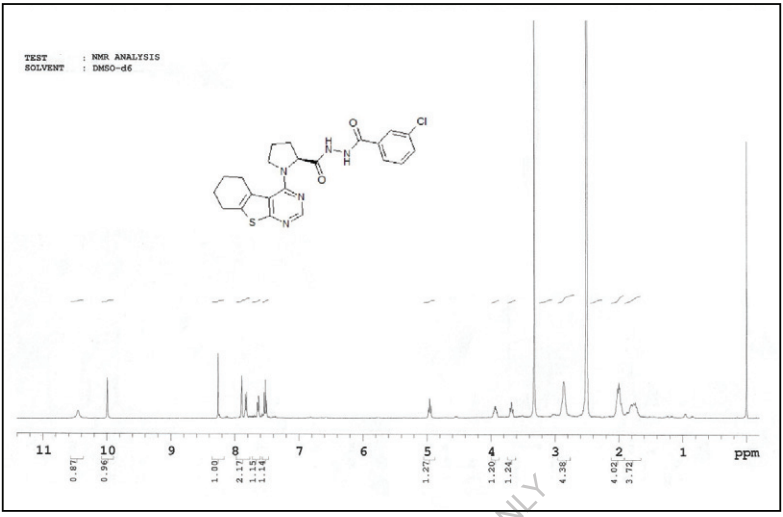
IR



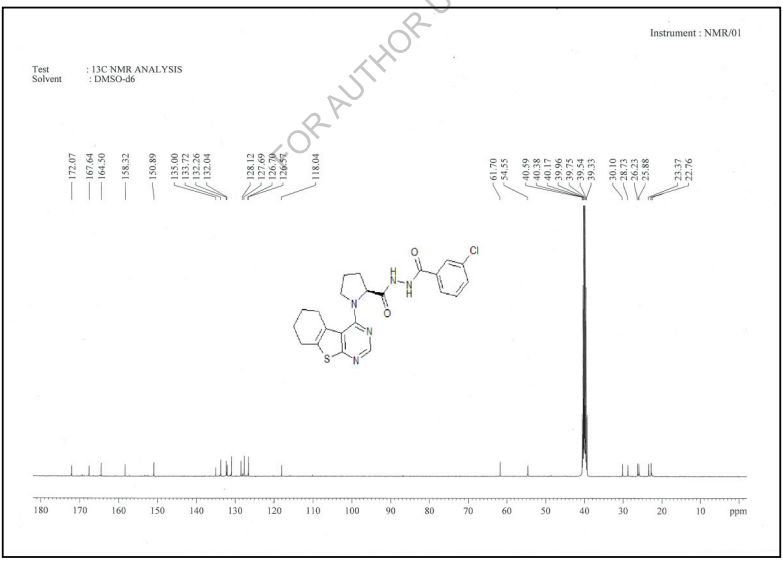
ES-MS



^{1}H NMR (DMSO-d_6, 400 MHz)



^{13}C NMR (DMSO-d_6, 100 MHz)



2.1.3.2. Biology

Antitumor activity

All the prepared target compounds **1-5, 8-13** and **15-20** were screened for their antitumor activities against breast MCF-7 cell line and colon HCT-15 cell lines at Anti-Cancer Drug screening facility (ACDSF), Tata memorial centre, Navi Mumbai.

Experimental procedure for SRB assay [9]

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37°C, 5%CO2, 95% air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent to prepare stock of 10^{-2} concentration. At the time of experiment four 10-fold serial dilutions were made using complete medium. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. The dose response parameters were calculated for each test article. Growth inhibition of 50 % (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured

by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

GI_{50}	Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] × 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
LC_{50}	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] × 100 = -50.

References

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SECTION B

2.2. Synthesis of *(R-phenyl)-[4-(5,6,7,8-tetrahydrobenzo[4,5]-thieno[2,3-d]pyrimidin-4-yl)-benzyl]-amine hydrochloride analogs*

2.2.1. AIM & OBJECTIVES

A number of thieno [2, 3-d] pyrimidine derivatives with different substituent at C-2 and C-4 positions were found to exert potential antitumor activity [1-4]. In this research work we prepared new thieno [2, 3-d] pyrimidines by introducing the 4-Formyl benzene group at C-4 position. We explore this small understanding by condensing substituted aniline with a novel C-4 substituted benzaldehyde core. Synthetic protocols for carbon-carbon bond formation reportedly [5] involve a multi step procedures. These procedures possess disadvantages, such as rigours conditions, long reaction time, complex handling and poor yields. Therefore, developing a facile process to produce C4- substituted thieno [2, 3-d] pyrimidine is necessary. We utilized Suzuki reaction to synthesized novel benzaldehyde core **5**, in more facile way by the use of Tetrakis (triphenylphosphine) palladium (0) and potassium acetate in water-ethanol (8:2) mixture to give 60% yield in 1.5h. Substituted anilines were selected to synthesized target molecules from this novel benzaldehyde core **5**. Intermediate step **ii** involving Schiff's base formation and its reduction were performed in one pot to offered crude product **6**. Target molecules were converted to their hydrochloride salts for the purpose of purity and stability for further studies. (**Scheme 5)**

2.2.2. RESULTS AND CISCUSSION

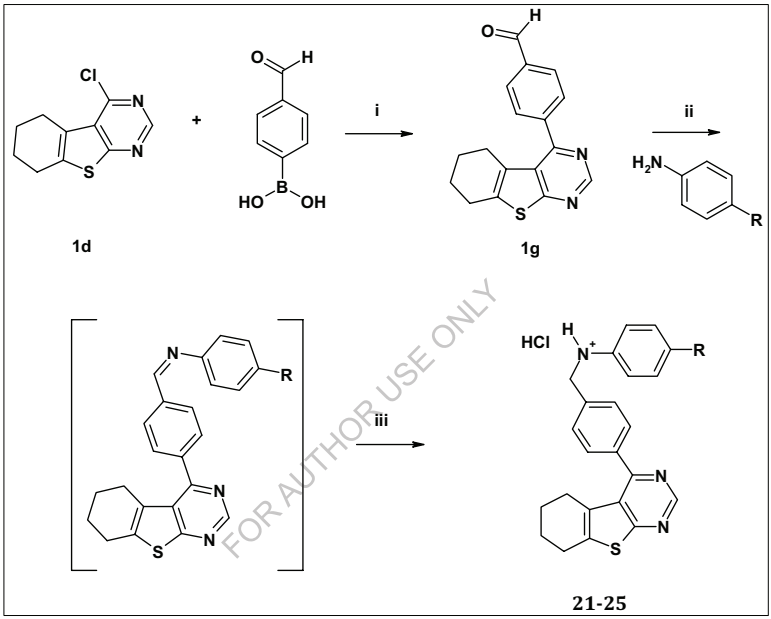
2.2.2.1. Chemistry

As a part of our ongoing research program on the application of Tetrahydrobenzo [4, 5] thieno Pyrimidine, we herein report the synthesis of various [4-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-d]pyrimidinew-4-yl)-benzyl]-amine hydrochlorides **21-25** from, a novel benzaldehyde intermediate **1g**. Synthesis was starts from cyclohexanone **1** by following the known reaction conditions to reach up to the Chloro intermediate **1d (Scheme 5)**. We have shown that Suzuki reaction adds a novel functionality at C-4 position, by using 4-formyl phenyl boronic acid. As a part of this project we were interested in developing a simple synthetic protocol to make large number of C-4 substituted aryl amine hydrochlorides. Herein we report the synthesis of [4-(5,6,7,8-tetrehydro-benzo[4,5]thieno[2,3-

d]pyrimidinew-4-yl)-benzyl]-amine hydrochlorides **21-25**from 4-(5, 6, 7, 8-Tetrahydrobenzo [4, 5] thieno [2, 3-d] pyrimidin-4-yl)-benzaldehyde **1g**.

Scheme 5

Synthetic route for ***Phenyl-[4-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-benzyl]-amine*** **(21-25)**



Reagents and conditions **: i) Pd[0] Tetrakis, $KHCO_3$, H_2O-EtOH, 4-Formyl boronic acid, 80°C ii) R-Aniline, $NaCNBH_3$, iii) MeOH. HCl (4 Molar)**

The targeted compounds were prepared as outlined in **Scheme 5**. The desired 4-chloro derivative **1d** was obtained via the reaction **3** with phosphorous oxychloride in reflux [18] condition. Compound **1g** is obtained by Suzuki coupling reaction [6] of 4-Formyl boronic acid with **1d** in water-ethanol (8:2) combination and Tetrakis (triphenylphosphine) Pd (0) as a catalyst with potassium acetate as a water soluble basic buffer.

^{1}H NMR spectrum of 1g revealed the presence of singlet signal at δ 10.13 ppm corresponding to the aldehyde proton obtained from the 4-formyl boronic acid. A doublet pattern at δ 7.77 & 8.06 ppm of the aromatic protons of phenyl ring. It is also observed that pyrimidine singlet proton of **1d** is shifted from 8.72 ppm to 9.04 in **1g**. Reaction of the 4-(5,6,7,8-tetrahydro-

benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-benzaldehyde derivative **1g** with substituted anilines formed Schiff base in methanol under reflux condition, and on *insitu* reduction with sodium cyanoborohydride gives corresponding crude benzyl amine derivatives, which on treatment with 4M Methanolic HCl formed hydrochloride salt of benzyl amine derivatives **21-25** (**Scheme 5**).

General Procedure

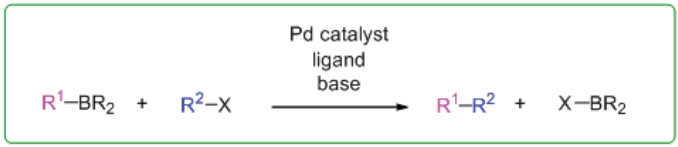
A solution of compound **1g** (1g, 34 mmol) in methanol (10ml) was added R-Aniline (438 mg, 34 mmol) and acetic acid (200 µL), refluxed the reaction mixture for 3h. TLC showed completion of reaction. Cooled the reaction mixture to room temperature and then to -10°C and Sodium cyanoborohydride (60mg, 0.87mol) was added and stirred for 2h. Distilled out volatiles under reduced pressure and product was taken in dichloromethane. Organic layer was washed with water and dried the over sodium sulphate. Evaporated volatiles under reduced pressure to obtained crude yellow gel (900 mg); ES-MS: *m/z* 408.3 $(M+H)^+$. To this was added 4M Methanolic-HCl solutions (5ml) under nitrogen and stirred for 30 min. Solid was precipitates out which was filtered off at Buchner funnel and washed with Diethyl ether to gave compound gave compounds (**21-25)** as a solid product.

^{1}H NMR spectrum of **23** revealed the presence of benzylic singlet at δ 4.42 ppm. Formation of Schiff base using corresponding anilines is monitored by TLC and also with ES-MS. **23** is the purified form corresponding amine intermediate. ^{1}H NMR spectrum also confirmed the formation of hydrochloride salt with broad singlet at δ 5.46 ppm.

Name reactions involved in the synthesis of thienopyrimidine

Suzuki reaction

The **Suzuki reaction** is an organic reaction, classified as a coupling reaction, where the coupling partners are a boronic acid and an organohalide catalyzed by a palladium(0) complex.[1][2][3] It was first published in 1979 by Akira Suzuki and he shared the 2010 Nobel Prize in Chemistry with Richard F. Heck and Ei-ichi Negishi for their effort for discovery and development of palladium-catalyzed cross couplings in organic synthesis.[4] In many publications this reaction also goes by the name **Suzuki–Miyaura reaction** and is also referred to as the **Suzuki coupling**.



The general scheme for the Suzuki reaction is shown below where a carbon-carbon single bond is formed by coupling an organoboron species (R_1-BY_2) with a halide (R_2-X) using a palladium catalyst and a base.

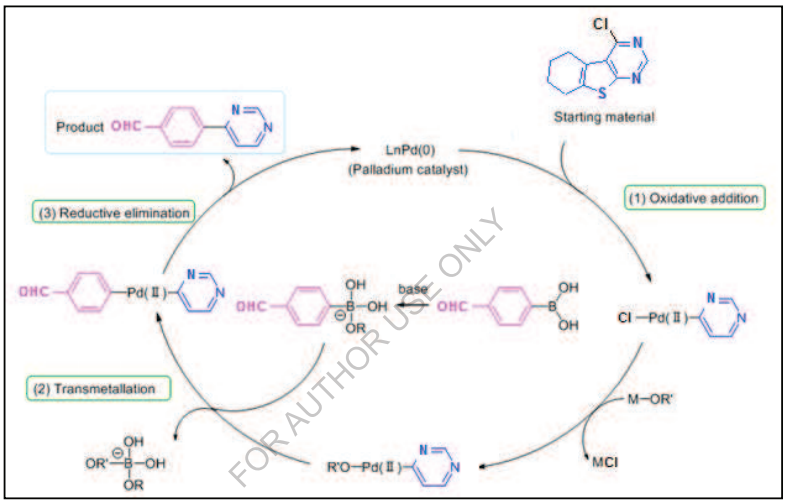
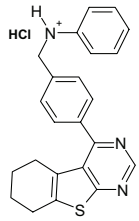
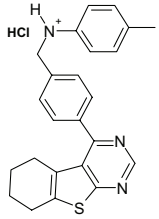
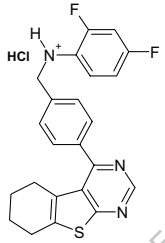
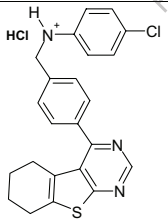
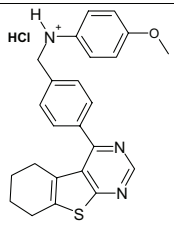


Fig.25: Mechanism of Suzuki reaction

Reaction Mechanism

1. Oxdative addition of palladium to aryl halide or vinyl halide.
2. Ligand change occurs between halide and boron. (transmetallation)
3. Reductive elimination occurs and coupling compound generates.

Table 9: Physical & ES-MS (*m/z*) data of synthesized compounds (21-25)

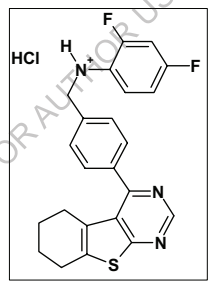
No.	R	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
21		**$C_{23}H_{21}N_3S.HCl$**	372.3	43	177-179
22		**$C_{24}H_{23}N_3S.HCl$**	386.3	45	166-168
23		**$C_{23}H_{19}F_2N_3S.HCl$**	408.3	33	186-188
24		**$C_{23}H_{20}ClN_3S.HCl$**	442.1	48	152-154
25		**$C_{23}H_{23}N_3OS.HCl$**	401.3	67	135-137

2.2.3. EXPERIMENTAL

***Synthesis of 4-(5, 6, 7, 8-Tetrahydrobenzo [4, 5] thieno [2, 3-d] pyrimidin-4-yl)-benzaldehyde* (1g)**

A suspension of compound **1d** (5g, 17 mmol) in Water-Ethanol (40ml-10ml) mixture was bubbled with Nitrogen gas for 30 min. Potassium acetate (2.52 g, 25 mmol) was added under nitrogen followed by the addition of 4-Fotmyl boronic acid (3.75 g, 25 mmol) and Tetrakis (triphenylphosphine) palladium [0] (200 mg, 0.17 mmol). Reaction mixture was heated to 80°C for 1.5h. Filtered off the catalyst at high flow bed, Filtrate was collected and purified by column chromatography using ($CHCl_3$: MeOH::2:8) to offered compound **1g** as off white solid; (3g, Yield= 60%); mp = 155-157°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.13 (s, 1H, Aldehyde-H), 9.04 (s, 1H, Pyrimidine-H), 8.06 (d, 2H, J = 8.4 Hz), 7.77(d, 2H, J = 8.4 Hz), 2.90 (t, 2H, J = 12 Hz, Cycl-CH_2), 2.08 (m, 2H, J = 12 Hz, Cycl-CH_2), 1.79-1.82 (m, 2H, Cycl-CH_2), 1.56-1.59 (m, 2H, Cycl-CH_2); ES-MS: *m/z* 295.1 $(M+H)^+$.

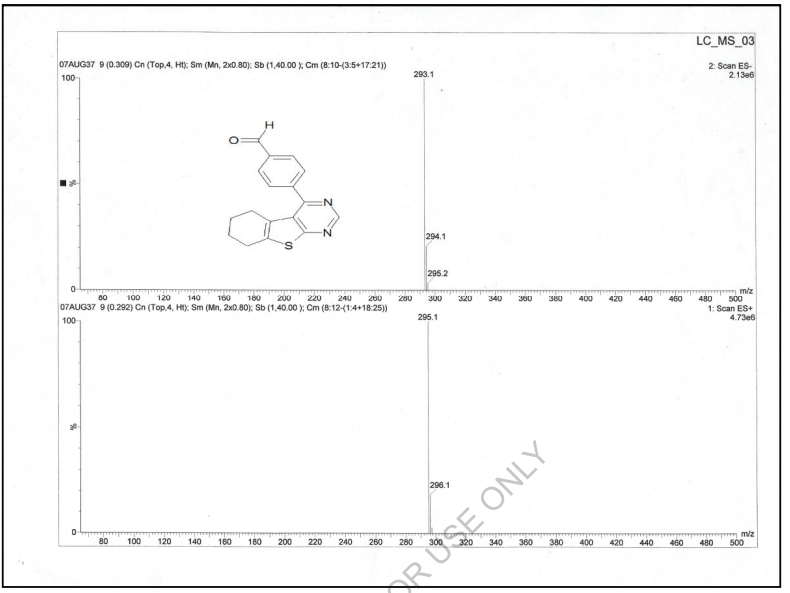
***(2,4-Difluoro-phenyl)-[4-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-benzyl]-aminehydrochloride* (23)**



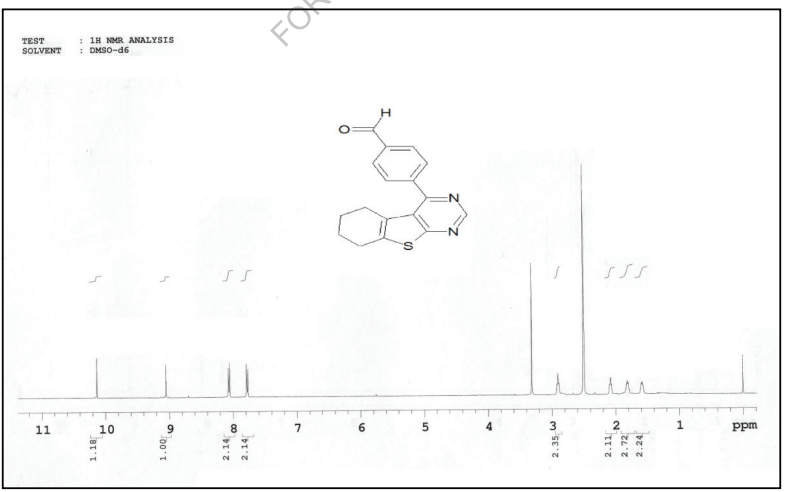
yellowish solid (500 mg 33%); (3g, Yield= 60%); mp = 186-188°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.96 (s, 1H, Pyrimidine-H), 7.44 (m, 4H, Ar-H), 7.04-7.10 (m, 1H, Ar-H), 6.75 (m, 1H, Ar-H), 6.51-6.58 (m, 3H, Ar-H), 5.46 (br s, 2H, HCl salt), 4.23 (s, 2H, -CH_2-Ar), 2.68 (t, 2H, Cycl-CH_2), 2.03-2.04(m, 2H, Cycl-CH_2), 1.77-1.79 (m, 2H, Cycl-CH_2), 1.52-1.54 (m, 2H, Cycl-CH_2) ; ES-MS: m/z 408.3 $(M+H)^+$; Anal. Calcd. for **$C_{23}H_{19}F_2N_3S.HCl$**; C, 67.79; H, 4.70; N, 10.31; S, 7.13; Found: C, 67.81; H, 4.78; N, 10.33.

Spectral Data for Compound No. 1g

ES-MS

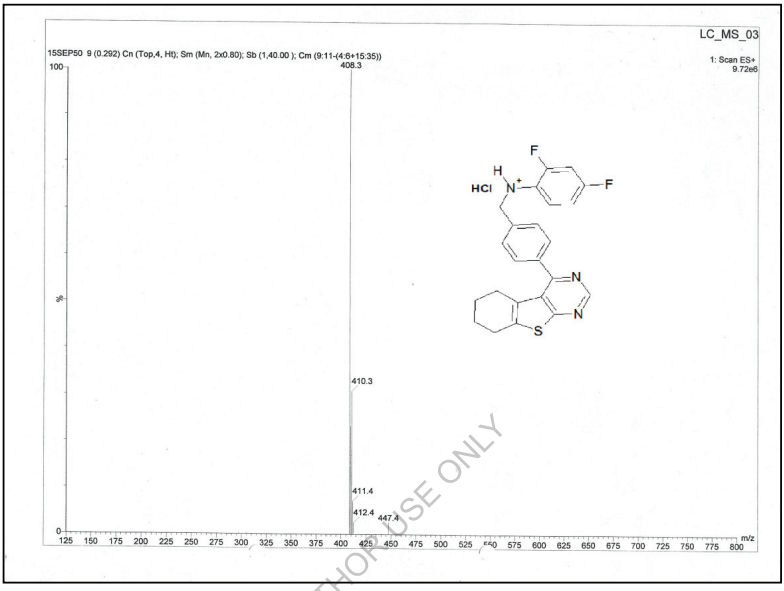


^{1}H NMR (DMSO-d_6, 400 MHz)

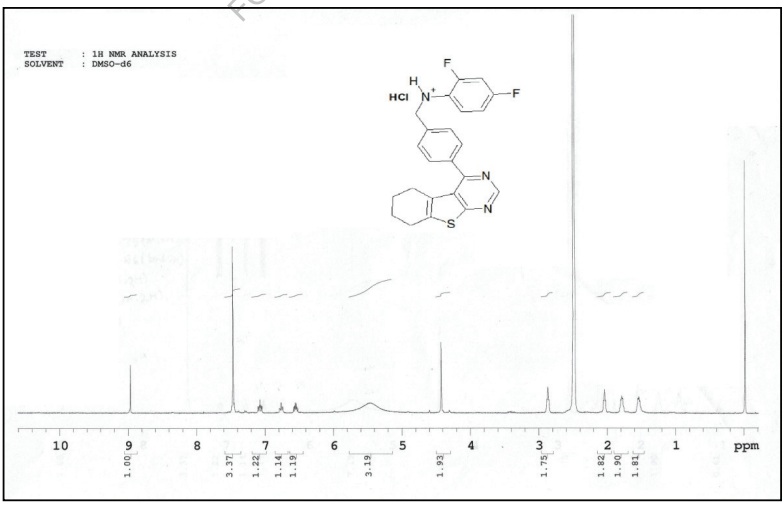


Spectral Data for Compound No. 23

ES-MS



^{1}H NMR (DMSO-d_6, 400 MHz)



References

[1] T. Horiuchi, M.Nagata, M.Kitagawa, K.Akahane, K.Uoto, *Biorg. Med.Chem.Lett*.17 (**2009**) 7850-7860

[2] A.G. Golub, V.G.Bdzohla, N.V.Briukhovetska, et.al. *Eur. J. Med. Chem*. 46(**2011**) 870-876.

[3] T. Beckers, A.Sellmer E.Eichhorn, et.al, *Bioorg.Med.Chem*.20 (**2012**) 125-136.

[4] S.E.Abbas, N.M.A.Gawad, R.F.George, Y.A.Akar, Eur.J.Med.Chem 65 (**2013**)195-204

[5] *J.Heterocycl.Chem*.46(3), 459-464,**2009**

SECTION C

2.3. Synthesis and antibacterial studies of *1-(2,3-Dihydro-1H-8-thia-5,7-diaza-cyclopenta[a]indene-4yl)-pyrrolidine-2-carboxylic acid* derivatives

2.3.1. AIM & OBJECTIVES

In an era of increasing bacterial resistance to classical antibacterial agents. It has been postulated that the development of resistance to known antibiotics could be overcome by identifying new drug targets via genomics, improving existing antibiotics and most importantly by identifying new antibacterial agents with novel structures and mode of action. [1] Pyrimidine derivatives play an imperative role in many transformation and biochemical processes. Pyrimidine and its fused ring system is present in Cytosine, adenine, guanine and thiamine, which form apart of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), vitamins and co-enzymes and other purines. Fused pyrimidine nucleus is used in the discovery of bioactive molecules. [2] Hydrazide and their hetero-cyclic products show evidence of diverse biological activities including antibacterial, antifungicidal, analgesic, antituberculosis, anticancer, anti-inflammatory properties [3-17]. In continuation of our research work on synthesis of thieno pyrimidines, we prepared new thieno[2, 3-d] pyrimidines by introducing a secondary nitrogen containing amino acid L- proline at C-4 position and synthesized structurally different hydrazides to explore the potential of thieno[2,3-d] pyrimidine as antibacterial agents. The synthesized compounds were screened for their antibacterial activities.

2.3.2. RESULTS AND DISCUSSION

2.3.2.1. Chemistry

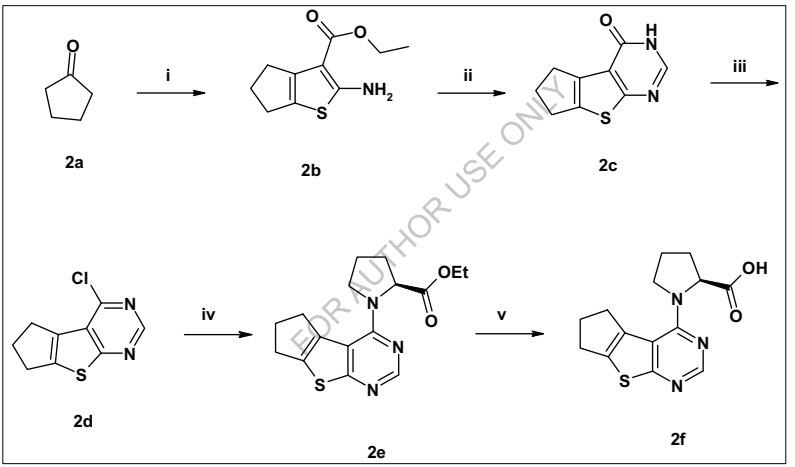
The targeted compounds were prepared as outlined in **Scheme 5** & **Scheme 6**. The starting material Ethyl-2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate **2b** was prepared following the method of Gewald [18-19] via the reaction of Cyclohexanone **2a** and sulfur with ethyl cynoacetate in the presence of morpholine. Cyclization of **2b** to **2c** is adopted using the reported reaction condition by refluxing it in Formamide [20]. The desired 4-chloro derivative **2d** was obtained via the reaction **2c** with phosphorous oxychloride in reflux [21] L-Proline ethyl ester was introduced by replacing Chloro group of thieno [2,3-d] pyrimidine **2d** in Methanol to obtained the Ester intermediate **2e**. Tetrahydrobenzo [4,5]

thieno [2,3-d] pyrimidine-4yl-pyrrolidine-2-carboxylic acid **2f** was obtained after the alkaline hydrolysis of ester intermediate **2e** by using lithium hydroxide.

^{1}H NMR spectrum of **2b** revealed the presence of triplet signal at δ 1.33 ppm and quartet signal at δ 4.25 ppm corresponds to ethyl group of ester, and singlet of two protons at δ 5.83 ppm corresponds to amino group. Similarly ^{1}H NMR spectrum of **2c** was identified by the broad singlet at δ 12.31 and singlet at δ 8.03 ppm of the pyrimidin-4(3H)-one. IR spectrum of **2c** showed bands at 3363, 3383 and 1639 cm^{-1} corresponding to the NH and C=O groups, respectively.

Scheme 6

Synthetic route for ***1-(2,3-Dihydro-1H-8-thia-5,7-diaza-cyclopenta[a]indene-4yl)-pyrrolidine-2-carboxylic acid*** **(2f)**

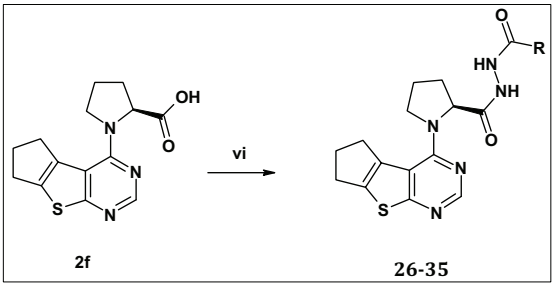


Reagents and conditions: **(i) Ethyl cynoacetate, sulphur, morpholine, EtOH; (ii) HCHO, reflux; (iii) $POCl_3$, reflux (iv) L-proline ethyl ester hydrochloride, Et_3N, MeOH (v) $LiOH.H_2O$, THF: H_2O**

Chloro intermediate **2d** was assigned by the shifted single signal to δ 8.70 ppm. Crude ester intermediate **2e** was used directly for hydrolysis reaction, its formation was confirmed by the ESI-MS spectrum showing ES-MS: *m/z* 318.3 $(M+H)^+$. Acid intermediate **2f** shows a characteristic triplet signal at δ 4.78 ppm corresponds to chiral proton of L-proline and pyrimidine proton at δ 8.32 ppm.

Scheme 7

Synthetic route for ***1-(5,6,7,8-tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine -2-carboxylic acid N'-aryl-hydrazide*** **(26-35)**

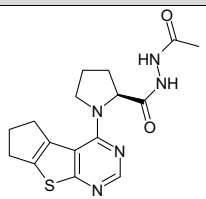
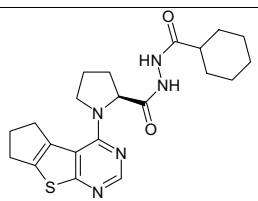


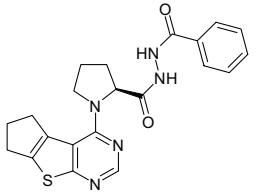
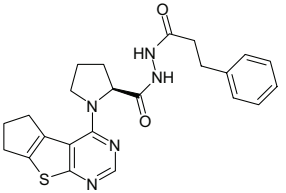
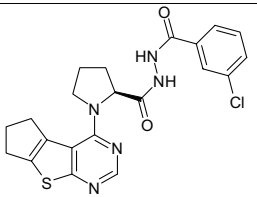
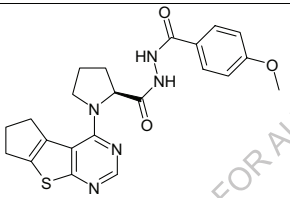
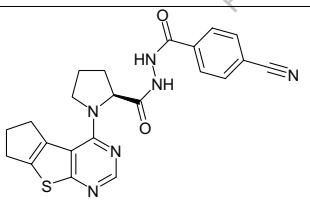
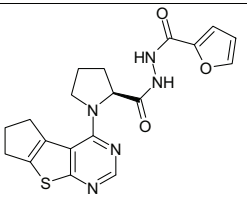
vi) R-Hydrazides, EDC.HCl/HOBt, DMF, RT

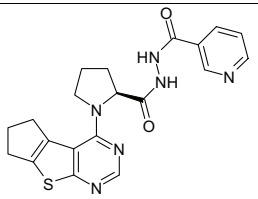
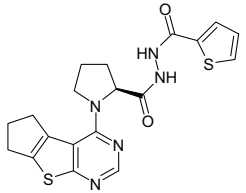
General Procedure

To a solution of 1-(2,3-Dihydro-1H-8-thia-5,7-diaza-cyclopenta[a]indene-4yl)-pyrrolidine-2-carboxylic acid **2f** (1g, 3.31 mmol) in DMF (5ml) was added EDC.HCl (950 mg, 4.96 mmol) and 3-Chloro-benzoic acid hydrazide (331 mg, 3.31mmol) followed by the HOBt (443mg, 3.31 mmol). The reaction mixture was stirred for 6 h and quenched with water (50 ml); the off-white colour solid that came out was filtered, dried, and washed with diethyl ether to give (**26-35**) as solid compounds; (**Scheme 7**).

Table 10: Physical & ES-MS (*m/z*) data of synthesized compounds (26-35)

No.	R	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
26		$C_{16}H_{19}N_5O_2S$	346.2	69	136-138
27		$C_{21}H_{27}N_5O_2S$	414.2	68	159-161

28		**$C_{21}H_{21}N_5O_2S$**	408.3	72	143-145
29		**$C_{23}H_{25}N_5O_2S$**	436.3	78	163-165
30		**$C_{21}H_{20}ClN_5O_2S$**	442.3	77	122-124
31		**$C_{22}H_{23}N_5O_3S$**	438.3	75	145-147
32		**$C_{22}H_{20}N_6O_2S$**	433.2	71	174-176
33		**$C_{19}H_{19}N_5O_3S$**	398.3	82	168-170

34		$C_{20}H_{20}N_6O_2S$	409.2	69	141-143
35		$C_{19}H_{19}N_5O_2S_2$	414.3	73	122-124

2.3.2.1. Biology

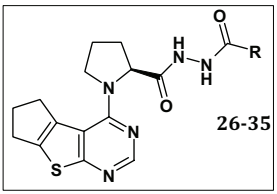
Antibacterial activity

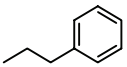
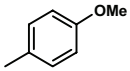
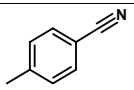
The evaluations of the synthesized compounds 26-35 for antibacterial activity was carried out by standard literature procedure using agar diffusion method by finding the zone of inhibition of the drug sample against the standard drugs and were reported in **Table 9**. The organisms employed in vitro testing of the compounds were *S. aurous* (Gram Positive), *S. albus* (Gram Positive) *S. faecalis* (Gram Positive), *Bacillus sp.* (Gram Positive) *Pseudomonasaeruginosa* (Gram Negative), *sp. Proteus sp.* (Gram Negative) *Klebsiella sp.* (Gram Negative) *Escherichia coli* (Gram Negative) (**Table 8**). All the cultures were maintained on Nutrient agar (Microbiology) grade, Hi Media) medium by periodic sub culturing. Ciprofloxacin was used as reference compound for antibacterial activity. The compounds were tested at a concentration of a 100µg/ml were prepared in Dimethylsulphoxide.

Table 11: *Gram positive* & *Gram Negative* species used for the study

Code No	Gram Positive Bacteria	Code No	Gram Negative Bacteria
A	*Staphylococcus aureus*	**E**	*Pseudomonas sp.*
B	*Staphylococcus albus*	**F**	*Proteus sp.*
C	*Staphylococcus faecalis*	**G**	*Klebsiella sp.*
D	*Bacillus species*	**H**	*Escherichia coli*

Table 12: Antibacterial activities (zone of inhibition) for the tested compounds



Comp. No.	R	Inhibition Zone Diameter (mm) Gram Positive Bacteria				Inhibition Zone Diameter (mm) Gram Negative Bacteria			
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
26	$-CH_3$	16	19	18	22	22	18	27	25
27		18	24	22	24	20	19	30	38
28		21	26	24	24	19	22	29	26
29		23	31	19	21	25	22	28	27
30		17	28	21	23	18	23	33	21
31		13	24	17	19	26	25	27	29
32		15	22	19	20	19	27	25	33
33		18	19	18	23	22	16	25	25
34		18	21	20	27	20	19	32	28
35		18	21	13	20	22	19	25	26
Std.	***Ciprofloxacin***	19	20	14	13	17	17	20	22

2.3.3. EXPERIMENTAL

***Ethyl 2-Amino-5, 6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate* (2b)**

In to a mixture of Cyclopentanone **2a** (49g, 0.5 mol), ethyl 2-cyanoacetate (56g, 0.5 mol,) and sulphur (16g, 0.5 mol) in 150ml of ethanol was added morpholine (44g, 0.5 mol). The mixture was stirred for 8 hr at room temperature. The reaction mixture was diluted with water and the precipitate was collected by filtration and recrystallized from ethanol. **2b** as yellow solid (65g, 61%) mp = 90°C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.83 (s, 2H, Ar-N$\underline{H}_2$), 4.25 (q, 2H, J = 7.1 Hz, -O-C$\underline{H}_2$), 2.85-2.80 (m, 2H, -CH_2-C$\underline{H}_2$-CH_2-), 2.74-2.69 (m,2H, -CH_2-C$\underline{H}_2$-CH_2-), 2.36-2.26 (m, 2H, -CH_2-C$\underline{H}_2$-CH_2-), 1.33 (t,3H, J = 7.1 Hz, -OCH_2-C$\underline{H}_3$); ES-MS:*m/z* 211.3$(M+H)^+$.

***3,5,6,7-Tetrahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one* (2c)**

The mixture of compound **2b** (35g, 0.16 mol) in 150 ml of formamide was heated at 180°C for 4h and then cooled down to room temperature. The mixture was poured into 200 ml water and filtered. The solid was collected and recrystallized from ethanol. Compound **2c** as yellow solid (21 g, 65%); ^{1}H NMR($CDCl_3$,400 MHz): δ 12.28 (br s, 1H,-CO-N$\underline{H}$-), 8.03 (s, 1H, =C$\underline{H}$-N-), 3.05-3.09 (m, 2H, CH_2-C$\underline{H}_2$-CH_2-), 2.95-2.98 (m, 2H,-CH_2-C$\underline{H}_2$-CH_2-), 2.47-2.49 (m, 4H,-CH_2-C$\underline{H}_2$-CH_2-); ES-MS: m/z 193.2$(M+H)^+$.

***4-Chloro-6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidine* (2d)**

A suspension of compound **2c** (25g, 0.12 mol) in 150 ml of $POCl_3$ was heated at reflux for 2h. $POCl_3$ was removed at reduced pressure and the residue was poured onto ice and filtered. The solid was washed with water and dried. Compound **2d** as brown solid (18g, 71%); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.70 (s, 1H, -N=C$\underline{H}$-N-), 3.13-3.17 (m, 2H,CH_2-C$\underline{H}_2$-CH_2-), 3.04-3.08 (m, 2H,CH_2-C$\underline{H}_2$-CH_2-), 2.48-2.56 (m, 4H, ,CH_2-C$\underline{H}_2$-CH_2-); ES-MS: *m/z* 225.3 $(M+H)^+$.

***1-(2, 3-Dihydro-1H-8-thia-5,7-diaza-cyclopenta[a] indene-4yl)-pyrrolidine-2-carboxylic acid ethyl ester* (2e)**

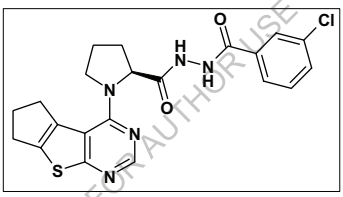
To a clear solution of compound **2d** (20 g, 0.095 mol) in Methanol 200 ml was added L-Proline ethyl ester hydrochloride (17 g, 0.095 mol) and triethylamine (33 ml, 0.237 mol), stirred the reaction mixture for 3h. Methanol was removed under reduced pressure and residue was taken in EtOAc, washed with water, 1N HCl solution in water and saturated $NaHCO_3$ solution in water. Collected Organic layer was dried over Na_2SO_4 and removed

under reduced pressure to yield Ester intermediate **2e** as a yellowish gel (22 g, 88%). This was used for without further purification. ES-MS: *m/z* 318.3 $(M+H)^+$.

1-(2,3-Dihydro-1H-8-thia-5,7-diaza-cyclopenta[a]indene-4yl)-pyrrolidine-2-carboxylic acid (2f)

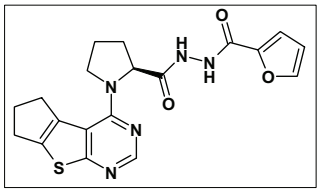
A suspension of ester compound **2e** (25g, 0.075 mol) in 225 ml THF and 25 ml water was added Lithium hydroxide monohydrate (4.2 g, 0.11 mol) at 0°C, and the reaction mixture for 12 h. Distilled out THF under vacuum and to the remaining aqueous residue was added 1N HCl solution in water to adjusted the solution P^H = 4, solid was precipitates out. Filtered the solid and dried Compound **2f** as yellowish solid (16 g, 70%); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.23 (s, 1H, Pyrimidine-H), 4.78 (t, 1H, J = 8 Hz, -N-CH-CO), 3.81-3.84 (m, 2H, -N-CH_2-CH_2-), 2.80-2.86 (m, 4H, -CH_2-CH_2-CH_2-), 1.75-1.98 (m, 6H, CH_2-CH_2-CH_2-), 1.49-1.50 (m, 2H, CH_2-CH_2-CH_2-); ES-MS: *m/z* 288.3 $(M+H)^-$.

3-Chloro-benzoic acid N'-[1-(2,3-dihydro-1H-8-thia-5,7-diaza-cyclopenta[a] -inden-4-yl)-pyrrolidine-2-carbonyl]-hydrazide (30)



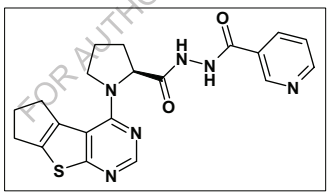
7a (800mg, 62%).1H NMR (DMSO-d_6, 400 MHz) δ 2.04-2.18 (m,2H),2.25-2.44 (m, 3H), 2.89-2.99 (m, 3H), 3.08-3.16 (m, 2H), 3.72-3.77 (m,1H), 3.88-3.92 (m, 1H), 4.84-4.87 (m, 1H,N-CH-CO), 7.50-7.54 (m, 1H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.82-7.90 (m, 2H, Ar-H), 8.24 (s, 1H, Ar-H), 9.99(s, 1H, -NH-NH-), 10.47 (s, 1H, -NH-NH-), ES-MS: *m/z* 442.4 $(M+H)^+$, mp= 122-124°C, Anal. Calcd. for $\mathbf{C_{21}H_{20}ClN_5O_2S}$: C, 57.07; H, 4.56; N, 15.85; S, 7.26. Found: C, 57.04; H, 4.53; N, 15.83; S, 7.22.

***Furan-2-carboxylic acid N′-[1-(2, 3-dihydro-1H-8-thia-5, 7-diaza-cyclopenta [a]inden-4yl)-pyrrolidine-2-carbonyl]-hydrazide* (33)**



^{1}HNMR (DMSO-d_6, 400 MHz) δ 10.22 (s, 1H, -NH-NH-), 9.89 (s, 1H, -NH-NH), 8.22 (s, 1H, Pyrimidine-H), 7.86 (s, 1H, Ar-H), 7.21 (d, 1H, J = 2.4 Hz, Ar-H), 6.62 (br s, 1H, Ar-H), 4.82-4.85 (m, 1H, -N-CH-CO), 3.87-3.90 (m,1H, -CH_2-CH_2-CH_2-), 3.73-3.76 (m,1H, -CH_2-CH_2-CH_2-), 3.10-3.15 (m, 2H, -CH_2-CH_2-CH_2-), 2.95-2.98 (m, 2H, -CH_2-CH_2-CH_2-), 2.25-2.45 (m, 3H, -CH_2-CH_2-CH_2-), 1.95-2.15 (m, 3H, -CH_2-CH_2-CH_2-); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 25.4, 27.9, 29.6, 29.9, 33.8, 50.8, 62.3, 112.1, 113.5, 114.7, 136.1, 137.5, 146.1, 146.6, 151.1, 155.7, 157.6, 172.4, 172.7; ES-MS: *m/z* 397.3 $(M+H)^+$; mp= 168-170°C. Anal.Calcd. for **$C_{19}H_{19}N_5O_3S$**: C, 57.42; H, 4.82; N, 17.62; Found: C, 57.39; H, 4.80; N, 17.60.

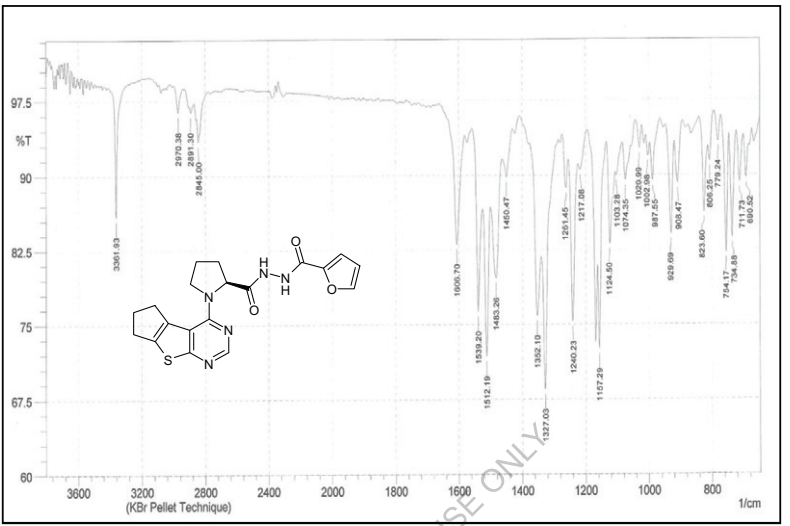
***Nicotinic acid N′-[1-(2, 3-dihydro-1H-8-thia-5, 7-diaza-cyclopenta[a]inden-4yl)-pyrrolidine-2-carbonyl]-hydrazide* (34)**



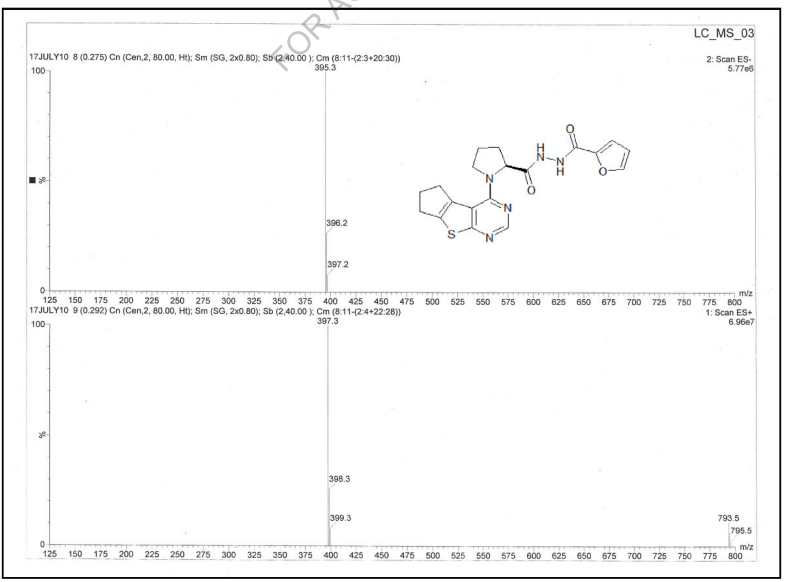
^{1}H NMR(DMSO-d_6, 400 MHz): δ 10.56 (s, 1H, -NH-NH-), 10.01 (s, 1H, -NH-NH-), 9.01 (s, 1H, Pyrimidine-H), 8.72-8.74 (m, 1H, Ar-H), 8.19-8.24 (m, 2H, Ar-H), 7.50-7.54 (m, 1H, Ar-H), 4.85-4.88 (m, 1H, -N-CH-CO), 3.89-3.93 (m, 1H, -CH_2-CH_2-CH_2-), 3.72-3.74 (m,2H, -CH_2-CH_2-CH_2-), 3.09-3.14 (m, 1H, -CH_2-CH_2-CH_2-), 2.95-2.99 (m, 3H, -CH_2-CH_2-CH_2-), 2.26-2.44 (m, 3H, -CH_2-CH_2-CH_2-), 2.07-2.18 (m, 2H, -CH_2-CH_2-CH_2-); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 25.4, 27.8, 29.6, 29.9, 33.8, 50.9, 62.4, 113.6, 124.0, 128.6, 135.6, 136.2, 137.6, 148.9, 151.2, 152.9, 155.8, 164.6, 172.4, 172.8.; ES-MS: m/z 409.1 $(M+H)^+$; m.p. = 141-143°C, Anal. Calcd. for **$C_{20}H_{20}N_6O_2S$**: C, 58.81; H, 4.94; N, 20.57; Found: C, 58.83; H, 4.91; N, 20.53.

Spectral Data for Compound No. 33

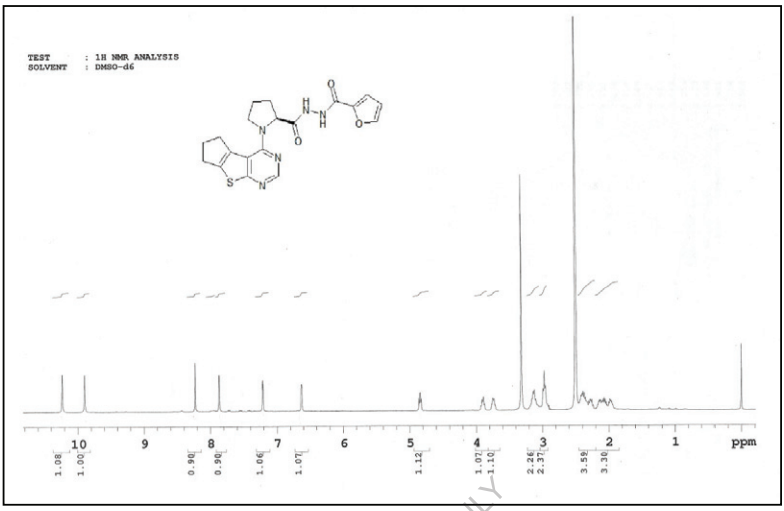
IR



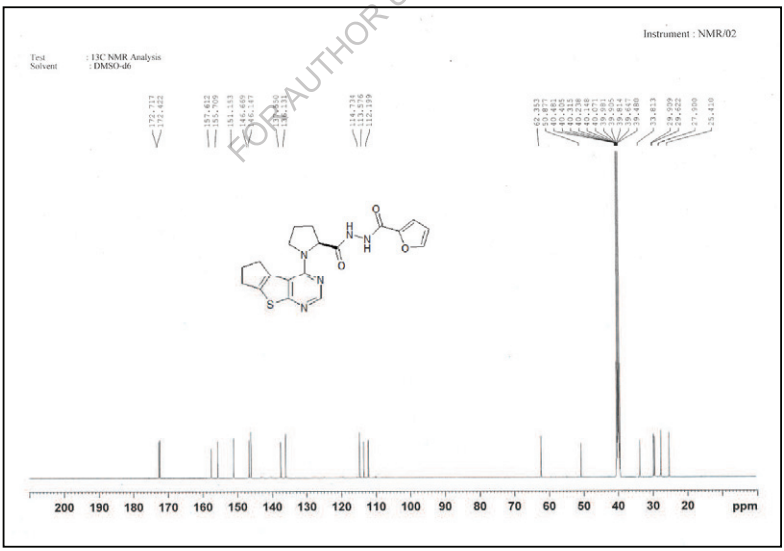
ES-MS



^{1}H NMR (DMSO-d_6, 400 MHz)



^{13}C NMR (DMSO-d_6, 125 MHz)



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CHAPTER 3
Synthesis and biological studies of diaryl pyrazoles

SECTION A

3.1. Synthesis and antibacterial studies of *3-methyl-1-phenyl-5-pyrrol-1-yl-1H-pyrazole-4-carboxilic acid hydrazides*

3.1.1. AIM & OBJECTIVES

Pyrazoles or azoles are five member ring heterocyclic compound having two adjacent nitrogen atoms [1]. The best described property of pyrazole is in the treatment of inflammation associated disorders, such as arthritis [2]. Pyrazole derivatives are the subject of many research studies because of their potential to exhibit biological activities such as antimicrobial [3], antitumor [4], antihistaminic [5], antiviral [6], fungicides [7], insecticides [8]. Substituted pyrazoles and its analogs have been used as precursors for synthesis of different biologically active molecules. Taking into consideration the importance of biological activities of pyrazoles, we have decided to synthesize some novel substituted pyrazoles and studied their antimicrobial activities.

3.1.2. RESULTS AND DISCUSSION

3.1.2.1. Chemistry

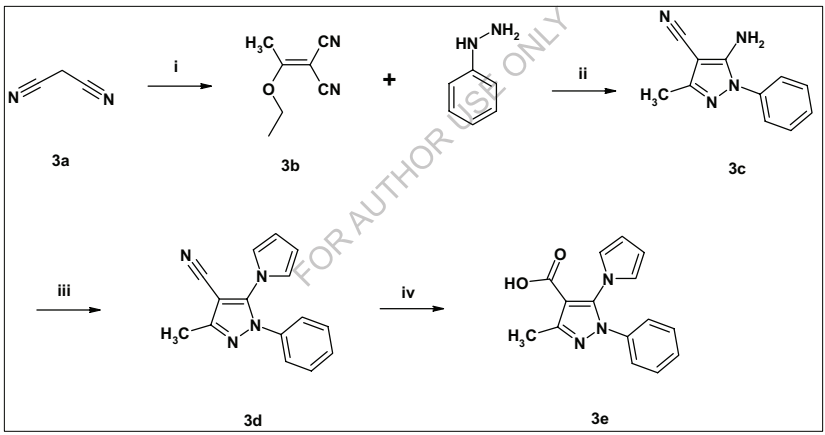
As a part of our ongoing research program on the application of substituted Pyrazole, we herein report the synthesis of novel 1-phenyl-5-(1H-pyrrol-1yl)-1H-pyrazole-4-carboxilic acid hydrazide (**36-45**). Synthesis of novel acid core was prepared as depicted in **Scheme 8**. Malononitrile **3a** when heated with triethylorthoacetate gave a reactive intermediate 2-(1-ethoxyethylidine)-malononitrile **3b** [9]. This on reaction with phenyl hydrazine in ethanol under reflux condition gave 5-amino-1-(4-phenyl)-3-methyl-1*H*-pyrazole-4-carbonitrile **3c** [10], which was transformed into the corresponding pyrrole derivative **3d** by reaction with 2,5 dimethoxytetrahydrofuran in glacial acetic acid according to Clauson-Kaas procedure [11]. Transformation of nitrile intermediate **3d** into the acid intermediate **3e** was achieved by alkaline hydrolysis using sodium hydroxide in refluxing ethylene glycol [12]. At last step,

pyrazole 4-carboxilic acid N'-acyl hydrazides **36-45** were obtained through coupling of acid intermediate **3e** with selected hydrazide side chains (**Scheme 9**).

^{1}H NMR spectrum of **3c** revealed the presence of triplet signal at δ 2.34 ppm corresponds to Pyrazolyl methyl group and broad singlet signal at δ 4.58 ppm corresponds to amino group. Similarly ^{1}H NMR spectrum of **3d** was identified by the triplet singlet at δ 6.73 ppm and δ 6.37 ppm of pyrrole moiety. Broad singlet at δ 12.36 ppm and ES-MS: *m/z* 268.1 $(M+H)^+$ confirmed the acid functional group present in **3e**. Novel hydrazide derivatives containing aromatic hydrazide **36-45** (**Scheme 9**) were prepared from **3e** and studies for antibacterial activities.

Scheme 8

Synthesis route for ***3-methyl-1-phenyl-5-pyrrol-1-yl-1H-pyrazole-4-carboxilic acid*** **(3e)**



Reagents and conditions:- **(i)Triethylorthoacetate, 95°C, 1.5h (ii) Ethanol, reflux, 3h (iii) 2,5 dimethoxytetrahydrofuran in glacial acetic acid, reflux 2h (iv) 3N NaOH, ethylene glycol, reflux, 24h.**

Name reactions involved in the synthesis of Pyrrole

Clauson-Kaas Reaction

The Knorr pyrazole synthesis is an organic reaction used to convert a hydrazine or its derivatives and a 1,3-dicarbonyl compound to a pyrazole using an acid catalyst. The mechanism begins with an acid catalyzed imine formation, where in the case of hydrazine derivatives the attack can happen on either carbonyl carbon and result in two possible products. The other nitrogen of the hydrazine derivative then attacks the other carbonyl group which has also been protonated by the

acid and forms a second imine group. This diimine compound gets deprotonated to regenerate the acid catalyst and provide the final pyrazole product. This protocol was reported by the Danish chemist Niels Clauson-Kaas in 1952. Pyrroles can be synthesized by the condensation of 2,5-dialkoxytetrahydrofurans with primary amines.

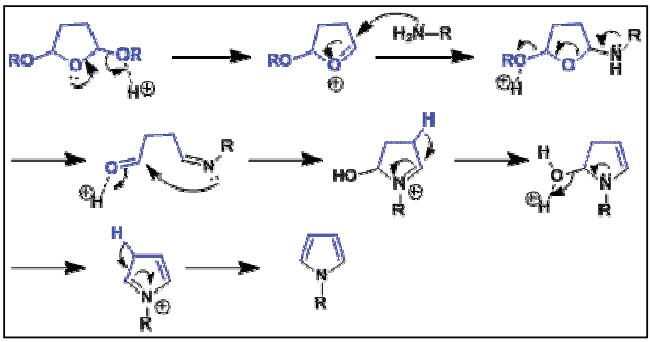
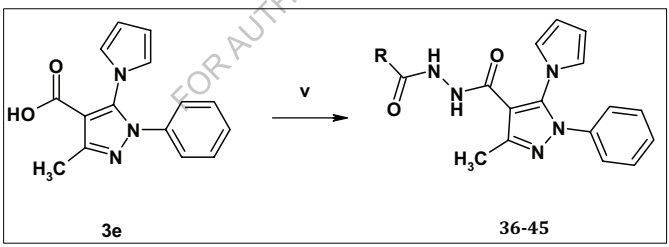


Fig.26: Mechanism of the Clauson-Kaas Reaction

Scheme 9

Synthesis route for ***R-acid N'-(3-methyl-1-phenyl-5-pyrrol-1-yl-1H-pyrazole-4-carbonyl)-hydrazide*** **(36-45)**

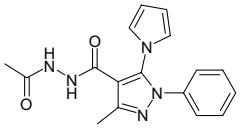
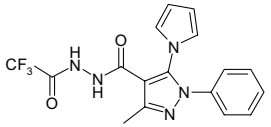
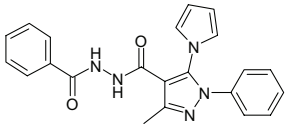
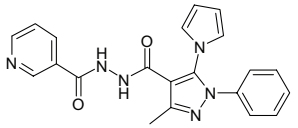
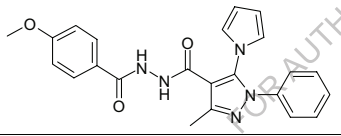
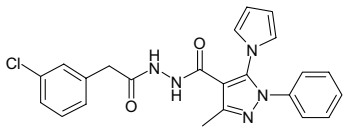
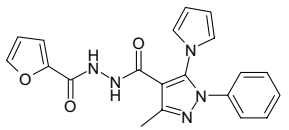
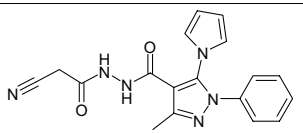


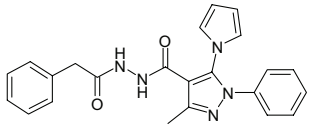
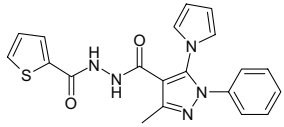
(iv)R-hydrazide, DMF, EDC.HCl, HOBt, TEA, 25°C

General Procedure

3-methyl-1-phenyl-5-pyrro1-yl-1H-pyrazole-4-carboxylic acid **3e** (500 mg, 1.87 mmol) was taken in DMF (5ml) and was added EDC.HCl (542 mg, 2.80 mmol), R-acid hydrazide (280 mg, 2.07 mmol), HOBt (253 mg, 1.87 mmol and TEA (787 µL, 5.61 mmol) under nitrogen. The reaction mixture was stirred for 6h. TLC showed completion of reaction. Water was added (50 ml), solid was comes out was filtered, dried and washed with diethyl ether to gave compounds **36-45** as solid products; **Scheme 9**.

Table 13: Physical & ES-MS (*m/z*) data of synthesized compounds (36-45)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
36		$C_{17}H_{17}N_5O_2$	324.2	72	132-134
37		$C_{17}H_{14}F_3N_5O_2$	375.2	68	157-159
38		$C_{22}H_{19}N_5O_2$	385.4	68	145-147
39		$C_{21}H_{18}N_6O_2$	386.4	58	153-155
40		$C_{23}H_{21}N_5O_3$	415.4	72	151-153
41		$C_{23}H_{20}ClN_5O_2$	433.9	70	168-169
42		$C_{20}H_{17}N_5O_3$	375.3	63	137-139
43		$C_{18}H_{16}N_6O_2$	348.3	56	127-129

44		$C_{23}H_{21}N_5O_2$	399.4	82	168-170
45		$C_{20}H_{17}N_5O_2S$	392.2	63	145-147

3.1.2.2. Biology

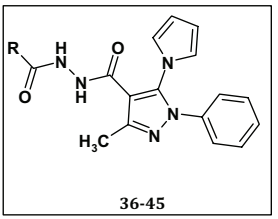
Antimicrobial activity

The evaluation of the synthesized compounds (**31-40**) for antibacterial activity was carried out by standard literature procedure using agar diffusion method by finding the zone of inhibition of the drug sample against the standard drugs. The organisms employed in vitro testing of the compounds were *S. aureus* (Gram Positive), *S albus* (Gram Positive) *S. faecalis* (Gram Positive), *Bacillus sp.* (Gram Positive) *Pseudomonas aeruginosa* (Gram Negative), *sp. Proteus sp.* (Gram Negative) *Klebsiella sp.* (Gram Negative) *Escherichia coli* (Gram Negative). All the cultures were maintained on Nutrient agar (Microbiology) grade, Hi Media) medium by periodic sub culturing. Ciprofloxacin was used as reference compound for antibacterial activity. The compounds were tested at a concentration of a 100 µg/ml were prepared in Dimethylsulphoxide.

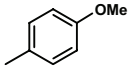
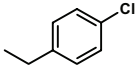
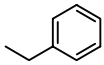
Table 14: *Gram positive* & *Gram Negative* species used for the study

Code No	Gram Positive Bacteria	Code No	Gram Negative Bacteria
A	*Staphylococcus aureus*	**E**	*Pseudomonas sp.*
B	*Staphylococcus albus*	**F**	*Proteus sp.*
C	*Staphylococcus faecalis*	**G**	*Klebsiella sp.*
D	*Bacillus species*	**H**	*Escherichia coli*

Table 15: Antibacterial activities (zone of inhibition) for the tested compounds



36-45

Comp. No.	R	Inhibition Zone Diameter (mm) Gram Positive Bacteria				Inhibition Zone Diameter (mm) Gram Negative Bacteria			
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
36	$-CH_3$	16	19	18	22	22	18	27	25
37	$-CF_3$	17	22	24	24	19	21	28	35
38		18	24	22	24	20	19	30	28
39		21	26	24	24	19	22	29	26
40		23	31	19	21	25	22	28	27
41		17	28	21	23	18	23	33	21
42		13	24	17	19	26	25	27	29
43		15	22	19	20	19	27	25	33
44		19	31	23	24	22	28	35	32
45		18	21	13	20	22	19	25	26
Std.	***Ciprofloxacin***	**19**	**20**	**14**	**13**	**17**	**17**	**20**	**22**

3.1.3. EXPERIMENTAL

2-(1-ethoxyethylidine)-malononitrile (3b)

A mixture of malononitrile **3a** (17g, 0.257 mol) and triethylorthoacetate (45.95g, 0.283 mol) was heated at 95°C FOR 1.5 h. The mixture was then evaporated in vacuo to give a solid that was filtered off yielding compound **3b** as yellowish solid (34g, 99%)

5-Amino-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile (3c)

2-(1-ethoxyethylidine)-malononitrile **3b** (10g, 0.072 mol) and phenyl hydrazine (7.76 g, 0.072 mol) was taken in ethanol (50 ml) and refluxed the reaction mixture for 3h. Cooled the content to room temperature, and obtained solid was collected by filtration. Solid bed was washed with cold ethanol (10 ml) to yield intermediate **3c** as yellowish solid**.** (8.54 g Yield = 60%), mp = 172-175°C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33-7.35 (m, 3H, Ar-H), 7.03-7.6 (m, 2H, Ar-H), 4.58 (br s, 2H, Ar-NH_2), 2.34 (s, 3H, Ar-CH_3); ES-MS: *m/z* 199.3 $(M+H)^+$.

3-methyl-1-phenyl-5-pyrrol-1-yl-1H-pyrazole-4-carbonitrile (3d)

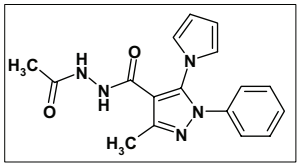
To a solution of nitrile intermediate **3c** (8.54g, 0.043 mol) in glacial acetic acid (85ml) was added 2, 5 dimethoxytetrahydrofuran (6.83 g, 0.051 mol). The reaction mixture was refluxed for 2h. After cooling water (10 ml) was added, and volatiles were removed under vacuum to obtained residue that was then taken in Ethyl acetate and washed with saturated sodium bicarbonate. Organic layers were collected, dried over sodium sulfate and removed under vacuum to give crude intermediate which was purified by column chromatography (chloroform as eluent) to furnish of reddish solid intermediate **3d**. (7.5 g Yield = 70%, mp = 142-146°C; ^{1}H NMR (DMSO-d6, 400 MHz): δ 7.35 (d, 2H, *J* = 6.8Hz, Ar-H), 7.03-7.6 (m, 2H, Ar-H), 6.73 (t, 2H, Pyrrole-H), 6.37 (t, 2H, Pyrrole-H), 2.49 (s, 3H, Ar-CH_3); ES-MS: *m/z* 249.1 $(M+H)^+$.

3-methyl-1-phenyl-5-pyrrol-1-yl-1H-pyrazole-4-carboxilic acid (3e)

Nitrile intermediate **3d** (7.5 g, 0.030 mol), 3N NaOH (45ml) and ethylene glycol (150 ml) was refluxed for 24 h. After completion of the reaction cooled it to 10°C and was quenched with water, then made acidic with 6N HCl until PH= 2. The product was extracted with ethyl acetate. The organic layers were washed with brine and dried. The evaporation of the organic layer under vacuum furnishes brownish solid acid intermediate **3e**. (6 g**,** Yield = 75%); mp = 232-235°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.36 (br, s, 1H, Ar-COOH), 7.34 (d, 3H, *J* = 6.8Hz, Ar-H), 7.08 (d, 2H, *J* = 8 Hz, Ar-H), 6.83 (s, 2H, Pyrrole-H), 6.1 (s, 2H, Pyrrole-H), 2.46 (s, 3H, Ar-CH_3); ES-MS: *m/z* 268.1 $(M+H)^+$.

3-Methyl-1-phenyl-5-pyrrol-1-yl-1H-pyrazole-4-carboxylicacid N'-acetyl-hydrazide

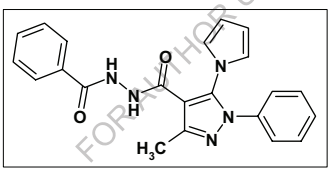
(36)



(0.520g, Yield= 72%) ; mp = 132-134°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.87 (s, 1H, -CO-NH-), 9.44 (s, 1H, -CO-NH-), 7.33-7.35 (m, 3H, Ar-H), 7.06 (d, 2H, J = 1.6 Hz, Ar-H), 6.84-6.85 (m,2H, Ar-H), 6.16-6.17 (d, 2H, J = 1.6 Hz, Ar-H),2.38 (s, 3H, Pyrazole-CH$_3$), 1.83 (s, 3H, -CO-CH$_3$); ^{13}C NMR (DMSO-d$_6$, 100 MHz): δ 13.1, 20.4, 110.3 (2×C), 110.3, 122.6 (3×C), 122.7, 123.3, 127.8, 129.1 (2×C), 137.4, 147.4, 161.0, 168.1; ES-MS: *m/z* 324.2 (M+H)$^+$; Anal. Calcd. for **$C_{17}H_{17}N_5O_2$**; C, 63.15; H, 5.30; N, 21.66; Found: C, 63.17; H, 5.33; N, 21.64.

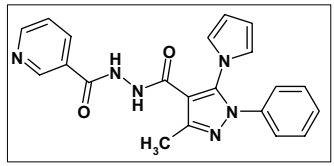
Benzoic acid N'-(3-methyl-1-phenyl-5-pyrrol-1-1H-pyrazole-4-carbonyl)-hydrazide

(38)



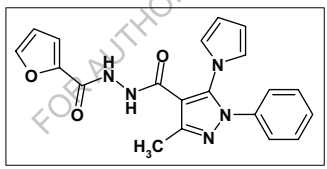
white solid (0.47g, Yield= 68%); mp=145-147°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.89 (s, 1H, -CO-NH-), 9.74 (s, 1H, -CO-NH-), 7.88 (d, 2H, J = 7.2 Hz, Ar-H), 7.46-7.56 (m, 3H, Ar-H), 7.32-7.36 (m, 3H, Ar-H), 7.05 (d, 2H, J = 1.6 Hz, Ar-H), 6.89 (d, 2H, J = 1.6 Hz, Ar-H), 6.19 (d, 2H, J = 1.6 Hz, Ar-H), 2.48 (s, 3H, Pyrazole -CH$_3$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 13.2, 109.8, 110.3, 111.4, 122.8 (3×C), 122.9, 123.1, 127.4 (2×C), 127.9 (2×C), 128.4 (2×C), 129.1 (2×C), 131.8, 132.2, 137.5, 147.5, 161.5, 165.4; ES-MS: *m/z* 386.1 (M+H)$^+$; Anal. Calcd. for **$C_{22}H_{19}N_5O_2$**; C, 68.56; H, 4.97; N, 18.17; Found: C, 68.53; H, 4.93; N, 18.19.

***Nicotinic acid N′-(3-methyl-1-phenyl-5-pyrrol-1-1H-pyrazole-4-carbonyl)-hydrazide* (39)**



brownish solid (0.45g, Yield= 64%); mp = 153-155°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.59 (s, 1H, -CO-NH-), 9.72 (s, 1H, -CO-NH-), 9.22 (s, 1H,Ar-H), 8.35-7.85 (m, 2H, Ar-H), 7.63 (m, 1H, Ar-H), 7.34-7.36 (m,3H, Ar-H), 7.05 (d, 2H, J = 7.2 Hz), 6.87 (d, 2H, Ar-H, J = 7.2 Hz,), 6.20 (d, 2H, Ar-H), 2.48 (s,3H, Pyrazole -CH$_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ13.2, 109.7, 110.5, 111.5, 125.1, 122.8 , 123.1, 123.4, 127.2, 127.6 , 128.4, 129.3, 131.8 (2×C), 132.3, 137.5, 138.0, 148.2, 147.5, 161.5, 165.4;ES-MS: *m/z* 387.2 (M+H)$^+$; Anal. Calcd. For **$C_{21}H_{18}N_6O_2$**; C, 65.28; H, 4.70; N, 21.75; Found: C, 65.26; H, 4.72; N, 21.78.

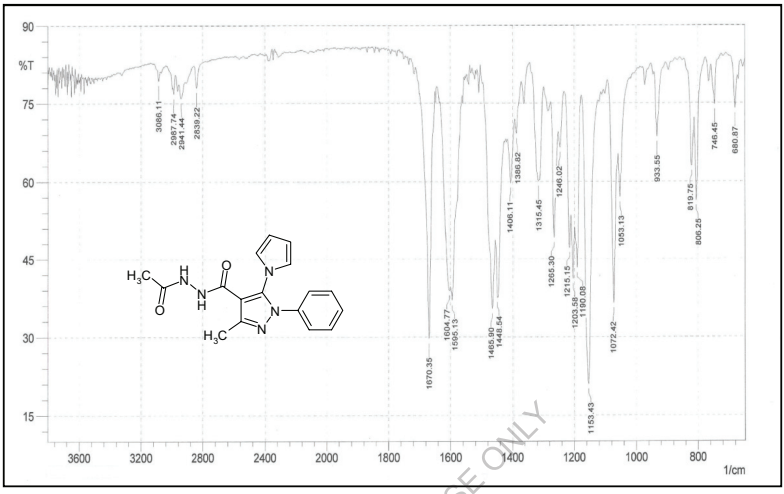
***Furan-2-carboxylic acid N′-(3-methyl-1-phenyl-5-pyrrol-1-1H-pyrazole-4-carbonyl)-hydrazide* (42)**



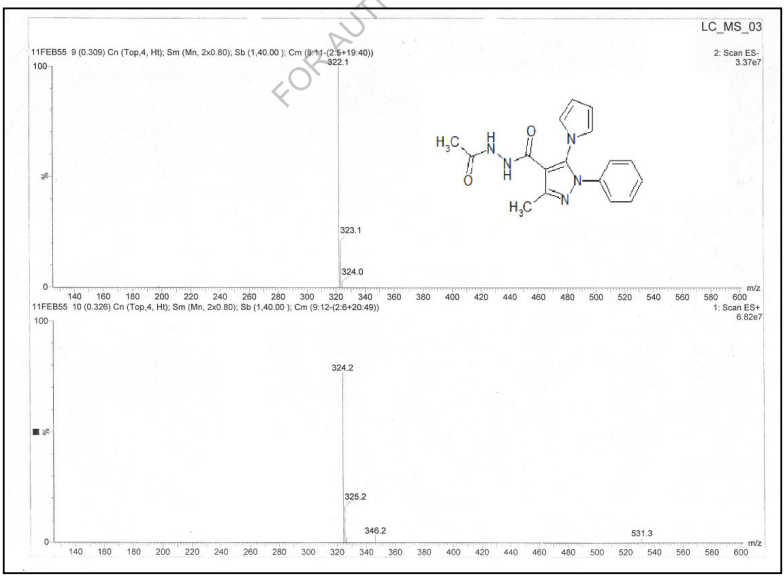
brownish solid (0.48g, Yield= 71%); mp = 137-139°C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.38 (s, 1H, -CO-NH-), 10.38 (s, 1H, -CO-NH-), 7.88 (s,1H, Ar-H), 7.33-7.35 (m, 3H, Ar-H), 7.04 (d, 2H, J = 1.6 Hz, Ar-H), 6.85 (t, 2H, J = 2.4 Hz, Ar-H), 6.17-6.18 (m, 2H, Ar-H), 2.48 (s, 3H, Pyrazole -CH$_3$); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 13.1, 109.6, 110.1, 111.4, 122.8 (2×C), 122.9, 123.1(2×C), 127.4 , 127.9, 128.4 (2×C), 129.1 (2×C), 132.2, 146.0, 147.5, 161.5, 165.4; ES-MS: *m/z* 376.2 (M+H)$^+$; Anal. Calcd. for $C_{20}H_{17}N_5O_3$; C, 63.99; H, 4.56; N, 18.66; Found: C, 64.01; H, 4.54; N, 18.64.

Spectral Data for Compound No. 36

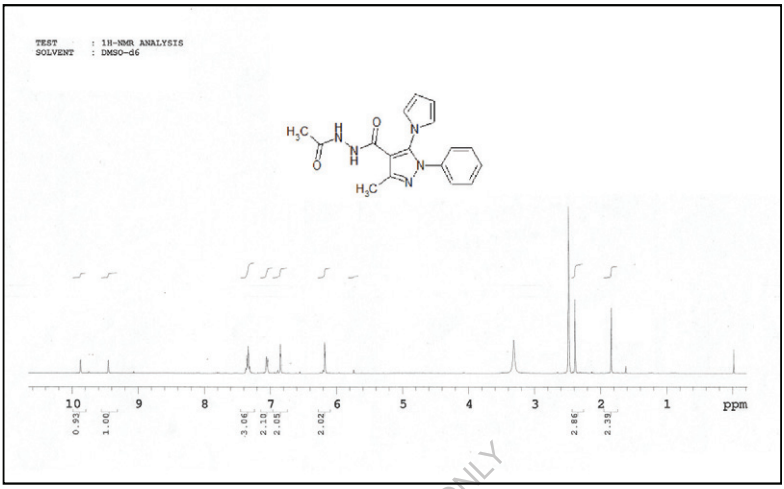
IR



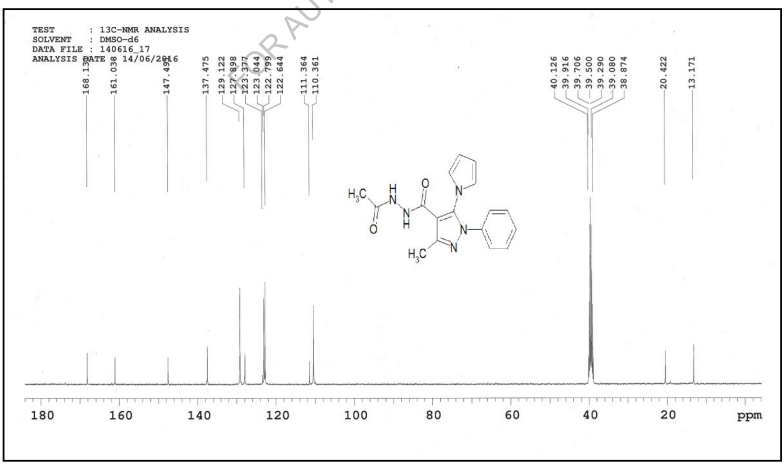
ES-MS



^{1}H NMR (DMSO-d_6, 400 MHz)



^{13}C NMR (DMSO-d_6, 100 MHz)



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SECTION B

3.2. Synthesis of *N-[5-Phenyl-2-(4-sulfamoyl-phenyl)-2H-pyrazol-3-yl]-amides* for the antibacterial & antiinflammatory studies

3.2.1. AIM & OBJECTIVES

Infectious diseases are the leaders among the challenging drug targets because of the multi-drug resist antimicrobial pathogens and continuous rise in the emerging infections from known and unknown sources. Though there is an availability of a large number of antibiotics and chemotherapeutics for medical use, the emerging resistance drives it for the search of new classes of antimicrobial agents [1, 2]. A potential approach to overcome the problem of antibiotic resistance is to design innovative agents with different modes of action so that no cross resistance with present drugs can occur [3]. Pyrazoles and their variously substituted derivatives exhibit wide range of biological activities and a significant amount of research activity has been directed towards this class. In particular, they are used as antitumor, antimicrobial, antitumor, antihistaminic, antiviral, fungicides, insecticides [4-8]. Some of these compounds also have antiinflammatory, anaesthetic and analgesic properties [9-11]. A typical procedure for the synthesis substituted pyrazoles involves base catalyzed aldol condensation followed by the cyclization with active hydrazines [12-15]. In continuation of our current antibacterial research [16], to obtain a potent and selective drug candidate, we herein reported the series of novel amides containing 1, 3-diaryl Pyrazoles.

We specifically design and synthesized these targeted molecules which were rationalized and have pharmacophores known for their biological activities. With the above facts we have brought variation across amine core **4d** with ten different acids to get the desired novel analogous (**46-55**) and tested for their antibacterial activities as discussed in **Table 13** at a concentration of 50 µg / ml and 100 µg / ml.

3.2.2. RESULTS AND DISCUSSION

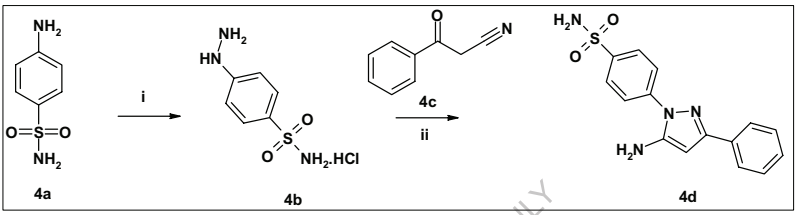
3.2.2.1. Chemistry

As a part of our ongoing research program on the application of substituted Pyrazole, we herein report the synthesis of novel N-[5-Phenyl-2-(4-sulfamoyl-phenyl)-2H-pyrazol-3-yl]-amide (**46-55**). Synthesis of novel amide derivative was prepared as depicted in **Scheme 11.** Reaction of sulfanilide **4a** with $SnCl_2$ and $NaNO_2$ in water under cooling condition gave 4-Hydrazino-benzenesulfonamide hydrochloride salt **4b** [17]. This was transformed into the

corresponding amino pyrrole derivative **4d** by reaction with 3-Oxo-3-phenyl-propionitrile **4c** in Ethanol at reflux condition **Scheme 11**. At last step, 4-(5-Amino-3-phenyl-pyrazol-1-yl)-benzenesulfonamide amide derivatives **46-55** were obtained through coupling of amine intermediate **4d** with selected acid side chains. ^{1}H NMR spectrum of **4b** revealed the presence of broad singlet signal at δ 10.43 ppm corresponding to two protons of hydrazine (-NH_2) forming HCl salt.

Scheme 10

Synthetic route for ***4-(5-Amino-3-phenyl-pyrazol-1-yl)-benzenesulfonamide*** **(4d)**

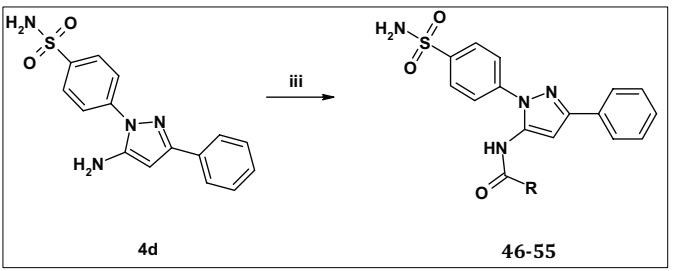


Reagents and conditions: **(i) $SnCl_2$, $NaNO_2$, Water, HCl 3h (ii) EtOH, reflux 4h (iii) R-Acid, DMF, EDC.HCl / HOBt, TEA, 25°C, 5h.**

It also showed singlet proton at δ 8.86 corresponding to hydrazine (-NH-). ES-MS spectrum showed *m/z* 188.1 $(M+H)^+$. Intermediate **4d**, was characterised by the singlet proton at δ 8.86 ppm corresponding to pyrazole ring and a broad singlet at δ 5.71 ppm for 5-amino pyrazole, mutilate for five protons at δ 7.33-7.42 was appeared for the newly added phenyl ring from the intermediate **4c**. ES-MS spectrum showed *m/z* 315.1 $(M+H)^+$ for the amine core **4d**.

Scheme 11

Synthesis route for ***N-[5-Phenyl-2-(4-sulfamoyl-phenyl)-2H-pyrazol-3-yl]-amides*** **(46-55)**

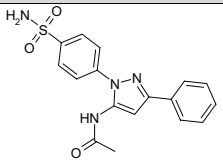
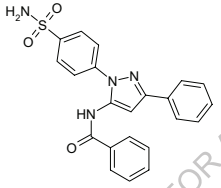
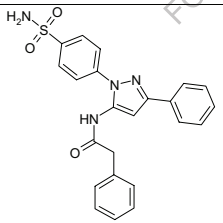
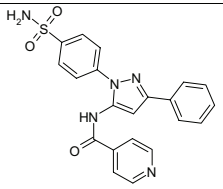


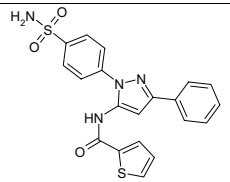
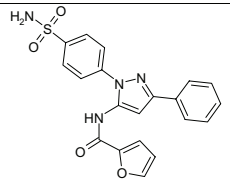
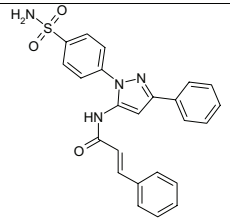
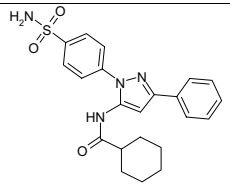
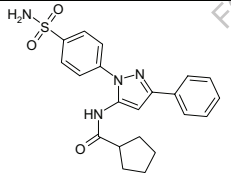
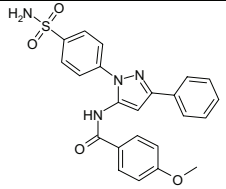
(iv) R-amine, DMF, EDC.HCl, HOBt, TEA, 25°C

General Procedure

To a solution of R-acid (32 mmol) in 5 ml DMF was added EDC.HCl (48 mmol) and 4-(5-Amino-3-phenyl-pyrazol-1-yl)-benzenesulfonamide **4d** (32 mmol) followed by the addition of triethylamine (96 mmol) and HOBt (32 mmol) under nitrogen atmosphere. The reaction mixture was stirred for 6h and quenched with water (50 ml), and adjust the pH = 4 of the reaction mixture with 1N HCl to obtained the solid which was filtered at Buchner funnel and washed with diethyl ether then dried under vacuum to yield desired solid product (**46-55**); **Scheme 11**.

Table 16: Physical & ES-MS (*m/z*) data of synthesized compounds (46-55)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
46		$C_{17}H_{16}N_4O_3S$	**357.2**	72	122-124
47		$C_{22}H_{18}N_4O_3S$	419.3	75	132-134
48		$C_{23}H_{20}N_4O_3S$	433.3	70	136-138
49		$C_{21}H_{17}N_5O_3S$	420.3	68	130-132

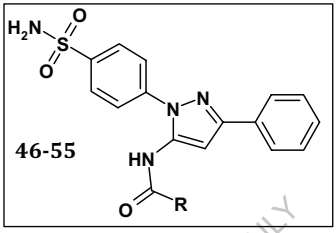
50		**$C_{20}H_{16}N_4O_4S_2$**	425.2	73	142-144
51		**$C_{20}H_{16}N_4O_4S$**	409.1	73	138-140
52		**$C_{24}H_{20}N_4O_3S$**	445.2	65	145-147
53		**$C_{22}H_{24}N_4O_3S$**	425.3	72	136-138
54		**$C_{21}H_{22}N_4O_3S$**	411.2	68	135-137
55		**$C_{23}H_{20}N_4O_4S$**	449.1	78	142-144

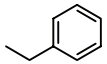
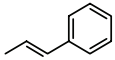
3.2.2.2. Biology

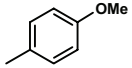
3.2.2.2.1. Evaluation of Antibacterial Activity

All the compounds (**Table 17**) were showed poor zone of inhibition in *E. coli* (Gram Negative) at both the tested concentrations as compared to standard drug. Aromatic amides **47**, **48, 52** and **55** are observed to showed poor zone of inhibition when compare to aliphatic amides **46**, **53** and **54.**

Table 17: Antibacterial activities for the tested compounds



Comp. No.	R	Inhibition Zone Diameter (mm) Gram Positive Bacteria (E.coli)		Inhibition Zone Diameter (mm) Gram Negative Bacteria (B. subtilis)	
		50µg/ml	100g/ml	50µg/ml	100µg/ml
46	$-CH_3$	14 ± 0.814	15.33± 0.471	21.66 ± .942	23.66 ± 0.942
47		13 ± 0.816	14.66 ± 0.942	21.33 ± 1.24	24.66 ± 0.471
48		12.66 ± .247	15.33 ± 0.471	22.66 ± .471	22.33 ± 0.942
49		14 ± 2.160	14.66 ± 1.247	19 ± 0.816	21 ± 1.414
50		12.33 ± 0.471	12 ± 0.816	20.66 ± .471	25.66 ± 0.942
51		**15.33 ± .247**	**17 ± 0.816**	**22.33 ±1.885**	**26.33 ± 0.942**
52		14 ± 1.414	16 ± 0.816	21 ± 1.414	21.66 ± 0.942

53		14 ± 0.816	15.66 ± 0.471	20.33 ± .885	20.66 ± 0.942
54		15.66 ± .471	17.66 ± 1.247	23.66 ± .471	23 ± 0.816
55		14 ± 0.816	13.66 ± 0.942	21.33 ± .471	23 ± 0.816
	Ciprofloxacin	**18.66 ± .471**	**20.66 ± 0.942**	**21.66 ± .471**	**27.66 ± 0.471**

Heterocyclic amides **49**, **50** and **51** showed intermittent activities. Five membered amide derivatives were showed better zone of inhibition among the studied aromatic, aliphatic and heterocyclic analogous. All the compounds were showed better to good zone of inhibition in *B. subtilis* (Gram Positive) at both the tested concentrations as compared to standard drug. Five membered heterocyclic amides **50** and **51** are observed to showed strong zone of inhibition when compare to aromatic amides **47**, **48** and **55**. Five membered ring containing amide **54** was found to better active analogue among the aliphatic amides **46** and **53**.

3.2.2.2.2. *In vitro* Antimicrobial Assay

Minimum inhibitory concentrations (MIC) assay of two superior molecules **45** and **46** was done according to CLSI standard protocol [18] by the micro dilution method in liquid medium distributed in 96 well plates. In each test a microbial culture control and sterility control (negative) were performed. The plates were incubated for 24 hours at 37°C. The lowest concentration to which inhibited the growth of test organism was considered as MIC (µg/ml) value for the tested compound. Ciprofloxacin was used as standard drug. The minimum inhibitory concentrations (MIC) values are presented in the **Table 18**.

Table 18: MIC values (µg/ml) of tested samples against tested microorganisms

Compound	MIC(µg/ml)	
	Escherichia coli	Bacillus Subtilis
50	**8**	**4**
51	**16**	**8**
Ciprofloxacin	**20**	**12**

Five membered amide derivatives were showed better MIC values (µg/ml) among the studied aromatic, aliphatic and heterocyclic analogous. This study concluded that all the tested compounds showed poor to better antibacterial activities in *E. coli* (Gram Negative) and B.

subtilis (Gram Positive). All the tested compounds (**46-55**) showed better activities in *B. subtilis* (Gram Positive) strain for both the concentrations than *E. coli* (Gram Negative) strain when compared with standard drug Ciprofloxacin.

3.2.2.2.2. *In-vitro* Cyclooxygenase Inhibition Assay

From the prepared target compounds five compounds (**47, 49-51** & **53**) were selected and screened for their anti-inflammatory activities against COX-1 & COX-2.

Table 19: *In-vitro* antiinflammatory COX-1 and COX-2 activities of compounds

Comp. No.	R	COX-1 IC_{50} (µM)[a]	COX-2 IC_{50} (µM) [a]	SI[b]
47		7.40	10.18	0.68
49		18.23	12.40	1.47
50		18.30	16.20	1.12
51		24.12	15.32	1.31
53		30.18	22.25	1.35
Celecoxib		**7.42**	**0.78**	**9.51**
Ibuprofen		**3.2**	**1.40**	**2.28**

[a]IC_{50} values represents concentration of test compound required to produce 50% inhibition, the result is the mean of two value obtained by assay of enzyme kits obtained from (Cayman Chemicals Inc., Ann Arbor, MI). [b]Selectivity index (COX-1 IC_{50} / COX-2 IC_{50}).

Inhibition by test compounds (**47**, **49-51** & **53**) of ovine COX-1 and human recombinant COX-2 [IC_{50} values (µM)] was assessed using a COX Fluorescent Inhibitor Screening Assay Kit (catalog number 700100, Cayman Chemical, Ann Arbor, MI, USA) and reported in **Table 19**. The *in vitro* COX-1/COX-2 isozyme inhibition studies measure the ability of tested compounds to inhibit ovine COX-1 and human recombinant COX-2 using an enzyme immunoassay (EIA). The obtained results (**Table 19**) showed that all the tested diaryl pyrazole are found to be weak inhibitors for COX-1 isoenzyme (IC_{50} = 7.40 µM - 30.18 µM range) and exhibited moderate COX-2 isoenzyme (IC_{50} = 10.18 µM-22.25µM range) with COX-2 selectivity's in the range of 0.68 - 1.47. The acquired data for the selected compounds (**47, 49-51** & **53**) showed that when the two phenyl rings attached to the central pyrazole

moiety are not vicinal (**47**, **49-51** & **53**), lower inhibitory activities against both COX-1 (IC_{50} = 7.40 µM - 30.18µM) and COX-2 (IC_{50} = 10.18 µM - 22.25 µM).

3.2.3. Experimental

3.2.3.1. Chemistry

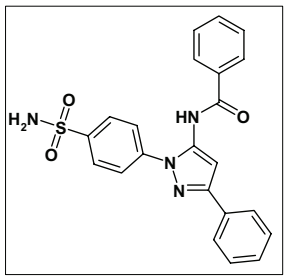
4-Hydrazino-benzenesulfonamidehydrochloride **(4b)**

To a solution of sodium nitrate (3.1 g, 0.44 mol) in 12.0 ml of water was added into a mixture of sulphanilamide (6.9 g, 0.40 mol) in conc. HCl (30 ml) over 15 min in ice-water bath. Then the mixture was rapidly added to a cooled (0°C) solution of tin (II) chloride dehydrate (27.1 g, 0.120 mol) in conc. HCl (30 ml). The resulting mixture was stirred for at 0°C for 1h, and then warmed to ambient temperature to stir overnight. The precipitate was collected by filtration and successively washed with cool water and Et_2O to give the relatively pure compound, as a hydrochloride salt which was used directly without further purification. Intermediate **4b** as white solid, (6.7g, yield=74.1%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.40 (br s, 3H,-N<u>H</u>$_2$.HCl), 8.86 (s, 1H, Ar-N<u>H</u>-NH$_2$), 7.68 (d, 2H, J = 8 Hz, -SO$_2$-N<u>H</u>$_2$-), 7.18 (s, 1H, Ar-<u>H</u>), 7.03 (d, 2H, J= 7.6Hz, Ar-<u>H</u>); ESI-MS (M-HCl)$^+$: m/z =188.1.

4-(5-Amino-3-phenyl-pyrazol-1-yl)-benzenesulfonamide **(4d)**

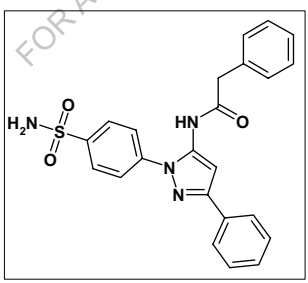
To a clear solution of 3-Oxo-3-phenyl-propionitrile (10g, 0.068 mol) in Ethanol (100ml) at 25°C was added hydrochloride salt of 4-hydrazinylbenzenesulfonamide (15.4g, 0.068 mol) to form a suspension which was then heated to refluxed temperature for 3h to become a clear solution. Reaction mixture was allowed to cool to 25°C. Obtained solid was collected by filtration and dries under vacuumed to yield the desired intermediate **4d** as a yellowish solid, (15g, yield= 70 %); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.87-7.94 (m, 4H, Ar-<u>H</u>), 7.77-7.79 (m, 2H,Ar-<u>H</u>), 7.33-7.42 (m, 5H, Ar-<u>H</u>), 6.02 (s, 1H, Pyrazole-<u>H</u>), 5.72 (br s, Ar-N<u>H</u>$_2$); ESI-MS (M+H)$^+$: m/z = 315.1.

***N-[5-Phenyl-2-(4-sulfamoyl-phenyl)-2H-pyrazol-3-yl]-benzamide* (47)**



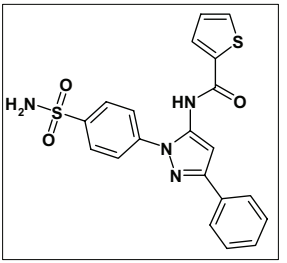
off-white solid; (1.0 g, yield = 75 %) ; mp= 132-134°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.11(d, 2H, J = 8.4 Hz, Ar-H), 7.99 (d, 2H, J = 8.8 Hz, Ar-H), 7.91 (d, 2H, J = 7.2 Hz, Ar-H), 7.62 (d, 2H, J = 7.2 Hz, Ar-H), 7.50 (t, 2H, J = 8.0 Hz, J = 7.2 Hz, Ar-H), 7.40 (t, 2H, J = 7.6 Hz, Ar-H), 7.33-7.34 (m, 3H, Ar-H), 5.97 (s,1H, Pyrazole-H), 5.77(br s, 2H,-SO_2-NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 94.7, 120.4 (2×C), 127.4 (2×C), 127.6(2×C), 127.8 (2×C), 127.9, 128.8, 128.9 (2×C), 129.4 (2×C), 133.1, 134.2, 137.2, 142.9, 147.9, 151.3, 164.8; ES-MS: m/z 420.3 $(M+H)^+$. Anal. Calcd. for **$C_{22}H_{18}N_4O_3S$**; C, 63.14; H, 4.34; N, 13.39; Found: C, 63.17; H, 4.33; N, 13.44.

***2-Phenyl-N-[5-phenyl-2-(4-sulfamoyl-phenyl)-2H-pyrazol-3-yl]-acetamide* (48)**



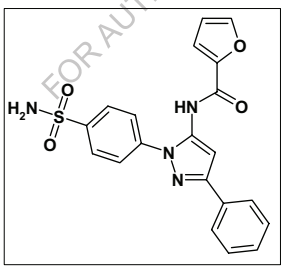
off-white solid (0.967g, yield= 70%) ; mp= 136-138°C;^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.14 (s, 1H, -CO-NH), 7.94 (d, 2H, J = 8.4 Hz, Ar-H), 7.84-7.86 (m, 4H, Ar-H), 7.78 (d, 2H, J = 8.0 Hz, Ar-H), 7.35-7.46 (m, 6H, Ar-H), 5.90 (s,1H, Pyrazole-H), 3.14 (s, 2H, Ar-CH_2-CO); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 42.0, 88.32, 121.9, 125.2, 126.9, 128.0, 128.3, 128.5, 128.8 (3×C),, 128.9, 129.2, 129.3 (2×C),, 133.0, 133.8, 135.6, 143.4, 149.1, 149.1, 151.3, 169; ES-MS: m/z 433.3 $(M+H)^+$. Anal. Calcd. for **$C_{23}H_{20}N_4O_3S$**; C, 63.87; H, 4.66; N, 12.95; Found: C, 63.83; H, 4.63; N, 12.94.

***Thiophene-2-carboxylic acid [5-phenyl-2-(4-sulfamoyl-phenyl)-2H-pyrazol-3-yl]-amide* (50):**



yellowish solid; (0.963 g, yield = 71 %) ; mp= 142-144°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.11(d, 2H, J = 8.8 Hz, Ar-H), 8.01 (d, 2H, J = 8.8 Hz, Ar-H), 7.96 (d, 2H, J = 8.8 Hz, Ar-H), 7.71-7.77 (m, 2H, Ar-H), 7.53-7.57 (m, 2H, Ar-H), 7.31-7.47 (m, 5H, Ar-H), 5.97 (s,1H, Pyrazole-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 94.9, 120.5 (2×C), 127.4 (2×C), 127.9 (2×C), 128.7, 128.9, 129.2 (2×C), 133.0, 137.2, 137.4, 137.7, 135.9, 142.9, 148.0, 151.3, 161.9; ES-MS: m/z 425.2 $(M+H)^+$. Anal. Calcd. for **$C_{20}H_{16}N_4O_3S_2$**; C, 56.59; H, 3.80; N, 13.20; Found: C, 56.61; H, 3.83; N, 13.23.

***Furan-2-carboxylic acid [5-phenyl-2-(4-sulfamoyl-phenyl)-2H-pyrazol-3-yl]-amide* (51)**



brownish solid; (0.955 g, yield = 73 %) ; mp= 138-140°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.13(d, 2H, J = 8.4 Hz, Ar-H), 7.96 (d, 2H, J = 8.4 Hz, Ar-H), 7.89 (d, 2H, J = 8.8 Hz, Ar-H), 7.71-7.77 (m, 2H, Ar-H), 7.53-7.57 (m, 2H, Ar-H), 7.31-7.47 (m, 5H, Ar-H), 5.99 (s,1H, Pyrazole-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 94.8, 111.7, 113.4, 120.6 (2×C), 127.5 (2×C), 127.9 (2×C), 128.7, 128.8, 129.2 (2×C), 133.0, 142.7, 146.1, 147.2, 148.0, 151.3, 161.9; ES-MS: m/z 409.1 $(M+H)^+$. Anal. Calcd. for $C_{20}H_{16}N_4O_4S_2$; C, 58.81; H, 3.95; N, 13.72; Found: C, 58.83; H, 3.97; N, 13.75.

3.2.3.2. *In-vitro* Antimicrobial activity

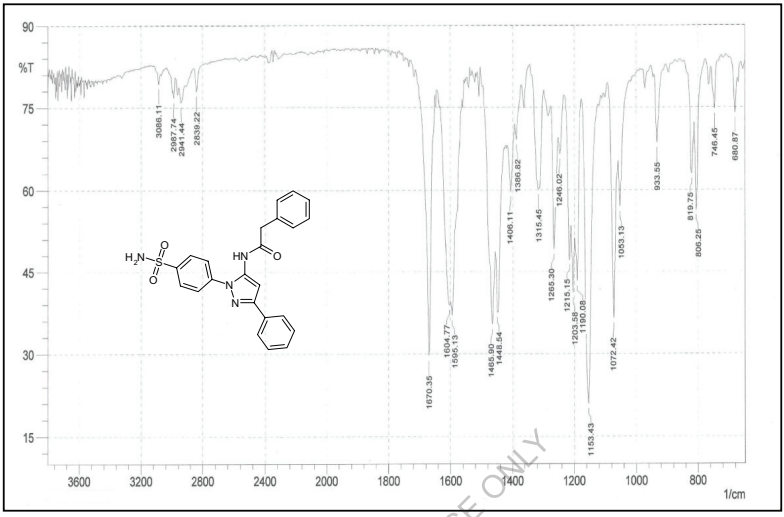
The evaluation of the synthesized compounds (**46-55**) for antibacterial activity was carried out by standard literature procedure [19] using agar diffusion method by finding the zone of inhibition of the drug sample against the standard drugs. The organisms employed in vitro testing of the compounds were *Escherichia coli* (Gram Negative) and *Bacillus subtilis* (Gram Positive). All the cultures were maintained on Nutrient agar (Microbiology) grade, Hi Media medium by periodic sub culturing. Ciprofloxacin was used as reference compound for antibacterial activity. The compounds were tested at a concentration of a 50µg/ml and 100µg/ml and were prepared in Dimethylsulphoxide. Obtained zone of inhibition at tested concentrations were tabulated in **Table 17**.

3.2.3.3. *In-vitro* Cyclooxygenase Inhibition Assay

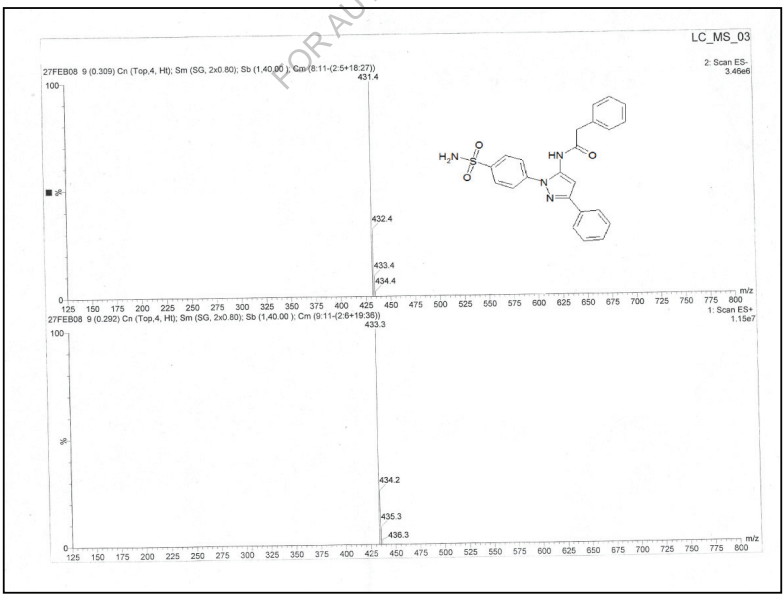
Stock solutions of test compounds were prepared in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (150 ml, 100 mMTrise-HCl, pH 8.0) with either COX-1 or COX-2(10 ml) enzyme in the presence of Heme (10 ml) and fluorometric substrate (10 ml) were added 10 ml of various concentrations of the test compound solutions (final between 0.01 and 100 mM). The reactions were initiated by quickly adding 10 ml of arachidonic acid solution and then incubated for 2 min at room temperature. Fluorescence of resorufin that is produced by the reaction between PGG2 and the fluorometric substrate, ADHP (10-acetyl-3, 7- dihydroxyphenoxazine) was read with an excitation wavelength of 535 nm and an emission wavelength of 590 nm. The intensity of this fluorescence is proportional to the amount of resorufin, which is proportional to the amount of PGG2 present in the well during the incubation. Percent inhibition was calculated by comparison from the 100% initial activity sample value (no inhibitor). The concentration of the test compound causing 50% inhibition of COX-1 and COX- 2 (IC50, mM) was calculated from the concentration-inhibition response curve (triplicate determinations).

Spectral Data for Compound No. 48

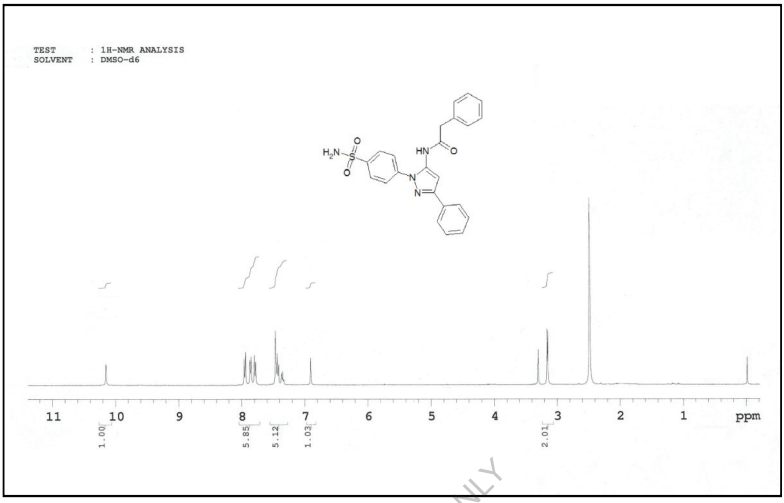
IR



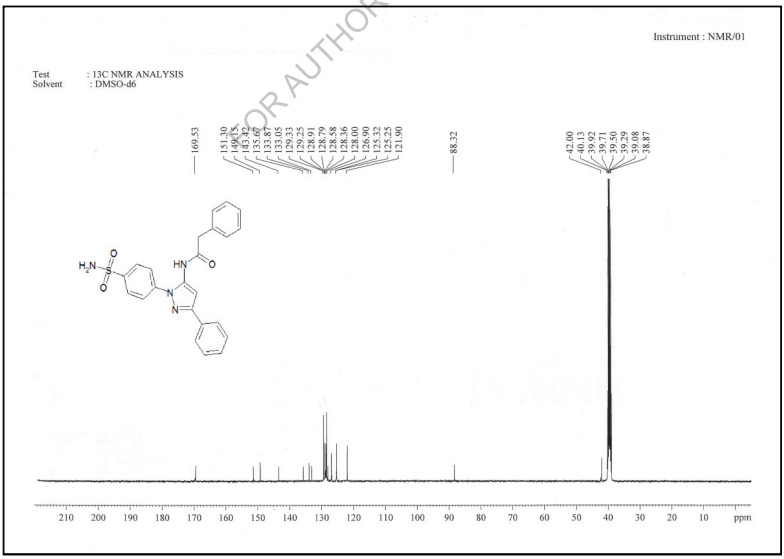
ES-MS



^{1}H NMR (DMSO-d_6, 400 MHz)



^{13}C NMR (DMSO-d_6, 125 MHz)



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SECTION C

3.3. Synthesis and anti-inflammatory studies of *4-(3-Hydrazino-carbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamide* derivatives

3.3.1. AIMS & OBJECTIVES

Fast and effective relief of pain and inflammation in the human being is continued to be a major task for the medicinal chemist. Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for the alleviation of pain and inflammation associated with a number of pathological conditions [1]. NSAIDs bestow their effect by inhibiting the catalytic activity of cyclooxygenase (COX), exists as two distinct isoforms (COX-1 and COX-2) [2]. Inhibition of COX-2 accounts for the anti-inflammatory effects of NSAIDs, whereas interruption of COX-1 leads to gastrointestinal toxicity ranging from ulcers to perforation and bleeding [3].

Celecoxib, in the 1, 5-diarylpyrazole class of compound was the first launched selective COX-2 inhibitor, and has excellent selectivity and potent antiinflammatory activity; having advantage of not associating with increased cardiovascular complications [4] but known to have gastrointestinal side effects [5].

The main part of our research has been devoted to synthetic methods containing the pyrazole nucleus, as a pharmacophoric moiety for potential drugs. Also hydrazide and their heterocyclic analogs showed evidence of diverse biological activities including anticancer [6] and antiinflammatory [7] properties. In particular, the pyrazole nucleus represents a very attractive scaffold to obtain new molecules endowed with anti-inflammatory activities. On the basis of these considerations, and in view of the reported COX-2 inhibitory activities of certain 4-(3-Hydrazinocarbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamidederivatives bearing two aryl moieties at 1- and 5-positions of the pyrazole ring and carrying different substituent on the 4-Hydrazinocarbony and 3-Hydrazinocarbony residue was synthesized. The synthesized compounds have a characteristic molecular pattern and bulk volume to fulfil the pharmacophoric requirements for better recognition at the COX-2 binding active site. The newly synthesized analogues were evaluated for their COX selectivity and their *in-vivo* antiinflammatory activity.

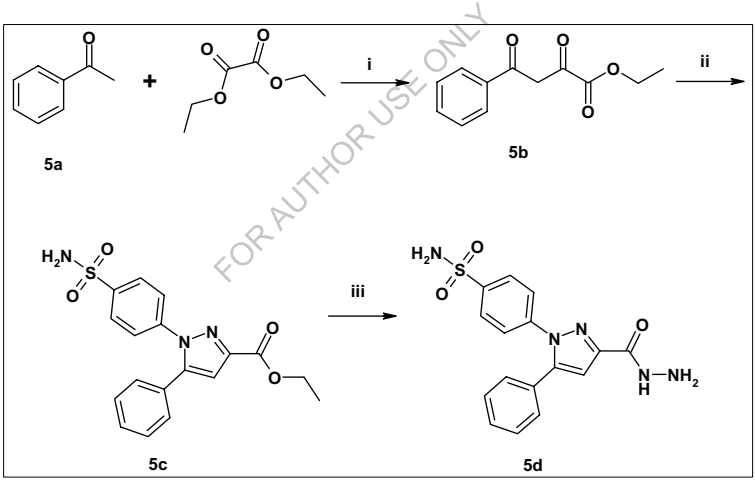
3.3.2. RESULTS AND DISCUSSION

3.3.2.1. Chemistry

The starting material, (**Scheme 12**) Ethyl-2, 4-dioxo-4-phenylbutanoate 5b was prepared from Acetophenone **5a** and Diethyl Oxalate in toluene using sodium hydride at 0°C to 50°C. Cyclization of ester intermediate **5b** to pyrazole **5c** was achieved using (4-sulfamoylphenyl) hydrazine hydrochloride in ethanol under reflux condition. Reaction of Hydrazine hydrate with the cyclized pyrazole **5c** convert its ester group to corresponding hydrazide intermediate **5d** in Ethanol with reflux condition. Coupling hydrazide intermediate **5d** with substituted acid side (**Scheme 13**) chains using EDC.HCl and HOBt in DMF resulted into proposed compounds (**56-65**) in 60-70% yield.

Scheme 12

Synthetic route for ***4-(3-Hydrazinocarbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamide*** **(5d)**



Reagents and conditions:- **(i) NaH/Toluene, N_2, 0°C to 50°C, 4h (ii) (4-sulfamoylphenyl) hydrazine hydrochloride, EtOH, refluxed 6h (iii) Hydrazine hydrate/ EtOH, refluxed 4h.**

^{1}H NMR spectrum of ester intermediate **5b** revealed the presence of triplet signal at δ 1.28 ppm (J = 7 Hz) and quartet signal at δ 4.32 ppm (J = 7 Hz) corresponds to ethyl group and two aromatic protons at δ 8.06 ppm (J = 8 Hz) corresponds to phenyl ring. Similarly ^{1}H NMR spectrum of pyrazole **5c** showed shift in triplet signals of ester intermediate **7** to δ 1.32 ppm (J = 7 Hz) and quartet signal to δ 4.34 ppm (J=7 Hz), an aromatic protons shifted to δ 7.85 ppm (J = 8 Hz), singlet of pyrazolyl proton was found at δ 7.13 ppm. Hydrazide intermediate

5d was confirmed by the disappeared signal of ester and by the shift of pyrazolyl proton to at δ 7.05 ppm.

Name reactions involved in the synthesis of thienopyrimidine

Knorr Pyrazole Reaction

The **Knorr pyrazole synthesis** is an organic reaction used to convert a hydrazine or its derivatives and a 1,3-dicarbonyl compound to a **pyrazole** using an acid catalyst.

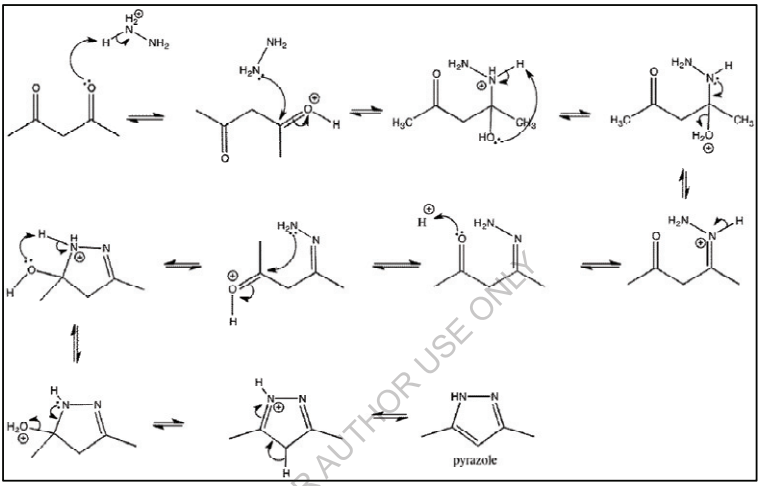
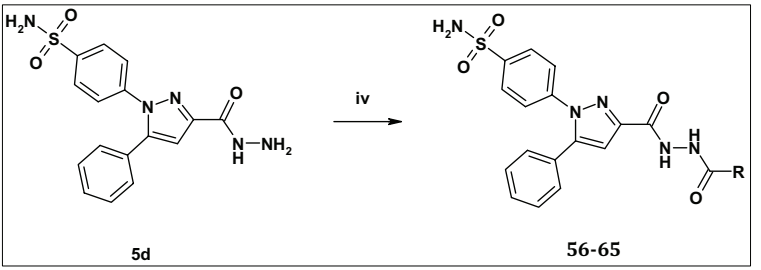


Fig.27: Mechanism of the Knorr Pyrazole Reaction

Scheme 13

Synthetic route for ***4-(3-Hydrazinocarbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamidehydrazides*** **(56-65)**

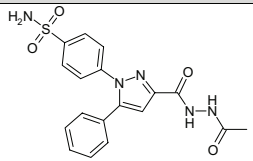
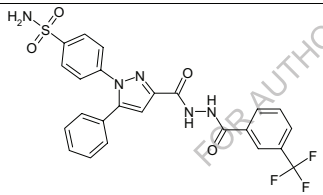
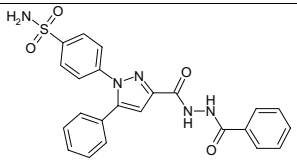
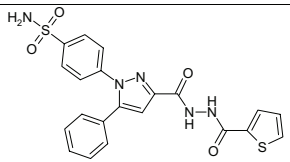


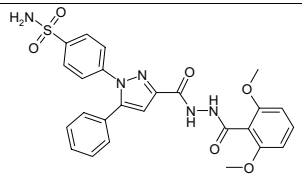
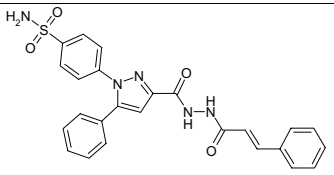
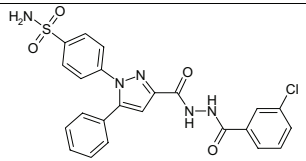
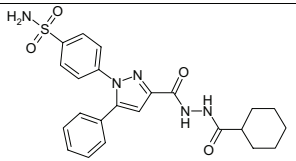
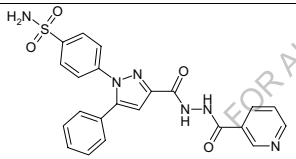
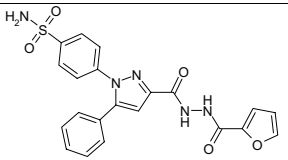
iv) R-Acid, EDC.HCl, HOBt, DIPEA, DMF, RT, 4h.

General procedure

To a stirred solution of benzoic acid (0.170g, 0.0014 mol) in dry Dimethylformamide (5ml) was added EDC.HCl (0.402g, 0.0021 mol) and 4-(3-Hydrazinocarbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamide **5d** (0.50g, 0.0014 mol), followed with the addition of HOBt (0.189g, 0.0014mol). Stirred for 6h.Water was added (50ml), and product was extracted in EtOAc (2×50ml), Organic layers was washed with $NaHCO_3$ solution in water, 10% aq. HCl, 25% aq. NH_4Cl solution in water, brine and dried over Na_2SO_4 and removed under vacuum to get crude residue which was stirred in diethyl ether and filtered to get title product (**56-65**) as white solid; **Scheme 13**.

Table 20: Physical & ES-MS (*m/z*) data of synthesized compounds (56-65)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
56		$C_{18}H_{17}N_5O_4S$	400.3	72	124-126
57		$C_{24}H_{18}F_3N_5O_4S$	528.5	74	136-138
58		$C_{23}H_{19}N_5O_4S$	462.3	83	128-130
59		$C_{21}H_{17}N_5O_4S_2$	468.2	79	122-124

60		**$C_{25}H_{23}N_5O_6S$**	522.4	69	132-134
61		**$C_{25}H_{21}N_5O_4S$**	488.2	80	128-130
62		**$C_{23}H_{18}ClN_5O_4S$**	496.3	76	125-127
63		**$C_{23}H_{25}N_5O_4S$**	468.3	76	134-136
64		**$C_{22}H_{18}N_6O_4S$**	463.3	75	148-150
65		**$C_{21}H_{17}N_5O_5S$**	451.2	85	138-140

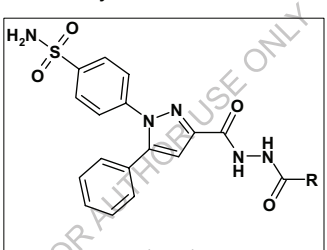
3.3.2.2. Biology

3.3.2.2.1. *In-vitro* cyclooxygenase inhibition assay

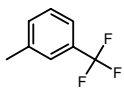
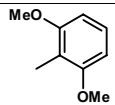
The *in vitro* COX-1/COX-2 isozyme inhibition studies measure the ability of tested compounds to inhibit ovine COX-1 and human recombinant COX-2 using an enzyme immunoassay (EIA). The obtained results (**Table 21**) showed that all the tested diaryl pyrazole are found to be weak inhibitors for COX-1 isoenzyme (IC_{50} = 3.35µM-10.42 µM

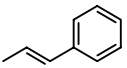
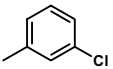
range) and exhibited moderate COX-2 isoenzyme (IC_{50}=0.52µM-3.70µM range) with COX-2 selectivity's in the range of 2.49-10.73. The acquired data for the diaryl series showed that when the two phenyl rings attached to the central heterocyclic pyrazole nucleus are vicinal (**56-65**) comparable inhibitory activities against both COX-1 (IC_{50} = 3.01µM- 10.42µM) and COX-2 (IC_{50}= 0.52 µM- 3.70µM) with celecoxib. In this series we replaced the trifluoromethyl group of the celecoxib with hydrazide and attached different alkyl and aryl carboxylic acid side chains. Within the biologically active series (**56-65**), the trifluoromethyl (**57**) and chloro (**62**) analogs were more selective COX-2 inhibitors (selectivity indexes=10.73 and 8.36 respectively) than unsubstituted phenyl (**58**), 3-pyridyl (**64**) and 2-thienyl (**59**) analogs (selectivity indexes = 7.93, 8.30 and 6.79 respectively) in comparison with celecoxib selectivity index = 9.51. Aliphatic acid side chains compounds (**56** & **63**) were stand inferior both in activity and COX-2 selectivity.

Table 21: *In-vitro* antiinflammatory COX-1 and COX-2 activities of compounds



56-65

Comp. No.	R	COX-1 IC_{50} (µM)[a]	COX-2 IC_{50} (µM)[a]	SI[b]
56	$-CH_3$	9.75	2.08	4.68
57		**5.58**	**0.52**	**10.73**
58		6.51	0.82	7.93
59		4.21	0.62	6.79
60		10.42	2.80	3.72

61		9.58	3.20	2.99
62		**7.52**	**0.90**	**8.36**
63		9.23	3.70	2.49
64		3.357	0.43	8.30
65		5.92	1.62	3.65
Celecoxib		7.42	0.78	9.51
Ibuprofen		3.2	1.40	2.28

[a]IC_{50} values represents concentration of test compound required to produce 50% inhibition, the result is the mean of two value obtained by assay of enzyme kits obtained from (Cayman Chemicals Inc., Ann Arbor, MI).
[b]Selectivity index (COX-1 IC_{50} / COX-2 IC_{50}).

In the active series it was observed that the aromatic acid compounds substituted with halogen at third position (**57** & **62**) were most active. Heterocyclic acid side chains (**59** & **64**) also contributed towards the activities and COX-2 selectivity than the benzoic acid derivative **58**.

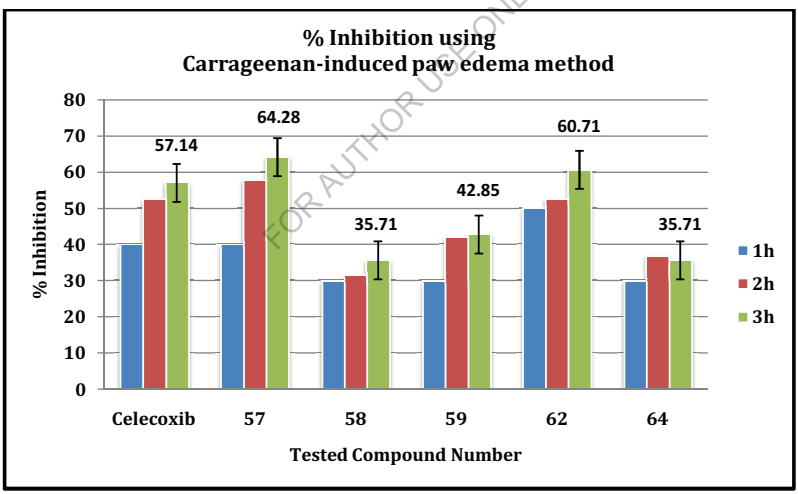
3.3.2.2.2. *In vivo* anti-inflammatory activity

The anti-inflammatory activities exhibited by selected five compounds (**57**, **58**, **59**, **62** and **64**) using dose 50mg/kg are listed in **Table 18**. The phenyl analog **58**, 2-thienyl analog **59** and 3-pyridyl analog **64** were found to possess antiinflammatory activity (35.71%, 42.85% and 35.71% reduction in inflammation after 3h) less than celecoxib (57.14% reduction in inflammation after 3h). While substituted phenyl derivatives **57** (3-trifluoromethyl) and **62** (3-chloro) which showed COX-2 activities *in vitro* comparable to celecoxib, showed *in vivo* activity (edema inhibition % = 64.28 & 60.71% respectively after 3h) higher than reference drug celecoxib (edema inhibition % = 57.14% after 3h) (**Graph 2**).

Table 22: Anti-inflammatory activity of the tested compounds using 50mg/kg dose employing carrageenan-induced paw edema method in mice

Comp. No.	Change in paw volume in (ml) after drug treatment (±SEM)				(% inhibition)		
	0h	1h	2h	3h	1h	2h	3h
Control	0.38±0.03*	0.48±0.04*	0.57±0.09*	0.66±0.04*	---	---	---
Celecoxib	0.27±0.10*	0.33±0.01*	0.36±0.10*	0.39±0.08*	40	52.63	57.14
57	**0.34±0.07***	**0.40±0.05***	**0.42±0.02***	**0.44±0.07***	**40**	**57.89**	**64.28**
58	0.37±0.02*	0.44±0.07*	0.50±0.07*	0.55±0.05*	30	31.57	35.71
59	0.30±0.07*	0.37±0.08*	0.41±0.09*	0.46±0.07*	30	42.10	42.85
62	**0.33±0.06***	**0.38±0.09***	**0.42±0.08***	**0.44±0.09***	**50**	**52.63**	**60.71**
64	0.35±0.03*	0.42±0.08*	0.47±0.06*	0.52±0.09*	30	36.84	35.71

*Data analyzed by one-way ANOVA followed by Dunnett's test, (n=6), *P <0.05 significant from control. Dose levels: Test compounds and Celecoxib (50 mg/kg, b. w. p. o.).*



Graph 2: % Inhibition at 50mg/kg dose of the tested compound

3.3.2.2.3. Evaluation of ulcerogenicity index

Compounds **57** and **62** with potential *in-vitro* cox-2 inhibitory activity and showed higher *in-vivo* anti-inflammatory activity than celecoxib were evaluated for their ulcerogenic liability according to a known method [8]. From the obtained results (**Table 23)**, compound **57** and **62** were found to be less ulcerogenic (Ulcer Index = 6.6 and 8.65 respectively) than the

Ibuprofen (Ulcer Index = 17.06) and displayed comparable ulcerogenic potentials with the celecoxib (Ulcer Index = 5.54). Hence **57**and **62** was found to be safe gastric profile.

Table 23: Ulcerogenic liability of compounds 57 & 62

Compound	Average severity	Average no. of ulcers	% incidence	Ulcer Index[a]
57	**0.5**	**0.6**	**5.5**	**6.6**
62	0.65	1.5	6.5	8.65
Celecoxib	0.54	0.5	4.5	5.54
Ibuprofen	2.26	6.5	8.3	17.06

[a]statistical analysis using one-way ANOVA followed by Dunnett's test, (n=6),
*** P < 0.01 significant from control*

3.3.2.2.4. Molecular Modelling Studies

The Significant correlations pertaining to the Protein inhibitory connections and structural characteristics of COX-2 active site can be established by molecular modelling studies as docking. COX-2 is the most versatile enzymatic target to recognize the inflammation and thereby the prediction of pharmacological potential of synthesized novel pyrazole derivatives. For this reason; to predict the COX-2 selectivity, the synthesized compounds **56-65** along with standard compounds diclofenac and potent COX-2 inhibitor celecoxib were subjected to molecular docking studies on COX-2 receptor using crystal structure of COX-2 (3LN1) [9]. For the purpose of assessment of docking of ligands to protein active sites for estimation of binding affinities of docked compounds, an advanced molecular docking programme GLIDE (Schrodinger Inc., USA) version 4.5 was used. GLIDE; the Grid-based ligand docking with energetics algorithm approximates a systematic search of positions, orientations and conformations and eliminates unwanted conformations using scoring in the enzyme pocket *via* a series of hierarchical filters. Finally the conformations were refined *via* Monte Carlo sampling of pose conformation [10-11]. X-ray crystal structure of COX-2 was taken from PDB entry COX-2 (3LN1) having resolution of 2.80 Å. Structural preparations for docking studies were accomplished using protein preparation wizard in Maestro 9.0. Protein preparation was carried out in two steps *viz.* preparation and refinement. Chemical correctness was ensured and water

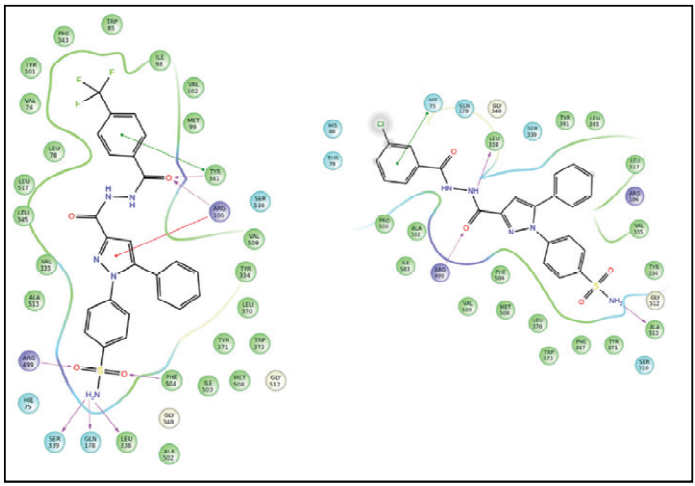


Fig.28: Interactions of 57 and 62 with the receptor

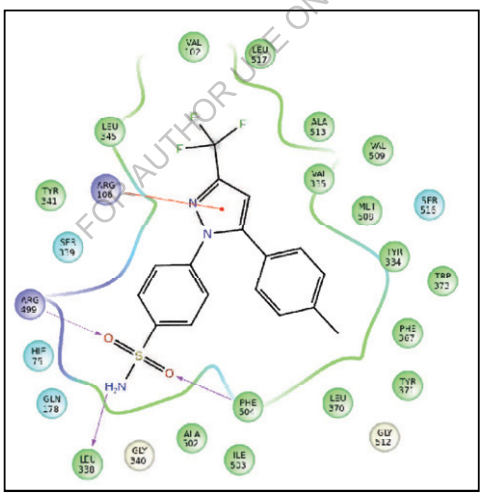


Fig.29: docking interactions of Celecoxib with COX-2

molecules in crystal structure were deleted and hydrogen atoms were added at missing positions and bond order for crystal ligand and protein was adjusted and minimized up to 0.30 Å RMSD. Ligprep 2.2 module in maestro 9.0 build panel was used for ligand preparation which produced low energy conformations of ligand using OPLS 2005 force field. Glide provides three different levels of docking precisions viz. High Throughput

Virtual Screening, HTVS; Standard precision, SP and Extra precision, XP. We carried out our calculations using XP docking mode as the tool is designed for better refinement in ligands. Molecular docking studies revealed that, all the designed molecules showed good interactions with receptor active site with glide scores in the range -13.130 to -10.624. Molecules **57** and **62**, the most potent COX-2 inhibitor in current series, showed good interactions with the receptor active site. (**Fig. 28**). Sulfonamide group of **57** showed hydrogen bonding with Leu338, Ser339, Arg499 and Phe504. Pyrazole ring of **57** showed pi stacking with Arg106. Compound **62** showed similar interactions with the receptor. All these interactions are also shown by celecoxib (**Fig. 29**). Thus, it can be concluded that these interactions contribute to the inhibitory activity of these molecules.

3.3.3. EXPERIMENTAL

3.3.3.1. Chemistry

2,4-Dioxo-4-phenyl-butyric acid ethyl ester **(5b)**

To a solution of Acetophenone **5a** (20g, 0.166 mol) in dry Toluene (200 ml) at -10°C was added sodium hydride (11.95g, 0.498 mol) and stirred under nitrogen atmosphere for 1hr. Diethyl oxalate (43.32g, 0.249 mol) in Toluene (100 ml) was added drop wise and allowed reaction mixture to room temperature. Reaction mixture then heated to 50°C for 4hr. Cooled the reaction mixture to -10°C was added water and (100ml) and crude product was extracted in Toluene. Organic layers were collected, dried over sodium sulfate and removed under vacuum to give crude intermediate which was purified by column chromatography (5% EtOAc in Hexane as eluent) to furnish yellow liquid intermediate **5b**. (28g, Yield= 76%); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.04 (d,2H, J =7.2 Hz, Ar-H), 7.93-7.94 (m, 1H, Ar-H), 7.67-7.70 (m, 1H, Ar-H), 7.54-7.58 (m,2H, Ar-H), 7.10 (s, 1H, Ar-H), 4.32 (q, 2H, J = 4.4Hz, -OCH_2-CH_3), 1.31 (t, 3H, -OCH_2-CH_3, J = 7.2Hz) ; ES-MS: *m/z* 221.1 $(M+H)^+$.

5-Phenyl-1-(4-sulfamoyl-phenyl)-1H-pyrazole-3-carboxylic acid ethyl ester **(5c)**

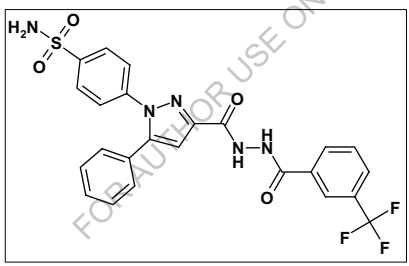
To a suspension of (4-sulfamoylphenyl) hydrazine hydrochloride (25.4g, 0.113mol) in Ethanol (100 ml) was added 2,4-Dioxo-4-phenyl-butyric acid ethyl ester **5b** (25g,0.113 mol) and refluxed the reaction mixture for 6h. Reaction mixture becomes clear at the completion of the reaction under reflux. Cooled the content to room temperature, precipitated solid was filtered and washed with chilled ethanol (10ml) and dried to get white solid as intermediate **5c** (30g, Yield= 69%); mp = 192-194° C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.84 (d,2H, J =8.4 Hz, Ar-H), 7.49-7.51 (m, 4H, Ar-H), 7.38-7.39 (m, 2H, Ar-H), 7.27-7.29 (m,2H, Ar-

H), 7.13 (s, 1H, Pyrazole-H), 4.34 (q, 2H, *J* = 6.8Hz, -OCH$_2$-CH$_3$), 1.30 (t, 3H, -OCH$_2$-CH$_3$, *J* =7.2 Hz) ; ES-MS: *m/z* 372.2 (M+H)$^+$

***4-(3-Hydrazinocarbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamide* (5d):**

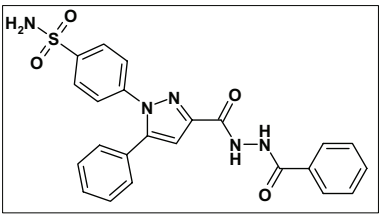
To a suspension of Ester intermediate (10g, 0.0269 mol) **5c** in Ethanol (50ml) was added hydrazine hydrate (6.72g , 0.134mol) and refluxed the reaction mixture for 6h. Reaction mixture becomes clear at the completion of the reaction under reflux. Cooled the content to room temperature, precipitated solid was filtered and washed with chilled ethanol (10ml) and dried to get white solid as hydrazide intermediate **5d** (8g, Yield= 83%); mp =187-189°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.73 (s, 1H, -NH-NH$_2$), 7.86 (d,2H, *J* =8.4 Hz, Ar-H), 7.54 (d, 2H, *J*=7.6 Hz, Ar-H), 7.38-7.44 (m, 4H, Ar-H), 7.27-7.29 (m,2H, Ar-H), 7.11 (s, 1H, Pyrazole-H),4.64 (br s, 2H,-NH-NH$_2$); ES-MS: *m/z* 358.1 (M+H)$^+$.

***4-{5-Phenyl-3-[N'-(3-trifluoromethyl-benzoyl)-hydrazinocarbonyl]-pyrazol-1-yl}-benzenesulfonamide* (57)**



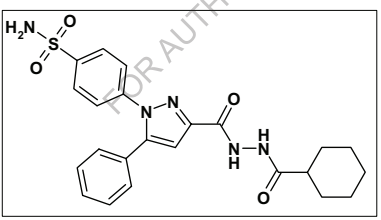
(0.55g, Yield:74%); greenish solid; mp = 136-138°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.81 (s, 1H, -CO-NH-), 10.51 (s, 1H, -CO-NH-), 8.27(s, 1H, Ar-H), 8.23 (d, 1H, *J* = 8Hz, Ar-H), 7.99 (d, 1H, *J* = 7.6 Hz, Ar-H), 7.88 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.78 (t, 1H, *J* = 7.6 Hz, Ar-H), 7.57 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.50 (s, 2H, Ar-SO$_2$-NH$_2$), 7.32-7.34 (m, 2H, Ar-H 7.42-7.44 (m, 3H, Ar-H), 7.34-7.35 (m, 2H, Ar-H), 7.16 (s, 1H, Pyrazole-H),^{13}C NMR (DMSO-d_6, 100 MHz) δ 108.7, 122.5, 125.2 (3×C), 125.7, 126.7, 128.5, 128.8 (2×C), 129.5, 129.9 (2×C), 131.6 (2×C), 133.3, 141.5, 143.6, 144.6, 146.0, 160.4, 164.3; ES-MS: *m/z* 528.5 (M+H)$^+$; Anal. Calcd. for **C$_{24}$H$_{18}$F$_3$N$_5$O$_4$S**: C, 54.44; H, 3.43; N, 13.23; Found:C, 54.47; H, 3.45; N, 13.25.

4-[3-(N'-Benzoyl-hydrazinocarbonyl)-5-phenyl-pyrazol-1-yl]-benzenesulfonamide **(58)**



(0.540g, Yield= 83%) ; mp= 128-130°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.49 (s, 1H,-CO-NH-), 10.36 (s, 1H, -CO-NH-), 7.91(d, 3H, J = 7.2 Hz, Ar-H), 7.86 (d, 2H, J = 8 Hz, Ar-H), 7.54-7.58 (m, 6H, Ar-H), 7.49 (s, 2H, Ar-SO_2-NH_2), 7.40-7.41 (br s, 2H, Ar-H), 7.31-7.32 (br s, 2H, Ar-H), 7.13 (s, 1H, Pyrazole -H), ^{13}C NMR (DMSO-d_6, 100 MHz) δ 108.7, 125.7(3×C), 126.7 (3×C), 127.5 (3×C), 128.5 (2×C),128.1, 128.8, 128.9 (2×C), 131.8, 132.5, 141.5, 143.5, 144.5, 146.1, 160.4, 165.7;ES-MS: *m/z* 462.3 $(M+H)^+$, mp= 128-130°C, Anal. Calcd. for **$C_{23}H_{19}N_5O_4S$**: C, 59.86; H, 4.15; N, 15.18; S, 6.95. Found: C, 59.83; H, 4.17; N, 15.16; S, 6.98.

4-[3-(N'-Cyclohexanecarbonyl-hydrazinocarbonyl)-5-phenyl-pyrazol-1-yl]-benzenesulfonamide **(63)**



white solid;(0.50g, Yield= 76%); mp = 134-136°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.11 (s, 1H, -CO-NH-), 9.78 (s,1H, -CO-NH-), 7.86 (d,2H, J = 9.2 Hz, Ar-H), 7.54 (d, 3H, J = 9.2 Hz, Ar-H), 7.48 (s, 1H, Ar-SO_2-NH_2), 7.41-7.43 (m, 3H, Ar-H), 7.29-7.32 (m, 2H, Ar-H), 7.09 (s, 1H, Pyrazole-H), 2.24 (m, 1H, -CH_2-CH_2-), 1.62-1.90 (m, 5H, -CH_2-CH_2-), 1.15-1.41 (m, 5H, -CH_2-CH_2-),^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.1(2×C), 25.4, 29.0 (2×C), 42.0, 108.6, 125.5 (3×C), 126.6 (3×C), 128.7, 128.8, 129.0, 129.1, 141.5, 143.5, 144.4, 146.1, 160.0, 174.5; ES-MS: *m/z* 468.3 $(M+H)^+$; Anal. Calcd. for **$C_{23}H_{25}N_5O_4S$**: C, 59.09; H, 5.39; N, 14.98; Found: C, 59.06; H, 4.37; N, 14.99.

Determination of anti-inflammatory activity

3.3.3.3.2. *In-vitro* Cyclooxygenase Inhibition Assay

Stock solutions of test compounds were prepared in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (150 ml, 100 mMTrise-HCl, pH 8.0) with either COX-1 or COX-2 (10 ml) enzyme in the presence of Heme (10 ml) and fluorometric substrate (10 ml) were added 10 ml of various concentrations of the test compound solutions (final between 0.01 and 100 mM). The reactions were initiated by quickly adding 10 ml of arachidonic acid solution and then incubated for 2 min at room temperature. Fluorescence of resorufin that is produced by the reaction between PGG2 and the fluorometric substrate, ADHP (10-acetyl-3, 7- dihydroxyphenoxazine) was read with an excitation wavelength of 535 nm and an emission wavelength of 590 nm. The intensity of this fluorescence is proportional to the amount of resorufin, which is proportional to the amount of PGG2 present in the well during the incubation. Percent inhibition was calculated by comparison from the 100% initial activity sample value (no inhibitor). The concentration of the test compound causing 50% inhibition of COX-1 and COX- 2 (IC_{50}, mM) was calculated from the concentration-inhibition response curve (triplicate determinations), results were tabulated in **Table 21**.

3.3.3.3. In-vivo Anti-inflammatory activity

Five compounds (**57**, **58**, **59**, **62** and **64**)) were studied further for in-vivo anti-inflammatory activity using carrageenan induced rat paw edema method (50mg/kg). A freshly prepared aqueous suspension of carrageenan (1.0% w/v, 0.1 ml) was injected in the sub planter region of right hind paw of each rat. One group was kept as control and the animals of the other group were pretreated with the test drugs and standard drug 1 h before the carrageenan treatment and is reported in **Table 2**. The paw volume of the all groups of rats were measured before injection of carrageenan for 0 minute and measured again after 1, 2 and 3 h after carrageenan injection with the help of digital plethysmometer (UGO BASIL, ITALY). The edema was expressed as a mean reduction in paw volume (ml) after treatment with test compounds and the percent of edema inhibition were obtained as follows:

$$\% \text{ inhibition} = \frac{(Vt - Vc)\text{control} - (Vt - Vc)\text{tested compound}}{(Vt - Vc)\text{control}} \times 100$$

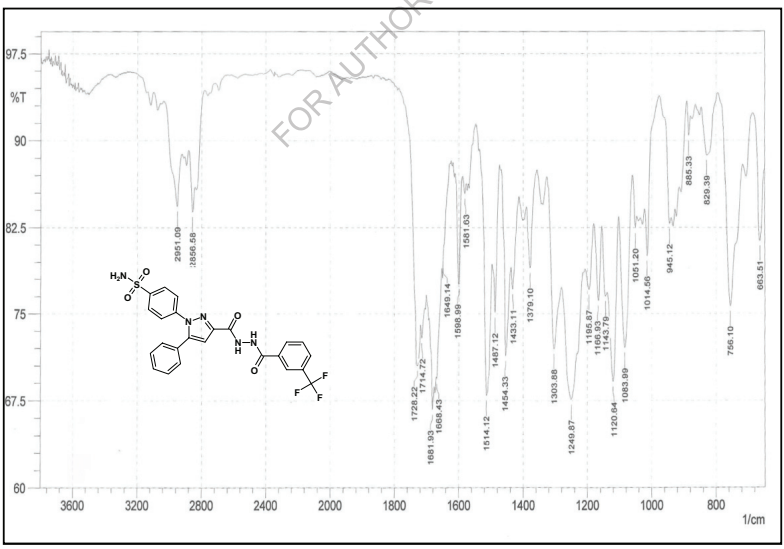
Where V_t = volume of edema at specific time interval and V_c = volume of edema at zero time interval.

3.3.3.4. Evaluation of ulcerogenicity index

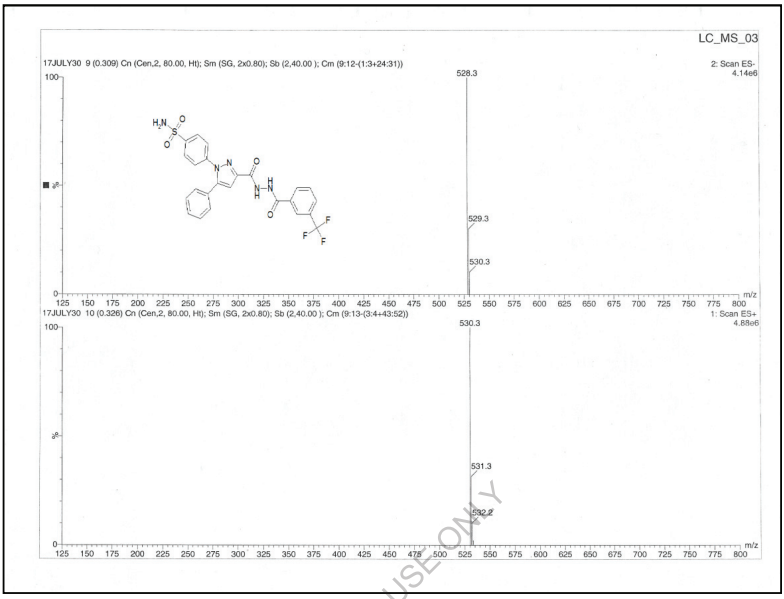
Compound **10b** and **10g** were studied further for ulcerogenicity index. Ulceration in rats was induced as described in known procedure [12]. Albino rats of the Wister strain weighing 150 - 200 g of either sex were divided into various groups, each of six animals. Control group of animals were administered only 1% carboxymethylcellulose solution in water. One group was administered with Ibuprofen at a dose of 100 mg/kg once daily for four days. The remaining group of animals was administered with test compound sat a dose of 100 mg/kg. On the fifth day, pylorus was legated as per the literature method [13]. Ten Animals were fasted for 24 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along with the greater curvature. Ulcer index was determined by the method of [8] and is recorded in **Table 23**.

Spectral Data of Compound 57

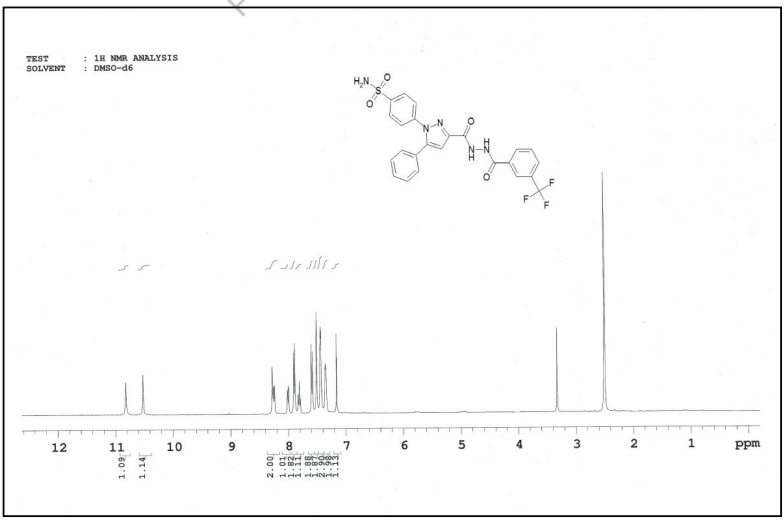
IR



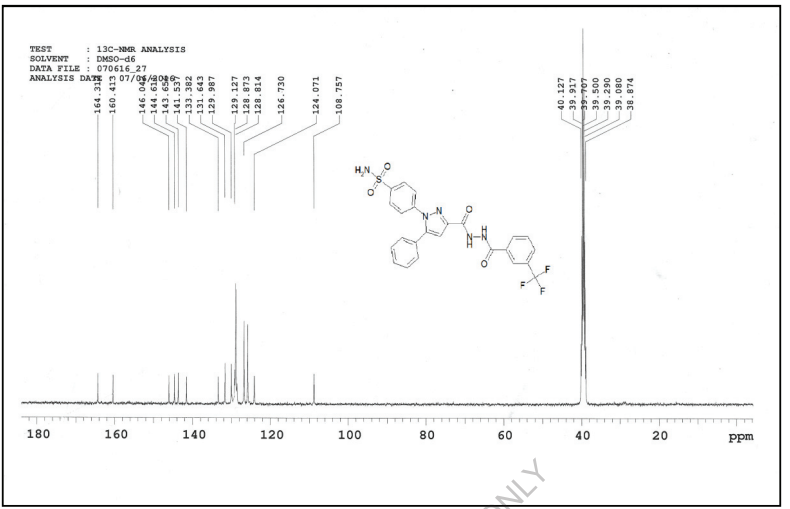
ESMS



^{1}H NMR



^{13}C NMR



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CHAPTER 4

Synthesis and biological studies of 4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl derivatives

SECTION A

4.1. Synthesis and anticancer studies of *1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid* derivatives

4.1.1. AIM & OBJECTIVES

The thiazolidine-2,4-dione (2,4-TZD) ring is a well-known scaffold in medicinal chemistry and has been used to develop new potential anticancer agents [1-3], such as the PI3Kα inhibitor GSK1059615 and its analogues [4]. Since 1,3,4-thiadiazole and thiazolidin-4-one moieties are biologically proven anticancer and antioxidant pharmacophores and substitution in these scaffolds may further enhance their activity, prompted us to undertake this problem. Moreover, the combination of two pharmacophores in a single molecule is a well established hypothesis for synthesis of more active drugs with dual activity [5]. Research into the antitumor efficacy of molecules which contain the 5-arylidene-thiazolidine-2,4-dione system has received significant attention over the last years [6-8].Thus, a series of novel sulphonamide substituted thiazolidine-4-ones were synthesized and evaluated for their antioxidant and anticancer activity. In our earlier anticancer research [9] we have found that L-proline containing amide and hydrazide derivatives showed potential anticancer activities in MCF-7 and HCT-15 cell line when tested *in-vitro*. Sulfonamide plays an essential role in biological activities [10]. In continuation to this research here we have combine the two pharmacophores derived from the preclinical candidates of GSK, i.e. aryl sulphonamides and thiazolidinedione. Here we have discussed the structure activity relationship observed after coupling this combination with the L-proline amide and hydrazide analogs. Dictated by the previous research results of thiazolidinone derivatives, the aim of the presented work was to

synthesize new substituted thiazolidinones with pyrazoline fragment and to investigate their anticancer activity.

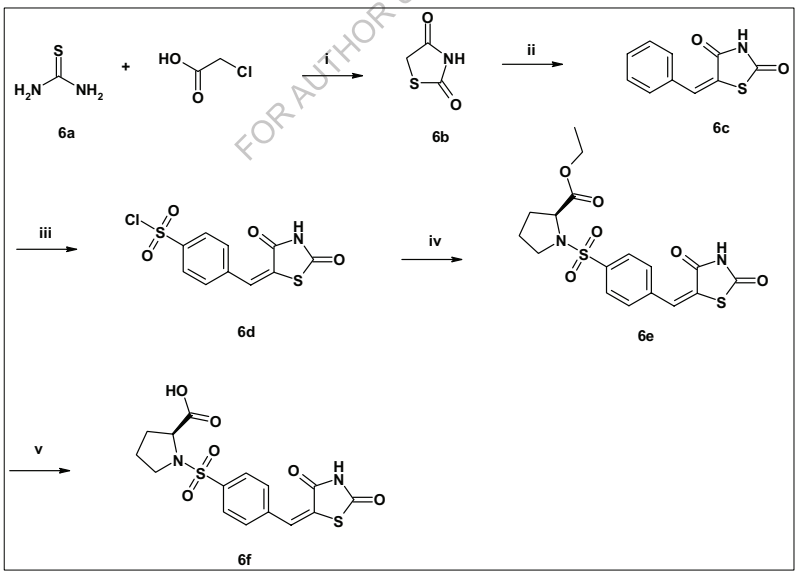
4.1.2. RESULTS AND DISCUSSION

4.1.2.1. Chemistry

The targeted compounds were prepared from acid intermediate **6** as outlined in **Scheme 14**. The starting material 2,4-thiazolidinedione **2** was prepared by following the method of [11] Thiourea **6a** and chloroacetic acid in water with the presence of Conc. Hydrochloric acid. Knoevenagel condensation was achieved to synthesized intermediate **6c** with 2,4-thiazolidinedione **6b** and benzaldehyde in Toluene with the presence of cat. piperidine. Aromatic sulfonylation was carried out on intermediate **6c** under cooling to reflux condition by using chlorosulfonic acid [12] to synthesized chloro sulfonyl intermediate **6d**.

Scheme 14

Synthetic route for ***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid*** **(6f)**



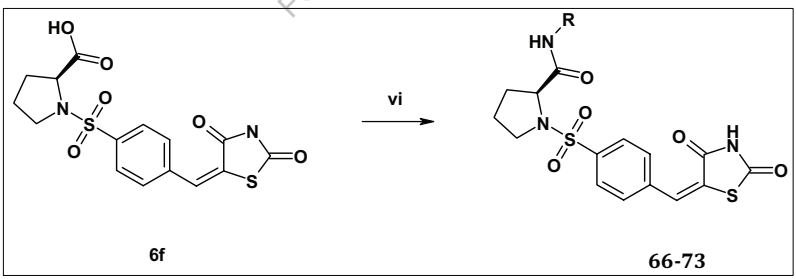
Reagents and conditions: **i) Conc. Hydrochloric acid, Water, Reflux; ii) benzaldehyde, Toluene, Reflux, 3h; iii) Chlorosulfonic acid; d. L-proline ethyl ester hydrochloride, Et_3N, 2,4-Dioxane; iv) $LiOH.H_2O$, THF: H_2O**

L-Proline ethyl ester was introduced by replacing chloro group of 5-benzylidine-2,4-thiazolidinedione **6d** in 2,4-dioxane and triethylamine to obtained the ester intermediate **6e**. Acid intermediate 1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid **6f** was obtained after the alkaline hydrolysis of ester intermediate **6e** by using lithium hydroxide in THF-water.

^{1}H NMR spectrum of **6b** revealed the presence of singlet for one proton at δ 12.0 ppm corresponding to the NH and singlet for two protons at δ 4.16 ppm corresponding to two methylene protons. Infrared (IR) spectrum of **6b** showed bands at 3387 cm^{-1} (NH), 1684 cm^{-1}(C=O) and 622 cm^{-1} (C-S) groups respectively. ^{1}H NMR spectrum of **6c** was identified by the characteristic multiplate of five aromatic protons at δ 7.49 to 7.62 ppm and singlet for benzylidine proton at δ 7.80 ppm for CH proton. Chloro intermediate **6d** was assigned by the shifted singlet at δ 7.77 ppm for CH proton and 2,4 thiazolidinone NH proton was shifted to δ 12.63 ppm. Crude ester intermediate **6e** was used directly for hydrolysis reaction; its formation was confirmed by the ESI-MS spectrum showing *m/z* 381.2. Acid intermediate **6f** showed a characteristic triplet signal at δ 4.14 ppm corresponding to chiral proton of L-proline and broad singlet at δ 12.74 ppm for the thiazolidinedione NH proton.

Scheme 15

Synthetic route for ***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid amides*** **(66-73)**



vi) R-Amine, EDC.HCl, HOBt, DMF, RT

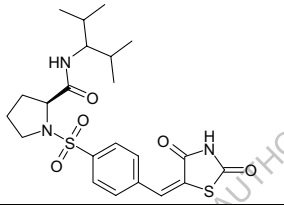
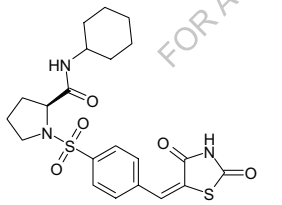
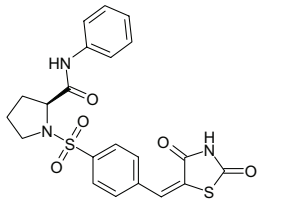
Novel amide compounds **66-73** were prepared by coupling selected amines with the acid core **6f** as outlined in **Scheme 15**. Substituted aromatic and aliphatic amines were selected to evaluate the structure activity relationship among the novel analogs.

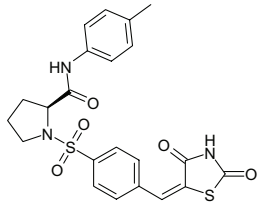
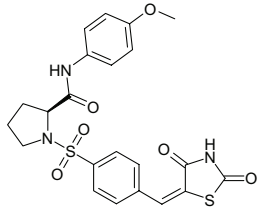
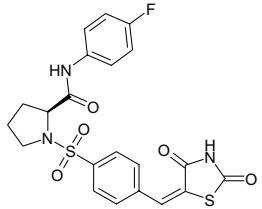
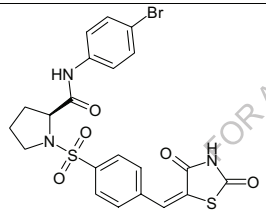
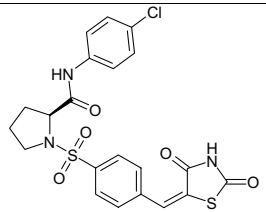
Coupling reactions were performed by using EDC.HCl and HOBt in DMF at 25°C. Purification of the desired product was achieved by crystallization and characterized by spectral and elemental analysis.

General procedure

To a solution of 1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid **6f** (1g, 0.0026 mol) in DMF (5ml) was added EDC.HCl (0.0.75 g, 0.0039 mol) and R-amine (0.257 g, 0.0026 mol) followed by the HOBt (0.351 g, 0.0026 mol) at 25°C. Reaction mixture was the stirred for the 6h. Quenched the reaction mixture with water (50ml), white solid was comes out was filtered, dried and washed with diethyl ether to gave amide products (**66-73**) as given in **Scheme 15**.

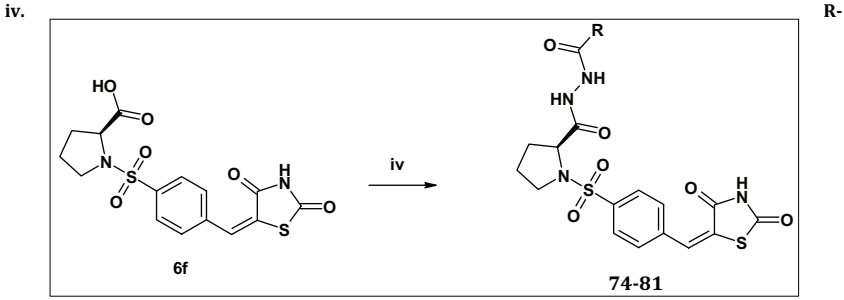
Table 24: Physical & ES-MS (*m/z*) data of synthesized compounds (66-73)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
66		$C_{22}H_{29}N_3O_5S_2$	480.3	65	147-149
67		$C_{21}H_{25}N_3O_5S_2$	463.2	68	137-139
68		$C_{21}H_{19}N_3O_5S_2$	458.2	76	147-149

69		$C_{22}H_{21}N_3O_5S_2$	472.1	72	153-155
70		$C_{22}H_{21}N_3O_6S_2$	488.3	61	161-163
71		$C_{21}H_{18}FN_3O_5S_2$	476.2	79	154-156
72		$C_{21}H_{18}BrN_3O_5S_2$	537.3	75	173-175
73		$C_{21}H_{18}ClN_3O_5S_2$	492.1	85	163-165

Scheme 16

Synthetic route for ***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid hydrazide*** (**74-81**)

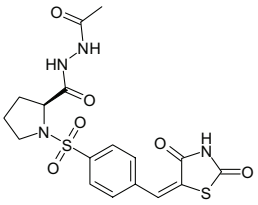
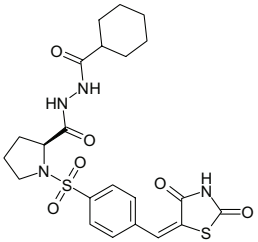
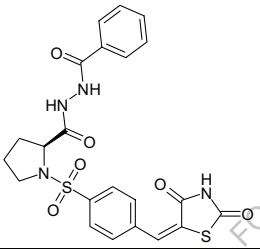
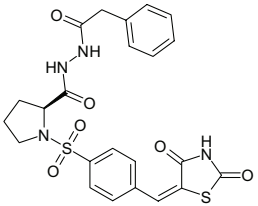
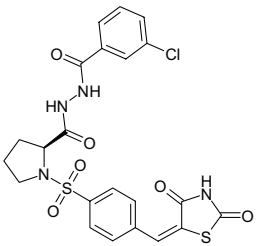


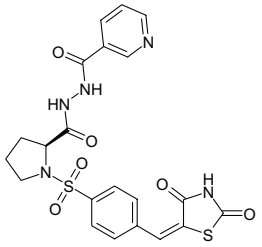
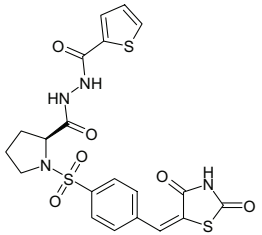
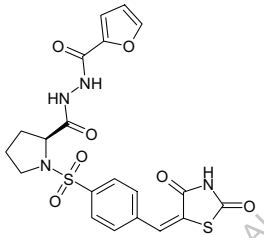
Similarly hydrazide derivatives containing aliphatic and aromatic hydrazide were also studied for anticancer activities. Synthesized compounds **74-81** by coupling selected hydrazide with the acid core **6f** as outlined in **Scheme 16**. Coupling reactions were performed by using EDC.HCl and HOBt in DMF at 25°C.

General procedure

To a solution of 1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid **6f** (1g, 0.0026 mol) in DMF (5ml) was added EDC.HCl (0.0.75 g, 0.0039 mol) and R-carboxylic acid hydrazide (0.369 g, 0.0026 mol) followed by the HOBt (0.351 g, 0.0026 mol). The solution was stirred for 6h. Quenched the reaction mixture with water (50ml), white solid was comes out was filtered, dried and washed with diethyl ether to gave hydrazide products, **(74-81)** as shown in Scheme **16**.

Table 25: Physical & ES-MS (*m/z*) data of synthesized compounds (74-81)

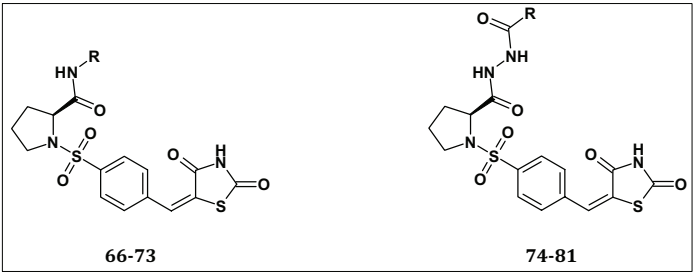
No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
74		$C_{17}H_{18}N_4O_6S_2$	439.1	80	163-165
75		$C_{22}H_{26}N_4O_6S_2$	507.3	72	173-175
76		$C_{22}H_{20}N_4O_6S_2$	501.4	79	187-189
77		$C_{23}H_{22}N_4O_6S_2$	515.4	78	193-195
78		$C_{22}H_{19}ClN_4O_6S_2$	436.3	75	185-187

79		$C_{21}H_{19}N_5O_6S_2$	502.4	68	201-203
80		$C_{20}H_{18}N_4O_6S_3$	507.3	76	189-191
81		$C_{21}H_{18}ClN_3O_5S_2$	491.3	74	192-194

2.1.2.2. Evaluation of Anticancer Activity

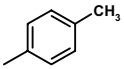
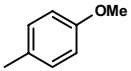
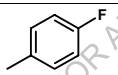
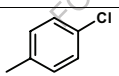
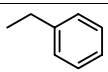
All tested compound, except **71** (TGI = 98.8), showed **TGI >100** and **LC_{50}>100** for both the breast MCF-7 and colon HCT- 15cell line.

We have designed and prepared eight novel compounds, (**66-73**). The results of the study were tabulated in **Table 22**. The variation was brought across acid core **6** with six different anilines to get the desired derivatives (**68-73**). In this series, 4-Fluoro aniline compound **71** has shown remarkable inhibitory activity in MCF-7 cell line (GI_{50} = 0.1 µmol/L) with good inhibitory activities in HCT-15 cell line (GI_{50} = 56.9 µmol/L) and Hep-G2 cell line (GI_{50} = 32.2 µmol/L). All the other aniline derivatives showed very poor inhibitory activity in HCT-15 cell line.



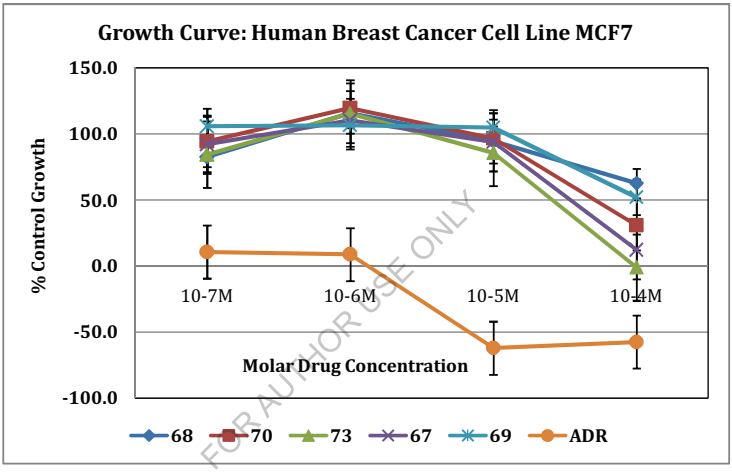
66-73 74-81

Table 26. GI_{50} of the tested compounds against human tumour cell line

No.	R	GI_{50} (µmol/L)		
		Breast MCF-7	Colon HCT-15	Hepatoma Hep-G2
67		58.1	> 80	99.9
68		>100	> 80	> 80
69		>100	> 80	> 80
70		74.7	> 80	> 80
71		**< 0.1**	24.0	32.2
73		49.0	71.8	> 80
75		>100	88.7	>100
76		>100	>100	>100
77		>100	>100	>100
79		>100	>100	>100

80		>100	>100	>100
81		>100	63.5	>100
Reference	***Adriamycin***	**< 0.1**	**< 0.1**	**<0.1**

4-Chloro aniline **73** revealed inferior activity in MCF-7 cell line than the 4-Fluoro aniline compound **71**. 4-Methoxyaniline **70** found to show better activity than 4-Methylaniline **69** in MCF-7 cell line, for HCT-15 cell line and Hep-G2 cell line both are equally inferior.



Graph 3: % Control Growth Vs Average Molar Drug Concentrations

Here, electron withdrawing group found to improving the potential of inhibiting the anticancer activities in MCF-7 cell line; therefore it was observed that compound **71** and compound **73** are superior to compound **69** and compound **70**, respectively. Only compound **71** and compound **73** were showed notable contribution among the compounds **68-73** in Human Colon Cancer Cell Line HCT-15. When we compare the activities of aniline **68** with the cyclohexyl amine **67**, we found that compound **67** showed better inhibition in MCF-7 cell line (GI_{50} = 58.1µmol/L) and Hep-G2 cell line (GI_{50} = 99.9 µmol/L) than **68**. In conclusion, between the three studied cell lines, compound **71** was found to be potent for MCF-7 cell line.

Hydrazide compounds **75-81** were also studied for the anticancer activities in MCF-7 cell line, HCT-15 and Hep-G2 cell line. All the tested compounds **75-81** was reportedly showed

(GI_{50} > 100μmol/L) in MCF-7 cell line and Hep-G2 cell line. Compound **75** (GI_{50} = 88.7μmol/L) and compound **81** (GI_{50} = 63.5μmol/L) exhibiting the anticancer activities for the human colon HCT-15 cell line. Compound **75** contains Cyclohexane carboxylic acid hydrazide side chain and compound **81** was with 2-Furoic acid hydrazide side chain. Between the two series of compounds containing substituted anilines **66-73** and hydrazide side chains **75-81**, it was found that substituted anilines resulted in improved activities in MCF-7 cell line than HCT-15 cell line and Hep-G2 cell line. Hydrazide compounds were very poor in all the three tested cell lines.

4.1.3. EXPERIMENTAL

4.1.3.1. Chemistry

***2,4-thiazolidinedione* (6b)**

To a solution of chloroacetic acid (60g, 0.6 mol) in 60 ml of water was added thiourea (50 g, 0.6 mol) dissolved in 60 ml of water was added. The mixture was stirred for 15 min to form a white precipitate, accompanied by considerable cooling. To the contents of the flask, 60 ml of concentrated hydrochloric acid was then added slowly from a dropping funnel, the flask was then connected with a reflux condenser and gentle heat applied to effect complete solution, after which the reaction mixture was stirred and refluxed for 8-10 h at 100-110°C. On cooling the contents of the flask solidified to a cluster of white needles, the product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was purified by recrystallized from ethyl alcohol to gave white crystalline solid (60g, Yield = 85%); mp = 122-127°C, IR (KBr disk) 3387 cm^{-1} (NH), 1684 cm^{-1} (C=O), 622 cm^{-1} (C-S); ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6) 12.00 (s, 1H, NH), 4.14 (s, 2H). ^{1}H-NMR (DMSO-d_6) D_2O Exchange experiment δ in ppm: 4.16 (s, 2H); ES-MS: *m/z* 118.2 $(M+H)^+$.

***5-benzylidine 2,4-thiazolidinedione* (6c)**

To a suspension of benzaldehyde (22.6g, 0.213 mol) and 2,4-thiazolidinedione (25g, 0.213 mol) in Toluene was added a catalytic amount of piperidine (1 mL) . Attached a dean-stark apparatus, and the mixture was stirred and refluxed. After the complete removal of water and when the temperature reached above 110 °C, the reaction mixture was stirred for a further 3 h. On cooling, the product precipitated out from Toluene. The compound was filtered and washed with cold toluene and dry ethanol to offered 40g yellowish solid Yield 93% m.p. 238-243°C, IR (KBr disk) 3360 cm^{-1} (NH), 1684 cm^{-1} (C=O), 628 cm^{-1} (C-S).IR (KBr) ν_{max}/cm^{-1}:

3135, 3029, 1737, 1690, 1603. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.49-7.62 (m, 5H, Ar-H), 7.80 (s, 1H, CH), 12.64 (s, 1H, NH); ES-MS: *m/z* 206.3 $(M+H)^+$.

***4'-chlorosulfonyl-5-benzylidine-2,4-thiazolidinedione* (6d)**

To a solid 5-Benzylidine 2,4-thiazolidinedione (35g, 0.170 mol) was added Chlorosulfonic acid (45ml, 0.682 mol) at room temperature using the dropping funnel and attached condenser. The reaction was found to be exothermic. After addition of chlorosulfonic acid completed, the reaction mass was refluxed for 1 h on a water bath. The reaction was cooled and poured in a thin stream with stirring into crushed ice contained in a 1L beaker. The product was filtered and dried. The product was purified by recrystallization from ethanol to furnish yellowish solid (35g Yield 68 %). m.p. 177-182°C, IR (KBr disk) 3360 cm^{-1} (NH), 1684 cm^{-1} (C=O), 1120 & 1310 cm^{-1} (SO_2 sym and asym), 763 cm^{-1} (Cl), 628 cm^{-1} (C-S); ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.56 (d, 2H, *J* = 8.4 Hz Ar-H), 7.71 (d, 2H, *J* = 8 Hz Ar-H), 7.77 (s, 1H, CH), 12.64 (s, 1H, NH); ES-MS: *m/z* 303.1 $(M+H)^+$.

***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid ethyl ester* (6e)**

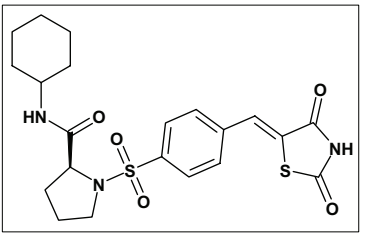
To a stirred solution of L-proline ethyl ester hydrochloride (12g, 0.066 mol) in Dioxane (120 ml) was added triethylamine (28 ml, 0.198 mol) followed by the drop wise addition of 4'-chlorosulfonyl-5-benzylidine-2,4-thiazolidinedione (20g, 0.066 mol) in Dioxane (100 ml) at 0°C. Allowed the reaction mixture to come to 25°C and stirred for 3h. Dioxane and other volatiles were removed under reduced pressure and residue was taken in EtOAc, washed with water, 1N HCl solution in water and saturated $NaHCO_3$ solution in water. Collected organic layers were dried over Na_2SO_4 and removed under vacuum to yield ester intermediate as a yellowish gel (22 g, 82 %) **6e**. This was used without further purification. ES-MS: *m/z* 411.3 $(M+H)^+$.

***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid* (6f)**

To a suspension of ester compound **5** (20 gm, 0.048 mol) in THF (160 ml) and water (40 ml) was added lithium hydroxide monohydrate (3 g, 0.072 mol) at 0°C, and the reaction mixture was stirred for 12 h. THF was distilled out under vacuum and to the remaining aqueous residue was added 1N HCl solution in water to adjust the solution P^H = 4, the solid was precipitated out. The solid was filtered and dried to obtained compound **6** as a yellowish solid (15 g, 79 %). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.74 (br, s 1H, -CO-NH-CO-), 7.93 (d, 2H, *J*

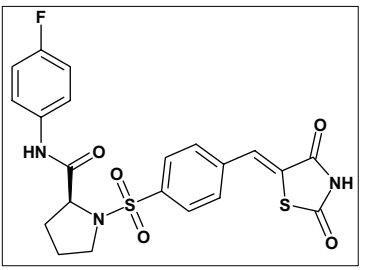
=8.8 Hz, Ar-H), 7.84 (s, 1H, Ar-H), 7.79 (d, 2H, *J* =8.8 Hz, Ar-H), 4.12-4.15 (m,1H,-N-CH-CO), 3.14-3.22 (m, 2H, -N-CH_2-CH_2), 1.77-1.94 (m, 3H, -N-CH_2-CH_2-), 1.58-1.61 (m, 1H, -CH_2-CH_2-CH_2-), ES-MS: *m/z* 381.2 $(M+H)^-$.

***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid cyclohexylamide* (67)**



off white solid (0.82g, Yield= 68%); mp= 137-139 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.75 (br, s 1H, -CO-NH-CO-), 7.96 (d, 2H, *J* =8.8 Hz, Ar-H), 7.86 (s, 1H, Ar-H), 7.81 (d, 2H, *J* =8.4 Hz, Ar-H), 7.71(s, 1H, *J* =8 Hz Ar-H), 4.04-4.07 (m,1H,-N-CH-CO), 3.40-3.49 (m, 2H, -N-CH_2-CH_2-), 3.20-3.25 (m, 1H, -N-CH_2-CH_2-), 1.66-1.82 (m,6H, -CH_2-CH_2-CH_2-), 1.51-1.55 (m, 2H, -CH_2-CH_2-CH_2-), 1.09-1.27 (m, 5H, -CH_2-CH_2-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.1(2×C), 30.9, 48.9, 59.8, 126.4, 126.7, 128.0, 128.1, 129.0 (2×C), 129.6, 130.5 (2×C), 135.6, 137.2, 167.0, 167.4, 168.6, 169.9; ES-MS: m/z 463.2 $(M+H)^+$; Anal. Calcd.for **$C_{21}H_{25}N_3O_5S_2$**; C, 54.41; H, 5.44; N, 9.06; Found: C, 54.45; H, 5.45; N, 9.03.

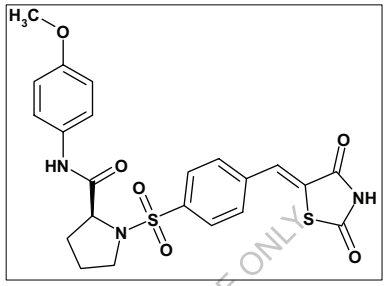
***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid (4-fluoro-phenyl)-amide* (71)**



Yellowish solid (0.98g, Yield = 79 %); mp = 154-156 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.68 (br, s 1H, -CO-NH-CO-), 10.09 (s, 1H, -CO-NH-), 7.96 (d, 2H, *J* =8.4 Hz, Ar-H), 7.80 (d, 2H, *J* =7.6 Hz, Ar-H), 7.58-7.61 (m, 2H, Ar-H), 7.04-7.16 (m, 3H, Ar-H), 4.20-4.23

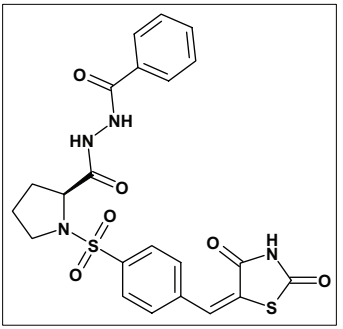
(m,1H,-N-C<u>H</u>-CO), 3.45-3.49 (m, 1H, -N-C<u>H</u>$_2$-CH$_2$-), 1.84-1.90 (m, 3H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-), 1.54-1.57 (m, 1H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-);^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.3, 31.1, 49.2, 61.7, 115.1, 115.3, 121.2, 121.3, 126.8, 127.9 (2×C), 130.2 (2×C), 130.8, 135.0, 137.0, 138.3,156.9, 159.3, 169.7 (2×C); ES-MS: m/z 476.X (M+H) $^+$; Anal. Calcd.for **$C_{21}H_{18}FN_3O_5S_2$**; C, 53.04; H, 3.82; N, 8.84; Found: C, 53.07; H, 3.85; N, 8.82.

***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid (4-methoxy-phenyl)-amide* (70)**



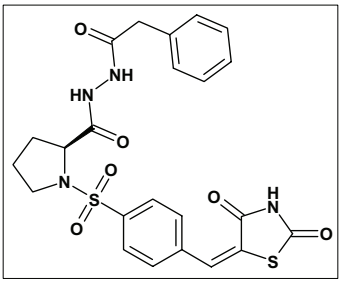
Brownish solid (0.78g, Yield= 61%); mp= 161-163 $^{\circ}$C;^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.65 (br, s 1H, -CO-N<u>H</u>-CO-), 9.84 (s, 1H, -CO-N<u>H</u>-), 7.96 (d, 2H, J =8.4 Hz, Ar-<u>H</u>), 7.84 (s, 1H, Ar-<u>H</u>), 7.80 (d, 2H, J =8.8 Hz, Ar-<u>H</u>), 7.47 (d, 2H, J =9.2 Hz, Ar-<u>H</u>), 6.87 (d, 2H, J =9.2 Hz, Ar-<u>H</u>), 4.20-4.22 (m,1H,-N-C<u>H</u>-CO), 3.70(s, 3H, Ar-OCH$_3$), 3.44-3.48 (m, 1H, -N-C<u>H</u>$_2$-CH$_2$-), 1.84-1.88 (m, 3H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-), 1.54-1.57 (m, 1H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-);^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.3, 31.0, 49.1, 55.1, 61.7, 113.8, 114.0, 119.7 (2XC), 127.2,128.0 (2XC), 129.3,130.5 (2XC), 131.8, 137.7, 137.8, 155.4, 167.6,167.7,169.3; ES-MS: m/z 488.X (M+H) $^+$; Anal. Calcd.for **$C_{22}H_{21}N_3O_6S_2$**; C, 54.20; H, 4.34; N, 8.62; Found: C, 54.23; H, 4.35; N, 8.59.

***Benzoicacid N'-{1-[4-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carbonyl}-hydrazide* (76)**



off white solid (1g, Yield= 79 %); mp= 187-189 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.52 (br, s 1H, -CO-NH-CO-), 10.49 (s, 1H, -CO-NH-NH-), 10.08 (s, 1H, -CO-NH-NH-), 7.99-8.01 (d, 2H, J = 8 Hz, Ar-H), 7.60-7.91 (m, 5H, Ar-H), 7.48-7.53 (m, 3H, Ar-H), 4.26-4.29 (m,1H,-N-CH-CO), 3.45-3.48 (m, 1H, -N-CH$_2$-CH$_2$-), 3.20-3.32 (m, 2H, -N-CH$_2$-CH$_2$-), 1.83-1.92 (m,2H, -CH$_2$-CH$_2$-CH$_2$-), 1.60-1.63 (m, 1H, -CH$_2$-CH$_2$-CH$_2$-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.2, 28.9, 55.6, 58.3,126.8 (2×C), 127.8 (2×C), 128.1(2×C), 128.8 (2×C), 129.6, 130.6(2×C), 131.6, 137.2, 137.6, 160.3, 167.2, 167.4, 170.7; ES-MS: m/z 501.4 (M+H)$^+$; Anal. Calcd.for **$C_{22}H_{20}N_4O_6S_2$**; C, 52.79; H, 4.03; N, 11.19; Found: C, 52.74; H, 4.06; N, 11.16.

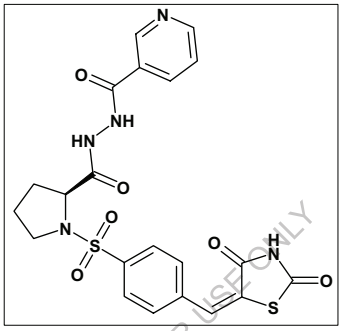
***1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid N'-phenylacetyl-hydrazide* (77)**



yellowish solid (1.0 g, Yield = 78%); mp = 193-195 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.74 (br, s 1H, -CO-NH-CO-), 10.21 (s, 1H, -CO-NH-NH-), 10.03 (s, 1H, -CO-NH-NH-), 7.77-7.96 (m, 5H,Ar-H), 7.26-7.29 (m, 2H, Ar-H), 7.20-7.23 (m, 1H,-C=CH-), 4.13-4.16 (m,

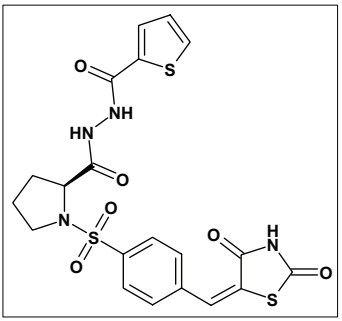
1H,-N-C<u>H</u>-CO), 3.45 (s, 2H, -CO-C<u>H</u>$_2$-Ar-), 3.14-3.19 (m, 1H, -N-C<u>H</u>$_2$-CH$_2$-), 2.71-2.75 (m, 1H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-), 1.78-1.86 (m, 3H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-), 1.52-1.55 (m, 3H,-CH$_2$-C<u>H</u>$_2$-CH$_2$-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.1, 25.1(2×C), 25.3, 28.9, 29.1, 30.9, 41.8,48.9, 59.8, 126.9, 128.0, 129.5, 130.5(2×C), 137.2, 137.7, 137.7, 167.2, 167.5, 169.8, 173.9; ES-MS: *m/z* 515.4 $(M+H)^+$; Anal. Calcd. for **$C_{23}H_{22}N_4O_6S_2$**; C, 53.69; H, 4.31; N, 10.89; Found: C, 53.73; H, 4.37; N, 10.86.

***Nicotinic acid N'-{1-[4-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carbonyl}-hydrazide* (79)**



brownish solid (0.88g, Yield = 68 %); mp= 201-203 $^{\circ}$C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.76 (br, s 1H, -CO-N<u>H</u>-CO-), 10.73 (s, 1H, -CO-N<u>H</u>-NH-), 10.19 (s, 1H, -CO-NH-N<u>H</u>-), 9.05 (s, 1H, Pyridine-<u>H</u>), 8.76 (d, 1H, *J*= 3.6Hz, Pyridine-<u>H</u>), 8.24(d, 1H, *J*= 7.6Hz, Pyridine-<u>H</u>), 7.99(d, 1H, *J*= 6.4 Hz, Pyridine-<u>H</u>), 7.82-7.84 (m, 3H, Ar-<u>H</u>), 7.53-7.57 (m, 1H, -C=C<u>H</u>-), 4.26-4.29 (m, 1H,-N-C<u>H</u>-CO), 3.33-3.49 (m, 1H, -N-C<u>H</u>$_2$-CH$_2$-), 3.20-3.25 (m, 1H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-), 1.84-1.93 (m, 3H, -CH$_2$-C<u>H</u>$_2$-CH$_2$), 1.60-1.63 (m, 1H, -CH$_2$-C<u>H</u>$_2$-CH$_2$-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.2, 28.9, 55.6, 58.3, 115.9, 125.1, 126.7 (2×C), 127.2 (2×C), 129.5, 130.7, 138.0, 138.3, 142.0, 148.3, 153.7, 167.2, 167.5, 169.8, 173.9; ES-MS: m/z 502.4 $(M+H)^+$; Anal. Calcd. for **$C_{21}H_{19}N_5O_6S_2$**; C, 50.29; H, 3.82; N, 13.96; Found: C, 50.33; H, 3.87; N, 13.98.

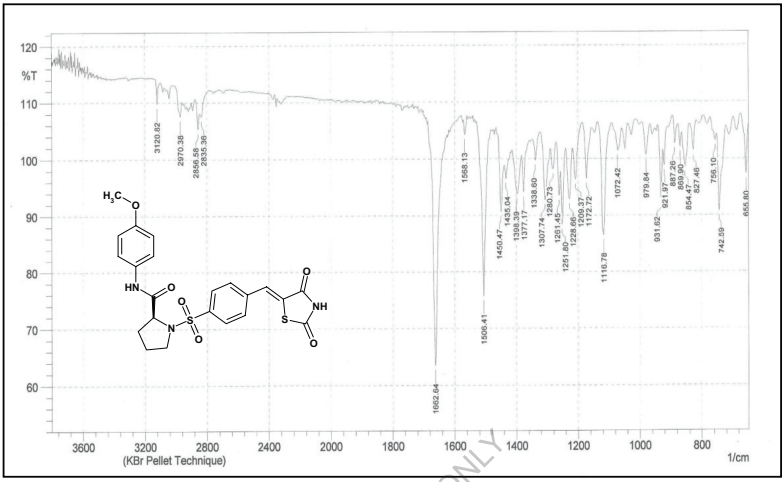
***Thiophene-2-carboxylic acid N'-{1-[4-(2,4-dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carbonyl}-hydrazide* (80)**



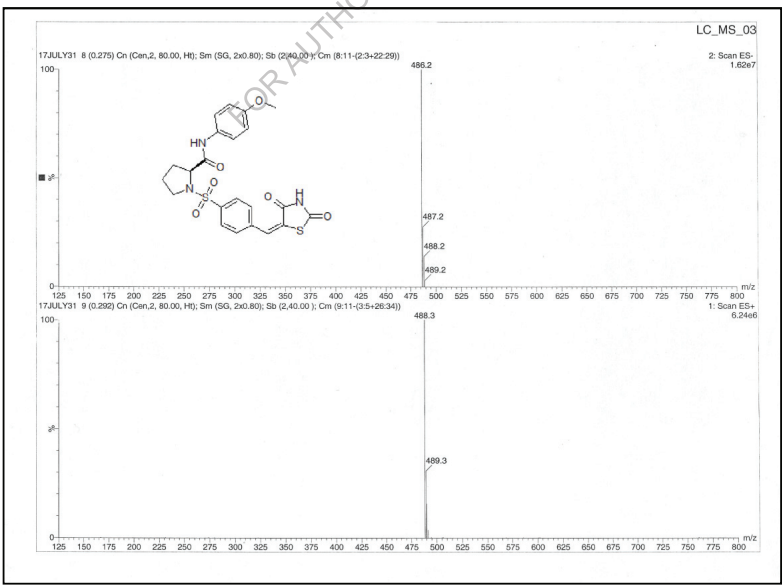
yellowish solid (1 g, Yield = 76 %); mp = 189-191°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.76 (br, s 1H, -CO-NH-CO-), 10.52 (s, 1H, -CO-NH-NH-), 10.10 (s, 1H, -CO-NH-NH-), 7.95-8.05 (m, 2H,Ar-H), 7.82-7.87 (m, 5H, Ar-H), 7.18-7.20 (m, 1H,-C=CH-), 4.22-4.26 (m, 1H,-N-CH-CO), 3.44-3.48 (m, 1H, -N-CH$_2$-CH$_2$-), 3.18-3.24 (m, 1H, -N-CH$_2$-CH$_2$-), 1.82-1.96 (m, 3H, -CH$_2$-CH$_2$-CH$_2$-), 1.59-1.62 (m, 1H, -CH$_2$-CH$_2$-CH$_2$-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.1, 30.9, 48.9, 59.8, 126.4, 126.7, 128.0, 128.1, 129.0 (2×C), 129.6, 130.6(2×C), 135.6, 137.2, 137.7, 167.0, 167.4, 168.6, 169.9; ES-MS: *m/z* 507.3 (M+H)$^{+}$; Anal. Calcd.for **$C_{20}H_{18}N_4O_6S_3$**; C, 47.42; H, 3.58; N, 11.06; Found: C, 47.45; H, 3.54; N, 11.02.

Spectral Data for Compound No. 70

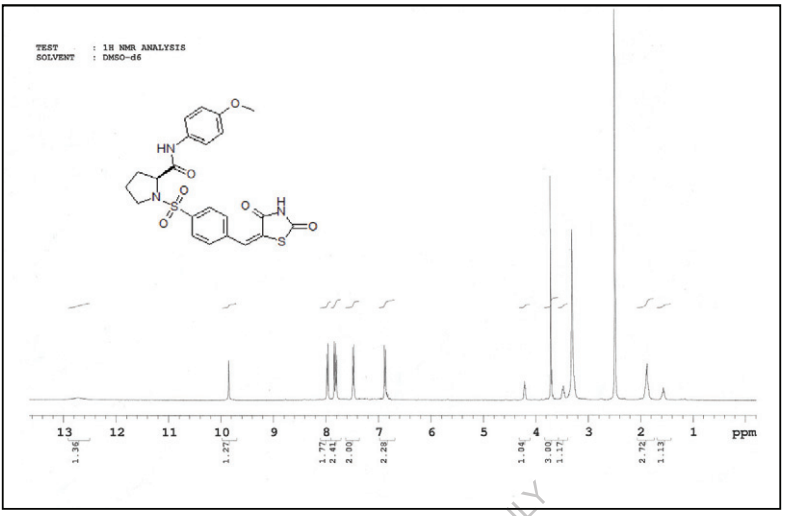
IR



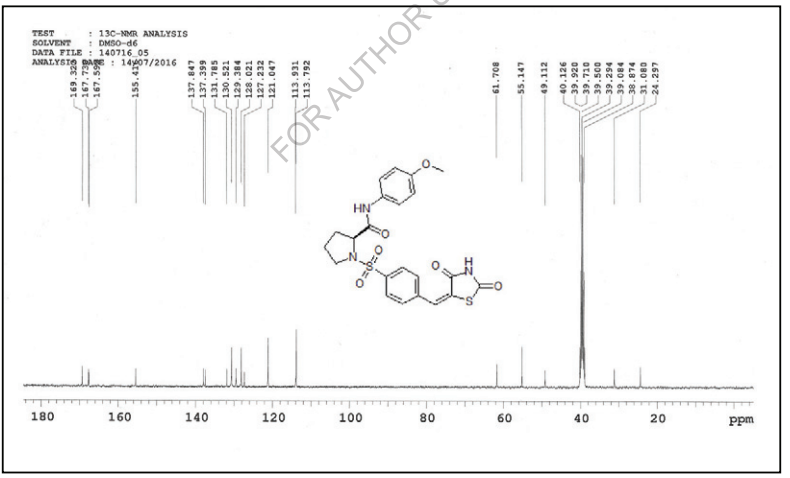
ES-MS



[1]HNMR

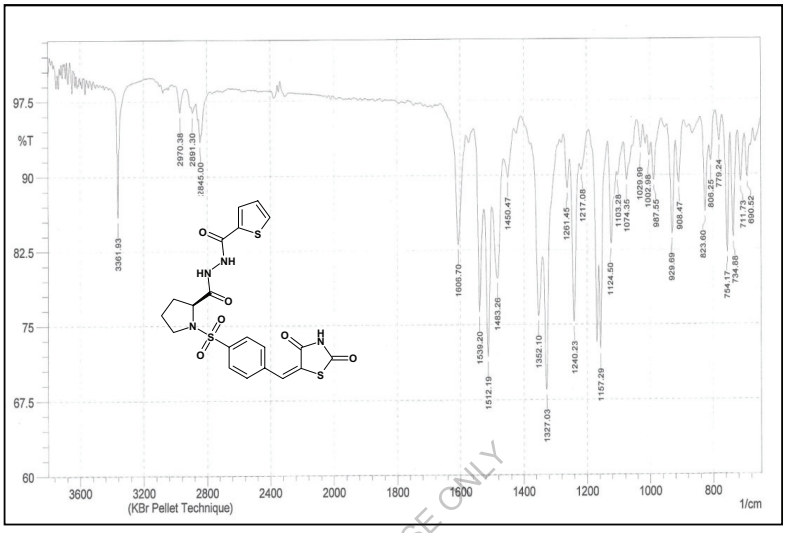


[13]CNMR

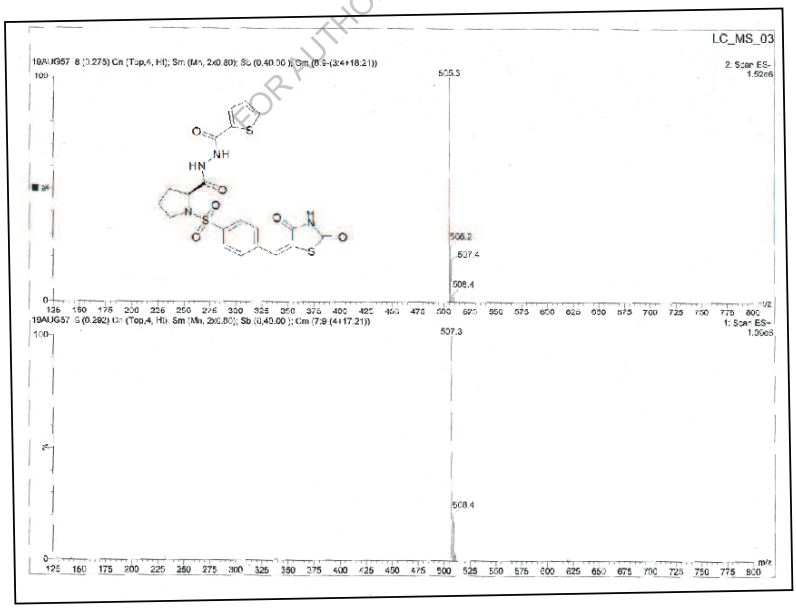


Spectral Data for Compound No. 80

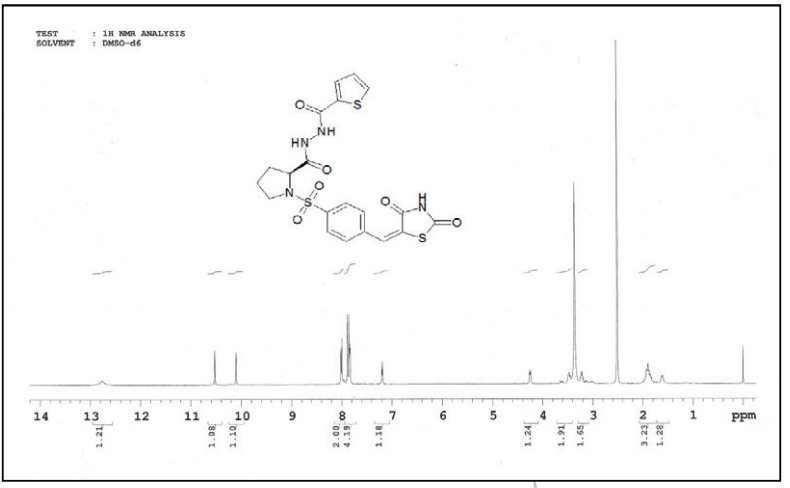
IR



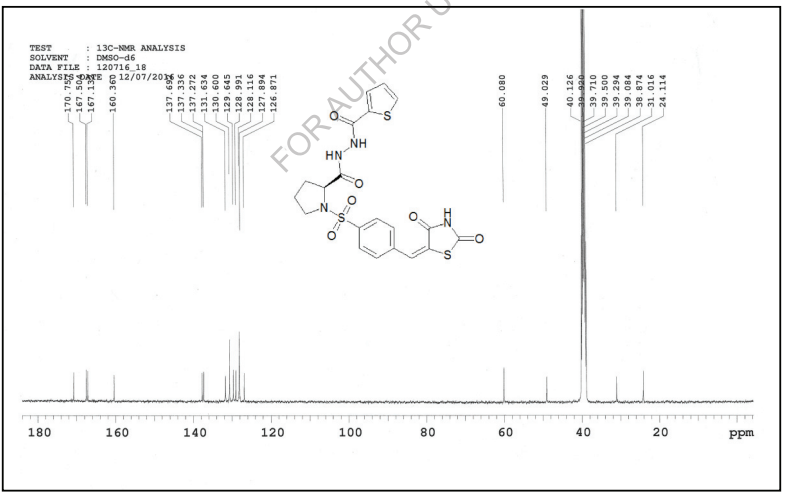
ES-MS



[1]HNMR



[13]CNMR



4.1.3.2. Biology

Antitumor activity

All the prepared target compounds **66-73** and **74-81** were screened for their antitumor activities against breast MCF-7 cell line and colon HCT-15 cell lines at Anti-Cancer Drug screening facility (ACDSF), Tata memorial centre, Navi Mumbai.

Experimental procedure for SRB assay [13]

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37°C, 5%CO2, 95% air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent to prepare stock of 10^{-2} concentration. At the time of

experiment four 10-fold serial dilutions were made using complete medium. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. The dose response parameters were calculated for each test article. Growth inhibition of 50 % (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured

by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

GI_{50}	Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] × 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
LC_{50}	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] × 100 = -50.

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SECTION B

4.2. Synthesis and Antidibetic studies of *4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonamide* analogous

4.2.1. AIM & OBJECTIVES

Sulfonylureas are generally undergone the chemical hydrolysis at ionizable hydrogen atom containing nitrogen which is situated between sulfonyl and carbonyl groups. The ionization leads the early cleavage of Sulfonylurea bridge, producing CO_2 and the corresponding sulfonamide and amine. Although the second generation sulfonylurea glibenclamide have longer duration of action but accumulates progressively in the β-cell. The efficacy and penetration of sulfonylureas can be enhanced by decreasing the rate of ionizable metabolism.

Thiazolidinone (TZDs) represent a new class of drugs with a new mechanism of action. In Europe, TZDs have been approved for type 2 diabetes mellitus, particularly for overweight patients who are inadequately controlled by diet and exercise alone, for whom metformin is inappropriate because of contraindications or intolerance. TZDs can be used in combination with metformin and sulphonylureas.

Since sulfonylureas and thiazolidinone moieties are biologically proven antidiabetic pharmacophores and substitution in these scaffolds may further enhance their activity, prompted us to undertake this problem. Moreover, the combination of two pharmacophores in a single molecule is a well established hypothesis for synthesis of more active drugs with dual activity. Thus, a series of novel sulfonylhydrazides substituted thiazolidine-4-ones were synthesized and evaluated for their hypoglycaemic activity.

This works presents the synthesis of some new thiazolidinedione sulfonylurea derivatives containing substitution at ionizable nitrogen atom and also with some aromatic and heteroaromatic hydrazides. Here we have discussed the structure activity relationship observed after coupling thiazolidinedione moieties with the sulfonylureas and sulfonyl hydrazides analogs. The aim of the presented work is to synthesis, substituted thiazolidinones containing sulfonylhydrazides and studies their hypoglycaemic activities.

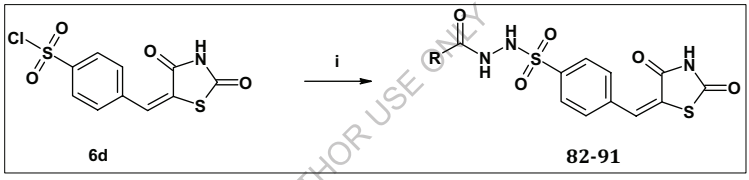
4.2.2. RESULTS AND DISCUSSION

4.1.2.1. Chemistry

The targeted compounds were prepared from chloro sulfonyl intermediate **6d** as outlined in **Scheme 17**. Different acid hydrazides were introduced by replacing chloro group of 5-benzylidine-2,4-thiazolidinedione **6d** in 2,4-dioxane and triethylamine to obtained the novel products **82-91**. Substituted aromatic and aliphatic hydrazides were selected to evaluate the structure activity relationship among the novel analogs. Purification of the desired product was achieved by crystallization and characterized by spectral and elemental analysis.

Scheme 17

Synthetic route for ***N'-({4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl} sulfonyl)R-hydrazide*** (**82-91**)

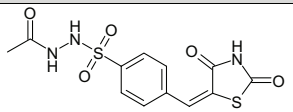
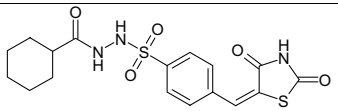
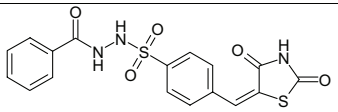
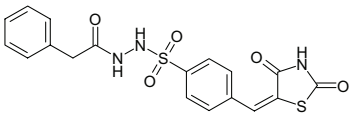
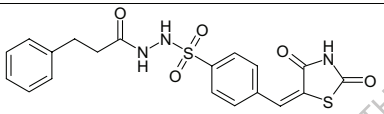
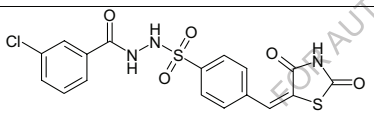
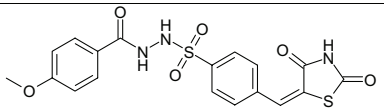
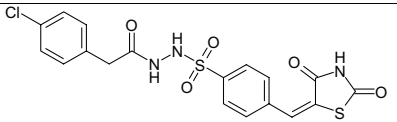
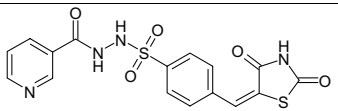
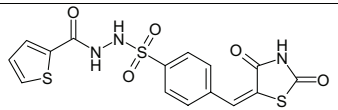


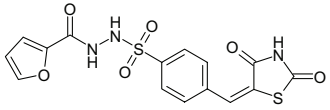
i) R-Hydrazide, TEA, Dioxane, 80°C

General procedure

To a solution of 4'-chlorosulfonyl-5-benzylidine-2,4-thiazolidinedione **6d** (1g, 3.30 mmol) in Dioxane (10 ml) was added triethylamine (925µL, 6.60 mmol) and R-hydrazide (3.30 mmol) at 25°C. Reaction mixture was then heated to 80°C for 3h. Completion of reaction was monitored by TLC and allowed the reaction mixture to cool to 25°C. Distilled out volatiles under vacuum and obtained residue was taken in water. Adjusted the reaction $P^H = 4$ with 1N HCl and stirred for 1h. Precipitated solid was filtered, washed with diethyl ether and dried under vacuum to give sulfonyl hydrazides **(82-92)**, as shown in **Scheme 17**.

Table 27: Physical & ES-MS (*m/z*) data of synthesized compounds (82-92)

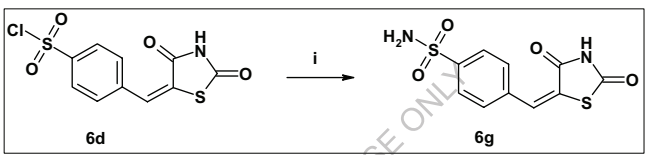
No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
82		$C_{12}H_{11}N_3O_5S_2$	342.2	80	173-175
83		$C_{17}H_{19}N_3O_5S_2$	410.4	72	188-190
84		$C_{17}H_{13}N_3O_5S_2$	404.2	78	196-198
85		$C_{19}H_{17}N_3O_5S_2$	417.2	70	208-210
86		$C_{19}H_{17}N_3O_5S_2$	432.2	70	178-180
87		$C_{17}H_{12}ClN_3O_5S_2$	438.2	78	196-198
88		$C_{18}H_{15}N_3O_6S_2$	434.1	75	218-220
89		$C_{18}H_{14}ClN_3O_5S_2$	452.2	78	196-198
90		$C_{16}H_{12}N_4O_5S_2$	405.3	68	183-185
91		$C_{15}H_{11}N_3O_6S_2$	394.2	68	203-205

92		$C_{15}H_{11}N_3O_5S_3$	410.2	82	179-181

The targeted compounds were prepared from sufonamide intermediate **6g** as outlined in **Scheme 18**. The starting material 4-(2,4-Dioxo-thiazolidin-5-lidenemethyl) -benzenesulfonamide **6g** was prepared by converting chloro group of **6d** to amino by using ammonia in water. Different isocynates and carbamic chlorides were used to synthesize sulfonylureas from **6g**.

Scheme 18

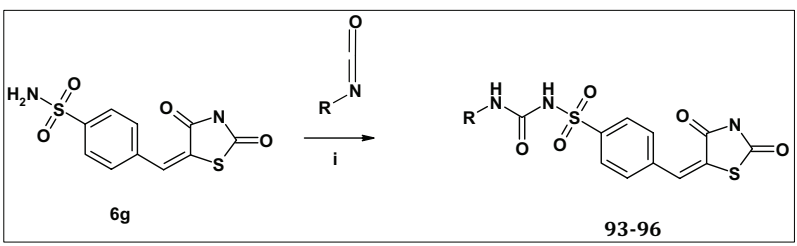
Synthetic route for ***4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonamide*** **(6g)**



i) Water, Ammonia gas

Scheme 19

Synthetic route for ***4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-N-(R-ylcarbamoyl)benzenesulfonamide*** **(93-96)**



i) R-Isocynate, NaH, Dioxane, 3h

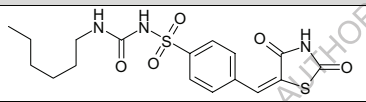
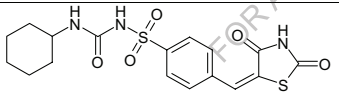
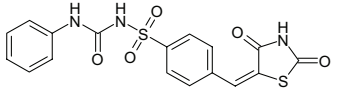
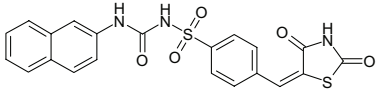
Novel sulfonylureas **93-96** were prepared by coupling selected isocynates with the sulfonamide core **6g** as outlined in **Scheme 19**. Substituted isocynates were selected to evaluate the structure activity relationship among the novel analogs. Coupling reactions were performed by using NaH in Dioxane at 0°C to 25°C for a period of 3h. Purification of the

desired product was achieved by crystallization and characterized by spectral and elemental analysis.

General procedure

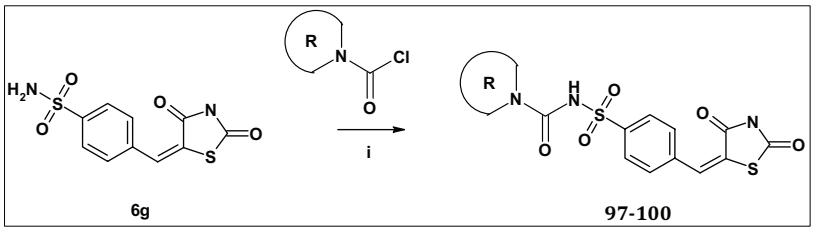
To a solution of, 4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonamide **6g** (1g, 3.52 mmol) in Dioxane (10 ml) was added Sodium hydride (169mg, 7.04 mol) and allowed to stir for 1h at 0°C. R-isocynate (5.28 mmol) was taken in 5 volume DMF and added drop wise to the reaction mixture over a period of 15 min. TLC was monitored and reaction was quenched with moist sodium sulphate. Distilled out volatiles under vacuum and obtained residue was taken in dichloromethane. Organic layers were washed with water, saturated solution of sodium bicarbonate and with 1N HCL solution. Collected organic layers were dried over sodium sulphate and evaporated under vacuum to yield solid products which was taken in diethyl ether and filtered, dried and washed with diethyl ether to give urea compounds **(93-96)**, as shown in **Scheme 19**.

Table 28: Physical & ES-MS (*m/z*) data of synthesized compounds (93-96)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
93		$C_{17}H_{21}N_3O_5S_2$	412.2	68	207-208
94		$C_{17}H_{19}N_3O_5S_2$	410.2	86	198-200
95		$C_{17}H_{13}N_3O_5S_2$	404.3	82	191-193
96		$C_{21}H_{15}N_3O_5S_2$	454.2	72	208-210

Scheme 20

Synthetic route for ***4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-N-(R-carbonyl)-benzenesulfonamide*** **(96-100)**



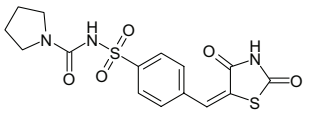
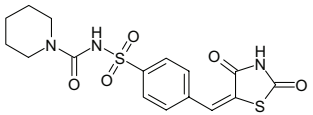
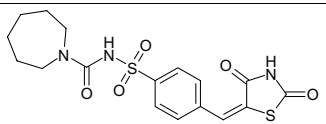
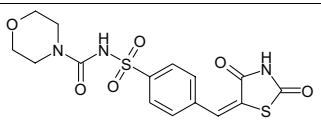
i) R-Carbamoyl chloride, NaH, Dioxane, 3h

Novel sulfonylureas **97-100** were prepared by coupling selected carbamoyl chlorides with the sulfonamide core **6g** as outlined in **Scheme 20**. Substituted carbamoyl chlorides were selected to evaluate the structure activity relationship among the novel analogs. Coupling reactions were performed by using NaH in Dioxane at 0°C to 25°C for a period of 3h. Purification of the desired product was achieved by crystallization and characterized by spectral and elemental analysis.

General procedure

To a solution of, 4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonamide **(6g)** (1g, 3.52 mmol) in Dioxane (10 ml) was added Sodium hydride (169 mg, 7.04 mol) and allowed to stir for 1h at 0°C. R-Carbamoyl chloride (5.28 mmol) was taken in 5 volume DMF and added drop wise to the reaction mixture over a period of 15 min. TLC was monitored and reaction was quenched with moist sodium sulphate. Distilled out volatiles under vacuum and obtained residue was taken in dichloromethane. Organic layers were washed with water, saturated solution of sodium bicarbonate and with 1N HCL solution. Collected organic layers were dried over sodium sulphate and evaporated under vacuum to yield solid products which was taken in diethyl ether and filtered, dried and washed with diethyl ether to give urea compounds **(97-100)**, as shown in **Scheme 20**.

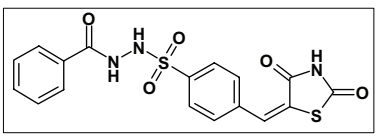
Table 29: Physical & ES-MS (*m/z*) data of synthesized compounds (97-100)

No.	Structure	Molecular Formula	ES-MS *m/z*	% Y	M.P. (°C)
97		$C_{15}H_{15}N_3O_5S_2$	382.2	67	183-185
98		$C_{16}H_{17}N_3O_5S_2$	396.2	74	191-193
99		$C_{17}H_{19}N_3O_5S_2$	410.2	63	206-208
100		$C_{15}H_{15}N_3O_6S_2$	398.2	78	179-181

4.2.3. EXPERIMENTAL

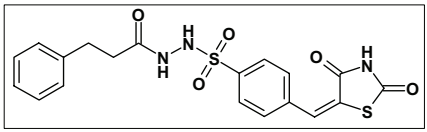
4.2.3.1. Chemistry

***N'-({4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl}sulfonyl) benzohydrazide* (84)**



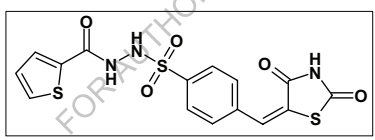
Off white solid (0.82g, Yield = 78%); mp = 196-198°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.68 (br, s, 1H, -CO-NH-CO-), 10.72 (s, 1H, -SO_2-NH-), 10.19 (s, 1H, -CO-NH-), 7.90 (d, 2H, J = 8Hz, Ar-H), 7.82 (s,1H, -C=CH-Ar), 7.73-7.76 (m, 2H, Ar-H), 7.68 (d, 2H, J = 7.6Hz, Ar-H), 7.52-7.56 (m,1H, Ar-H), 7.42-7.46 (m, 2H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 126.7, 127.4 (2×C), 128.5 (4×C), 129.8, 130.1, 131.9 (2×C), 132.0, 137.1, 140.0, 165.6, 167.1, 167.6; ES-MS: *m/z* 404.2 $(M+H)^+$; Anal.Calcd.for $C_{17}H_{13}N_3O_5S_2$; C, 50.61; H, 3.25; N, 10.42; Found: C, 50.63; H, 3.27; N, 10.45.

***N'-({4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl}sulfonyl)-3-phenylpropanehydrazide* (86)**



Yellowish solid (0.73g, Yield = 70%); mp = 172-174 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.68 (br,s,1H, -CO-NH-CO-), 10.08 (s, 1H, -SO_2-NH-) , 9.99 (s,1H, -CO-NH-), 7.83-7.85 (m, 2H, Ar-H) , 7.72 (d, 2H, *J* = 8Hz, Ar-H), 7.23 - 7.27 (m, 2H, Ar-H), 7.18(d, 2H, *J* = 6.8 Hz, Ar-H), 7.11(d, 2H, *J*=7.6Hz, Ar-H), 2.65(t, 2H, *J* = 8Hz, -CH_2-CH_2-CO-), 2.29 (t, 2H, *J* = 8 Hz, Ar- CH_2-CH_2-); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 30.43, 34.31, 126.0, 126.7, 128.2 (2×C), 128.3 (4×C), 128.4 (2×C), 130.0, 130.1, 139.9, 140.7, 167.1, 167.6, 170.3; ES-MS: *m/z* 432.2 (M+H) $^+$; Anal.Calcd.for **$C_{19}H_{17}N_3O_5S_2$**; C, 52.89; H, 3.97; N, 9.74; Found: C, 52.91; H, 3.99; N, 9.72.

***N'-({4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenyl} sulfonyl) thiophene-2-carbohydrazide* (91)**



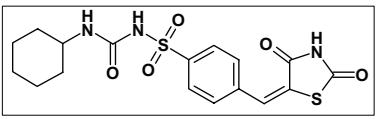
Yellowish solid (0.62g, Yield = 68%); mp = 165-167°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.78 (br, s, 1H, -CO-NH-CO-), 10.73 (s,1H, -SO_2-NH-), 10.24 (s, 1H, -CO-NH-), 7.92 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.82 (s, 1H, -CO-CH-CH_2-) , 7.76 - 7.79 (m, 2H, Ar-H) , 7.13-7.15(m, 1H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 126.8, 128.2, 128.3 (2×C), 129.5, 129.7, 130.3 (2×C), 132.0, 136.4, 137.2, 140.2, 160.4, 167.2, 167.6; ES-MS: *m/z* 410.2 (M+H) $^+$; Anal.Calcd.for **$C_{15}H_{11}N_3O_5S_3$**; C, 44.00; H, 2.71; N, 10.26; Found: C, 44.04; H, 2.72; N, 10.24.

***4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonamide* (6g)**

To a suspension of 4'-chlorosulfonyl-5-benzylidine-2,4-thiazolidinedione **6d** (10g, 33 mmol) in water (100ml) was purged ammonia gas (generated from heating ammonium hydraxide in a separate RB flask) at 0°C. Reaction mixture turns into a clear solution & after completion of reaction again turns into a suspension form. This suspension was filtered, washed with

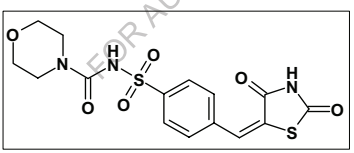
diethyl ether and dried under vacuum to give 4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonamide **6g** as a off white solid (8 g, 82 %). This was used without further purification. ES-MS: *m/z* 411.3 $(M+H)^+$.

***N-(cyclohexylcarbamoyl)-4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl] benzenesulfonamide* (94)**



Off white solid (0.79g, Yield = 86 %); mp = 127-129 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.6 (br s, 2H, -CO-NH-CO-), 7.88 (d, 2H, J = 8.8 Hz, Ar-H), 7.68 (d, 2H, J = 8.8Hz, Ar-H), 7.38 (s,1H, Ar-H), 1.49-1.73 (m, 4H, Cyclohexane-CH_2), 1.19-1.25 (m, 2H, Cyclohexane-CH_2), 1.10-1.17 (m, 3H, Cyclohexane-CH_2), 1.04-1.10 (m, 2H, Cyclohexane-CH_2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.4, 25.0, 32.2, 33.3, 32.5, 48.2, 127.3, 128.1 (2×C), 129.4, 130.2 (2×C), 137.4, 140.8, 150.4, 167.5, 167.8; ES-MS: m/z 410.2 $(M+H)^+$; Anal.Calcd.for **$C_{17}H_{19}N_3O_5S_2$**; C, 49.86; H, 4.68; N, 10.26; Found: C, 49.88; H, 4.64; N, 10.23.

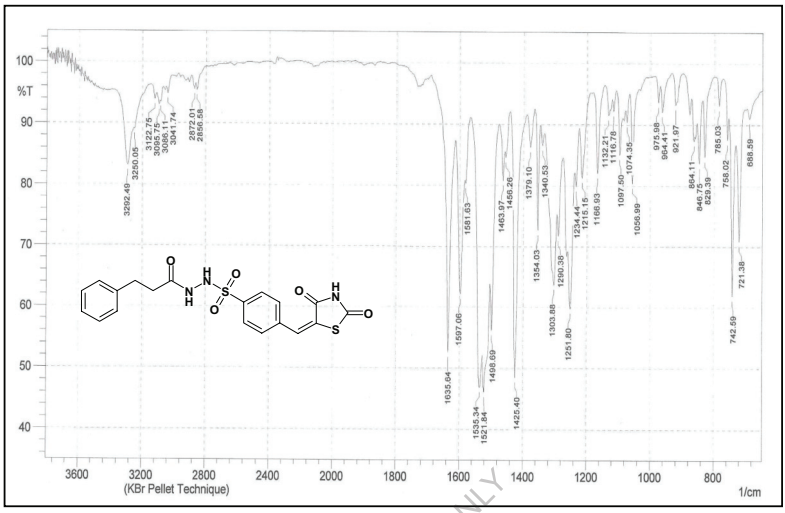
***4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-N-(morpholine-4-carbonyl)-benzenesulfonamide* (100)**



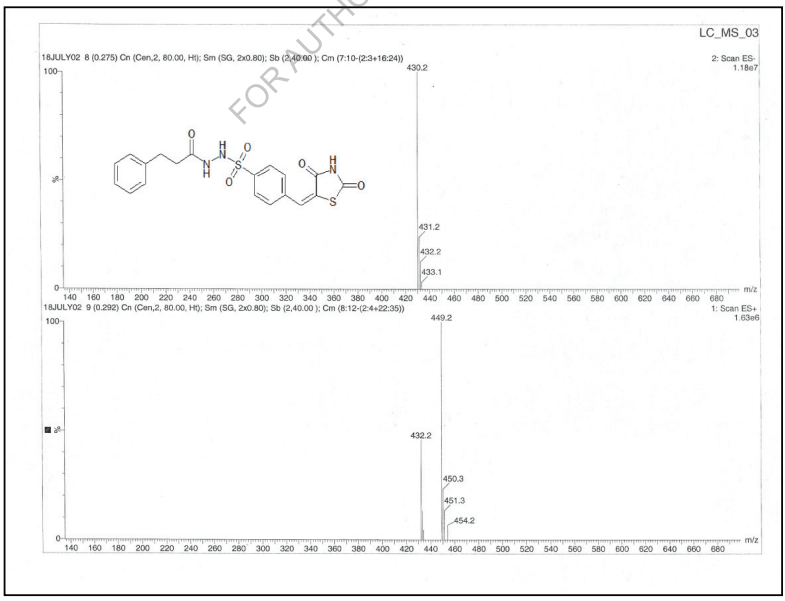
Off white solid (0.82g, Yield = 78%); mp = 179-181 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 12.68 (br s,1H, -CO-NH-CO-), 11.18 (br s,1H, -SO_2-NH-CO-), 7.98 (d, 2H, J = 8.4 Hz, Ar-H), 7.82 (s,1H, -C=CH-Ar), 7.78 (d, 2H, J = 8.4 Hz, Ar-H), 3.43-3.50 (m, 4H, -OCH_2-CH_2-), 3.29-3.32 (m, 4H, -N-CH_2-CH_2-); ES-MS: m/z 398.2 $(M+H)^+$; Anal. Calcd. for **$C_{15}H_{15}N_3O_6S_2$**; C, 45.33; H, 3.80; N, 10.57; Found: C, 45.31; H, 3.83; N, 10.54.

Spectral Data for Compound No. 86

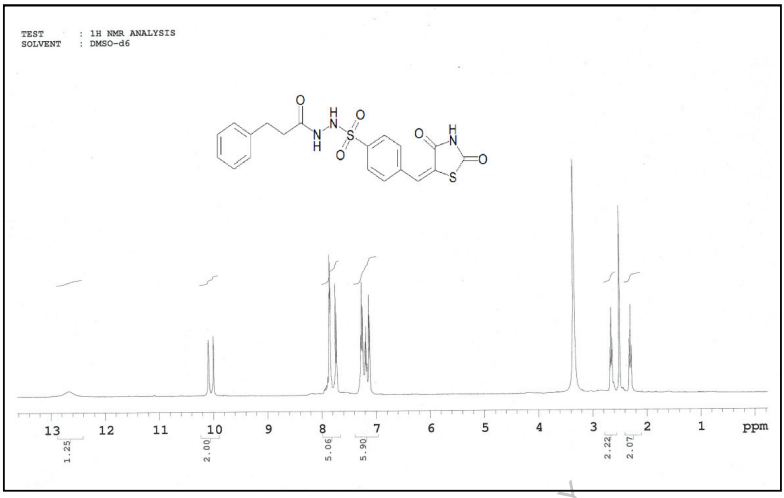
IR



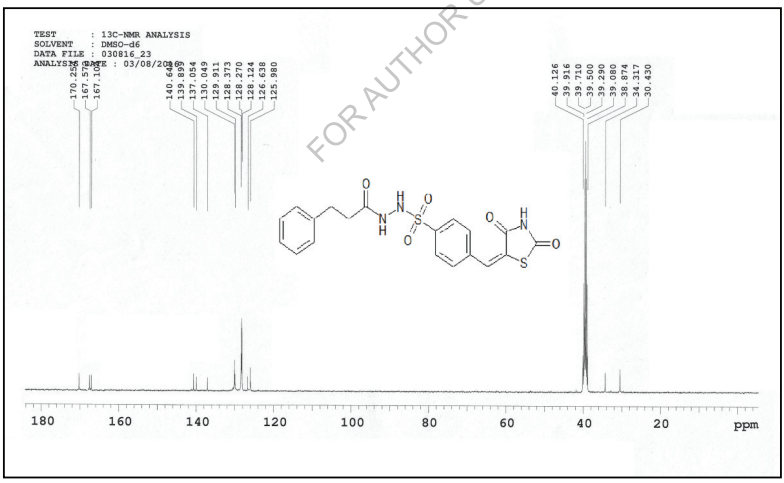
ES-MS



^{1}H NMR

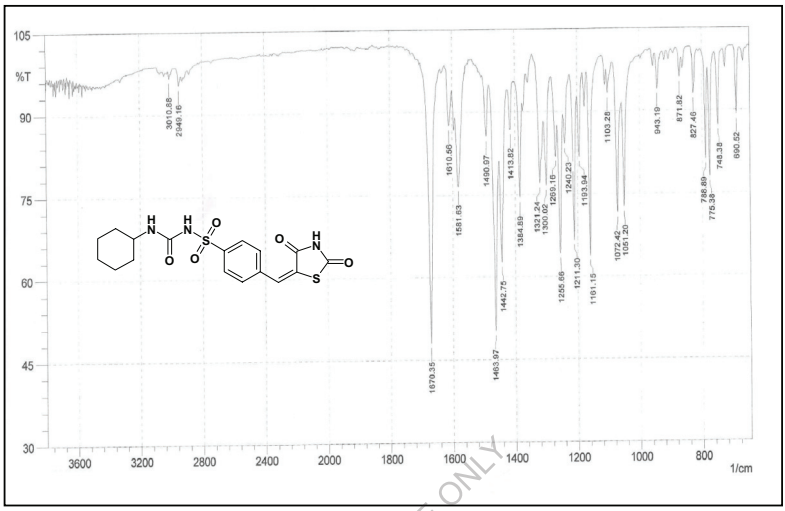


^{13}C NMR

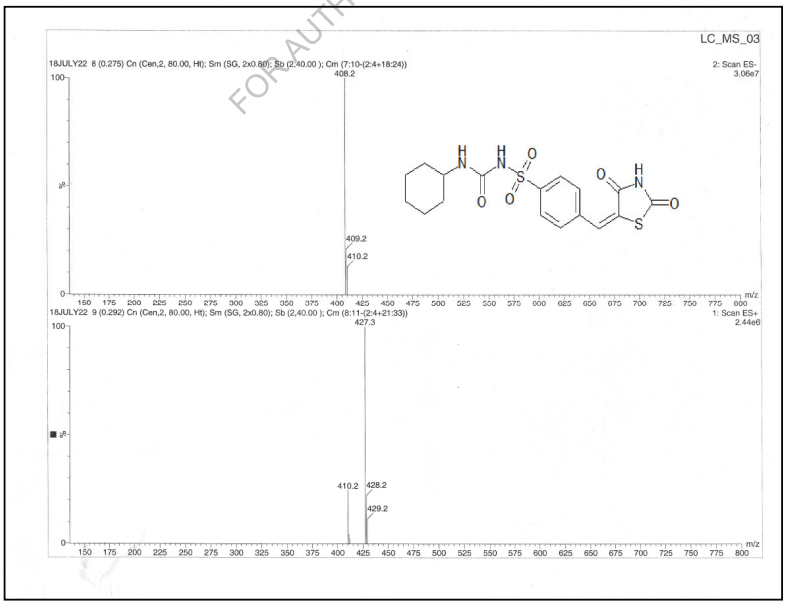


Spectral Data for Compound No. 94

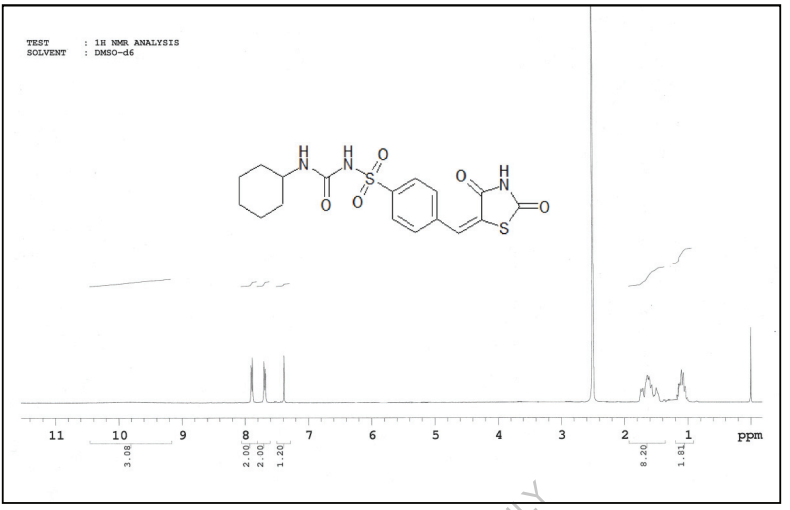
IR



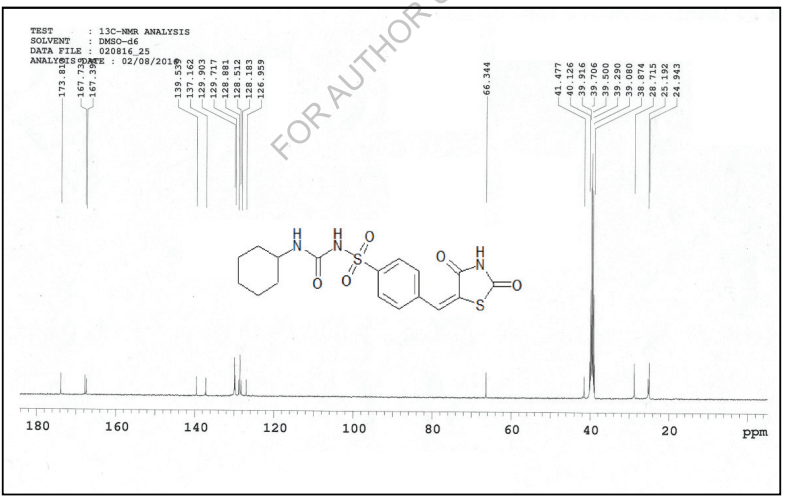
ES-MS



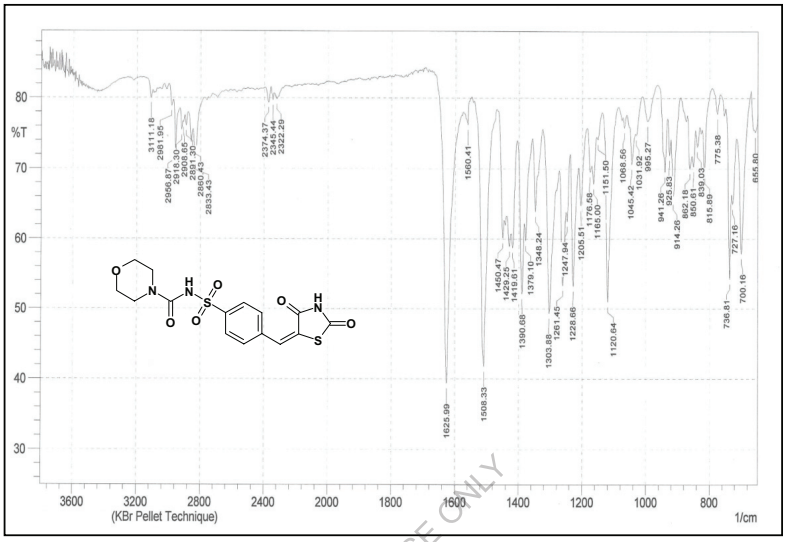
^{1}H NMR



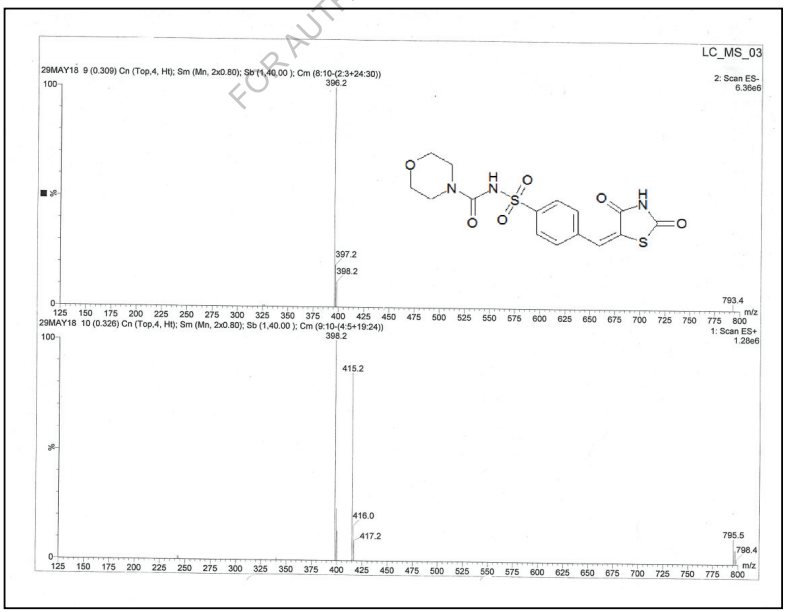
^{13}C NMR



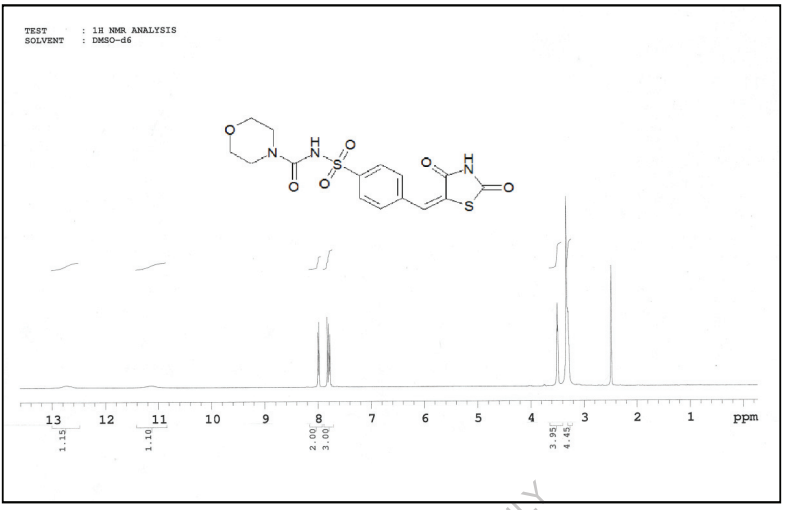
Spectral Data for Compound No. 100
IR



ES-MS



^{1}H NMR



SECTION C

4.3. Antidibetic studies of *4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonamide* analogous

4.3.1. AIM & OBJECTIVES

In continuation with the research work as described in previous section, here we studied the different sulfonylureas with thiazolidinone moieties to enhance their Antidiabetic activities. Thus, a series of novel sulfonylureas substituted with thiazolidine-4-ones were synthesized and selected molecules were evaluated for their hypoglycaemic activity. This works presents the synthesis of some new thiazolidinedione sulfonylurea derivatives substituted with some aliphatic and aromatic alkyl groups. Here we have discussed the structure activity relationship observed after coupling thiazolidinedione moieties with the sulfonylureas analogs. The aim of the presented work is to synthesis, substituted thiazolidinones containing sulfonylureas and studies their hypoglycaemic activities.

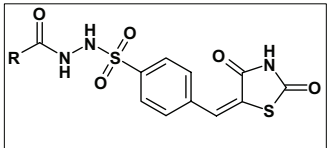
4.3.2.1. Chemistry

Selected eight (**82, 83, 84, 86, 91, 92, 94, 100**) compounds from the following three series were studied for their antidiabetic properties using *Intraperitonial Glucose Tolerance Test* **(IPGTT)**

Scheme 17

N'-({4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl] phenyl} sulfonyl) R-hydrazide

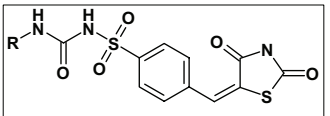
(**82-91**)



82-91

Scheme 19

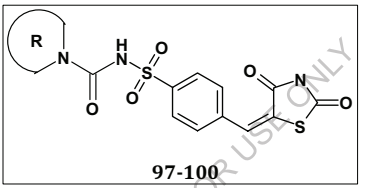
4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-N-(R-ylcarbamoyl) benzenesulfonamide (**93-96**)



93-96

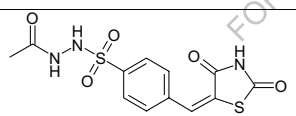
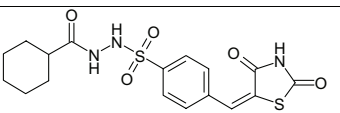
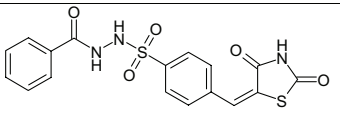
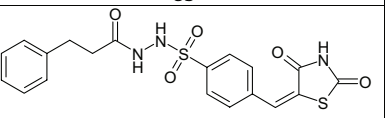
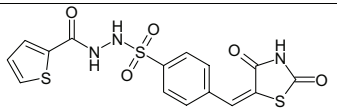
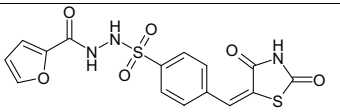
Scheme 20

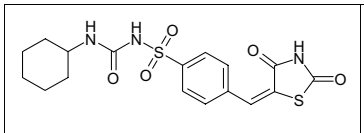
4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-N-(R-carbonyl)-benzenesulfnamide (**96-100**)



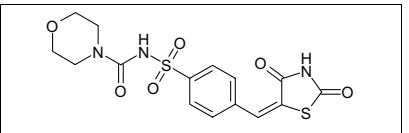
97-100

Following compounds were studied for the IPGTT.

 **82**	 **83**
 **84**	 **86**
 **91**	 **92**



97



100

4.3.2. RESULTS AND DISCUSSION

4.3.2.2. Biology

Intraperitonial glucose tolerance test (IPGTT)

Procedure:

1) Male Wistar rats 11-12 weeks of age were procured from the Animal House Facility, Wockhardt Research Centre, and housed in the experimental animal room. Animals will be acclimatized at least for a week prior to start of the experiment. During the acclimatization animals were provided *adlibitum* feed and water.
2) The rats were fasted over night before the start of an experiment. During fasting and throughout the experiment, animals were kept on fasting grills not more than 3 animals/cages with free access to the water throughout the study period.
3) Following fasting, the blood is withdrawn from tail-vein by nicking of tail-tip with surgical blade/scissor and whole blood glucose is measured using glucometer which is called as basal or –0.5h time point blood glucose. Rats will be randomized into treatment arms (n = 5-6 variable number) based upon the basal blood glucose levels.
4) At 0 h or 0.5 h after placebo or test drug administration (per oral) glucose was administered (intraperitonial) and blood glucose levels will be measured at 0.25, 0.5, 1.0, 1.5, 2 h.
5) The data obtained were entered in MS-Office excel sheets. The respective graphs are prepared and statistical analysis is performed by using one-way analysis of variance (one-way ANOVA) followed by post-hoc Dennett's multiple comparison test, and expressed as of $p < 0.05$ are significance.
6) All statistical analyses are carried out using Graph Pad Prism version 5.00 for Windows (Graph Pad Software, San Diego, CA, USA).

Table 30: Placebo_ Blood glucose (mg/dL) measured at 0h to 2h.

Placebo_ Blood glucose (mg/dL)						
Sr No	**0 Hr**	**0.25 h**	**0.5h**	**1 h**	**1.5 h**	**2 h**
1	83	348	414	331	210	144
2	79	230	311	207	157	104
3	72	369	339	150	139	99
4	82	333	395	266	191	129
5	81	333	335	132	117	103
Mean	**79.4**	**322.6**	**358.8**	**217.2**	**162.8**	**115.8**
SEM	**2.0**	**24.1**	**19.5**	**36.9**	**16.9**	**8.8**

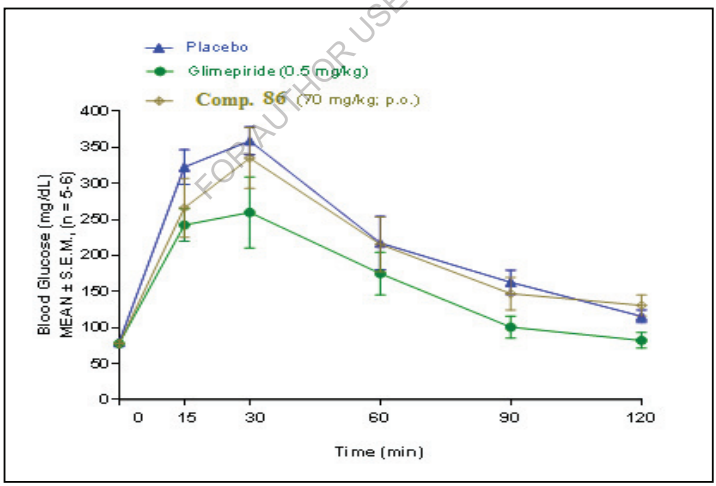
Table 31: Glimepiride (0.5 mg/Kg) _Blood glucose (mg/dL) measured at 0h to 2h.

Glimepiride (0.5 mg/Kg)_Blood glucose (mg/dL)						
Sr No	**0 Hr**	**0.25 h**	**0.5h**	**1 h**	**1.5 h**	**2 h**
1	82	222	152	124	108	85
3	80	317	340	224	147	114
4	73	259	406	264	115	97
5	74	182	166	152	70	55
6	80	231	234	111	64	61
Mean	**77.8**	**242.2**	**259.6**	**175.0**	**100.8**	**82.4**
SEM	**1.8**	**22.4**	**49.4**	**29.6**	**15.3**	**11.0**

Table 32: Compound 86 (70 mg/Kg)_Blood glucose (mg/dL) measured at 0h to 2h.

Compound 86 (70 mg/Kg)_Blood glucose (mg/dL)						
Sr No	**0 Hr**	**0.25 h**	**0.5h**	**1 h**	**1.5 h**	**2 h**
1	81	115	156	118	110	106
2	83	191	289	186	132	102
3	75	380	387	182	110	132
4	75	283	344	185	131	116
5	78	361	376	232	143	129
6	75	267	458	390	257	200
Mean	**77.8**	**266.2**	**335.0**	**215.5**	**147.2**	**130.8**
SEM	**1.4**	**41.1**	**42.3**	**37.9**	**22.6**	**14.7**

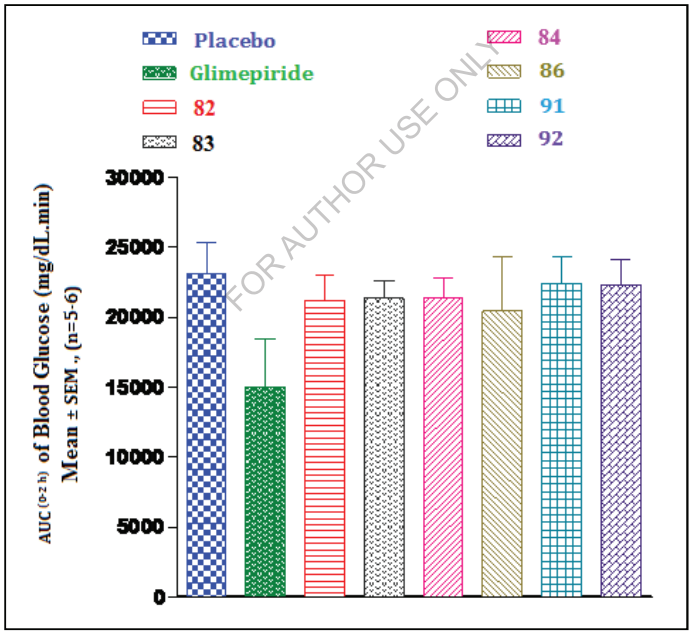
Blood Glucose during intraperitonial glucose tolerance test (IPGTT) in healthy rats for compound 86



Graph 4: Time (min) Vs Blood Glucose (mg/dl)

Table 33: AUC $^{(0\text{-}2\ h)}$ of Blood Glucose mg/dL.min ± SEM for the tested compounds

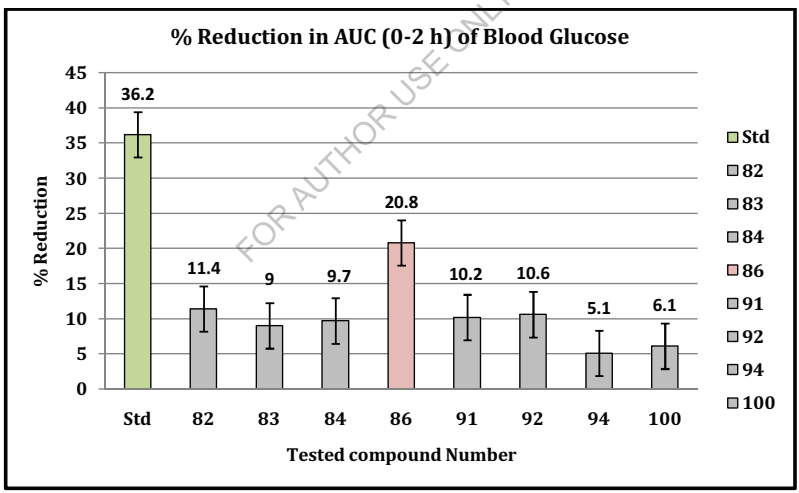
AUC $^{(0\text{-}2\ h)}$ of Blood Glucose mg/dL.min										
No	**Placebo**	**Std**	**82**	**83**	**84**	**86**	**91**	**92**	**94**	**100**
1	29708	8475	21923	24960	26085	5378	25110	28823	24125	26945
2	18240	22185	26745	22110	22965	14790	24548	19245	22210	21230
3	22268	24428	16913	20040	17333	24585	19230	20963	19825	20147
4	26423	8973	23588	22380	22665	20468	29573	27240	21380	22808
5	19155	11077	22868	22785	22418	25260	17513	18375	21870	27963
6	-	-	15105	15923	16995	32303	18615	19320	28342	17850
Mean	**23158.8**	**15027.6**	**21190.3**	**21366.3**	**21410.2**	**20464.0**	**22431.5**	**22327.7**	**22958.6**	**22823.8**
SEM	**2174.5**	**3426.4**	**1782.1**	**1263.5**	**1448.9**	**3829.4**	**1929.3**	**1847.1**	**1216.5**	**1610.7**



Graph 5: AUC $^{(0\text{-}2\ h)}$ of Blood Glucose mg/dL.min ± SEM) for the tested compounds

Table 34: % Reduction in AUC $^{(0\text{-}2\ h)}$ of Blood Glucose ± SEM for the tested compounds

% Reduction in AUC $^{(0\text{-}2\ h)}$ of Blood Glucose									
Sr. No	**Std**	**82**	**83**	**84**	**86**	**91**	**92**	**94**	**100**
1	63	5	0	0	77	0	0	0	0
2	4	0	5	1	36	0	17	4	8
3	0	27	13	25	0	17	9	14	2
4	61	0	3	2	12	0	0	7	5
5	52	1	2	3	0	24	21	6	0
6	-	35	31	27	0	20	17	0	22
Mean	**36.2**	**11.4**	**9.0**	**9.7**	**20.8**	**10.2**	**10.6**	**5.1**	**6.16**
SEM	**14.1**	**6.3**	**4.8**	**5.2**	**12.6**	**4.6**	**3.7**	**2.12**	**3.40**



Graph 6: % reduction in blood glucose AUC for the tested compounds

4.3.2.3. Structure Activity Relationship

The sulphonylureas act to enhance the sensitivity of the beta-cell to glucose, while thiazolidinone drugs results from stimulation of a nuclear PPAR-γ, which culminate an increase in insulin sensitivity. Moreover, the combination of two pharmacophores in a single molecule is a well-established hypothesis for synthesis of more active drugs with dual activity. Thus, a series of novel sulphonamide substituted thiazolidine-4-ones were synthesized and evaluated for their anti-diabetic activity. We have prepared two different series, sulfonylhydrazides and sulfonylureas. The anti-inflammatory activities exhibited by selected eight compounds (**82**, **83**, **84**, **86**, **91**, **92, 94**, **100**) using dose 70 mg/kg are listed in **Table 27**. Alkyl and aryl hydrazides to replace sulfonyl chloride core were selected to evaluate structure activity relationship among the novel the hydrazide derivaties (**82, 83**, **84, 86**, **91**, **92**). While to study the sulfonylurea's properties with thiazolidinone moiety, two compounds (**94** &**100**) with Secondary amines such as cyclohexyl and morpholine substituents were selected to evaluate the structure activity relationship among the novel analogue.

The variation was brought across acid core **6d** with five different hydrazides to get the desired derivatives (**82-92**). In this series, 3-phenylpropanehydrazidecompound **86** has shown remarkable inhibitory activity inIntraperitonial Glucose Tolerance Test (IPGTT) in healthy ratswith 20.8% reduction in blood glucose AUC, while standard drug Glimepiride showed 36.2% reduction. Alkyl hydrazide sidechain containing derivatives **82** (acetic acid hydrazide) and **83** (cyclohexyl carboxylic acid hydrazide) showed moderate % of reduction in AUC with 11.4% and 9% reduction. Compound **83** showed better % of reduction than the aromatic hydrazide analogous 84, 91 and 92. Benzoic acid hydrazide derivative **84**, showed 9% reduction while aromatic heterocyclic compounds **91**(Thiophene) and **92** (Furan) showed 10.2% and 10.6% reduction in AUC. Increase in lipophilicity increases the potency of compound towards the % reduction of AUC, and this trend continued to obtain a 20.8% reduction for most lipophilic compound **86**.

Among the two compounds (**94** & **100**) from urea derivatives it was observed that the combination of the two pharmacophores were not fruitful and results into the most poor % reduction in AUC (5.1 % & 6.1% respectively) within the studied compounds.

Chapter 5

Summary and Future scope

Summary

In our research program, we had synthesised **100** novel heterocyclic analogs with Thienopyrimidines, Pyrazoles and Thiazolidine-2,4-diones were synthesized and studied for their anticancer, anti-inflammatory, anti-diabetic and antibacterial activities. We had explored ***thirteen*** different series in this thesis. Compounds from five different series were studied for their anticancer activities in diverse human cancer cell lines. Two series were explored for anti-inflammatory activities, three series were evaluated for anti-diabetic studies and three series were synthesized to examine antibacterial properties of heterocycles. To conclude the thesis, the compounds which demonstrated promising activities in their respective therapeutic area are summarised herewith. Also, the future scope of the current work has been discussed in details in this chapter.

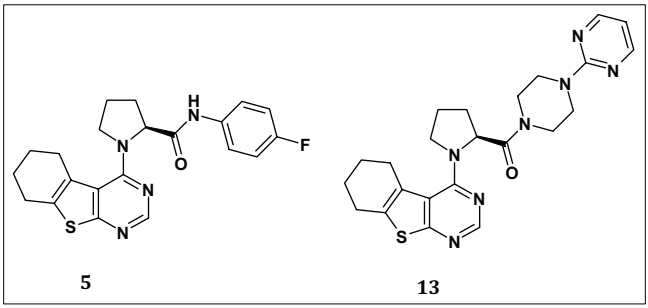
5.1. Conclusion of Tyrosine kinase inhibitors as anticancer agents

In our research project to develop newer anticancer molecules, we have adopted two different strategies using two different pharmacophores. First we used thieno [2, 3-d] pyrimidine nucleus to designed and synthesized newer amide and hydrazide molecules of target. Secondly, we designed the novel amide and hydrazide molecules combining the two pharmacophores such as thiazolidine-2,4-dione (2,4-TZD) and sulphonamides. We have synthesized five different series of compounds and studied their anticancer activities in different human cell lines. We end-up with some promising compounds that will help in future drug development of cancer treatment. Here we have discovered three lead molecules that showed $GI_{50} < 0.1$ µmol/L in human.

5.1.1. Design & synthesis of novel thieno [2, 3-d] pyrimidine

In **Chapter 2**, Section A discussed the Synthesis and anticancer studies of *1-(5,6,7,8-Tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine-2-carboxylic acid* derivatives. It includes the synthetic schemes (**Scheme 1** to **Scheme 4**), experimental details and spectral and elemental analysis for the synthesized novel thieno[2,3-d]pyrimidine

containing pyrolidine-2-carboxylic acid derivatives **1-20** followed by the anticancer studies in MCF-7 (Breast) and HCT-15(Colon) human cancer cell lines.

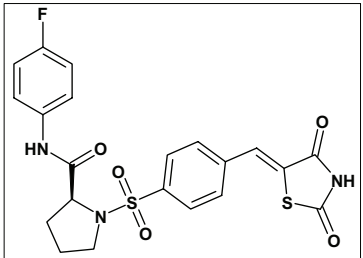


Between the three series of compounds containing substituted anilines **1-7**, substituted piperazine **8-14** and hydrazide side chains **15-20** it was found that secondary substituted piperazine derivatives are exhibiting good inhibitory in both the MCF-7 and HCT-15 cell lines. Primary substituted anilines resulted in improved activities in MCF-7 cell line than HCT-15 cell line and hydrazide compounds were very poor in both the MCF-7 and HCT-15 cell lines. Compound **5** and compound **13** were the new finding from this research work (**GI_{50} < 0.1 µmol/L**; MCF-7 cell line) and it will be studied further in near future.

5.1.2. Design & synthesis of novel thiazolidine-2,4-dione

In continuation to this research here we have combine the two pharmacophores derived from the preclinical candidates of GSK, i.e. aryl sulphonamides and thiazolidinedione. In **Chapter 4**, Section A discussed the Synthesis and anticancer studies of *1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid* derivatives.

It includes the synthetic schemes (**Scheme 14** to **Scheme 16**), experimental details and spectral and elemental analysis for the synthesized novel thiazolidine-2,4-dione containing pyrolidine-2-carboxylic acid derivatives **66-81** followed by the anticancer studies in MCF-7 (Breast), HCT-15(Colon) and Hep-G2 (liver) human cancer cell lines.



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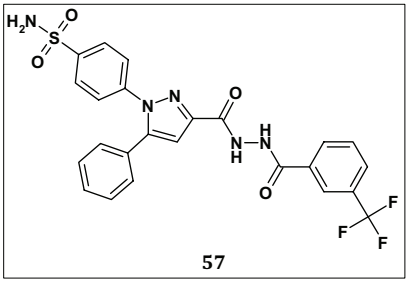
Between the two series of compounds containing substituted anilines **66-73** and hydrazide side chains **75-81**, it was found that substituted anilines resulted in improved activities in MCF-7 cell line than HCT-15 cell line and Hep-G2 cell line. Hydrazide compounds were very poor in all the three tested cell lines. All tested compound, except **71** (**TGI = 98.8**), showed **TGI >100** and **LC_{50}>100** for both the breast MCF-7 and colon HCT- 15cell line. Compound **71** was the new finding from this research work (**GI_{50} < 0.1 µmol/L**; MCF-7 cell line) and it will be studied further in near future.

5.2. Conclusion of Anti-inflammatory study with pyrazole nucleus

The pyrazole nucleus represents a very attractive scaffold to obtain new molecules endowed with anti-inflammatory activities. In **Chapter 3,** Section B *&* Section C , (**Scheme 10** to **Scheme 13**), discussed the Synthesis and anti-inflammatory studies of certain *4-(3-Hydrazinocarbonyl-5-phenyl-pyrazol-1-yl)-benzenesulfonamide* derivatives **56-65**, bearing two aryl moieties at 1- and 5-positions of the pyrazole ring and carrying different substituent on the 5-amino and 3-hydrazinocarbony residue were synthesized and were subsequently tested for their in-vitro cyclooxygenase inhibitory assay. Compounds that showed promising in-vitro COX-2 IC_{50} values and selectivity indices were then evaluated for their in-vivo anti-inflammatory inhibition assay using standard carrageenan induced rat paw edema method. Two promising inhibitors were evaluated for ulcerogenic liability.

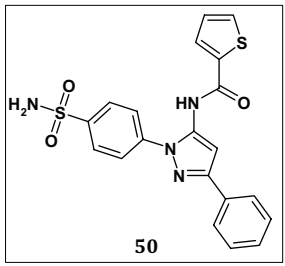


57

X-ray crystal structure of COX-2 was taken from PDB entry COX-2 (3LN1) having resolution of 2.80 Å (Angstroms). Structural preparations for docking studies were accomplished using protein preparation wizard in Maestro 9.0. Compounds **57** displayed reasonable COX-2 inhibition (COX-2 IC_{50}=0.52 μM) and COX-2 selectivity index (SI=10.73) when compared to Celecoxib (COX-2 IC_{50}=0.78 μM) and (SI=9.51). In-vivo anti-inflammatory studies demonstrated **64.28% inhibition** for **57** in comparison with the 57.14% for that of *Celecoxib* itself. The results of ulcerogenic liability were also found comparable with standard celecoxib. Molecular docking studies revealed that, all the designed molecules showed good interactions with receptor active site with glide scores in the range -13.130 to -10.624.

5.3. Conclusion of Antibacterial agents study

Infectious diseases are the leaders among the challenging drug targets because of the multi-drug resist antimicrobial pathogens and continuous rise in the emerging infections from known and unknown sources. Though there is an availability of a large number of antibiotics and chemotherapeutics for medical use, the emerging resistance drives it for the search of new classes of antimicrobial agents. In **Chapter 3,** Section B, (**Scheme 10** to **Scheme 11**), we discussed, a series of novel amides **46-55** containing 1, 3-diaryl pyrazoles were synthesized, characterized and evaluated for their anti-bacterial properties against gram - positive organisms (*B. subtilis*) and gram - negative organisms (*E. coli*).

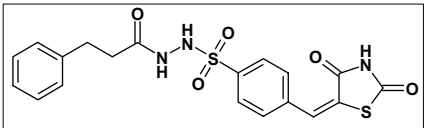


50

The structures of these novel compounds were confirmed by 1H NMR, ES-MS and elemental analysis. Ciprofloxacin was used as standard reference compound. In the initial inhibitory study at 100µg/ml, compounds **50** (12 ± 0.816) demonstrated comparable zone of inhibition with ciprofloxacin (20.66 ± 0.942) in *E. coli* strain, while for *B. subtilis,* at 100µg/ml, compounds **50** (25.66 ± 0.942) was found to be equipotent as compared to standard ciprofloxacin (27.66 ± 0.471). Hence **50** was tested for its MIC values (µg/ml) using *E. coli* and *B. subtilis* bacterial strains. To summarize, **50** (MIC = 8µg/ml for *E. coli* and MIC = 4 µg/ml for *B. subtilis*) showed better MIC values than the standard *Ciprofloxacin* (MIC = 20 µg/ml for *E. coli* and MIC = 12 µg/ml for *B. subtilis*).

5.4. Conclusion of Sulfonylureas as Antidiabetic agents

Since sulfonylureas and thiazolidinone moieties are biologically proven antidiabetic pharmacophores and substitution in these scaffolds may further enhance their activity, prompted us to undertake this problem. Moreover, the combination of two pharmacophores in a single molecule is a well established hypothesis for synthesis of more active drugs with dual activity. Thus, in **Chapter 4,** Section B, (**Scheme 17** to **Scheme 20**), we discussed, a series of novel sulfonylhydrazides **82-92** and sulfonylureas **93-100**, substituted with thiazolidine-4-ones were synthesized and evaluated for their hypoglycaemic activity.



86

In this series, 3-phenylpropanehydrazide compound **86** has shown remarkable inhibitory activity in *Intraperitonial Glucose Tolerance Test* (**IPGTT**) in healthy rats with **20.8**% reduction in blood glucose AUC, while standard drug *Glimepiride* showed 36.2% reduction.

Future Scope

With rapid advances in rational-design approaches in recent decades, the 'drug-discovery' process has become a 'drug-creation' process, at the center of which are medicinal chemists. They have become multidimensional puzzle solvers, bringing to bear a body of science that has vastly expanded over the past couple of decades, and will continue to rapidly grow and evolve. Cheminformatics continues to evolve in its applications to the design or selection of molecules for inclusion in compound libraries (including virtual ones) for screening, for designing diversity-oriented synthesis programs, and for similarity searching for lead identification and scaffold-hopping.

Publications

1. ***Laxmikant S Pavase*** *& Dhananjay V Mane*; Synthesis of some novel Tetrahydrobenzo[4,5]thieno pyrimidine derivatives by using Suzuki coupling reaction; Conference: Pre Science Congress at Dr. Babasaheb Ambedkar Marathwada University, Aurangabad; **2014**.

2. ***Laxmikant S Pavase****, Dhananjay V Mane, Prashant P Dixit*; Synthesis & antibacterial activities of 1-phenyl-5-(1H-pyrrol-1yl)-1H-pyrazole-4-carboxilic acid N'-acyl hydrazides; *Am J Pharm Tech Research*; **2015**; 5(3), **ISSN: 2249-3387**.

3. ***Laxmikant S Pavase*** *& Dhananjay V Mane*; Synthesis and antibacterial activity of novel hydrazides containing thienopyrimidine, *J Chem Pharma Res*; **2015**, 7(9): 670-675, **ISSN: 0975-7384**.

4. ***Laxmikant S Pavase*** *& Dhananjay V Mane*; Synthesis and anticancer activities of novel (tetrahydrobenzo [4,5] thieno[2,3-d] pyrimidine-4-yl)-pyrolidine-2-carboxylic acid derivatives; *Medicinal Chemistry Research*; **2016**, 25: 2380-2391.

5. ***Laxmikant S Pavase****, Dhananjay V Mane, Kamalkishor Baheti*; Synthesis and antibacterial activities of novel sulphonamide containing 1, 3-diarylpyrazolyl amides; *Current Bioactive Compounds*; **2017**, 13: 1-5.

6. ***Laxmikant S Pavase*** *& Dhananjay V Mane*; Synthesis and anticancer studies of 1-[4-(2,4-Dioxo-thiazolidin-5-ylidenemethyl)-benzenesulfonyl]-pyrrolidine-2-carboxylic acid derivatives; *Chemistry Biology Interface*; **2017**, 7(3):183-194.

7. ***Laxmikant S Pavase****, Dhananjay V Mane, Kamalkishor Baheti*; Anti-inflammatory Exploration of sulfonamide containing diaryl Pyrazoles with Promising COX-2 selectivity and Enhanced Gastric Safety Profile; *Journal of Heterocyclic Chemistry*; Feb **2018**, *https://doi.org/10.1002/jhet.3118*.

Conferences

1. Attended, 21st ISCB International Conference (ISCBC-2015) Current Trends in Drug Discovery and Developments, Central Drug Research Institute, Lucknow, U.P., India, 25th - 28th February, **2015**.

2. Attended, International Conference on Contemporary Antimicrobial Research 2016, Assam University, Silchar, India, 14th-17th November, **2016**.

3. 24st ISCB International Conference (ISCBC-2018) Frontier Research in Chemistry & Biology Interface, Manipal University, Jaipur, India, 11th -13th February, **2018**, *Abstract accepted for poster presentation*.