DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NOVEL LIGANDS FOR ADENOSINE RECEPTORS

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In

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BY

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GUJARAT, INDIA

October 2014

Certificate

This is to certify that the thesis entitled **"Design, synthesis and pharmacological evaluation of novel ligands for adenosine receptors"** has been prepared by Mr. Dhaivatkumar Harikrishnabhai Pandya under my supervision and guidance. The thesis is his own original work completed after careful research and investigation. The work of the thesis is of the standard expected of a candidate for Ph.D. programme in Science and I recommend that it be sent for evaluation.

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I do hereby further declare that the text, diagrams or any other material taken from other sources have been acknowledged, referred and cited to the best of my knowledge and understanding.

Date: 15|10|2014

Signature of Student

Dedicated to

Pappa-Mummy,

My Family and MANSI

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DHAIVAT H. PANDYA

Abstract

The thesis deals with design, synthesis and pharmacological evaluation of novel ligands for adenosine receptors with their computational studies. It starts with introduction related to adenosine, adenosine receptors and various classes of adenosine receptor ligands. As there is great and extensive roles of adenosine receptor subtypes in both physiological and pathophysiological events, these receptors are becoming important drug targets in the treatment of a variety of diseases like cardiovascular disorders (A_1 adenosine receptor antagonist), Parkinson disease (A_{2A} adenosine receptor antagonist), inflammation (A_{2B}/A_3 adenosine receptor antagonist) etc. With all the studies it has emerged that adenosine receptors can be safely targeted by various ligands and various highly specific agonists and antagonists of adenosine receptors can be generated. As a result, increasing numbers of clinical trials testing of novel molecules in various indications have been initiated during the past decade.

For design of molecules, fragment based drug design approach was used and novel compounds thiophenyl-thiazole carboxamides with amied spacer were developed which was further validated by molecular docking study. Library of compound were synthesized and evaluated for their biological activity against adenosine receptors. All the molecules had shown good affinity and selectivity to adenosine receptors.

Keeping in mind the importance of thiophene and to see the SAR around 2-amino thiophenes, modification of the thiophenyl-thiazole carboxamides into thiophenyl-thiophene carboxamides with amide spacer were carried out for the new series of compounds. Synthetic methodology for the synthesis of this series of molecule was established and library of molecules were synthesized and evaluated for their biological activity against adenosine receptors. As per our findings, this is first time such a scaffold has been prepared and checked for activity against adenosine receptors.

Various reported 2-cyclicamino thiazoles have shown good affinity to adenosine receptors. 2-cyclicaminothiophene prepared in our lab was active as anti-inflammatory compounds. So in the new series of compounds we have tried to design similar structural feature with replacement of 2-cyclicaminothiophens with 2-cyclicaminothiazoles to come up at good adenosine receptor ligands. Here, novel synthetic methodology was developed

for the synthesis of novel 2,4- and 2,5- disubstituted 2-cyclicaminothiazoles. To best of our knowledge 2,4- and 2,5-disubstituted 2-cycloaminothiazoles has been checked first time against adenosine receptors.

In continuation of previous series of work, we designed some new 2-aminothiazole derivatives with major focus on aminothiazoles. Our previous work on 2-aminothiazole derivatives had shown good affinity and selectivity towards adenosine receptor. So, in this series of molecules we have kept aminothiazole moiety intact to see the change in biological activity from previous series of molecules. Library of compounds with structural diversification was synthesized and evaluated for their biological activity against adenosine receptors. The molecules showed remarkable affinity, selectivity and activity to adenosine receptors. The selective and active molecules were further taken for molecular docking studies into the adenosine receptors to see their possible binding modes.

Further to see the structure–activity relationship and identify structural features influencing the biological activity 3D QSAR was carried out. The satisfactory predictive ability of 3D QSAR models observed for compounds indicates that these models could be successfully used for predicting activity of the antagonists and can guide the further modification of these compounds.

In addition to the designed molecules, we have also developed novel synthetic methodology for the synthesis of 2-aminothiadiazine and 2-cycloaminothiadiazine from various substituted thiosemicarbazide derivatives and phenacyl bromides.

In search of potential antagonist for adenosine receptors here we have designed novel molecules which were further validated with the molecular docking study. Synthetic methodology was developed and library of compounds with structural diversification was synthesized from various starting materials. Further all the molecules were characterized by spectral techniques like NMR and Mass. All the molecules were screened for their binding affinity to the adenosine receptor subtypes and the molecules were found to be selective and active. 3D QSAR study of adenosine receptor ligands shown a statistically significant result which is helpful for further modification.

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List of abbreviations and symbols

%	Percent
°C	Degree Celsius
δ	Delta
ACN/MeCN	Acetonitrile
ARs	Adenosine receptors
COPD	Chronic obstructive pulmonary disorder
DCM	Dichloromethane
DMF	*N*,*N*-Dimethyl formamide
DMFDMA	*N*,*N*-dimethylformamide dimethyl acetal
EA	Ethyl acetate
GPCRs	G-protein-coupled receptors
Hz	Hertz
[^{3}H]CCPA	[^{3}H]2-chloro-N^6-cyclopentyladenosine
[^{3}H]NECA	[^{3}H]5'-(*N*-ethylcarboxamido)adenosine
[^{3}H]HEMADO	[^{3}H] 2-hexyn-1-yl-N^6-methyladenosine
h	Hour
IR	Infra red
LC-MS	Liquid chromatography-mass spectrometry
μM	Micromolar
mp	Melting Point
MeOH	Methanol
mL	Milliliter
nM	Nanomolar
NMR	Nuclear magnetic resonance
R_f	Retardation factor
TEA	Triethyl amine
THF	Tetrahydrofuran
TLC	Thin layer chromatography

List of materials and equipments

Solvents and chemicals

Chloroform (LR grade, Ranbaxy Fine Chem Ltd., New Delhi)

Dichloromethane (LR grade, Qualigens Fine Chemicals, Mumbai)

Ethyl acetate (LR grade, Ranbaxy Fine Chem Ltd., New Delhi)

Hexane (LR grade, Ranbaxy Fine Chem Ltd., New Delhi)

Methanol (LR grade, Qualigens Fine Chemicals, Mumbai)

Anhydrous Sodium sulphate (LR, Ranbaxy Fine Chem Ltd., New Delhi)

Sodium hydroxide (LR, Himedia Laboratories, Mumbai)

Triethyl amine (LR grade, Qualigens Fine Chemicals, Mumbai)

Equipment

Corousel 6 stirrer (Radleys Discovery technologies)

Rotary evaporator (Buchi, Switzerland/Heidolph, Germany)

Magnetic Stirrer (Deepali United Private Ltd, Mumbai)

Overheal Stirrer (Deepali United Private Ltd, Mumbai)

CHAPTER-1

Adenosine receptors and its ligands

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Chapter 1

Adenosine receptors and its ligands

1.1 Introduction to Adenosine Receptors

Purines are the most widespread chemical messengers in animal and plant kingdoms. Adenosine, a purine nucleoside is an endogenous ligand composed of adenine attached to ribose. Adenosine plays an important role in biochemical processes, such as energy transfer—as adenosine triphosphate (ATP) and adenosine diphosphate (ADP)—as well as in signal transduction as cyclic adenosine monophosphate (cAMP). It is identified as a major local regulator of tissue function especially when energy supply fails to meet cellular energy demand. Due to its ability to equalize energy intake to metabolic demand, it earned the reputation of a **"retaliatory metabolite"** (Sattin & Rall 1970).

Adenosine is a signaling molecule whose physiological functions are mediated by its interaction with four G-protein-coupled receptor (GPCR) subtypes, A_1, A_{2A}, A_{2B} and A_3 respectively. Extracellular adenosine acts as a local modulator with a generally cytoprotective function in the body. Its effects on tissue protection and repair fall into four categories: increasing the ratio of oxygen supply to demand; protecting against ischaemic damage by cell conditioning; triggering anti-inflammatory responses; and the promotion of angiogenesis.

The adenosine receptors (ARs) differ in their affinity for adenosine. The A_1 and A_3 adenosine receptor subtypes have high and low affinity for adenosine, respectively. They couple to Gi protein to inhibit adenylate cyclase and thus lead to decrease the cyclic AMP (cAMP). By contrast, high affinity A_{2A} and low affinity A_{2B} adenosine receptor subtypes couples to Gs and stimulates adenylyl cyclase leading to an increase of cyclic AMP (cAMP) levels (Ham & Evans 2012) (Figure-1.1). These two subtype pairs also share higher sequence identity: the human A_1 and A_3 ARs are 49% identical, and the human A_{2A} and A_{2B} ARs are 59% identical.

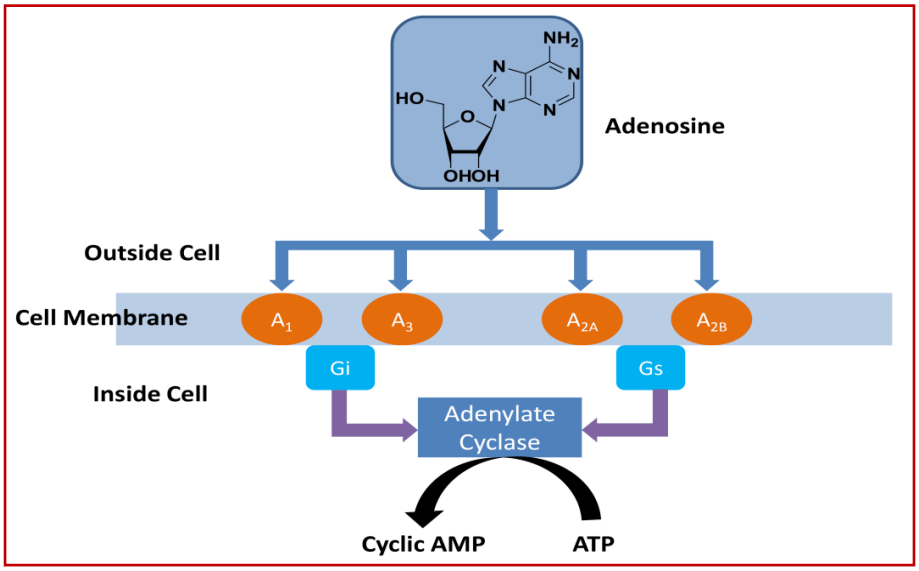


Figure 1.1 Adenosine receptor signaling

The four adenosine receptors have been cloned from several mammalian species, including human. There is extensive sequence similarity between species for the A_1, A_{2A} and A_{2B} receptors, whereas A_3 receptors are more variable (Londos et al. 1980). Each adenosine receptor has different but overlapping functions. Each of them is unique in pharmacological profile, tissue distribution and binding partners. The greatest challenge in developing adenosine receptor ligands for specific clinical applications is that adenosine signaling is so widespread. Adenosine itself is present ubiquitously, adenosine receptors are widely distributed throughout the body and adenosine acting at these receptors exerts a broad spectrum of physiological and pathophysiological functions (Chen et al. 2013). By designing synthesizing and screening the molecules against adenosine receptors there is hope to be able to target a disease specifically by a selective compound.

1.2 Adenosine receptors as therapeutic target

As there is great and extensive roles of adenosine receptor subtypes in both physiologic and pathophysiologic events, these receptors are becoming important drug targets in the treatment of a variety of diseases. With more and more research going on in the field of adenosine receptors it has been possible to find out a number of physiological and pathophysiological processes where one or more adenosine receptors are involved. The

list of such processes is quite wide, and since it is increasing each year it is likely that it will further lengthen.

ARs have been targets for drug development which is schematically shown in Figure 1.2 (Fredholm 2010). By far the most serious attempts have been made in the development of A_{2A} antagonists for neurodegeneration where several drugs companies have candidate drugs in late phases of clinical trial and one of them has been approved (Dungo & Deeks 2013). Adenosine receptors are found on almost all the cells and so the agonists are likely to produce unwanted side effects. On contrast selective antagonists will only affect those sites where receptors are active. The fact that a majority of humans already consume an adenosine antagonist, caffeine, on a daily basis of course also makes one wonder how much can be derived by additional blockade (Fredholm 2010).

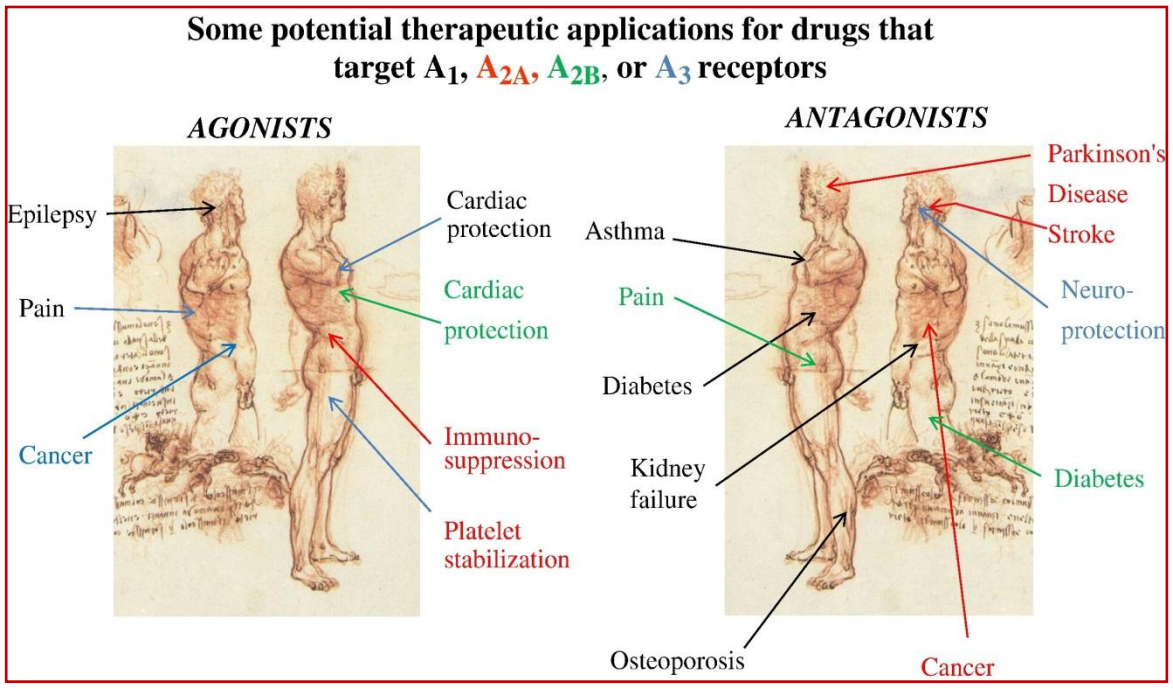


Figure 1.2 Some of the potential uses of drugs that act as agonists (left) and antagonists (right) at the four different adenosine receptors are indicated (Fredholm 2010)

1.2.1 The A_1 adenosine receptor

The adenosine A_1AR is widely distributed in varying levels of expression about many different tissues in the human body, ranging from the colon to the brain. The highest levels found in the brain, especially at excitatory nerve endings (Daly & Padgett 1992). Activation of the A_1AR inhibits adenylyl cyclase activity, that activates potassium channels (including KATP channels in neurons and the myocardium), blocks transient calcium channels and increases intracellular calcium and inositol-1,4,5-trisphosphate[Ins(1,4,5)P3] levels by activating phospholipase C (PLC). A_1AR modulates neuronal activity by blocking neurotransmitter release and reducing the firing rate. The A_1AR mediates negative chronotropic and inotropic effects in the heart but they also exert effects in many other organs and cells. The physiology and pathophysiological effect are implicated in decreased renal blood flow, tubuloglomerular feedback, inhibition of renin release, inhibition of lipolysis, vasoconstriction, bronchoconstriction, inhibition of neurotransmitter release, inhibition of insulin and glucagon release, reduced heart rate, osteoclast activation and bone resportion, reduced respiration, sleep, analgesia, cardiac preconditioning (Fredholm 2010). Considerable advances have been made recently in the pharmacological and molecular characterization of A_1AR, which had been proposed as targets for drug design and discovery. Xanthine and xanthine derivatives, including the natural compounds theophylline and caffeine, constitute the prototypical group of antagonists at all ARs, and modifications of the xanthine structure have given various derivatives which have shown good subtype selectivity. Most of the selective A_1AR antagonists are xanthine-based derivatives (Baraldi, Tabrizi, Gessi, et al. 2008).

1.2.2 The A_{2A} adenosine receptor

The A_{2A}AR has been most widely studied receptor among the all the adenosine receptors structurally as well as therapeutically. A_{2A}AR are highly expressed in the spleen, thymus, leukocytes, blood platelets, striatopallidal GABAergic neurons and the olfactory bulb and expressed to a lesser extent in the heart, lung, blood vessels, and other brain regions. The A_{2A}AR is important in mediating vasodilation, supporting the synthesis of new blood

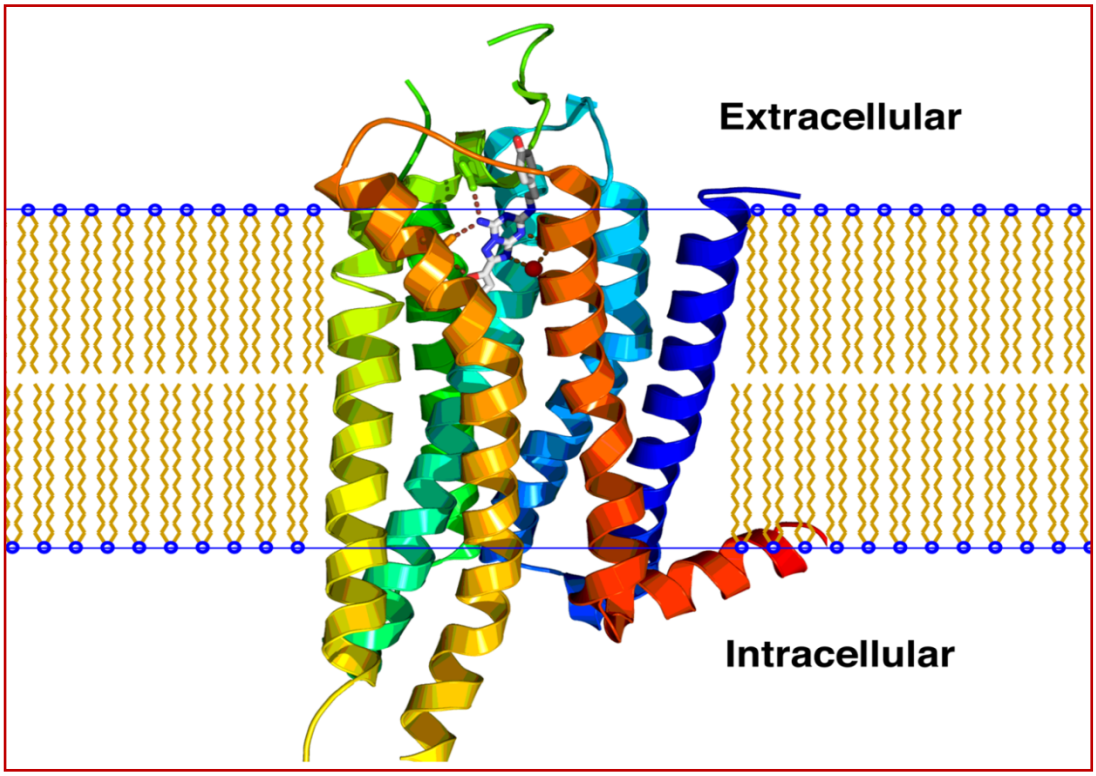


Figure 1.3 X-Ray crystallographic structure of the A_{2A}AR bound to ZM241385 (Jaakola et al. 2008)

vessels and protecting tissues from collateral inflammatory damage. In the brain, A_{2A}AR influences the activity of the indirect pathway of the basal ganglia. Various investigations are being conducted on a number of compounds to treat inflammation, cancer, ischemia reperfusion injury. A_{2A}AR is highly expressed in the spleen, thymus, infectious diseases, wakefulness, neurodegeneration (including Parkinson's disease and Alzheimer's disease) and other CNS disorders (de Lera Ruiz et al. 2013). To understand the insights of receptor and its ligand binding interaction, the A_{2A}AR has been resolved by X-ray crystallographic techniques (Jaakola et al. 2008; Lebon et al. 2011; Doré et al. 2011; Xu et al. 2011; Congreve et al. 2012). Normally, adenosine receptors have a common central core consisting of seven transmembrane helices (TM1−7), each TM being mainly α-helical and composed of 20−27 amino acids. Each TM domain is linked by three intracellular (IL1, IL2, and IL3) and three extracellular (EL1, EL2, and EL3) loops. ARs differ in the length and function of their *N*-terminal extracellular domain, their *C*-terminal intracellular

domain, and their intracellular/extracellular loops. Each of these areas provides very specific properties that are critical for achieving ligand selectivity among the different receptor subtypes. Figure 1.3 shows X-ray crystallographic structure of A_{2A}AR bound to ZM241385 (Jaakola et al. 2008). Various agonist and antagonists synthesized are based on adenosine derivatives, xanthine derivatives, tricyclic molecules and many other derivatives and they have proved to be highly active and selective to A_{2A}AR proving to be important in many diseases specifically neurological disorders (Shook & Jackson 2011).

1.2.3 The A_{2B} adenosine receptor

The A_{2B} AR is highly expressed in the gastrointestinal tract, bladder, lung, mast cells, eye, adipose tissue, brain, kidney, liver and other tissues. The A_{2B}AR is structurally closely related to the A_{2A}AR and able to activate adenylate cyclase but it is functionally very different from A_{2A}AR. It has been postulated that this subtype may utilize signal transduction systems other than adenylate cyclase because of these functional differences (Livingston et al. 2004). Among all the adenosine receptors, the A_{2B}AR is a low affinity receptor that is thought to remain silent under physiological conditions and to be activated in consequence of increased extracellular adenosine levels. As such there is no crystal structure of A_{2B}AR available; but the available structures of A_{2A}AR are suitable as templates for the study of A_{2B} AR models. Despite the similarity between the A_{2A} and A_{2B} ARs, there are many differences between the two receptor subtypes, e.g., the longer extracellular loop 2 of the A_{2B}AR or the fact that the A_{2A}AR possesses four disulfide bonds in the extracellularly oriented part of the protein whereas the A_{2B}AR has been found to have only one disulfide bond. The adenosine A_{2B}AR's physiological role is very less understood, still it has been implicated to play key roles in various processes like modulation of arterial blood pressure and heart rate, glucose metabolism, angiogenesis induction, growth of some tumors, intestinal inflammation, myocardial ischemia, acute lung and kidney injury, inflammatory response and much involvement in the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD) (Thimm et al. 2013). Several class of ligands found have shown good affinity towards A_{2B}AR are adenosine

derivatives, xanthine derivaties, pyrrolopyrimidines, pyrazolotriazolopyrimidines and 2-aminopyrazines (Taliani et al. 2013).

1.2.4 The A_3 adenosine receptor

The A_3AR is the last member of the adenosine receptors family to have been cloned (Jacobson & Gao 2006). Considering receptor distribution, the highest levels of human A_3AR mRNA have been found in lung and liver. However, A_3ARs have been detected in various tissues including testis, lung, kidney, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, aorta, proximal colon and eyes (Borea et al. 2009). A_3ARs, via the interaction with Gi proteins, inhibit adenylate cyclase, decreasing cyclic AMP accumulation and protein kinase A (PKA) activity. In addition, A_3ARs, by coupling with Gq proteins, stimulate phospholypase C (PLC), causing an increase of calcium levels from intracellular stores, and modulate the protein kinase C (PKC) activity. In case of adenosine A_3AR also there is no crystal structure available, but for molecular modeling studies, the homology modeled structure of adenosine A_3AR is used. The functional role of A_3AR is implicated in many disorders like joint disorder, eye disorders, cancer, respiratory disorders and cardiovascular disorders. Majority of the A_3AR receptor agonists are nucleoside derivatives. Apart from nucleoside derivatives some of xanthine and pyridine derivatives are also reported to play agonist's role. Derivatives of pyrimidines, thiazoles, thiadiazoles, quinazolinones, quinoxalines, adenines, triazoloquinzoline, pyrazoloquinolines, pyridotriazolopyrazines have been reported to possess antagonist activity against A_3AR.

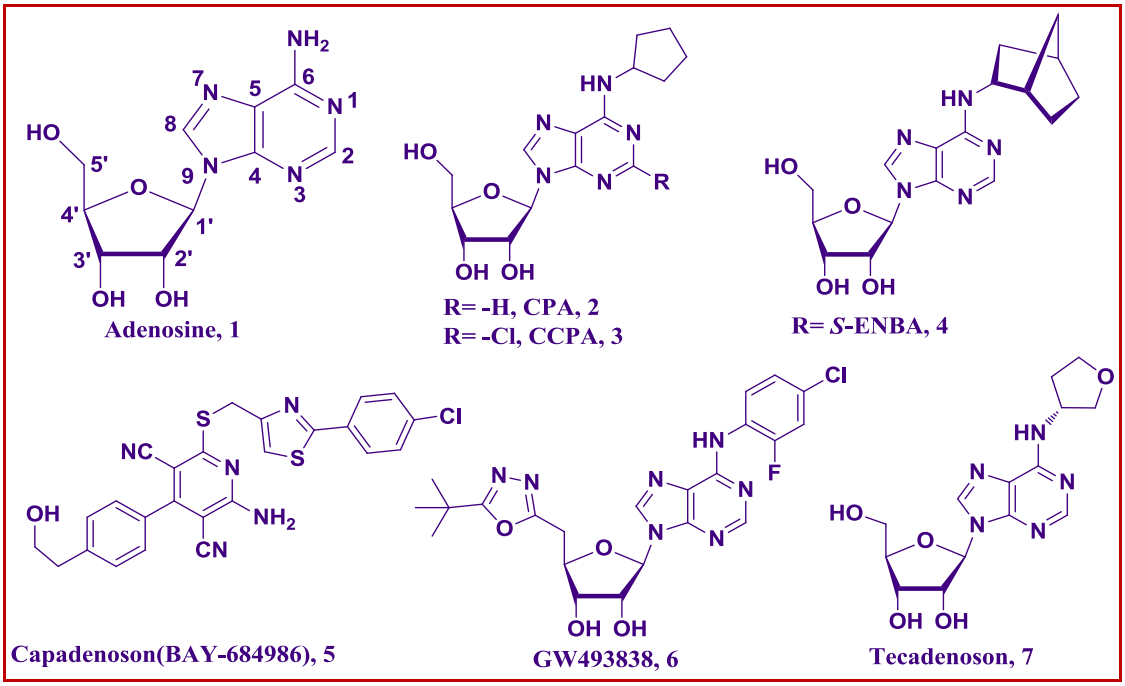
1.3 Adenosine Receptors agonists

Adenosine is an endogenous natural agonist which binds to all the ARs, so modification of adenosine is main approach for discovering AR agonists. Apart from adenosine xanthosine derivatives have been also good agonists. The structure-activity relationships of adenosine **(1)** at ARs have been extensively probed (Yan et al. 2003). Most of the useful analogues are modified in the N^6-or 2-position of the adenine moiety and in the 3′-, 4′- or 5′-position of the ribose moiety. Highly selective agonists of the various receptor

subtypes have been designed through both empirical approaches and a semi-rational approach based on molecular modeling (Kim et al. 2003; Tchilibon et al. 2005).

1.3.1 A_1 adenosine receptor agonists

To get A_1 adenosine receptor agonists, generally substitution of adenosine **(1)** at the N^6-position with a wide range of alkyl, cycloalkyl, and arylalkyl groups increases selectivity. The most successful selective agonists of the A_1AR were achieved by substituting adenosine at N^6 position with cycloalkyl group. N^6-Cyclopentyladenosine **(2, CPA)** and its 2-chloro analogue **(3, CCPA)** are among the most potent and selective A_1AR agonists and they are in wide use as pharmacological agents. *S*(–)-ENBA **(4)** is an even more potent and selective agonist for both human and rat A_1ARs compared with the three other AR subtypes (Gao et al. 2003). Bayer Co.(Germany) discovered 2-amino-3, 5-dicyanopyridine derivatives e.g. capadenoson **(5)**, as non-nucleoside-derived adenosine receptor agonists (Rosentreter et al. 2009). Other than these derivatives, several selective adenosine derivatives, including GW493838 **(6)** and Tecadenoson **(7)** have been evaluated in clinical trials for various indications (Müller & Jacobson 2011) (Figure 1.4).



Figurse 1.4 A_1 adenosine receptor agonists

1.3.2 A_{2A} adenosine receptor agonists

Substitution of adenosine at the 2-position, especially with (thio) ethers, secondary amines, and alkynes, has resulted in many synthetic analogues selective for the A_{2A}AR. NECA (**8**) is a potent nonselective agonist has a 5′-*N*-alkyluronamide modification, and a 5′-*N*-ethyluronamide modification has maintained or enhanced the selectivity for the A_{2A}AR which is present in case of CGS21680 (**12**). 2-(2- phenylethyl) amino is also important modification in case of CGS21680 for enhancing the affinity at A_{2A}AR. UK-432097 (**11**) is N^6-(2, 2- diphenylethyl) adenosine analogues, so some N^6-position substitutions have also been found to increase the affinity at the A_{2A}AR. Regadenoson (**9**, Lexiscan™) and Apadenoson (**10**) has been developed for vasodialation (Müller & Jacobson 2011) (Figure 1.5).

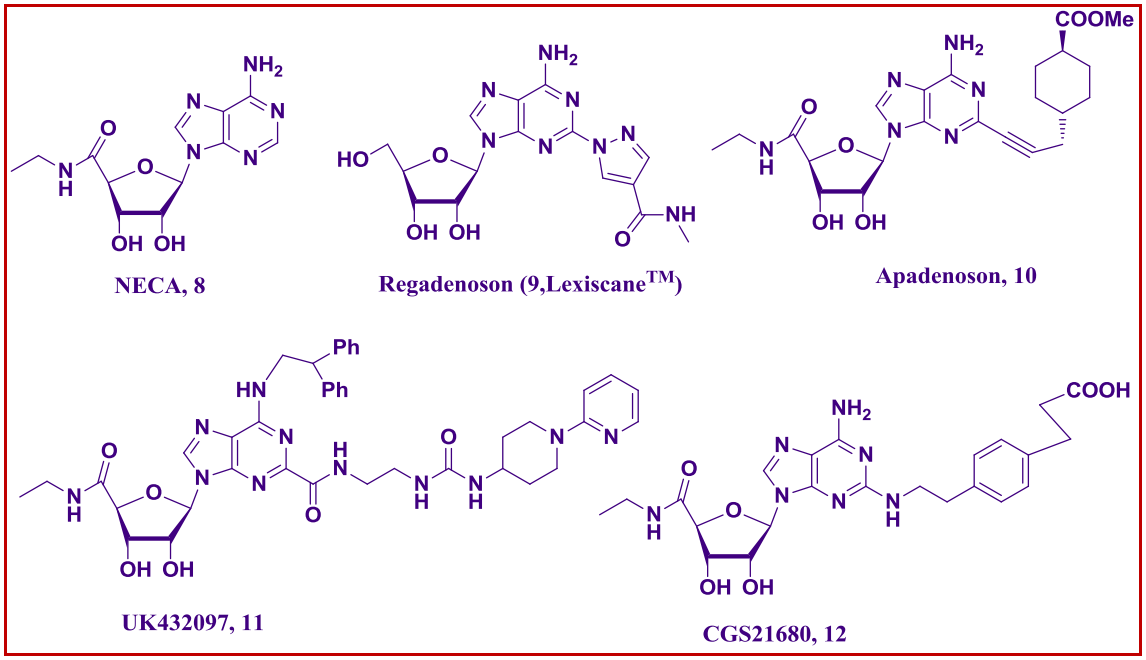


Figure 1.5 A_{2A} adenosine receptor agonists

1.3.3 A_{2B} adenosine receptor agonists

The adenosine A_{2B}AR is least characterized subtype in the AR family. Combinations of changes at adenosine have resulted in compounds that activated the A_{2B}AR with good selectivity. N^6-substituted adenosines, N^6-substituted-5′-*N*-alkyl-carboxamido adenosines, C^2-substituted adenosines and C^2-substituted-5′-*N*-alkyl-carboxamido adenosines have

been reported to show good affinity and selectivity towards $A_{2B}AR$ (**13, 14**). Apart from adenosine derivatives, BAY 60-6583 (**15**) is one of the 2-aminopyridine-3, 5-dicarbonitrilederivatives which is found to activate the $A_{2B}AR$ (Baraldi, Tabrizi, Fruttarolo, et al. 2008; Müller & Jacobson 2011) (Figure-1.6).

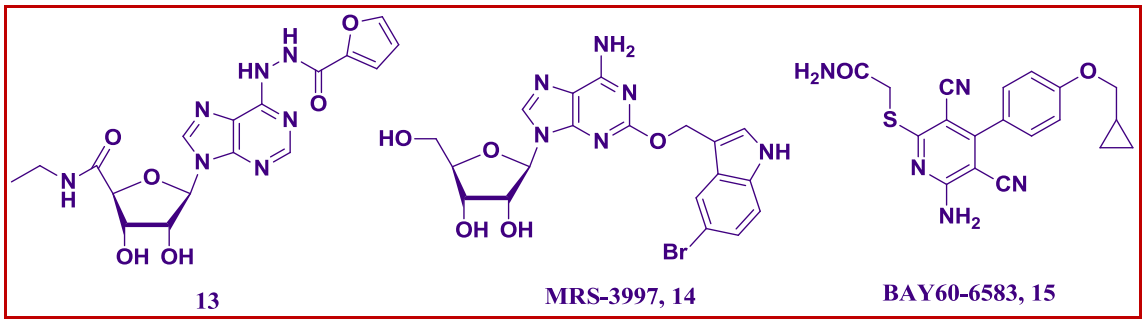


Figure 1.6 A_{2B} adenosine receptor agonists

1.3.4 A_3 adenosine receptor agonists

The vast majority of A_3AR agonists reported to date reflects the nucleoside structure of the endogenous ligand, adenosine. The most successful structural modification of the adenosine skeleton in enhancing A_3AR potency and selectivity involve N^6-, C^2-, and 5′-substitutions or combination of these. Substitution with an N^6-benzyl group or substituted benzyl group increases selectivity for the A_3AR. IB-MECA (CF101, **16**) and the more selective agonist Cl-IB-MECA(CF102, **17**) have been widely used as pharmacological probes. The 4′-thioadenosine derivative LJ-529 (**18**) also acts as a highly potent and selective A_3AR agonist with a subnanomolar affinity.

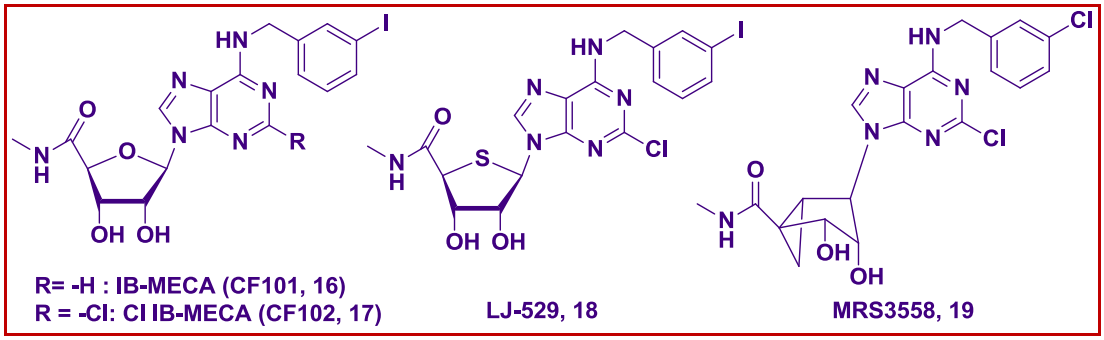


Figure 1.7 A_3 adenosine receptor agonists

The new design which includes [3.1.0]bicyclohexane ring system in place of the ribose 5-membered ring was utilized to get more potent and selective analogues such as

MRS3558 (**19**), which displays nanomolar affinity at the A_3AR (Müller & Jacobson 2011; Baraldi et al. 2012) (Figure 1.7).

1.4 Adenosine receptors antagonists

Traditionally, the adenosine receptor antagonists have been xanthine derivatives. The natural products like caffeine and theophylline behaves as weak and nonselective AR antagonists. The structure activity relationship (SAR) of xanthine derivatives as AR antagonists has been the core research area to get selective AR antagonists. The effects of receptor subtype selectivity of substitution at the 1-, 3-, 7-, and 8-positions have been explored in detail. However, many new nonxanthine and non-purine derivatives have been developed as highly selective AR antagonists.

1.4.1 A_1 adenosine receptor antagonists

Potent and selective antagonists for A_1AR have been developed by modification of the xanthines at 8-position with aryl or cycloalkyl groups and these modification has led to high affinity and selectivity (Moro et al. 2006). For example, the 8-cyclopentyl derivative DPCPXor CPX (8-cyclopentyl-1, 3-dipropylxanthine, **20**) is highly selective at the human A_1AR.

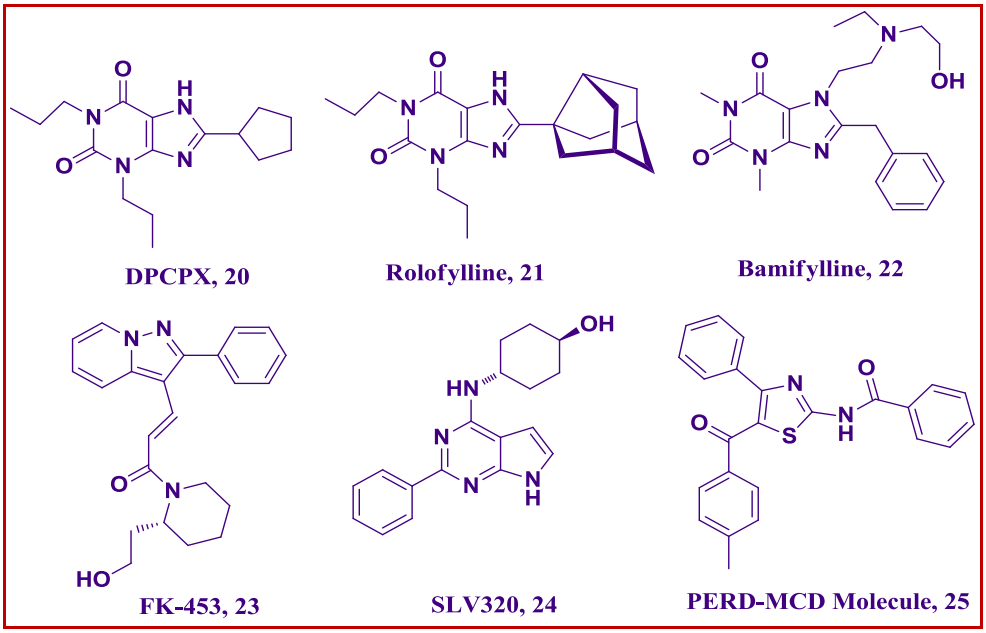


Figure 1.8 A_1 adenosine receptor antagonists

Rolofylline(KW-3902, **21**) has a bicycloalkyl group present at 8th position of xanthine ring and has a good selectivity. Bamifylline (**22**) is another selective derivative with benzyl group at 8th position on xanthine ring. Apart from the xanthine derivatives, non-xanthine structures are also reported to show high affinity and selectivity towards A_1AR (Kiesman et al. 2009). FK-453 (**23**) is a pyrazole derivative and SLV-320 (**24**) is a pyrrolopyrimidine derivative, both are highly selective. New 2-aminothiazole derivatives which are PERD-MCD (**25**) derivatives have shown high A_1AR affinity and selectivity (Scheiff et al. 2010) (Figure 1.8).

1.4.2 A_{2A} adenosine receptor antagonists

Historically, A_{2A} AR antagonists have been divided into xanthine-based and non-xanthine-based derivatives. Modification of xanthines at the 8-position with alkenes has led to selectivity for the A_{2A}AR. Istradefylline (KW-6002, **26**) is a xanthine based, selective A_{2A}AR antagonist that was approved recently for Parkinson's Disease (Dungo & Deeks 2013). MSX-2 (**27**) is selective A_{2A}AR inhibitor which was further converted into two different prodrugs phosphate prodrug (MSX-3, **28**) and L-valine ester prodrug (MSX-4, **29**) to make them water soluble (Sauer et al. 2000; Vollmann et al. 2008).

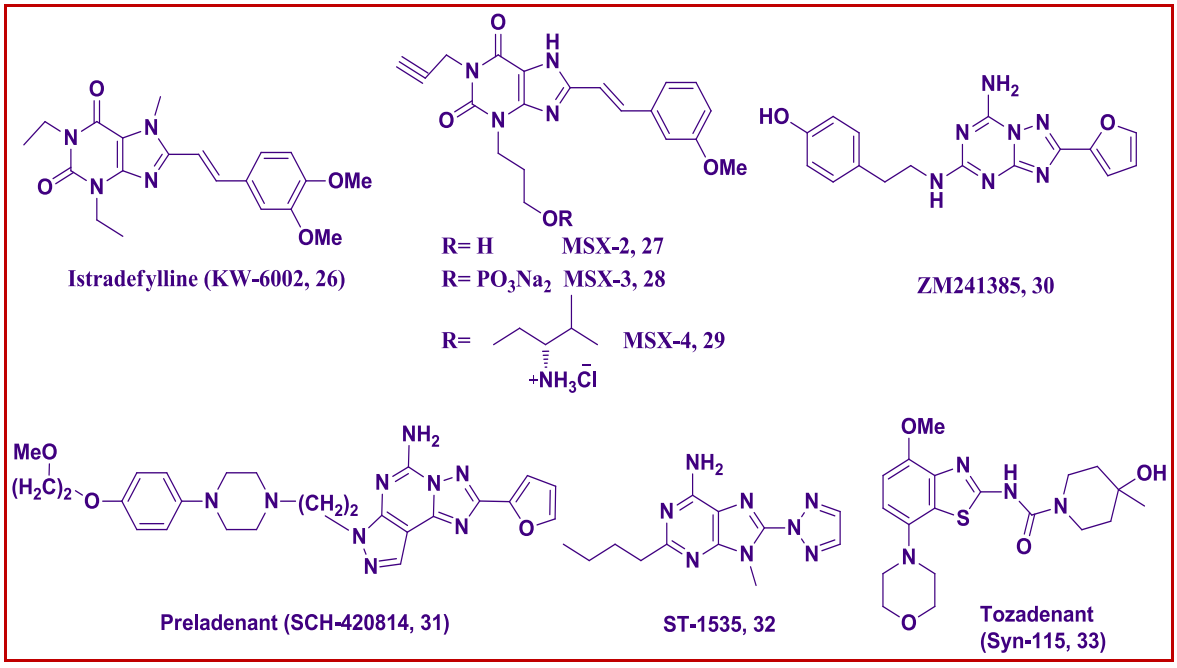


Figure 1.9 A_{2A} adenosine receptor antagonists

Both are now broadly used as pharmacological tools in particular for *in vivo* studies. Xanthine derivatives however, have several limitations as pharmacologic tools because of poor pharmacokinetic profile. So number of monocyclic, fused bicyclic and tricyclic derivatives other than xanthine derivatives has been developed as adenosine A_{2A}AR antagonists (de Lera Ruiz et al. 2013). ZM-241385 (**30**) is highly selective triazolopyrimidine derivative which may become useful for neurodegenerative diseases. Triazolopyrzaolopyrimidine derivatives like Preladenant (**31**) and benzothiazole derivatives like Tozadenant (**33**) have reached late stage clinical trials for Parkinson's Disease (Figure 1.9).

1.4.3 A_{2B} adenosine receptor antagonists

One of the first compounds as A_{2B}AR antagonist was the xanthine derivative MRS1754 (**34**) which is potent and selective in humans. Furthermore potent and A_{2B}AR selective xanthine derivatives include MRE-2029-F20 (**35**) and PSB-1115 (**36**) which have been used as radioligands. Besides xanthines derivatives, nonxanthine A_{2B}AR antagonists have recently been developed is LAS38096 (**37**), a pyrimidine derivative. Other non-xanthine derivative includes thiazole in QAF 805 (**38**) which is Novartis compound. All of these A_{2B}AR antagonists (Figure 1.10) have been in the development for various inflammatory diseases and asthma.(Baraldi, Tabrizi, Fruttarolo, et al. 2008)

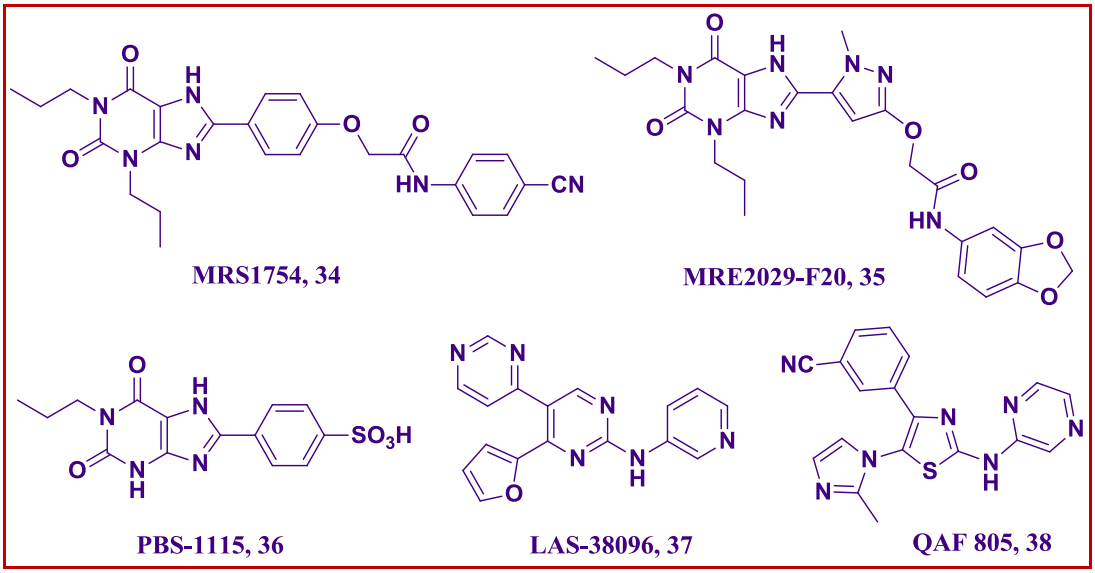


Figure 1.10 A_{2B} adenosine receptor antagonists

1.4.4 A_3 adenosine receptor antagonists

Unlike other three adenosine receptor subtypes (A_1, A_{2A} and A_{2B}), the A_3AR has low binding affinity towards xanthine derivatives. Still, some of the cyclized xanthine derivatives like KF-26777 (**39**) showed good affinity and selectivity towards A_3AR. Because of low affinity towards xanthine derivatives, various classes of non-xanthine monocyclic, bicyclic and tricyclic heterocyclic derivatives were developed as A_3AR antagonists. Pyridine derivative like MRS1523 (**40**) and 2-amino thiazole derivative like CGH2466 (**41**) had shown nanomolar affinity towards A_3AR. VUF5574 (**42**) is a quinazoline derivative and LJ1251 (**43**) is thioadenosine derivatives which had shown antagonist activity towards A_3AR. Triazoloimidazolepyrimidine derivative OT-7999 (**44**) is developed for the treatment of glaucoma. The A_3AR antagonists are under consideration for treatment of cancer, stroke, asthma, COPD and inflammation (Baraldi et al. 2012) (Figure 1.11).

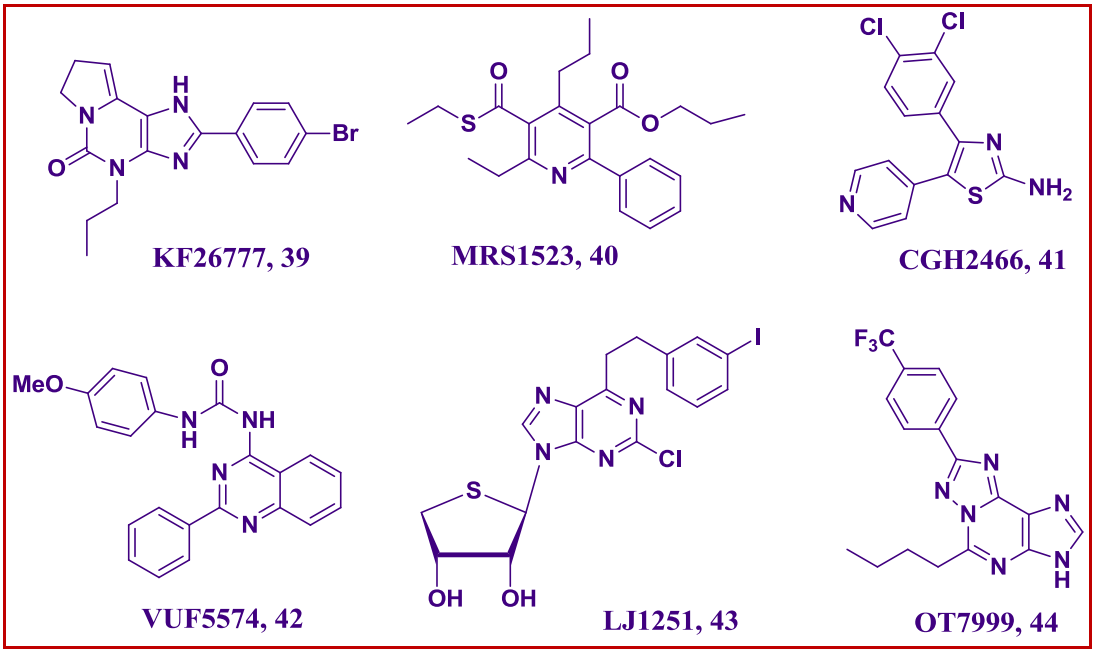


Figure 1.11 A_3 adenosine receptor antagonists

1.5 Allosteric modulation of adenosine receptors

Allosteric modulators of adenosine receptors are the alternative to direct acting AR agonist and antagonists. Allosteric modulators bind at a distinct site other than the natural

ligand binding site. They exert their effect only in the presence of the orthosteric ligand. A positive allosteric modulator (PAM) induces an enhancement of effects of the orthosteric ligand, while a negative allosteric modulator (NAM) attenuates those effects (Göblyös & Ijzerman 2009).

***A_1 AR*:** Bruns and colleagues introduced the first allosteric modulators of A_1AR in 1990s (Bruns & Fergus 1990; Bruns et al. 1990). They described various 2-amino thiophene derivatives such as PD 71605 (**45**), PD 81723 (**46**) and PD 117975 (**47**) as allosteric modulators of A_1AR. Other thiophene derivative T62 (**48**) is clinically evaluated for treatment of neuropathic pain. Allosteric enhancers at the A_1AR have received attention as anti-arrhythmic cardiac agents, and, more recently, as anti-lipolytic agents. In addition, this class of compounds has therapeutic potential as analgesics and neuroprotective agents (Figure 1.12).

***A_{2A} AR*:** Allosteric modulation for the A_{2A}AR has not been much developed; still amiloride (**49**) and analogues developed by Gao and Ijerman were demonstrated to be allosteric inhibitors for the A_{2A}AR. However, these compounds are not selective for this subtype and they also allosterically modulate action at both A_1 and A_3 ARs (Göblyös & Ijzerman 2009).

***A_{2B} AR*:** Allosteric modulators for A_{2B}AR have also been not much reported. Despite that, a recent report has shown the allosteric modulation of adenosine A_{2B}AR by indole derivatives (Trincavelli et al. 2014).

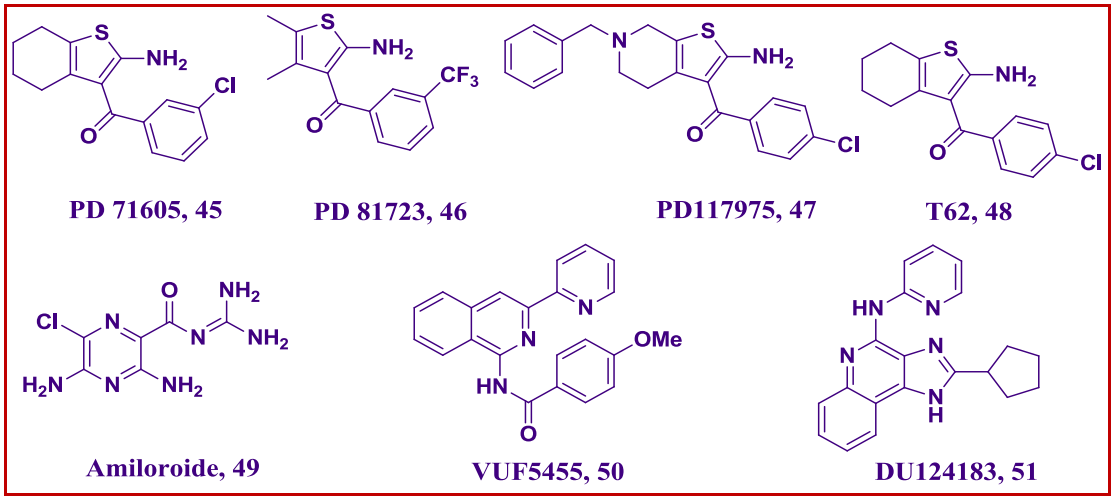


Figure 1.12 Allosteric modulators of adenosine receptors

***A_3 AR*:** Allosteric modulation of the A_3AR was first observed by Gao et al with VUF5455 (**50**) and other 2-pyridinyl isoquinoline derivatives which were previously reported as A_3AR antagonists. Imidazoquinolinamines are another structural class of A_3AR modulators, which were originally A_1AR antagonists. Imidazoquinlinamine derivative DU124183 (**51**) is an allosteric enhancer of radioligand binding at the A_3AR (Baraldi et al. 2012).

1.6 Selective disease targets for Adenosine receptors

The various disorders targeted by drugs that are in pre or advanced clinical trials modulating adenosine receptors include CNS-disorders, cardiovascular disorders, antinflammatory, autoimmune disorders and cancer.

CNS disorders: Several pharmacological studies suggest that the A_{2A}AR is involved in motor activity. In particular, adenosine A_{2A}AR antagonists have been demonstrated to restore the deficits caused by degeneration of the striatonigral dopamine system, and therefore offer a possible treatment for Parkinson's Disease.

The current treatment for Parkinson's Disease is primarily based on dopamine replacement therapy. Levodopa (L-DOPA), a metabolic precursor of dopamine, has been used for the treatment of Parkinson's Disease for decades. The A_{2A}AR is present in good concentration in striatum, which interacts with the D_2 receptor. Preclinical studies of A_{2A}AR antagonist is demonstrating motor benefit in rodent and non-human primate models of Parkinson's disease (Richardson et al. 1997; Schwarzschild et al. 2006; Shook & Jackson 2011). So A_{2A}AR antagonists have emerged as leading non-dopaminergic drugs for the treatment of Parkinson's Disease. Over the past 8 years, a total of 25 clinical trials have been conducted. Six double-blind, placebo-controlled clinical Phase IIb and Phase III trials of Istradefylline (KW-6002, **26**), involving a total of >2,000 patients with advanced Parkinson's Disease, and one Phase IIb trial with Preladenant (SCH420814, **31**), involving 253 patients with advanced Parkinson's Disease, have been reported (Hauser et al. 2011). These clinical Phase IIb and Phase III trials have shown a modest but significant reduction in the average 'off-time' by about 1.7 hours compared to the optimal

L-DOPA (levodopa) dose regimen. However, Preladenant did not prove to be effective in phase-III clinical trials so it was discontinued in May 2013, whereas Kyowa Hakko Kirin got the first global approval of drug Istradefylline (**26**) for Parkinson's Disease.

Adenosine as a contributing element in the pathophysiology of schizophrenia embraces several neurotransmitter systems and brain regions due to its multiple and widespread modulatory actions (Lara et al. 2006).

Cardiovascular disorders: The A_1AR is potential therapeutic target for a number of disorders including atrioventricular (AV) node block and supraventricular tachyarrhythmia (AR agonist); AV block of cardiac arrest (AR antagonist); bradyarrhythmias in transplanted hearts (AR antagonists); diuresis (AR antagonists). In patients with documented paroxysmal supraventricular tachycardias involving the AV node, 99% are successfully terminated with standard doses of adenosine (Strickberger et al. 1997).

Ellenbogen et al. in 2005 found that Tecadenoson (**7**) is a potent selective A_1AR agonist with a dose-dependent negative dromotropic effect on the AV node. They evaluated tecadenoson, a selective A_1AR agonist, for the acute termination of paroxysmal supraventricular tachycardia (PSVT). In the atrial-paced guinea pig heart model, tecadenoson caused an A_1AR receptor mediated negative dromotropic effect on the AV node and lengthening of the AV nodal refractory period, leading to termination of reentrant PSVT at doses that did not affect blood pressure (BP), sinus cycle length, or the His-ventricular interval. Side effects mediated by the A_{2A}, A_{2B} and A_3 ARs such as flushing, chest pressure, hypotension, and bronchospasm were infrequent, consistent with the A_1AR selectivity of the drug (Ellenbogen et al. 2005).

Impaired renal function is common in patients with acute heart failure; it directly contributes to deterioration of the heart and is associated with an adverse outcome, including increased mortality. Local adenosine production in the kidney is increased in patients with heart failure as a result of hypoxia caused by reduced renal perfusion and by stimulation with diuretics. Based on our understanding of the mechanisms associated with

renal dysfunction and the demonstrated control of renal function via A_1AR, A_1AR antagonists were developed. These antagonists reduced the risk of persistent worsening renal failure by >50% in a Phase IIb study involving 301 patients with acute heart failure, and improved renal plasma flow in 63 ambulatory patients with chronic heart failure. Based on these promising results, a placebo-controlled, randomized Phase III trial involving 2,033 patients with acute heart failure (the PROTECT study) was carried out with the A_1AR antagonist rolofylline; this was the largest study to date involving the use of A_1AR antagonists to target renal function (Cotter et al. 2008). Unfortunately, the results were disappointing and rolofylline did not prevent persistent worsening renal function. The reason for this absence of renoprotective effects is likely to be due to an enhanced diuretic effect in the rolofylline group, which may have offset the effects of rolofylline on the preservation of renal function. Moreover, pharmacological and genetic studies have clearly demonstrated that A_1AR mediate protective effects against ischaemic kidney injury and brain injury, which is consistent with the increased frequency of stroke and seizure activity in clinical trials of A_1AR antagonists. Thus, the development of A_1 AR antagonists for the treatment of disorders associated with impaired fluid retention, such as congestive heart failure, should proceed with caution.

The A_{2A}AR is involved in vasodilation in the aorta and coronary artery. It was suggested that the tachycardic effect of A_{2A}AR activation is mediated by centrally located receptors, whereas its hypotensive effect is mediated by the peripheral A_{2A}AR (Schindler et al. 2005). In the late 1960s and 1970s, metabolically stable AR agonists were tested clinically as antihypertensives, and this was an intended use of the A_{2A}AR agonist CGS21680 (**12**); however its clinical path was stopped due to non selectivity towards adenosine receptors. In platelets, an A_{2A}AR agonist was shown to inhibit aggregation by increasing intracellular cAMP levels, suggesting that adenosine agonists might have utility as antithrombotic agents. Then after various efforts has been carried out to further improve subtype-selectivity of A_{2A}AR agonists for novel therapeutic applications, including imaging. Adenosine (**1**), under the name Adenoscan (Astellas Pharma), is used in myocardial stress imaging to evaluate coronary artery disease by achieving vasodilation in patients unable to exercise adequately. Regadenoson (CVT- 3146, **9**), a

potent and selective A_{2A}AR agonist, is approved drug for myocardial perfusion imaging (Hendel et al. 2005).

Inflammatory diseases, autoimmune disorders and cancer: The adenosine receptor subtypes are highly expressed in all the cells of immune system, the adenosine A_1, A_{2A}, A_{2B} and A_3 receptors are being actively engaged as therapeutic targets for autoimmune diseases, chronic inflammatory disorders and cancer (Voors et al. 2011).

The potent A_3AR antagonists have been developed for therapeutic treatment of inflammatory diseases such as asthma and glaucoma. Activation of A_3AR has been shown to stimulate phospholipase C and to inhibit adenylate cyclase. A_3AR agonists also cause stimulation of phospholipase D and the release of inflammatory mediators, such as histamine from mast cells, which are responsible for inflammation and hypotension. For these reasons, the clinical use of A_3AR antagonists for the treatment of asthma and inflammatory disease has been suggested. Another suggestion says that this effect is mediated by the A_{2B}AR in human and canine mast cells. A bioavailable thiazole derivative that acts as a mixed A_{2B}/A_3 AR antagonist, has failed to attenuate bronchial hyper responsiveness to inhaled AMP in a phase Ib clinical trial in asthmatics, but has also been investigated for other indications.

Based on preclinical pharmacology and encouraging safety data in Phase I studies, the A_3AR agonists CF101 (**16**) and CF102 (**17**) have been tested in several Phase II trials for rheumatoid arthritis (Gessi et al. 2011). Based on anecdotal findings from this trial indicating that CF101 also improved indicators of dry eye syndrome, a follow-up Phase II trial was carried out, which determined that CF101 improved the clearance of corneal staining, tear break-up time and tear meniscus height with no side effects. In addition, active Phase II clinical trials are underway to test the efficacy of A_3AR agonists for the treatment of hepatocellular carcinoma and hepatitis (Fishman et al. 2012). Furthermore, experimental studies in mice suggest a possible use of A_3AR agonists in suppressing melanoma growth by inducing T cell-mediated adoptive immunity and in the control of chronic neuropathological pain (Fishman et al. 2012; Chen et al. 2012). These therapeutic

effects of CF101 are believed to be mediated by its inhibition (via cAMP and calcium signaling) of the oxidative burst and its anti-inflammatory activity (Gessi et al. 2002).

Conclusion:

It has been proved by different studies that extracellular adenosine is an important modulator of various physiological and pathological processes. With all these studies, it has emerged that adenosine receptors can be safely targeted by various ligands and various highly specific agonists and antagonists of adenosine receptors can be generated. As a result, increasing numbers of clinical trials testing of novel adenosine-based drugs in various indications have been initiated during the past decade.

Activation of adenosine receptors is beneficial in the treatment of various inflammatory and autoimmune disorders, pain, arrhythmia as well as sleep disorders and some metabolic disorders. Because of adenosine receptors are widely distributed in the body adenosine receptor agonists produces effects in almost all the tissues which make them difficult to use.

Selective antagonists of the adenosine receptors are more important. So, inhibition of adenosine receptors is having larger effects in treatment of the diseases like asthma, neuroprotection, diabetes, pain and cancer. The fact that a majority of humans already consume an adenosine antagonist, caffeine, on a daily basis of course also makes one wonder how much benefit can be derived by additional blockade.

CHAPTER-2

Rationale of the present work

PAGE NO. 21 TO 28

Chapter 2

Rationale of the present work

2.1 Importance of Drug discovery

"Drug discovery" is the process of finding new drugs. It has evolved from early serendipitous discovery from natural sources to today's industrial-scale screening projects. Modern drug discovery starts with the identification of a biological target that can be modulated to induce the desired therapeutic effect. To search for potential drugs, compounds are tested for their ability to modulate the target. High-throughput screening (HTS) and combinatorial chemistry facilitate screening of libraries of millions of compounds. Historically, drug discovery has been a stronghold of chemistry, especially natural products chemistry and synthetic organic chemistry. So, chemistry has a major role on drug discovery, since other disciplines do not create the novel small molecules.

The exclusive role of heterocyclic compounds for drug discovery is best documented by the occurrence of a heterocyclic moiety in current drugs; the majority of drugs are heterocyclic compounds. Since there is no doubt that heterocyclic compounds are relevant targets for drug discovery, a substantial effort has been dedicated to the development of chemistries, both solid- and solution-phase, for combinatorial synthesis of heterocyclic libraries. The relevance of compounds composed from two or more heterocyclic rings for drug discovery, regardless of the target, can be best documented by the frequency with which bisheterocyclic compounds were identified as the most potent ones (Soural et al. 2008).

As mentioned in chapter-1, adenosine receptors are of great interest for new drug development. Agonist and antagonists of adenosine receptors have been developed and they are implicated in many diseases like cardiovascular diseases, diabetes, asthma and COPD, inflammation and cancer etc. Earlier, number of different derivatives of adenosine and xanthine were developed as selective and specific ligands for adenosine receptors.

Xanthine derivatives were mostly used as antagonists of adenosine receptors. However the problems associated with xanthine derivatives are limited creative possibilities on such a rigid core and poor water solubility. Because of these problems various nonxanthine mono, bi and tricyclic derivatives of thiazoles, thiophenes and triazolopyrimidines has been developed as adenosine receptor antagonists. Here based on fragment based drug design approach we have designed thiophenyl-thiazole carboxamides and further the design was modified into thiophenyl-thiophene carboxamides, *N*-cyclicaminothiazoles and substituted aminothiazoles.

2.2 Fragment based drug design approach : Design of new thiophenyl-thiazole carboxamides as adenosine receptor ligands

The fragment-based drug design (FBDD) approach has been established as an efficient tool in the search for new drugs (Erlanson et al. 2004; Rees et al. 2004) and few of them are in clinical trial (Hajduk & Greer 2007). FBDD has emerged as an alternative approach to traditional lead identification via high-throughput screening (HTS). Unlike HTS, FBDD identifies smaller compounds, the "fragments", which bind to different parts of a biological target, and linking of these "fragments" via linker results in good leads. Fragment evolution, fragment linking, fragment self assembly and fragment optimization are the main four fragment based drug design approaches (Rees et al. 2004). Here we have used fragment linking approach for the design of our molecule which involves two (or more) fragments, which are active against one receptor are joined together to give a higher affinity molecule (Figure 2.1).

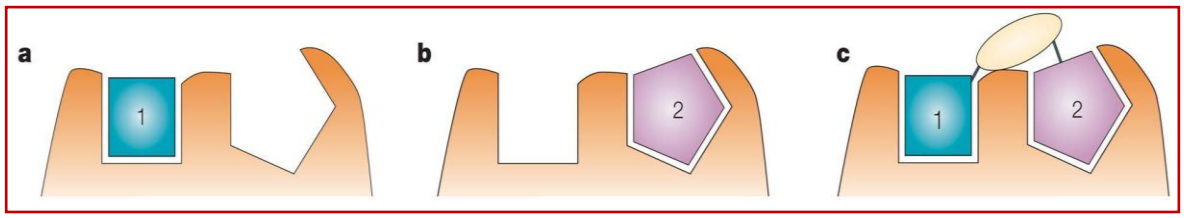


Figure 2.1 Fragment linking approach for drug design (Rees et al. 2004)

The example of fragment based drug design is given in Figure-2.2, where matrix metalloproteinase inhibitor was developed by linking the two fragments biphenyl ring and acetamide derivative. Both the fragments were having biological activity in micromolar range. These fragments were merged into one molecule to give better biological activity (Figure 2.2).

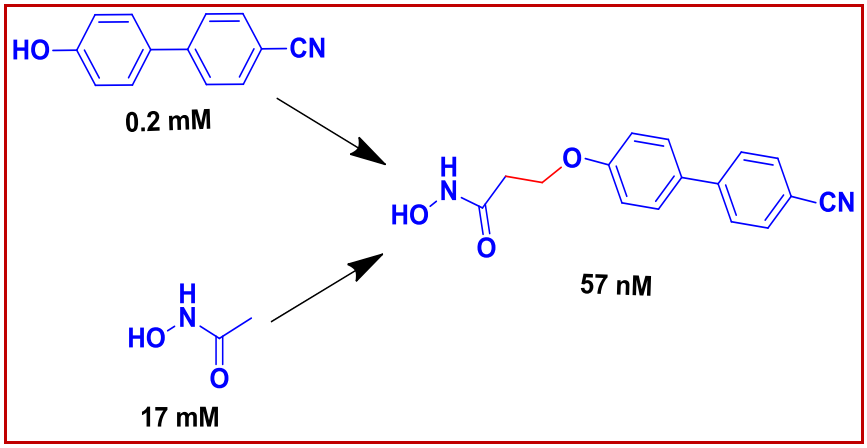


Figure 2.2 Example of Fragment linking approach: Development of Matrix Metalloproteinase Inhibitors (Rees et al. 2004)

The drug Vemurafenib is the first drug designed through fragment based drug design approach to get regulatory approval. Selected clinical stage compounds developed through fragment based drug design approach are given in Table 2.1 (Baker 2013).

Table 2.1 Selected clinical-stage compounds originating from fragment-based lead discovery (Baker 2013)

Drug	Target	Company	Status
Vemurafenib	Mutant BRAF Kinase (Metastatic melanoma)	Plexxikon	Approved
MK-8931	β-secretase 1(Alzheimer's Disease)	Merck	Phase II/III
ABT-263	BLC-2/BLC-X_L(Leukaemia)	Abbot	Phase II
AT 13387	HSP90 (Gastrointestinal stromal tumours)	Astrex	Phase II
AT9283	Janus Kinase 2/Aurora (multiple myeloma)	Astex	Phase II

The thiazole ring is an important heterocycle which plays a prominent role in nature and has broad therapeutic applications. Specifically, 2-aminothiazoles are an important class of heterocycles found in numerous biologically active compounds. They have been reported to possess antiviral, antibacterial, antiprion, and psychotropic activities. Compounds containing the aminothiazole moiety are also known to be a ligand of estrogen receptors, adenosine receptor antagonists, while other analogs exhibit antitumor properties (Fink et al. 1999; van Muijlwijk-Koezen et al. 2001; Kumar et al. 1993).

Thiophene is also equally important structural motif. It has been observed that the substitution of different heterocyclic moieties with thiophene nucleus modulates anti-inflammatory, anti-cancer (Giordano et al. 2012; Giri et al. 2009) and act as allosteric adenosine modulators (Göblyös & Ijzerman 2011).

The synthesis of new thiazole derivatives such as thiazoles substituted with thiophene as fused heterocycles have been reported to exhibit affinity towards adenosine receptors. Our group has been working on new aminothiazoles and thiophenes and the outcome of the work resulted in many hits and leads (Giordano et al. 2012; Giri et al. 2009) for adenosine receptors (Inamdar 2007; Yerande 2008). Moreover this literature has shown that amide spacer plays important role in showing good biological activity against adenosine receptor. Keeping the biological importance of the both scaffold in view, we had designed the molecule as thiophenyl-thiazole carboxamides which is having fragments thiazole and thiophene scaffold in single molecule via amide linker (Figure 2.3).

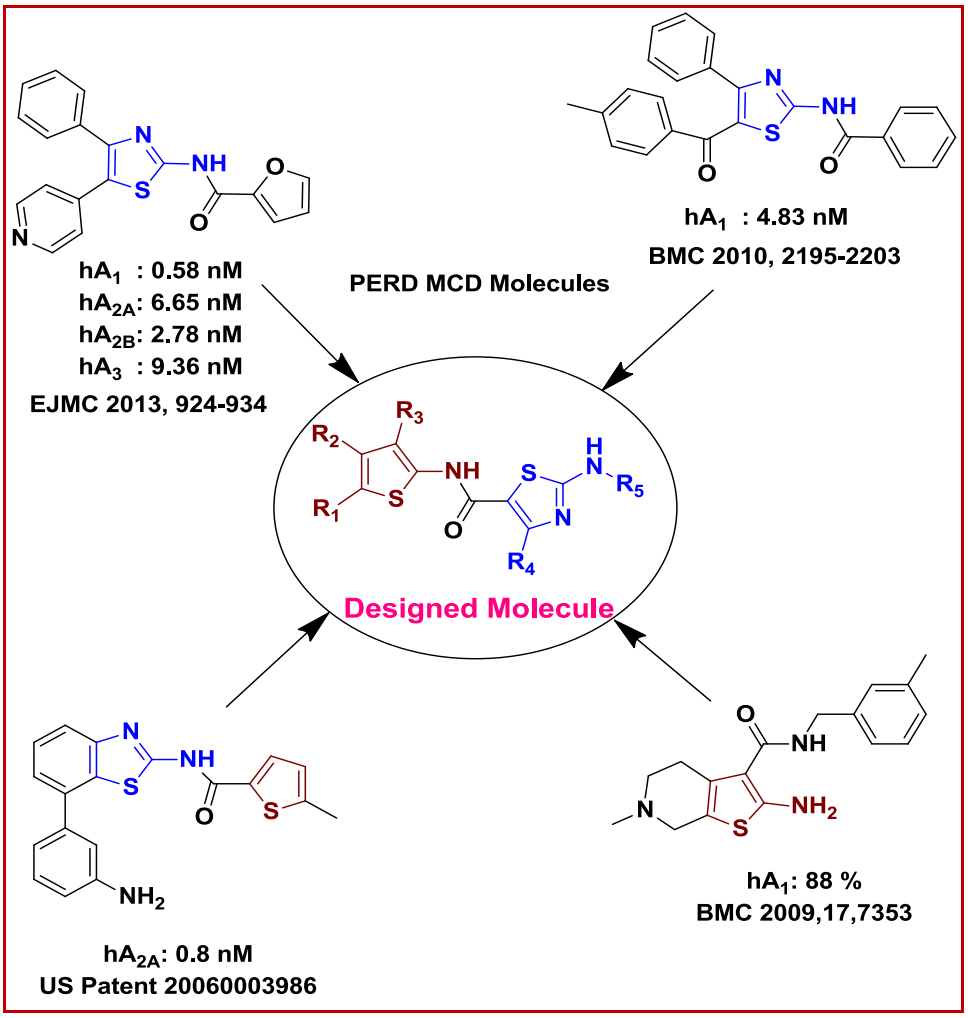


Figure 2.3 Fragment based drug design approach for design of molecule

2.3 New 2-aminothiophene linked to substituted thiophenes

A number of reports have shown 2-aminothiophene molecules as active moiety against adenosine receptors (Aurelio et al. 2011; Göblyös & Ijzerman 2011). New thiophene derivatives synthesized in our lab had shown good biological activity in inflammation and cancer. Keeping in mind the importance of thiophene and to see the SAR around 2-amino thiophenes, modification of the thiophenyl-thiazole carboxamides into thiophenyl-thiophene carboxamides with amide spacer were carried out (Figure 2.4). As per our findings, this is first time such a scaffold has been prepared and checked for activity against adenosine receptors. Our older reports had shown that thiophenyl-thiophenes with

keto spacer were highly active in cancer which was further patented (Giordano et al. 2012).

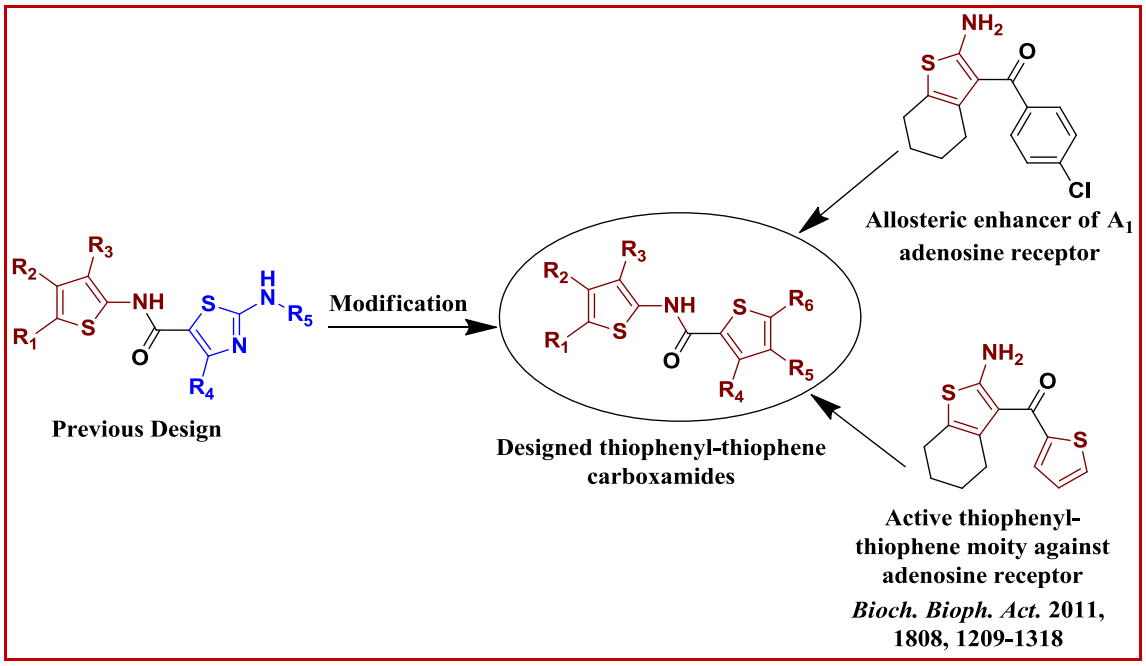


Figure 2.4 Design of thiophenyl-thiophene conjugate

2.4 New di/tri substituted *N*-cyclicaminothiazoles

As mentioned earlier, thiazoles have been highly active against adenosine receptors. 2-cycloaminothiophene prepared in our lab was active as anti-inflammatory compounds (Pillai et al. 2004). So here we have tried to design similar structural feature with replacement of 2-cyclicaminothiophens with 2-cyclicaminothiazoles to come up at good adenosine receptor ligands. Here, novel synthetic methodology was developed for the synthesis of novel 2,4- and 2,5- disubstituted 2-cyclicaminothiazoles. To best of our knowledge 2,4- and 2,5- disubstituted 2-cycloaminothiazoles has been checked first time against adenosine receptors. To see the SAR on thiazole moeity, here we have designed and synthesized new di/tri-substituted 2-cyclicaminothiazoles (Figure 2.5).

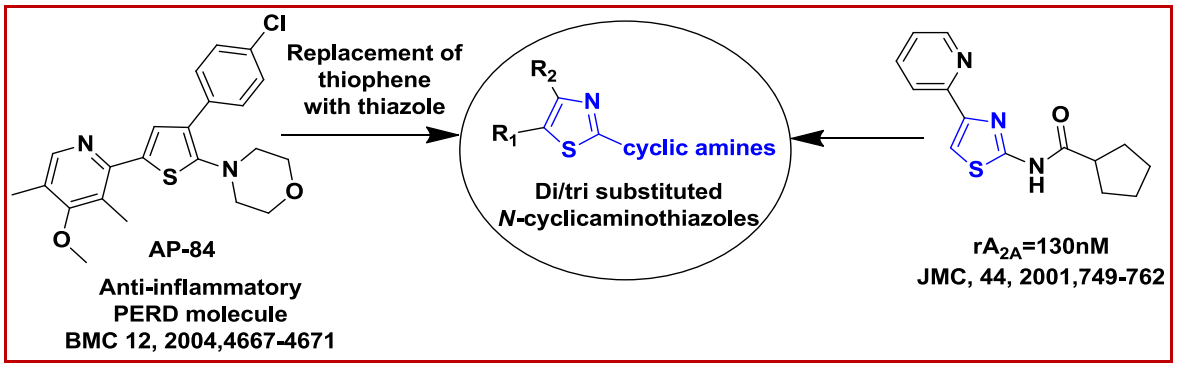


Figure 2.5 Design of di/trisubsituted *N*-cyclicaminothiazoles

2.5 2-aminothiazoles as adenosine receptor ligands

In continuation of previous series of work, here we have design new 2-aminothiazole derivatives with major focus on aminothiazoles. In the last few decades 2-aminothiazoles had proved to be good non-xanthine adenosine receptor ligands. Our previous work on 2-aminothiazole derivatives had shown good affinity and selectivity towards adenosine receptor (Scheiff et al. 2010; Inamdar et al. 2013). So, in this series of molecules we have kept aminothiazole moiety intact to see the change in biological activity from previous series of molecules (Figure 2.6).

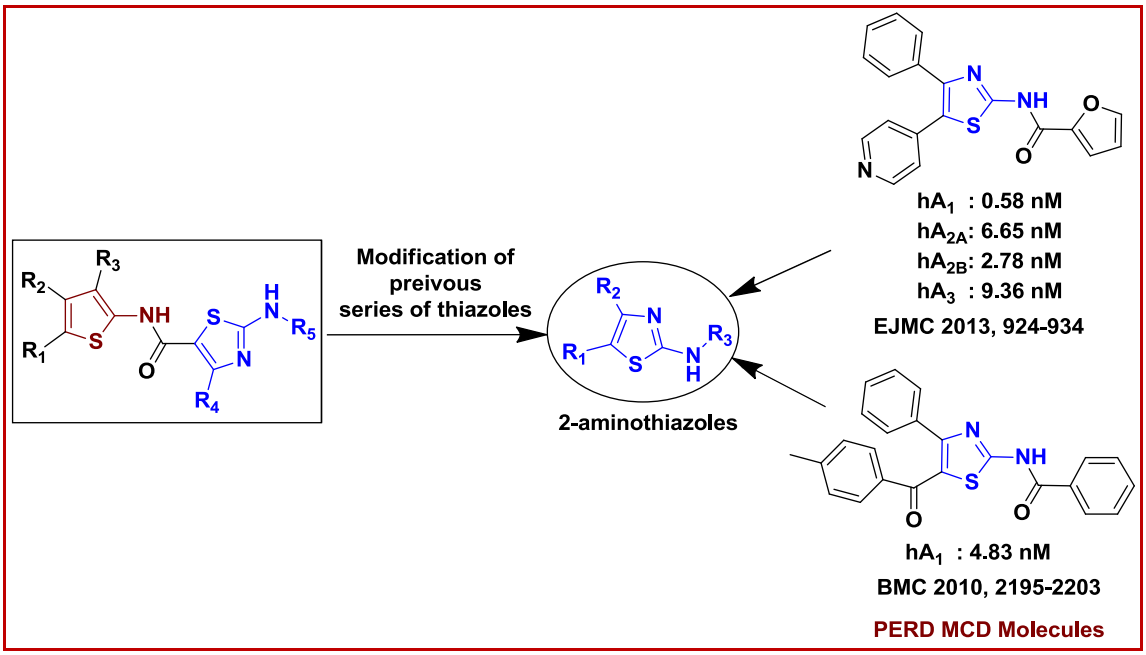


Figure 2.6 Design of subsituted 2-aminothiazoles

2.6 Novel synthetic methodology: Synthesis of 2-amino/cycloamino thiadiazines

In addition to the designed molecules, here we have developed novel synthetic methodology for the synthesis of 2-aminothiadiazine and 2-cycloaminothiadiazine from various substituted thiosemicarbazide derivatives and phenacyl bromides (Figure 2.7).

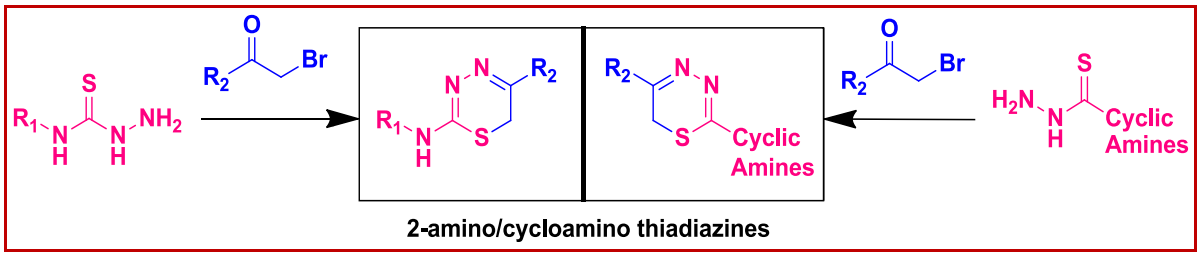


Figure 2.7 Novel 2-amino/cycloamino thiadiazines

CHAPTER-3

Design, synthesis and biological evaluation of thiophenyl-thiazole carboxamides as adenosine receptor antagonists

PAGE NO. 29 TO 58

Chapter 3

Design, synthesis and biological evaluation of thiophenyl-thiazole carboxamides as adenosine receptor antagonists

3.1 Introduction

Thiazoles and thiophenes have been reported as important structural feature as an individual heterocycle because they are having many pharmaceutical applications. 2-aminothiazole is an important and classic heterocyclic scaffold used in the drug discovery programs. All the described biological and physico-chemical properties of aminothioazole are probably due to its small ring structure with nitrogen atom behaving as hydrogen bond acceptor which makes the aminothiazole ring having Π excessive and Π deficient properties. The broad spectrum biological activities exhibited by this structure include anticancer (Lee et al. 2014), antiprion (Gallardo-Godoy et al. 2011), antimicrobial (Annadurai et al. 2012) and antituberculosis (Pieroni et al. 2014) activities that assign it as an indispensible heterocyclic feature in drug design. In addition to this, recently, our group has successfully employed 2-aminothiazole scaffolds in the design of anti-inflammatory agents as well as adenosine receptor antagonist (Scheiff et al. 2010; Inamdar et al. 2013).

Thiophene is an important structural motif in medicinal chemistry and it is considered as a classical bioisostere for the benzene ring and due to its small ring structure it is found in many therapeutically active substances. Multisubstituted 2-aminothiophenes are privileged structures, which attracted considerable attention in the designing of biologically active molecules. Moreover, they were found to have various biological applications such as antihypertensive, antipsychotic, as potent apoptosis inducer (Kemnitzer et al. 2009), a potential anti-inflammatory agent (Katada et al. 1999), anti-osteoporosis agents and allosteric agonists and modulators of the A_1 adenosine receptor (Valant et al. 2012).

3.2 Present Work

Keeping in mind importance of thiazoles and thiophenes, the present work is focused on designing and synthesizing the molecules which incorporates both the biologically important heterocycles. Fragment based drug design approach is used to design the conjugated new thiophenyl-thiazoles with amide spacer as adenosine receptor ligands (Figure 3.1).

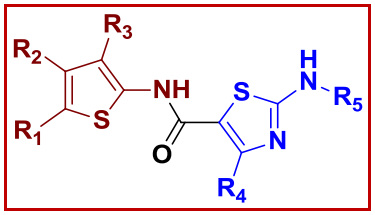


Figure 3.1 Designed thiophenyl-thiazole carboxamides

Molecular Docking for validation of designed molecule

In order to validate the designed molecule we have carried out the molecular docking study with adenosine A_{2A} adenosine receptor of our designed molecule **(b)** and compared with a reference molecule from US Patent **20060003986 (a)** (Alanine et al. 2006). The reference molecule **(a)** was found to have hydrophobic interactions with the amino acids PHE168, LEU267, LEU249, MET 270 and TYR271 with binding energy of -8.70 kcal/mol. Whereas our designed molecule **(b)** was found to have hydrophobic interactions with the amino acids ILE 251, ILE 252, TRP268 and LEU269 with binding energy of -10.49 kcal/mol. We find that our designed molecule and reported molecule have comparable binding energy. This suggests that such type of compounds may have a good potential as A_{2A} adenosine receptors antagonists (Figure 3.2).

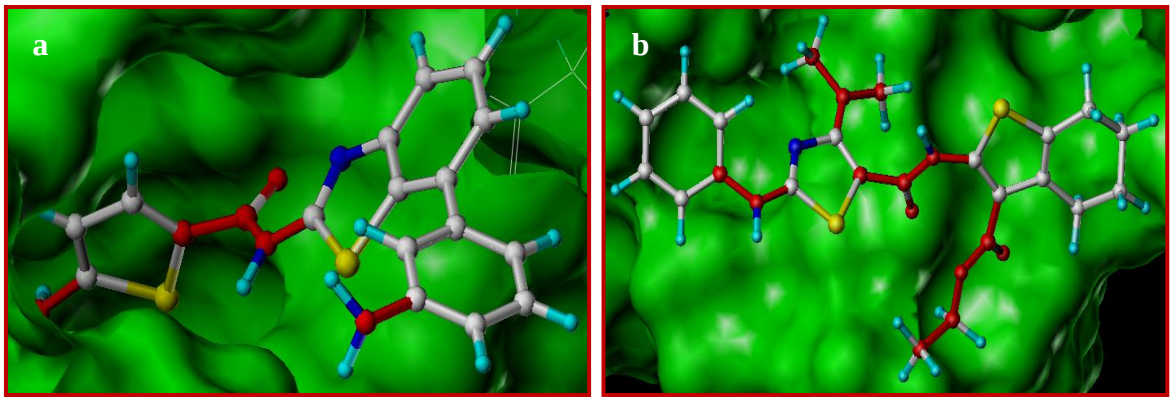


Figure 3.2 Docking study with A_{2A} adenosine receptor (a) Reference molecule in the binding pocket (b) Designed molecule in the binding pocket

Retrosynthetic analysis of designed molecule

In order to design the synthetic route, retrosynthetic analysis was carried out (Figure 3.3).

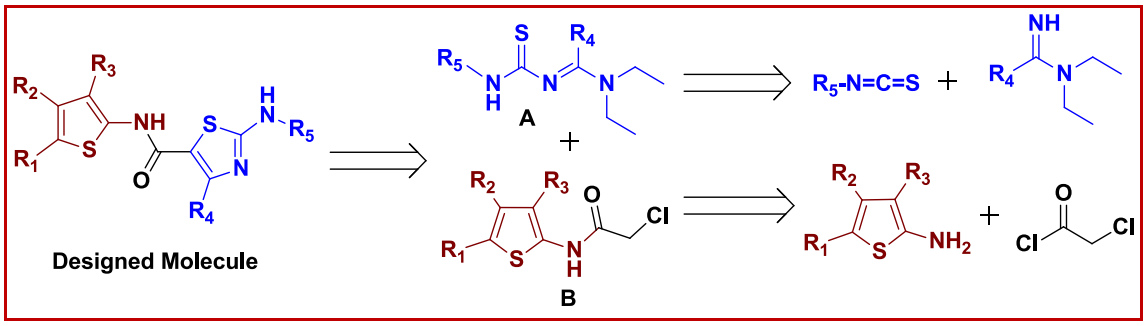


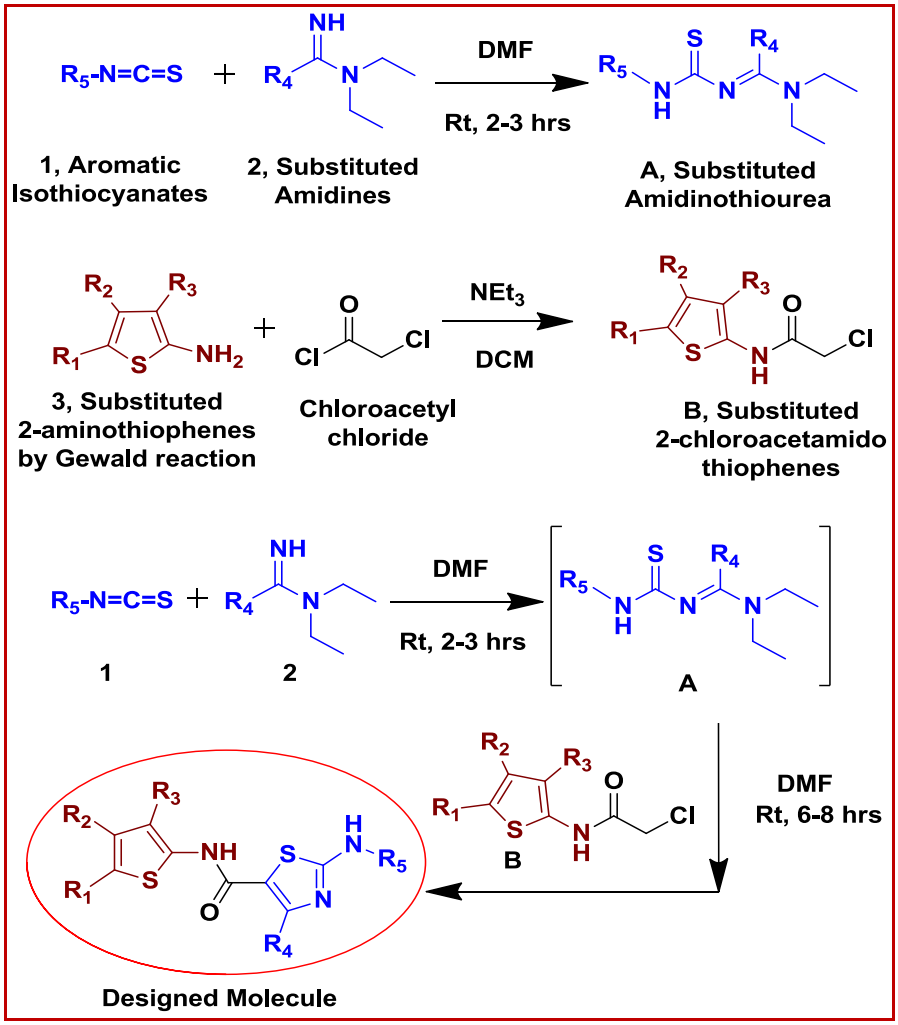
Figure 3.3 Retrosynthetic analysis of designed molecule

In continuation of our synthesis of biologically active new heterocycles from amidinothiourea **(A)** (Kaila et al. 2008; Kaila et al. 2010), here we have synthesized series of thiophenyl-thiazole carboxamides **(Table-1)** from 2-chloroacetamidothiophenes **(B)** and amidinothioureas **(A)** as potential antagonists of adenosine receptors. The synthetic methodology for the formation of 2-aminothiazole was further published by our group (Jalani et al. 2013).

Amidinothioureas **(B)** can be can be obtained by reacting various isothiocyanates with amidines. This reaction is general for wide range of isothiocyanates and amidines. 2-chloroacetamidothiophenes **(A)** can be generated by the reaction between 2-aminothiophenes and chloroacetyl chloride. The 2-aminothiophene is prepared by

Gewald's multicomponent reaction. The various amidinothioureas were reacted with substituted 2-chloroacetamido thiophenes under mild conditions for the synthesis of final designed molecule. Later on a sequential one-pot multicomponent reaction methodology was established by reacting isothiocyanates with amidines and 2-chloroacetamidothiophenes to furnish final designed molecule with good yields.

So, herein we report, a versatile sequencial one pot multicomponent reaction leading to thiophenyl-thiazole carboxamides by reacting different amidines, isothiocyanates and substituted 2-chloroacetamido thiophenes to furnish in good to excellent yields (Scheme 3.1).



Scheme 3.1 A sequential one pot multicomponent synthesis of new thiophenyl-thiazole carboxamides

As an example of reaction, we started the reaction with phenyl isothiocyanate (1 mmol) and tetramethyl guanidine (1 mmol) in DMF. The reaction mixture was stirred for 2 hrs and formation of amidinothiurea was checked with TLC. After the formation of amidinothiourea, Ethyl 2-(2-chloroacetamido)-4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (1 mmol) in DMF was added and allowed to stir for another 6 hrs at room temperature. Completion of the reaction was checked by TLC which showed different spot then the starting materials. The reaction mixture was added into the cold water and the resulted solid was filtered and dissolved in ethyl acetate followed by drying over sodium sulfate. The organic layer was then concentrated under reduced pressure to give the crude compound which was further treated with diethyl ether and/or hexane to produce the pure light yellow solid (**PMCDP-1**). The structure of this compound was assigned with the help of LC-MS, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The mass spectrum of **PMCDP-1** displayed the molecular ion peak at *m/z* 471.1 (M+1) which confirm the molecular weight of the compound 470.14 calculated for $C_{23}H_{26}N_4O_3S_2$ (Figure 3.4).

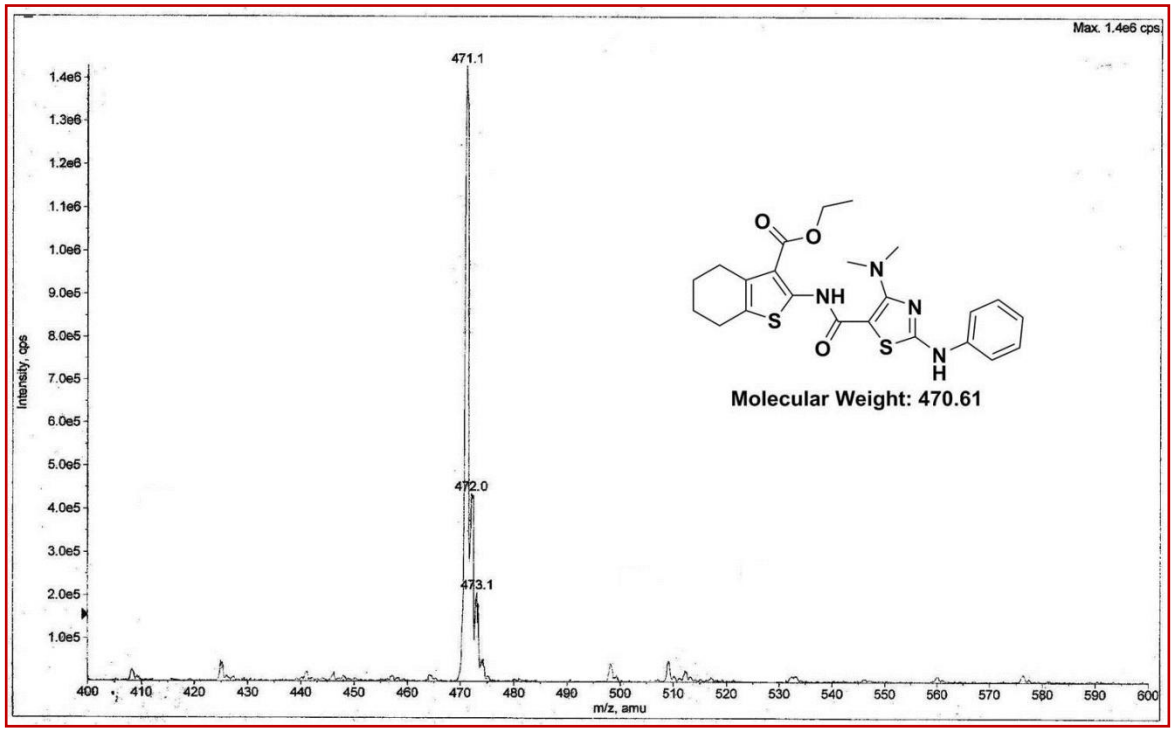


Figure 3.4 LC-MS $(M+1)^+$ of **PMCDP-1**

Further the structure of the molecule was confirmed by ^{1}H NMR (400 MHz, DMSO-d_6), which gave the characteristic peak of all the hydrogen present. The amide proton –NH singlet came at 12.28 δ and the thaizole –NH proton came at 10.75 δ. The protons of ester methyl group (-CH_3) were observe as triplet at 1.28-1.32 δ due to presence of adjacent methylene group (–CH_2). The eight protons of four methylene group were found in the range 1.74-2.71 δ. The two methyl group protons of –N(CH_3) were found as singlet at 2.88 δ. The ester methylene group present at the 3rd position of thiophene gave a quartet at 4.27-4.32 δ. All the five aromatic protons were observed from 7.02-7.61 δ (Figure 3.5).

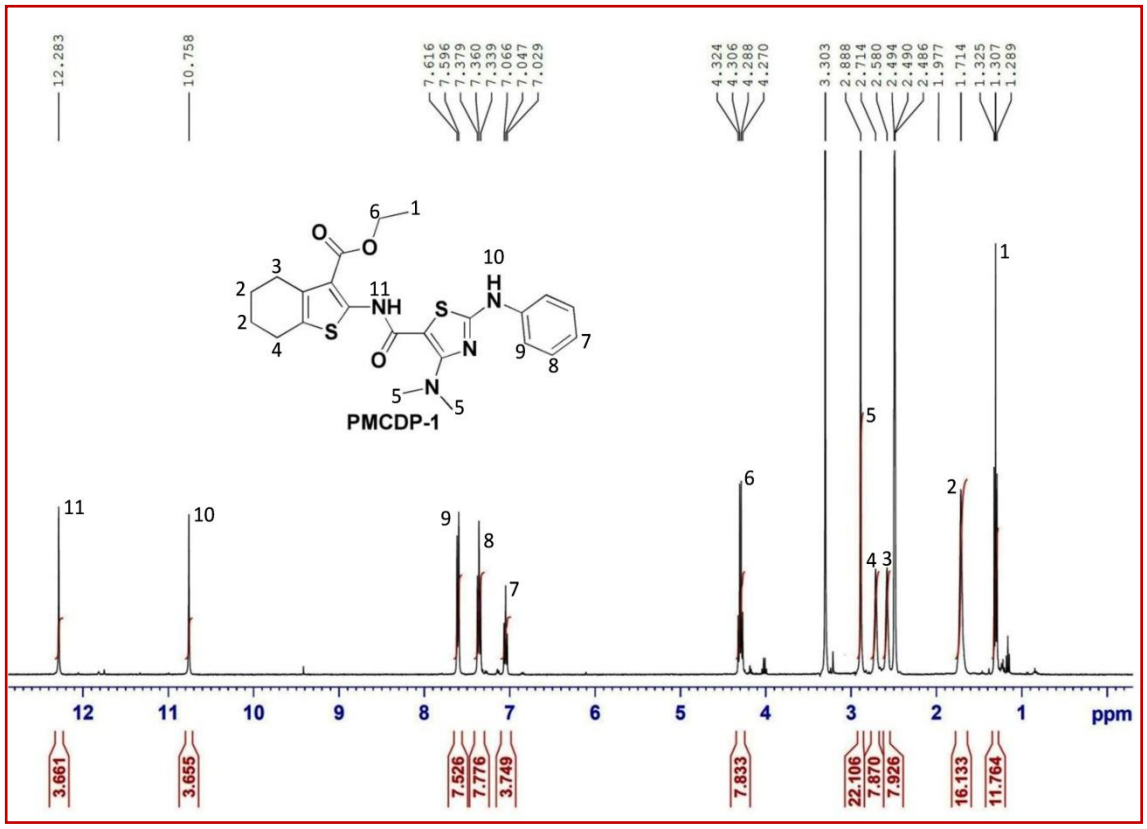


Figure 3.5 ^{1}H NMR of **PMCDP-1**

Further, the structure was confirmed by ^{13}C NMR (400 MH_Z in DMSO-d_6). The carbonyl carbon present in the ester group of thiophene is observed most downfield at 165.17 δ. The other carbonyl carbon of amide was observed at 163.65 δ. The carbon of methyl group present on ester of thiophene was observed most upfield at 14.12 δ. The four methylene group carbons of cyclohexane attached to thiophene was observed in the range

of 22-26 δ. And the two methyl group carbons present in –N(Me)$_2$ of thiazole was observed at 43.45 δ. All the aromatic carbon present on phenyl, thiophene and thiazole rings were observed in the range of 98-161 δ.

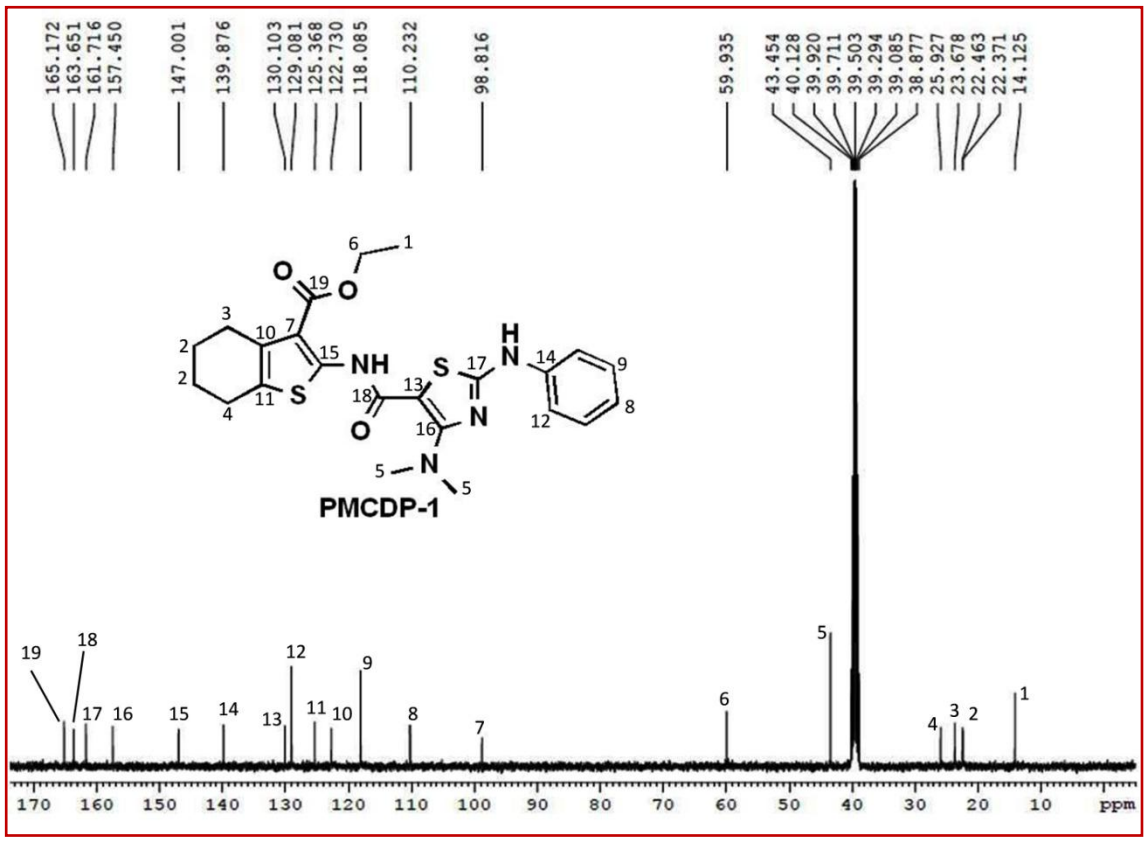
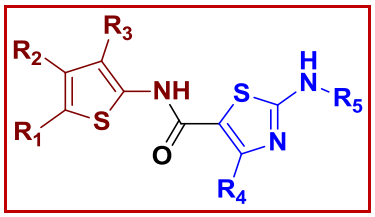


Figure 3.6 ^{13}C NMR of **PMCDP-1**

As the established one-pot synthetic methodology was in hand, we synthesized 18 molecules with structural diversification in good to excellent yields (PMCDP-n, Table-1).

Table 3.1 Synthesis of thiophenyl-thiazole carboxamide derivatives



No	Code	R_1	R_2	R_3	R_4	R_5
1	PMCDP-1	$-(CH_2)_4$		-COOEt	$-N(Me)_2$	C_6H_4
2	PMCDP-2	$-(CH_2)_4$		-COOEt	Me	$4\text{-}MeC_6H_4$
3	PMCDP-3	$-(CH_2)_4$		-COOEt	$4\text{-}MeC_6H_4$	$4\text{-}MeC_6H_4$
4	PMCDP-4	$-(CH_2)_4$		-COOEt	$4\text{-}MeC_6H_4$	4-OMe C_6H_4
5	PMCDP-5	$-(CH_2)_4$		-COOEt	$4\text{-}MeC_6H_4$	C_6H_4
6	PMCDP-6	$-(CH_2)_4$		-COOEt	$-N(Me)_2$	4-Me C_6H_4
7	PMCDP-7	$-(CH_2)_4$		-COOEt	$-N(Me)_2$	CH_3OCO
8	PMCDP-8	$-(CH_2)_4$		-CN	$-N(Me)_2$	C_6H_4
9	PMCDP-10	$-(CH_2)_4$		-CN	$-N(Me)_2$	$4\text{-}MeC_6H_4$
10	PMCDP-11	$-(CH_2)_4$		$-CONH_2$	$-N(Me)_2$	$4\text{-}MeC_6H_4$
11	PMCDP-12	$-(CH_2)_4$		$-CONH_2$	$4\text{-}MeC_6H_4$	C_6H_4
12	PMCDP-13	$-(CH_2)_4$		-CN	$4\text{-}MeC_6H_4$	$4\text{-}MeC_6H_4$
13	PMCDP-14	$-(CH_2)_4$		-CN	$4\text{-}MeC_6H_4$	$4\text{-}OMeC_6H_4$
14	PMCDP-19	$-(CH_2)_4$		$-CONH_2$	$4\text{-}MeC_6H_4$	$4\text{-}MeC_6H_4$
15	PMCDP-20	$-(CH_2)_4$		-CN	Me	$4\text{-}MeC_6H_4$
16	PMCDP-21	-COOMe	Me	-COOEt	$-N(Me)_2$	$4\text{-}OMeC_6H_4$
17	PMCDP-22	-COOMe	Me	-COOEt	Me	$4\text{-}MeC_6H_4$
18	PMCDP-25	-COOMe	Me	-COOEt	$-N(Me)_2$	$4\text{-}MeC_6H_4$

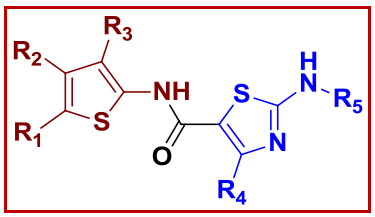
3.3 Biological results and discussion

The binding affinities of the newly synthesized compounds were evaluated by measuring the displacement of selective radioligands which were previously bound to the receptor expressed [Chinese hamster ovary cells (CHO) for hA_1AR, $hA_{2A}AR$ and hA_3AR] at the cellular surface. In this assay, the displacement of specific [^{3}H]CCPA binding at hA_1AR, specific [^{3}H]NECA binding at the $hA_{2A}AR$ and [^{3}H]HEMADO at the hA_3AR were evaluated. Due to the lack of a suitable radioligand for $hA_{2B}AR$, the antagonist activity was determined in adenylyl cyclase experiments in CHO cells expressing the $hA_{2B}AR$ (Klotz et al. 1985; Klotz et al. 1997). Ki (dissociation constant) value of the data was calculated using Cheng and Prusoff equation (Yung-Chi & Prusoff 1973), with geometric means of at least three experiments including 95% confidence intervals.

All the compounds showed affinity towards A_1, A_{2A} and A_3 adenosine receptor subtypes. To be more specific **PMCDP-3, PMCDP-4, PMCDP-12 and PMCDP-19** showed good affinity towards A_1, A_{2A} and A_3 adenosine receptor subtypes with the Ki values in low micromolar range **(Table-2)**. The compound **PMCDP-12** showed relatively less (hA_1/hA_3=7 and hA_{2A}/hA_3=10 fold) selectivity against adenosine receptors. The other

three compounds **PMCDP-3, PMCDP-4 and PMCDP-19** were more selective towards A_3 adenosine receptor. The compound **PMCDP-3** was hA_1/hA_3=13 and hA_{2A}/hA_3=13 fold selective and **PMCDP-4** was hA_1/hA_3=19 and hA_{2A}/hA_3=19 fold selective, which was good. The most active compound **PMCDP-19** was found to be hA_1/hA_3=>90 and hA_{2A}/hA_3=>90 fold selective. Presence of phenyl or p-tolyl group on the 4th position of thiazole ring was found to be optimum for good activity for this series of compounds (Table 3.2).

Table 3.2 Binding affinity of synthesized molecules



No	Compound	Ki in µM at 95 % Confidence Limits				Selectivity	
		hA_1[a]	hA_{2A}[b]	hA_{2B}[d]	hA_3[c]	hA_1/hA_3	hA_{2A}/hA_3
1	**PMCDP-1**	40	6.4	>10	4.1	9.75	1.56
2	**PMCDP-2**	>30	>30	>10	8.2	>3.65	>3.65
3	**PMCDP-3**	>30	>30	>10	**2.2**	>13.63	>13.63
4	**PMCDP-4**	>10	>10	>10	**0.52**	>19.23	>19.23
5	**PMCDP-5**	7.7	28.4	>10	4.1	1.87	6.92
6	**PMCDP-6**	23.5	18	>10	4.7	5	3.82
7	**PMCDP-7**	>100	>100	>10	>30	>3.33	>3.33
8	**PMCDP-8**	>30	>30	>10	35.6	>0.84	>0.84
9	**PMCDP-10**	>100	>10	>10	34	>2.94	>0.2941
10	**PMCDP-11**	32.4	22.3	>10	7.1	4.56	3.14
11	**PMCDP-12**	2.3	3.4	>10	**0.33**	6.96	10.30
12	**PMCDP-13**	>10	>10	>10	>10	>1	>1
13	**PMCDP-14**	>30	>30	>10	9.5	>9.15	>1.05
14	**PMCDP-19**	>30	>10	>10	**0.33**	**>90**	**>90**
15	**PMCDP-20**	>30	5.6	>10	3.9	>7.69	1.43
16	**PMCDP-21**	14	9.6	>10	2.7	5.18	3.55
17	**PMCDP-22**	>30	>30	>10	>30	>1	>0.33
18	**PMCDP-25**	19	26.6	>10	3.9	4.87	6.82

Data are expressed as geometric means with 95% confidence limits.
[a]Displacement of specific [^{3}H]CCPA binding at human A_1 receptors expressed in CHO cells.
[b]Displacement of specific [^{3}H]NECA binding at human A_{2A} receptors expressed in CHO cells.
[c] Displacement of specific [^{3}H]HEMADO binding at human A_3 receptors expressed in CHO cells.
[d]Inhibition of NECA-stimulated adenylyl cyclase activity at human A_{2B} receptors expressed in CHO cells.

3.4 Conclusion

In conclusion, we have designed new series of thiophenyl-thiazole carboxamides as adenosine receptor antagonists, which was further validated with the docking study. The designed molecules were synthesized using newly developed one pot synthesis. All the compounds were investigated for their radio ligand binding assay in adenosine receptors. The compounds were found to have good affinity towards adenosine receptors. Particularly the compounds were active against A_3 adenosine receptor with high selectivity.

3.5 Experimental

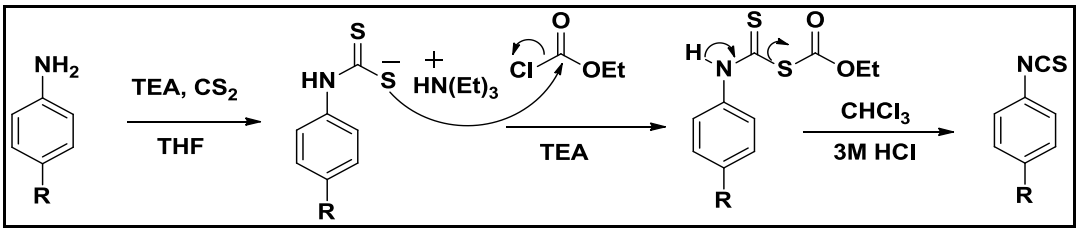
Melting points were recorded on scientific melting point apparatus (Veego; Model: VMP-DS) and are uncorrected. The ^{1}H NMR spectra were recorded on Bruker NMR spectrometer (400 MHz) using TMS as an internal standard and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer at 75 MHz. Proton chemical shifts are expressed in ppm relative to internal tetramethylsilane. Mass spectra were recorded on Perkin Elmer Sciex API 165. TLC was carried out on Merck Kieselgel 60 PF_{254}. IUPAC name of the compounds were generated using Cambridge soft ChemBioDraw ultra 12.0. Preparation of starting material amidinothiourea was carried out using the procedure described in the literatures (Rajappa, Sudarsanam & Yadav 1982; Rajappa, Sudarsanam, Advani, et al. 1982; Rajasekharan et al. 1986; Franklin et al. 2008). The molecular docking study was carried out in the flexidock tool available in software SYBYL 6.9.1.

3.5.1 Protocol for molecular docking study

The molecular docking study of our designed molecule and reference molecule with A_{2A} adenosine receptor was carried out by FlexiDock tool available in the SYBYL 6.9.1. The ligands were drawn using the SKETCH module available in the SYBYL 6.9.1. The

molecules were given Gasteigere-Huckel charges and minimized to a minimum energy conformation. The A_{2A} adenosine receptor was downloaded from protein data bank (PDB ID: 3EML). The protein was already having a bound ligand, so that ligand was extracted and that site was taken as binding site. Flexible docking was facilitated through the FlexiDock utility in the Biopolymer module of SYBYL 6.9.1. During flexible docking, the ligand and the side chains of hydrophilic amino acids in the putative binding site were defined as rotatable bonds. After the hydrogen atoms were added to the receptor, atomic charges were recalculated by using Kollman All-atom for the protein and Gasteigere-Huckel for the ligand. H-bonding sites were marked for all residues in the active site and ligands with H-bond donor or acceptor. Ligands were pre-positioned in the putative binding cavity guided by several superimposition results. Default FlexiDock parameters were set at 3000-generations for genetic algorithms. To increase the binding interaction, the torsion angles of the side chains within 5 Å of the ligands were manually adjusted from the results of FlexiDock. Finally, the complexes were minimized by using the powell method with a fixed dielectric constant (4.0), until the conjugate gradient reached 0.001 kcal $mol^{-1}A^{-1}$.

3.5.2 General procedure for the synthesis of isothiocyanates



Solution of aryl amines (0.0392 mole), triethylamine (0.129 mole) in THF (25 mL) was cooled to 0-5 ºC. To the cold reaction mixture carbon disulfide (0.0431 mole) was added dropwise through addition funnel within 1h. The reaction mixture was allowed to stir at room temperature for 15-18h. Completion of dithiocarbamate salt formation was checked by TLC (MeOH: Hexane: Ethyl acetate- 0.5:3:1.5). The dithiocarbamate salt was filtered and washed with hexane (2 x 25 mL).

The air dried dithiocarbamate salt was dissolved in chloroform (40 mL) and triethyl amine (0.0392) was added to the reaction mixture. After stirring at 0-5 ºC for 10 min,

ethyl chloroformate (0.047) was added to the reaction mixture and allowed to stir at room temperature for 1.5 h. To the reaction mixture 3M HCl was added and stirred for 10 min. Chloroform layer was separated and washed with 3 x 50 ml of water. The chloroform layer was dried over anhydrous sodium sulfate, and evaporated to give isothiocyanates. In case of impure isothiocyanates, it was purified by column chromatography using hexane as a mobile phase.

3.5.2.1 Synthesis of Phenyl isothiocyanate (1a)

Phenyl isothiocyanate was synthesized using the procedure as described in 3.5.2 by the reaction of aniline, carbon disulfide and ethyl chloroformate to afford the title compound as light yellow solid. Yield: 76.0%, bp 221-222°C, Rf: 0.77, MW: 135.19; LC-MS found (m/z): 136.1 $(M+1)^+$.

3.5.2.2 Synthesis of 4-Methyl phenyl isothiocyanate(1b)

4-Methyl phenyl isothiocyanate was synthesized using the procedure as described in 3.5.2 by the reaction of p-toluidine, carbon disulfide and ethyl chloroformate to afford the title compound as light yellow semi solid. Yield: 39%, mp 25-26^{0}C, Rf = 0.62 (Dichloromethane) MW: 149.21; LC-MS found (m/z): 150.1 $(M+1)^+$.

3.5.2.3 Synthesis of 4-Methoxy phenyl isothiocyanate(1c)

4-Methoxy phenyl isothiocyanate was synthesized using the procedure as described in 3.5.2 by the reaction of 4-methoxy aniline, carbon disulfide and ethyl chloroformate to afford the title compound as light yellow semi solid. Yield: 69.0%, mp 18-19^{0}C, Rf: 0.60, MW: 165.21; LC-MS found (m/z): 166.1 $(M+1)^+$.

3.5.2.4 Synthesis of Methoxy carbonyl isothiocyanate(1d)

To a 50% aqueous solution of potassium thiocyanate (17.58gm, 0.2 moles) 0.736ml of quinoline was added. Then methyl chloroformate (20gm, 0.184 moles) was added drop wise with stirring at 8-12 °C, which was continued at same temperature for 7.5 hours. Then 12ml of petroleum ether and 22ml of water (both chilled to 2-4°C) were added followed by 0.6ml chilled concentrated hydrochloric acid to remove quinoline. Care was taken to maintain the temperature between 8-10 °C during the separation of the layers. The organic layer was dried over with sodium sulfate and distilled under vacuum to yield

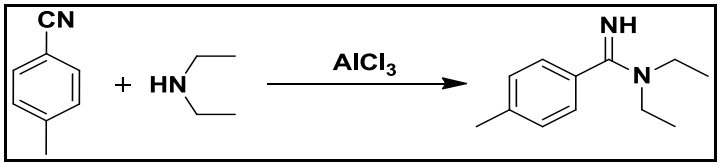
(14gm, 0.107 moles, 58 %) methoxy carbonyl isothiocyanate as colourless, strong lachrymatory liquid.

3.5.3 Procedure for the synthesis of acetamidine and benzamidine

3.5.3.1 Synthesis of N,N-Diethyl acetamidine(2a) [CAS 14277-06-6]

37.12 g (0.9 mole) of acetonitrile was cooled to 10^0C and 60 g of anhydrous aluminium chloride was added during the course of 30 min, maintaining the temperature between 10^0C and 30^0C. Then 33.44g (0.45 mole) of diethylamine was added rapidly with cooling in 20 min; the internal temperature was raised to 60-70^0C. Later, the reaction mixture was cooled to 10^0C, and another lot of 60 g of anhydrous aluminium chloride was added, followed by 33.44(0.45 mole) g of diethyl amine as before. The inside temperature was maintained at 120^0C for 30 min. and then at 140-145^0C for 1 hr. The mixture was cooled to 70^0C and poured into ice with stirring, the temp not being allowed to rise above 15^0C. Then 400 mL of dichloromethane was added, followed by slow addition of a solution of 162 g sodium hydroxide in 400 mL of water. After stirring for 15 min. the dichloromethane layer was separated. The aqueous layer was again extracted with another 400 mL. of dichloromethane. The dichloromethane extracts were combined, dried over anhydrous sodium sulfate, and the solvent distilled off. The residual *N,N*-diethyl acetamidine was vacuum-distilled. B.P.50-55^0C/ 5-6 mm Hg.

3.5.3.2 Synthesis of N,N-Diethyl 4-methylbenzamidines(2b)

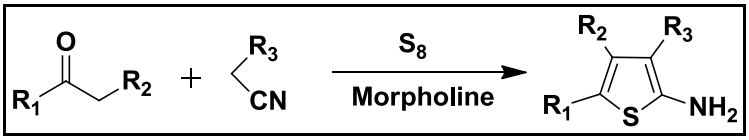


30 g of 4-methyl benzonitrile was cooled to 10°C and 21.5 g of anhydrous aluminum chloride added during the course of 30 min, maintaining the temperature between 10° and

30°C. Then 11.77g of diethylamine was added rapidly with cooling in 20 min; the internal temperature raise to 60-70°C. Later, the reaction mixture was cooled to 10°C, and another lot of 21.5 g of anhydrous aluminum chloride was added, followed by 11.77 g of diethyl amine as before. The inside temp was maintained at 120°C for 30 min. and then at 140-145°C for 1h. The mixture was cooled to 70°C and poured on ice with stirring, the temp not being allowed to rise above 15°C. Then 200 mL of dichloromethane was added, followed by slow addition of a solution of 38.64 g sodium hydroxide in 200 mL of water. After stirring for 15 min the dichloromethane layer was separated. The aqueous layer was again extracted with another 200 mL of dichloromethane. The dichloromethane extracts were combined, dried over anhydrous sodium sulfate, and the solvent distilled off. The residual N, N-Diethyl 4-methyl benzamidine was vacuum-distilled. B.P. 100-105^{0}C/ 5-6 mm Hg.

3.5.4 General procedure for the synthesis of substituted 2-Amino thiophenes (Huang & Dömling 2011)

An equimolar (5 mmol) mixture of powdered sulfur and morpholine was stirred until total dissolution of the sulfur. The reaction is cooled to 0-10^{0}C. Then active methylene nitrile(5 mmol) and the ketone (5 mmol) were added to the reaction mixture and the reaction was stirred at room temperature for the time 5-7 hrs. After completion of the reaction, as monitored by TLC, the reaction mixture was poured into ice cold water to get solid. This solid was filtered, dried and recrystallized from methanol to give pure 2-aminothiophenes.



3.5.4.1 Synthesis of ethyl 2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate(3a) [CAS :4506-71-2]

It was synthesized using the procedure as described in 3.5.4 by the reaction of cyclohexanone, ethyl cyanoacetate, sulphur and morpholine to afford the title compound as light yellow solid. Yield: 75 %, mp. 112-113 °C, M. W. = 225.08, LC-MS found (m/z): 226.1 $(M+1)^+$.

3.5.4.2 Synthesis of 2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile(3b) [CAS :4651-91-6]

It was synthesized using the procedure as described in 3.5.4 by the reaction of cyclohexanone, malononitrile, sulphur and morpholine to afford the title compound as White solid. Yield: 79 %, mp. 151-153 °C.M.W.= 178.25, LC-MS (m/z): 179.2 (M+1).

3.5.4.3 Synthesis of 2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide(3c) [CAS :4815-28-5]

It was synthesized using the procedure as described in 3.5.4 by the reaction of cyclohexanone, cyanoacetamide, sulphur and morpholine to afford the title compound as light yellow solid. Yield: 70 %, mp. 195-197 °C M.W. = 196.30; LC-MS (m/z): 197.2 (M+1).

3.5.4.4 4-Ethyl 2-methyl 5-amino-3-methylthiophene-2,4-dicarboxylate (3D)

It was synthesized using the procedure as described in 3.5.4 by the reaction of methylacetoacetate, ethylcyanoacetate, sulphur and morpholine to afford the title compound as light yellow solid. Yield: 80 %, mp. 115-116 °C M.W. = 243.4; LC-MS (m/z): 245.6 (M+1).

3.5.4.5 2-Amino-5-methylthiophene-3-carbonitrile (3E)[138564-58-6]

It was synthesized using the procedure as described in 3.5.4 by the reaction of acetone, malononitrile, sulphur and morpholine to afford the title compound as light yellow solid. Yield: 63 %. It was directly used for the next step.

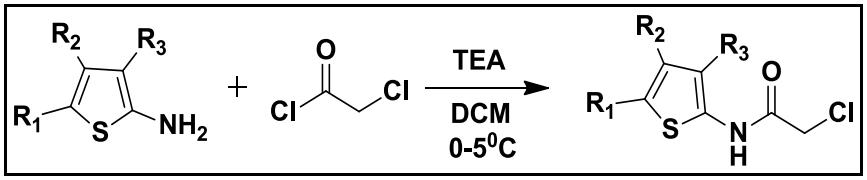
3.5.4.6 2-Amino-6,6-dimethyl-4-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (3F)

It was synthesized using the procedure as described in 3.5.4 by the reaction of dimedone, malononitrile, sulphur and morpholine to afford the title compound as light yellow solid. Yield: 52 %. It was directly used for the next step.

3.5.5 General procedure for the synthesis of substituted 2-Chloroacetamido thiophene

Substituted 2-amino thiophene (1 mmol) is dissolved into the dichloromethane (10 ml). To this triethyl amine (2.2 mmol) is added. The reaction is cooled to 0 ^{0}C and stirred for 10 minutes. While maintaining the temperature, chloroacetyl chloride (1.1 mmol)

suspended in dichloromethane (5 ml) is added dropwise in 15 minutes. The reaction mixture is stirred at room temperature for 4 hrs. The completion of the reaction is checked by TLC. The reaction mixture is poured into the ice cold water and the layers are separated. The organic layer is washed with water (3 X 25). The organic layer is dried over anhydrous sodium sulfate, and then distilled off under reduced pressure to give crude 2-chloracetamido thiophene.



3.5.5.1 Ethyl 2-(2-chloroacetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (B1)[CAS 60442-41-3]

This intermediate was prepared using the procedure described in 3.5.5 using **3a** and chloroacetyl chloride to give light brown solid. M.W. 301.7, LC-MS (m/z): 302.20 (M+1).

3.5.5.2 2-(2-Chloroacetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (B2)

This intermediate was prepared using the procedure described in 3.5.5 using **3b** and chloroacetyl chloride to give brownish white solid. M.W. 254.7, LC-MS (m/z): 255.3 (M+1).

3.5.5.3 2-(2-Chloroacetamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (B3)

This intermediate was prepared using the procedure described in 3.5.5 using **3C** and chloroacetyl chloride to give light yellow solid. M.W. 272.7, LC-MS (m/z): 273.4 (M+1).

3.5.5.4 4-Ethyl 2-methyl 5-(2-chloroacetamido)-3-methylthiophene-2,4-dicarboxylate (B4)

This intermediate was prepared using the procedure described in 3.5.5 using **3D** and chloroacetyl chloride to give light yellow solid. LC-MS (m/z): 320.5 (M+1).

3.5.5.5 2-Chloro-N-(3-cyano-5-methylthiophen-2-yl)acetamide (B5)

This intermediate was prepared using the procedure described in 3.5.5 using **3E** and chloroacetyl chloride to give light yellow solid.

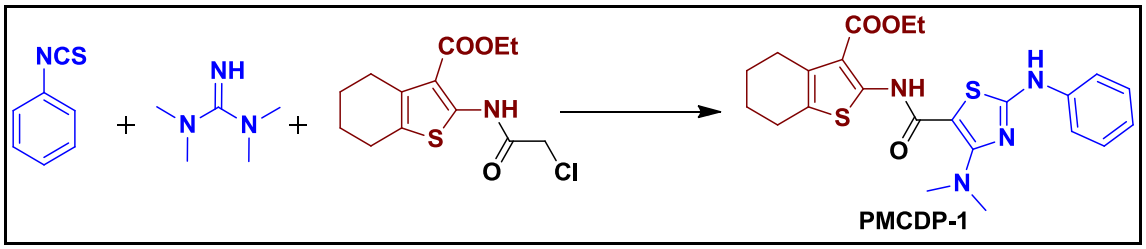
3.5.5.6 2-(2-Chloroacetamido)-6,6-dimethyl-4-oxo-4,5,6,7-tetrahydrobenzo[b] thiophene-3-carboxamides (B6)

This intermediate was prepared using the procedure described in 3.5.5 using **3E** and chloroacetyl chloride to give white solid.

3.5.6 General procedure for the synthesis of the synthesis of Thiophene-2-yl thiazole carboxamides

To a hot air dried round bottomed flask, containing a solution of isothiocyanate (2.0 mmol) in DMF (5 mL), amidine/guanidine (2.0 mmol) was added at 20–25 ^{0}C and the solution was stirred for 2–3 h. To the above solution, 2-chloroacetamido thiophene compound (2.0 mmol) in DMF (5 mL) was added at ambient temperature and the reaction was further stirred for 6-8 h with stirring. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice cold water. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through Buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to yield pure solid compounds. The structures of compounds **(PMCDP-n)** were assigned with the help of NMR and mass spectra.

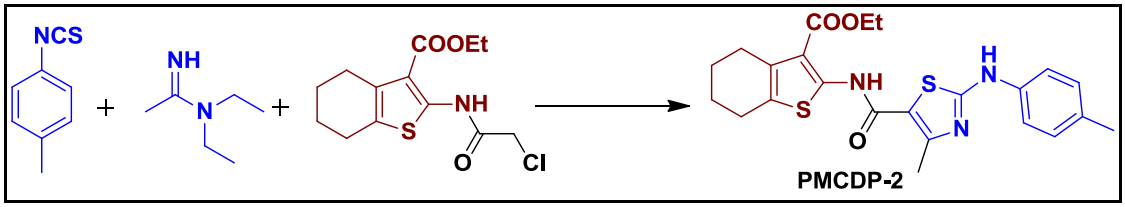
PMCDP-1: Ethyl 2-(4-(dimethylamino)-2-(phenylamino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



Yield: 59%, yellow solid, mp : 202-205 ^{0}C, **Molecular formula: $C_{23}H_{26}N_4O_3S_2$,** LC-MS calculated: 470.6, found: 471.3$(M+1)^+$,**^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.28-1.32(t, 3H), 1.71(s, 4H), 2.58(s, 2H), 2.71(s, 2H), 2.88(s, 6H), 4.27-4.32(q, 4H), 7.02(t, 1H), 7.33(t, 2H), 7.59(d, 2H), 10.75(s, 1H), 12.28(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ:

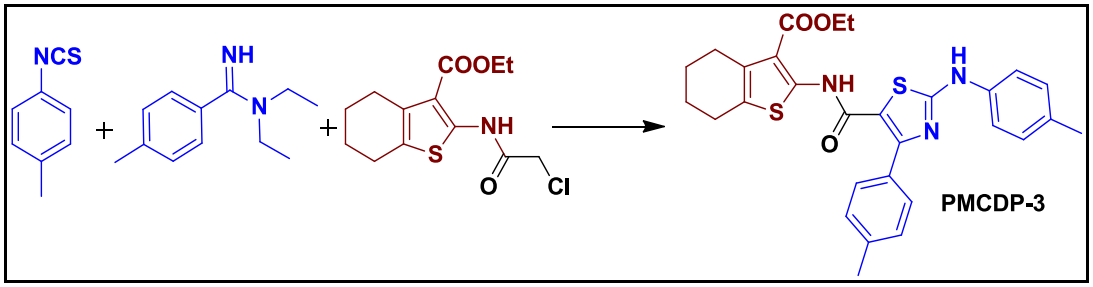
14.12, 22.31, 22.46, 22.67, 43.54, 59.93, 98.81, 110.23, 118.08, 122.73, 125.36, 129.08, 130.10, 139.87, 147.00, 157.45, 161.71, 163.65, 165.17

PMCDP-2: Ethyl 2-(4-methyl-2-(p-tolylamino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



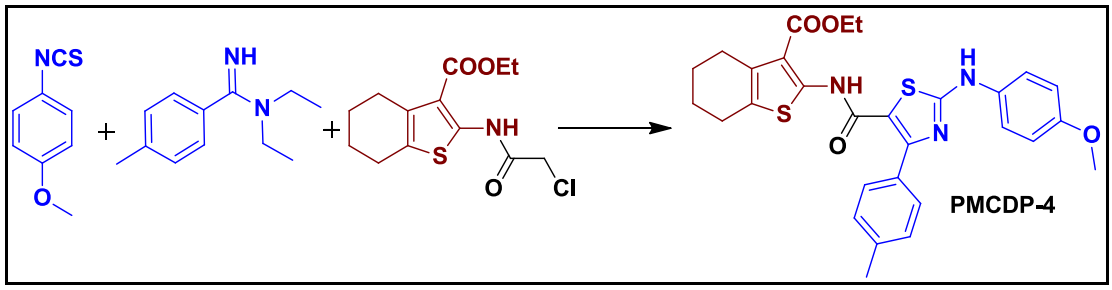
Yield: 63%, buff solid, mp: 220-223 ^{0}C, **Molecular formula: $C_{23}H_{25}N_3O_3S_2$**, LC-MS calculated: 455.6, found: 476.6(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.29(t, 3H), 1.71(s, 4H), 2.26(s, 3H), 2.60(s, 3H), 2.60(s, 2H), 2.70(s, 2H), 4.26(q, 2H), 7.14(d, 2H), 7.46(d, 2H), 7.59(d, 2h), 10.61(s, 1H), 11.45(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 14.04, 17.62, 20.33, 22.22, 22.40, 23.64, 25.79, 60.44, 110.88, 118.23, 126.12, 129.47, 130.35, 131.84, 137.48, 146.76, 157.71, 165.75.

PMCDP-3: Ethyl 2-(4-(p-tolyl)-2-(p-tolylamino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



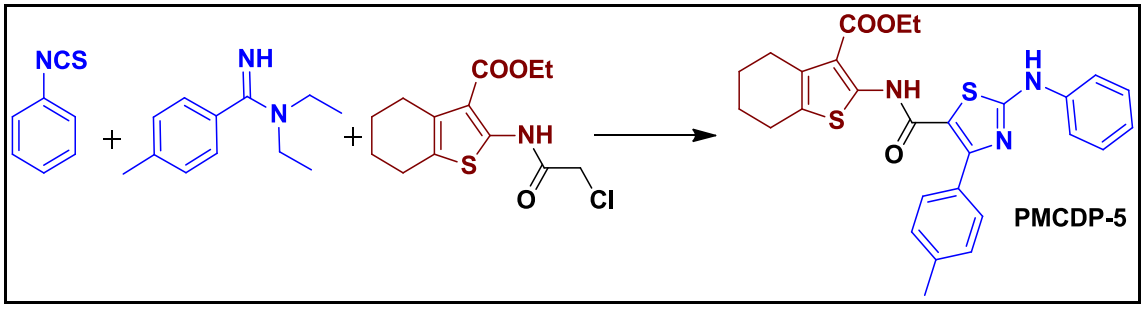
Yield: 72%, white solid, mp: 231-234 ^{0}C, **Molecular formula: $C_{29}H_{29}N_3O_3S_2$**, LC-MS calculated: 531.7, found: 532.4(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.16(t, 3H), 1.68(s, 4H), 2.26(s, 3H), 2.34(s, 3H), 2.57(s, 2H), 2.63(s, 2H), 3.99(q, 2H),7.14(d, 2H), 7.24(d, 2H), 7.48(q, 4h), 10.68(s, 1H), 11.07(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 13.99, 20.33, 20.87, 22.18, 22.37, 23.62, 25.69, 54.81, 59.95, 111.10, 113.30, 118.13, 126.23, 129.09, 129.21, 129.49, 130.35, 130.69 131.75, 137.58, 139.08, 145.79, 154.75, 157.65, 164.27, 164.61.

PMCDP-4: Ethyl 2-(2-((4-methoxyphenyl)amino)-4-(p-tolyl)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



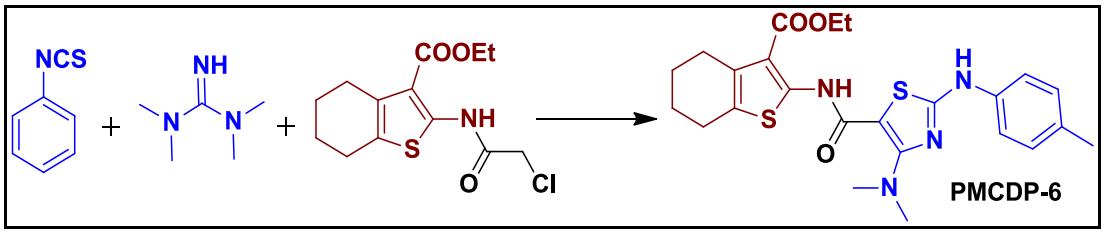
Yield: 70%, white solid, mp: 225-228 ^{0}C, **Molecular formula: $C_{29}H_{29}N_3O_4S_2$,** LC-MS calculated: 547.5, found: 548.1(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.17(t, 3H), 1.67(d, 4H), 2.34(s, 3H), 2.55(s, 2H), 2.62(s, 2H), 3.72(s, 3H), 4.00(q, 2H), 6.92(d, 2H), 7.22(d, 2H), 7.50(d, 4H), 10.60(s, 1H), 11.02(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 14.10, 20.39, 20.87, 22.20, 22.42, 23.59, 25.71, 55.18, 60.13, 110.12, 114.31, 118.21, 126.23, 128.99, 129.33, 129.53, 130.33, 130.73, 131.78, 137.62, 139.18, 145.82, 154.83, 157.68, 164.37, 164.70.

PMCDP-5: Ethyl 2-(2-(phenylamino)-4-(p-tolyl)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



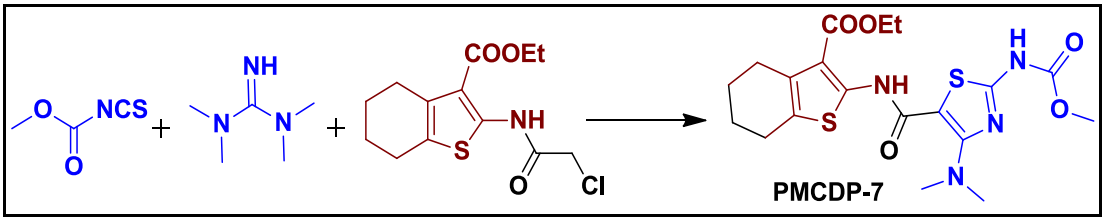
Yield: 76%, off white solid, mp: 235-237 ^{0}C, **Molecular formula: $C_{28}H_{27}N_3O_3S_2$,** LC-MS calculated: 517.00, found: 518.6(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.18(t, 3H), 1.70(d, 4H), 2.38(s, 3H), 2.58(s, 2H), 2.65(s, 2H), 4.00(q, 2H), 7.03(t, 1H), 7.27(d, 2H), 7.34(t, 2H), 7.54(d, 2H) 7.64(d, 2H) 10.81(s, 1H), 11.12(s, 1H).

PMCDP-6: Ethyl 2-(4-(dimethylamino)-2-(p-tolylamino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



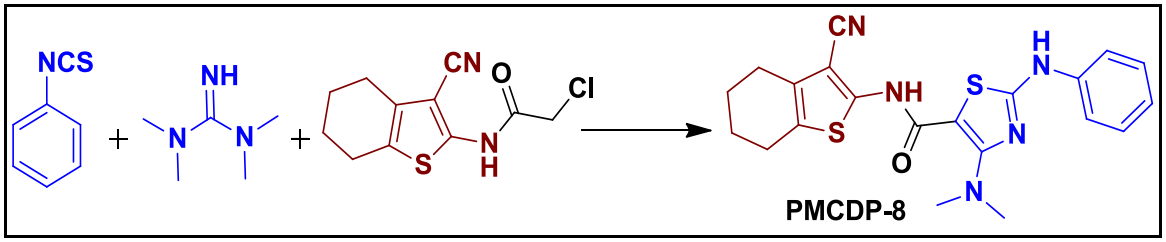
Yield: 65%, off white solid, mp: 218-221 ^{0}C, **Molecular formula: $C_{24}H_{28}N_4O_3S_2$**, LC-MS calculated: 484.63, found: 485.3(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.28(t, 3H), 1.71(d, 4H), 2.26(s, 3H), 2.57(s, 2H), 2.71(s, 2H), 2.87(s, 6H), 4.26(q, 2H), 7.15(d, 2H), 7.45(d, 2H), 10.67(s, 1H), 12.25(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 14.12, 20.32, 22.36, 22.45, 23.66, 25.91, 59.91, 98.36, 110.16, 118.37, 125.32, 129.49, 130.08, 131.97, 137.36, 147.03, 157.45, 161.88, 163.95, 165.19

PMCDP-7: Ethyl 2-(4-(dimethylamino)-2-((methoxycarbonyl)amino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



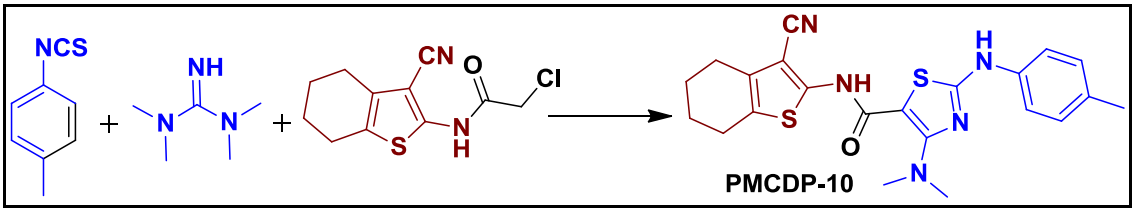
Yield: 55%, white solid, **Molecular formula: $C_{19}H_{24}N_4O_5S_2$**, LC-MS calculated: 452.5, found: 453.7(M+1)$^{+}$,**^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.35(t, 3H), 1.78(t, 4H), 2.65(s, 2H), 2.78(s, 2H), 2.88(s, 6H), 3.88(s 3H), 4.30(q, 2H), 8.33(s, 1H), 12.67(s, 1H).

PMCDP-8: *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-(dimethylamino) -2-(phenylamino)thiazole-5-carboxamide



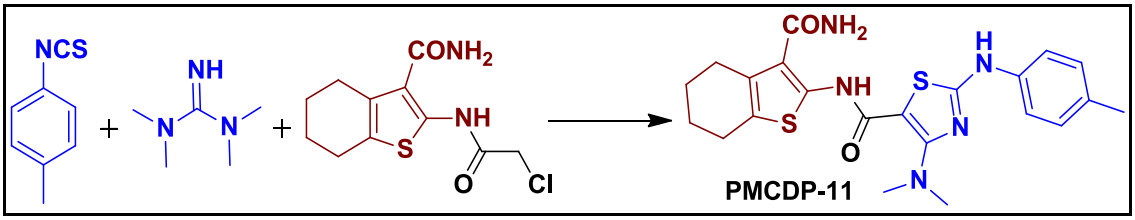
Yield: 60%, white solid, mp: 220-222 ^{0}C, **Molecular formula: $C_{21}H_{21}N_5OS_2$**, LC-MS calculated: 423.3, found: 454.4(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.75(s, 4H), 2.59(s, 2H), 2.72(s, 2H), 2.88(s, 6H), 7.04(t, 1H), 7.35(t, 2H), 7.60(d, 2H), 10.85(s, 1H), 12.21(s, 1H).

PMCDP-10:*N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-(dimethylamino) -2- (p-tolylamino)thiazole-5-carboxamide



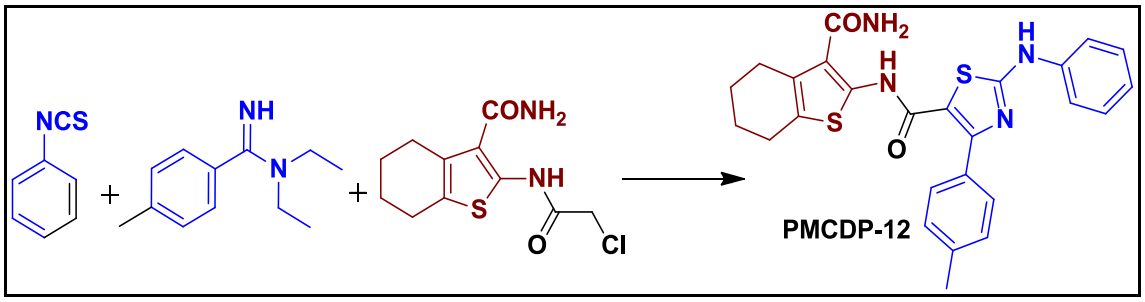
Yield: 60%, white solid, **Molecular formula: $C_{22}H_{23}N_5OS_2$**, LC-MS calculated: 437.6, found: 438.7(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.76(s, 4H), 2.59(s, 2H), 2.73(s, 2H), 2.87(s, 6H), 7.17(d, 2H), 7.46(d, 2H), 10.76(s, 1H), 12.20(s, 1H).

PMCDP-11: *N*-(3-Carbamoyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-(dimethylamino)-2-(p-tolylamino)thiazole-5-carboxamide



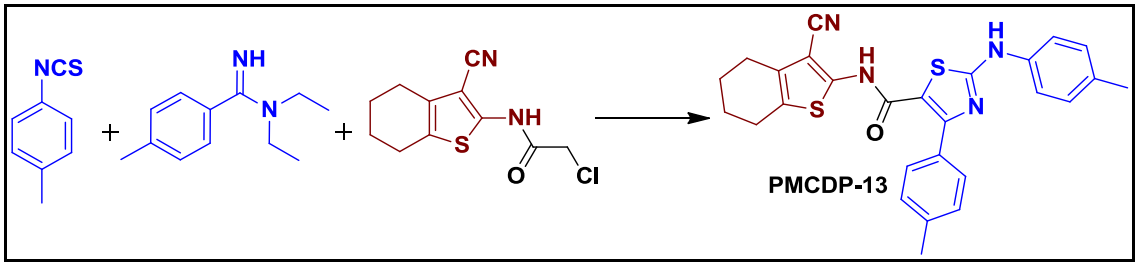
Yield: 62%, Light yellow solid, mp: 175-180 ^{0}C, **Molecular formula: $C_{22}H_{25}N_5O_2S_2$**, LC-MS calculated: 455.3, found: 456.3(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.77(s, 4H), 2.28(s, 3H), 2.62(s, 2H), 2.72(s, 2H), 2.92(s, 6H), 6.80(d, 1H-NH_2), 7.11(d, 2H), 7.46(d, 2H), 10.48(s, 1H), 12.49(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 13.95, 20.39, 22.63, 22.52, 23.84, 25.34, 59.61, 98.47, 114.77, 118.12, 125.21, 128.42, 129.25, 131.54, 137.53, 143.19, 157.46, 161.21, 163.65, 167.41

PMCDP-12: *N*-(3-Carbamoyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-2-(phenylamino)-4-(p-tolyl)thiazole-5-carboxamide



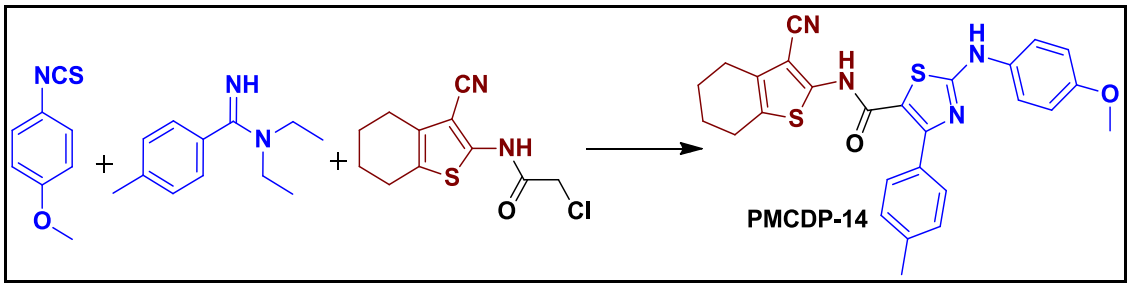
Yield: 80%, Yellow solid, mp: 237-240 ^{0}C, **Molecular formula: $C_{26}H_{24}N_4O_2S_2$,** LC-MS calculated: 488.1, found: 489.6(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ: 1.76(s, 4H), 2.38(s, 3H), 2.62(s, 2H), 2.68(s, 2H), 3.41(s, 2H, -NH_2), 6.97(t, 1H), 7.19(d, 2H), 7.28(d, 2H), 7.60-7.66(m, 4H), 10.55(s, 1H), 12.19(s, 1H). **^{13}C NMR (400 MHz, DMSO- *d*$_6$)** δ: 13.92, 20.62, 22.40, 22.52, 23.89, 25.35, 59.63, 113.20, 114.97, 117.67, 122.11, 126.26, 128.41, 129.17, 131.04, 133.46, 138.45, 140.18, 143.36, 154.51, 157.51, 163.19, 167.29.

PMCDP-13: *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-(p-tolyl)-2-(p-tolylamino)thiazole-5-carboxamide



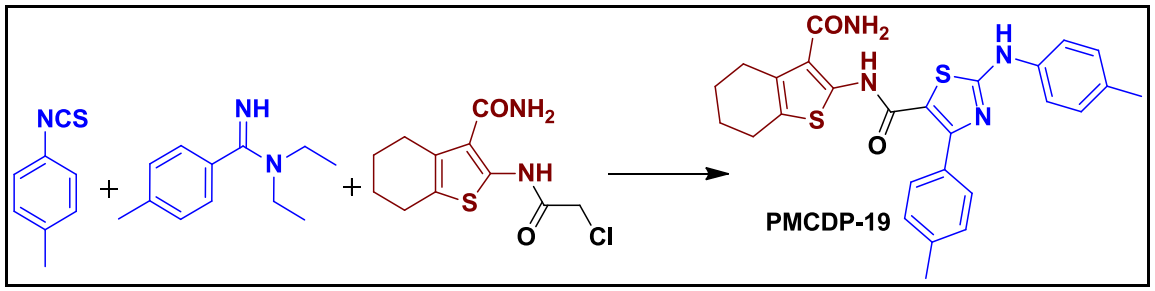
Yield: 75%, White solid, **Molecular formula: $C_{27}H_{24}N_4OS_2$.** LC-MS calculated: 484.7, found: 485.4(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ: 1.77(s, 4H), 2.29(s, 3H), 2.40(s, 3H), 2.66(s, 2H), 2.68(s, 2H), 7.14(d, 2H), 7.24(d, 2H), 7.48(q, 4H), 10.70(s, 1H), 11.67(s, 1H).

PMCDP-14: *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-2-((p-methoxyphenyl)amino)-4-(p-tolyl)thiazole-5-carboxamide



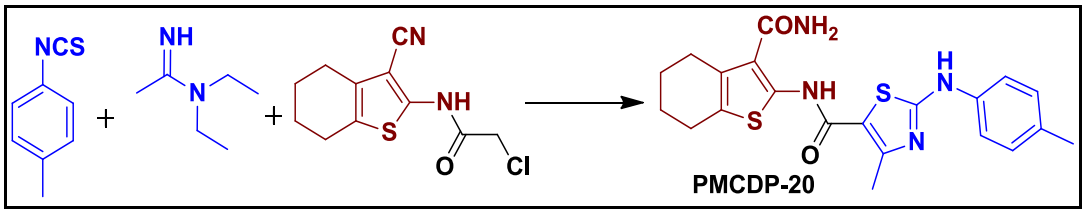
Yield: 75%, White solid, **Molecular formula: $C_{27}H_{24}N_4O_2S_2$**, LC-MS calculated: 500.13, found: 501.3(M+1)$^+$, **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ: 1.67(d, 4H), 2.34(s, 3H), 2.55(s, 2H), 2.62(s, 2H), 3.72(s, 3H), 6.90(d, 2H), 7.21(d, 2H), 7.48(d, 4H), 10.54(s, 1H), 11.78(s, 1H).

PMCDP-19: *N*-(3-Carbamoyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-(p-tolyl)-2-(p-tolylamino)thiazole-5-carboxamide



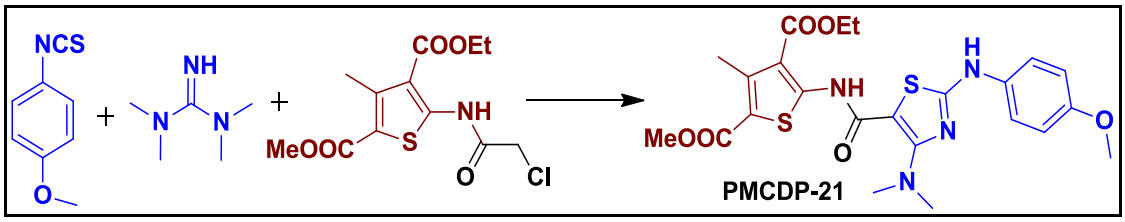
Yield: 73%, Yellow solid, mp: 241-244 ^{0}C, **Molecular formula: $C_{27}H_{26}N_4O_2S_2$**, LC-MS calculated: 502.6, found: 503.4(M+1)$^+$, **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ: 1.75(s, 4H), 2.28(s, 3H), 2.37(s, 3H), 2.62(s, 2H), 2.68(s, 2H), 6.76(d, 1H-NH_2), 7.11(d, 2H), 7.19(d, 2H), 7.50(d, 2H), 7.58(d, 2H), 10.51(s, 1H), 12.12(s, 1H). **^{13}C NMR (400 MHz, DMSO-*d*$_6$)** δ: 14.02, 20.41, 21.04, 22.40, 23.87, 25.26, 112.79, 115.19, 117.95, 126.11, 128.57, 129.17, 129.28, 131.08, 131.39, 137.74, 138.44, 143.11, 154.58, 157.45, 163.46, 167.24.

PMCDP-20: *N*-(3-Carbamoyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-methyl-2-(p-tolylamino)thiazole-5-carboxamide



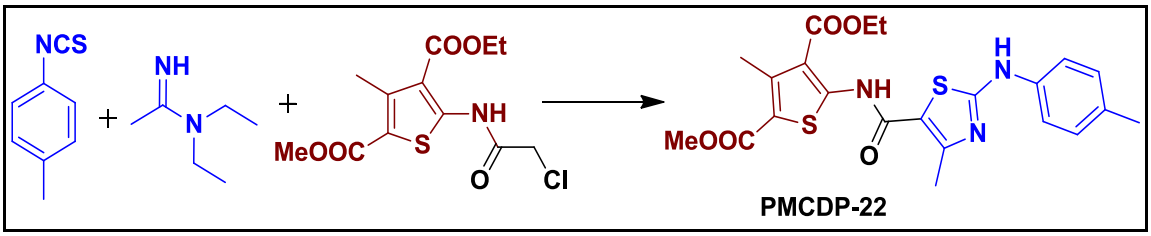
Yield: 73%, White solid, **Molecular formula: $C_{21}H_{22}N_4O_2S_2$**, LC-MS calculated: 426.5, found: 427.4$(M+1)^+$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.80(s, 4H), 2.28(s, 3H), 2.52(d, 2H) 2.58(s, 3H), 2.61(s, 2H), 7.12(d, 2H), 7.46(d, 2H), 10.47(s, 1H), 10.82(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 17.78, 20.40, 21.73, 22.65, 23.49, 23.58, 95.06, 111.31, 114.25, 118.13, 127.99, 129.29, 130.80, 131.54, 137.67, 146.55, 156.47, 159.43, 164.56.

PMCDP-21: 4-Ethyl 2-methyl 5-(4-(dimethylamino)-2-((4-methoxyphenyl)amino) thiazole-5-carboxamido)-3-methylthiophene-2,4-dicarboxylate



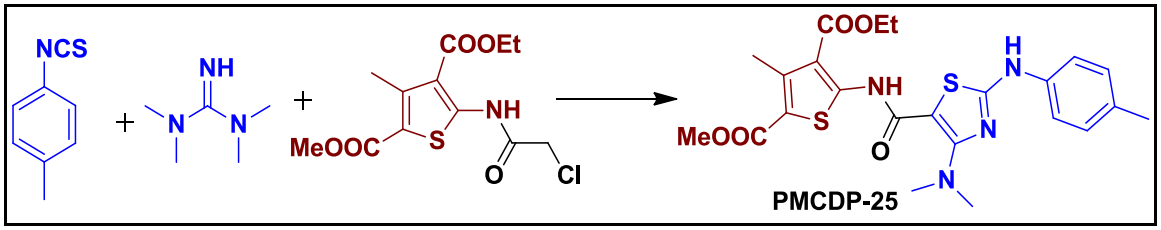
Yield: 66%, White solid, **Molecular formula: $C_{23}H_{26}N_4O_6S_2$**, LC-MS calculated: 502.6, found: 503.4$(M+1)^+$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.36(t, 3H), 2.68(s, 3H), 2.94(s, 6H), 3.76(s, 6H, 2x-CH_3), 4.33(q, 2H), 6.89(d, 2H), 7.50(d, 2H), 10.57(s, 1H), 12.53(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 13.97, 15.18, 43.40, 51.26, 55.03, 60.38, 112.73, 114.05, 115.37, 120.24 132.96, 144.03, 152.63, 155.27, 158.02, 162.62, 163.18, 164.90, 165.04.

PMCDP-22: 4-Ethyl 2-methyl 3-methyl-5-(4-methyl-2-(p-tolylamino)thiazole-5-carboxamido)thiophene-2,4-dicarboxylate



Yield: 70%, White solid, mp: >250 ^{0}C, **Molecular formula: $C_{22}H_{23}N_3O_5S_2$**, LC-MS calculated: 473.3, found: 474.6(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.36(t, 3H), 2.29(s, 3H), 2.64(s, 3H), 2.71(s, 3H), 3.80(s, 3H), 4.35(q, 2H), 7.14(d, 2H), 7.48(d, 2H), 10.68(s, 1H), 11.74(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 13.97, 15.18, 43.40, 51.26, 55.03, 60.38, 112.73, 114.05, 115.37, 120.24 132.96, 144.03, 152.63, 155.27, 158.02, 162.62, 163.18, 164.90, 165.04.

PMCDP-25: 4-Ethyl 2-methyl 5-(4-(dimethylamino)-2-(p-tolylamino)thiazole-5-carboxamido)-3-methylthiophene-2,4-dicarboxylate



Yield: 73%, Yellow solid, **Molecular formula: $C_{23}H_{26}N_4O_5S_2$**, LC-MS calculated: 502.6, found: 503.5(M+1)$^{+}$, **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.33(t, 3H), 2.28(s, 3H), 2.92(s, 6H), 3.78(s, 3H), 4.33(q, 2H), 7.17(d, 2H), 7.47(d, 2H), 10.80(s, 1H), 12.47(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 14.05, 15.23, 43.48, 51.30, 53.73, 60.20, 113.33, 114.50, 115.49, 121.44 133.26, 143.58, 152, 154.24, 158.12, 162, 163.29, 164.90, 165.33.

3.5.7 Method used for pharmacological evaluation

All pharmacological methods followed the procedures as described earlier. In brief, membranes for radioligand binding were prepared from CHO cells stably transfected with human adenosine receptor subtypes in a two-step procedure. In a first low speed step (1000 x g) cell fragments and nuclei were removed. The crude membrane fraction was sedimented from the supernatant at 100,000 x g. The membrane pellet was resuspended in the buffer used for the respective binding experiments, frozen in liquid nitrogen and stored at -80 ^{0}C. For the measurement of adenylyl cyclase activity only one high speed centrifugation of the homogenate was used. The resulting crude membrane pellet was resuspended in 50 mM Tris/HCl, pH 7.4 and immediately used for the cyclase assay. For radioligand binding 1 nM [^{3}H]CCPA at A_1AR, 30 nM [^{3}H]NECA at A_{2A}AR, 1 nM [^{3}H]HEMADO were used. Non-specific binding of [^{3}H]CCPA was determined in the

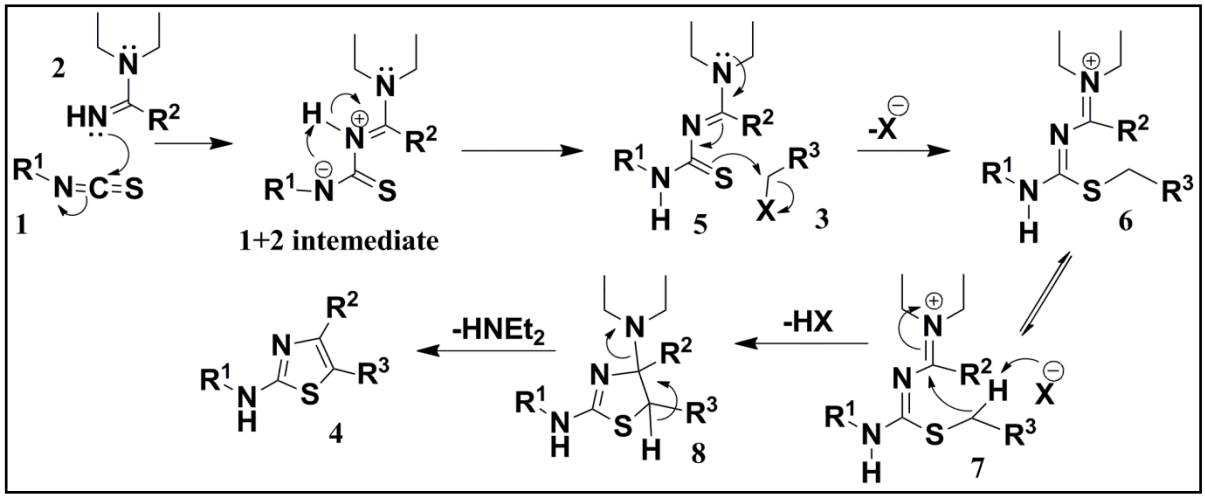
presence of 1mM theophylline, in the case of [^{3}H]NECA and [^{3}H]HEMADO 100 μM R-PIA was used. Ki-values from competition experiments were calculated with the program SCTFIT (De Lean et al. 1982). Inhibition of NECA-stimulated adenylyl cyclase activity was determined as a measurement of affinity of compounds. EC50-values from these experiments were converted to Ki-values with the Cheng and Prusoff equation.

Note: All the biological assays were carried out in collaboration with Prof. Karl-Norbert Klotz, Institut für Pharmakologie und Toxikologie, Julius-Maximilians-Universität Würzburg, Würzburg, Germany. The biological assays were performed as per the standard protocols published by Prof. Klotz **(Ref. pg 36)**.

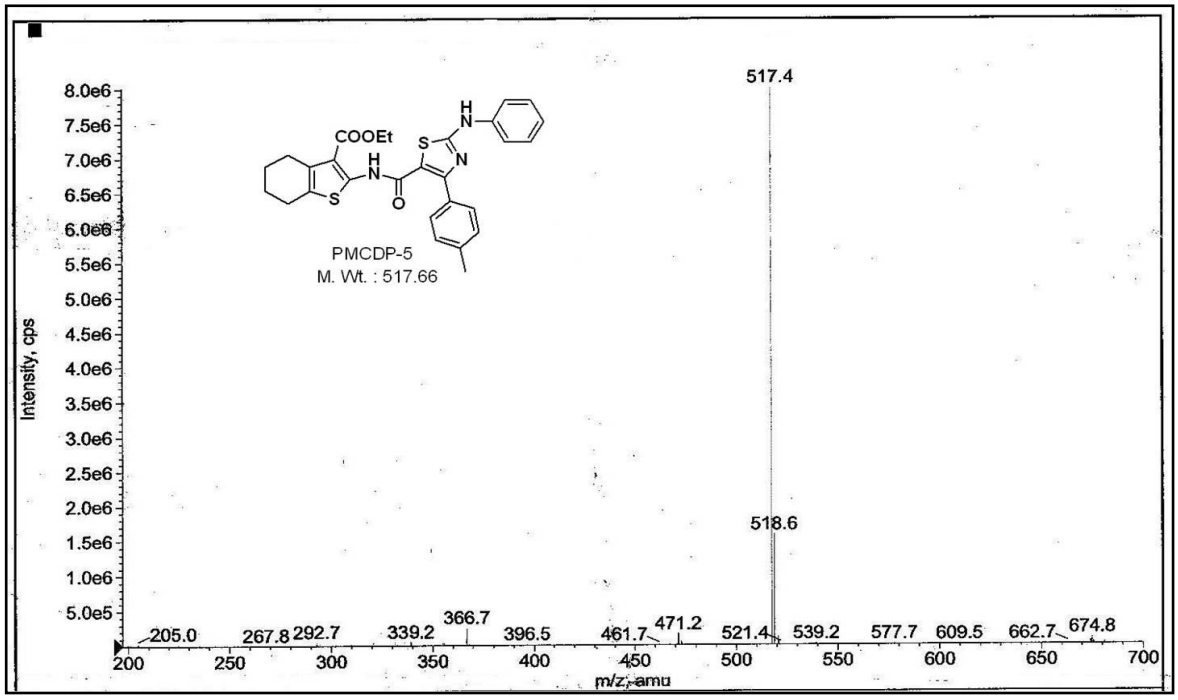
Supporting Information

(General Reaction Mechanism, LC-MS, ^{1}H-NMR and ^{13}C NMR of representative compounds)

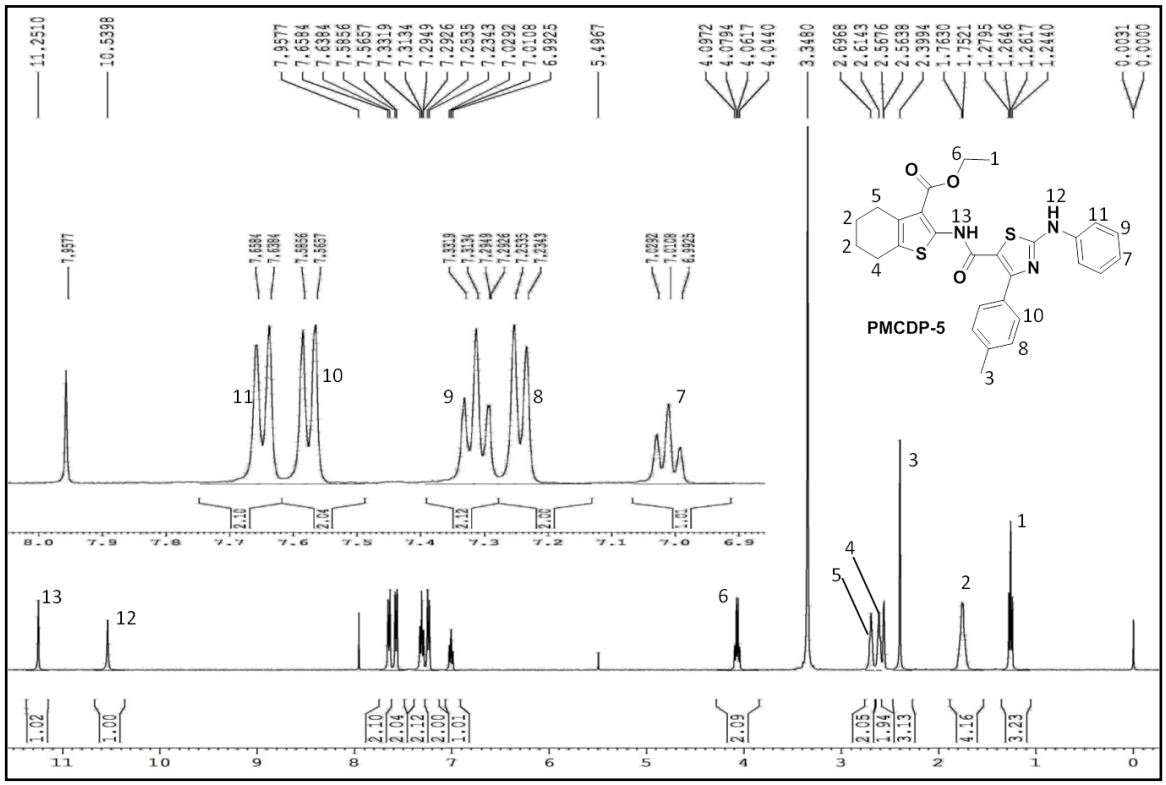
General Reaction Mechanism for the formation of Thiazole



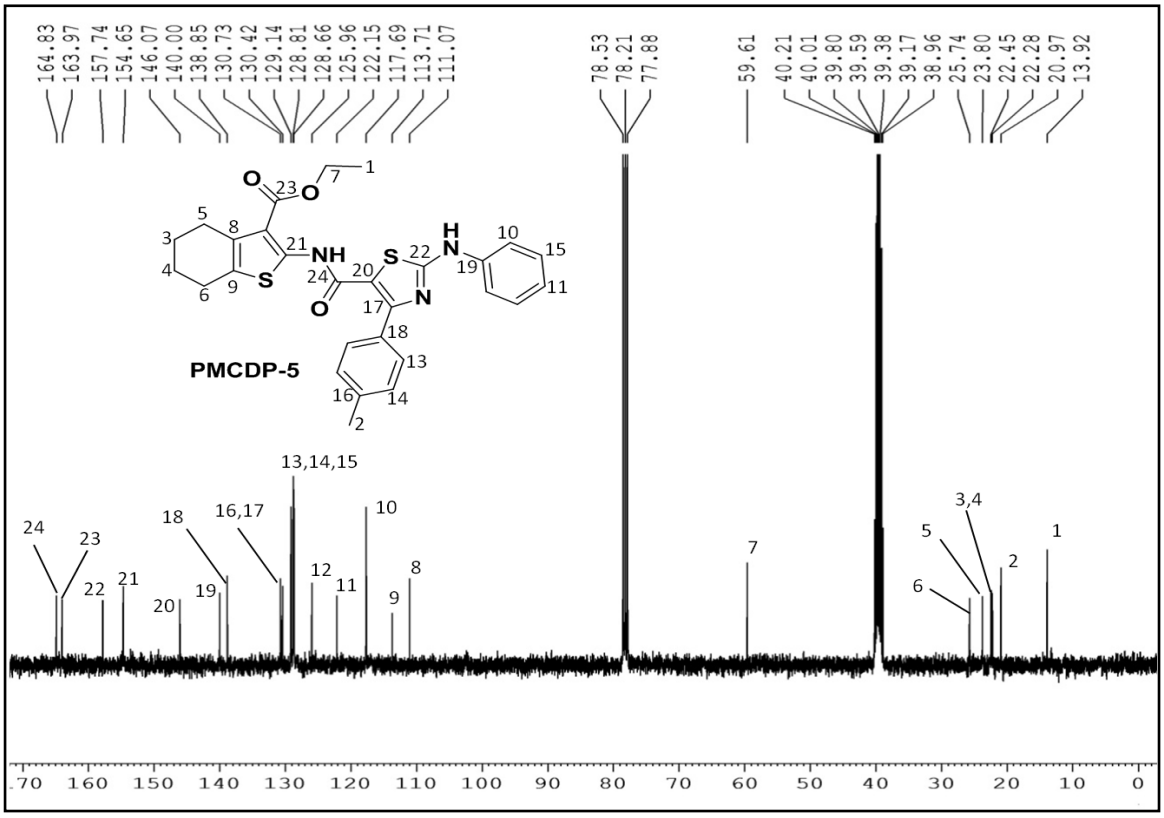
LC-MS spectrum of PMCDP-5



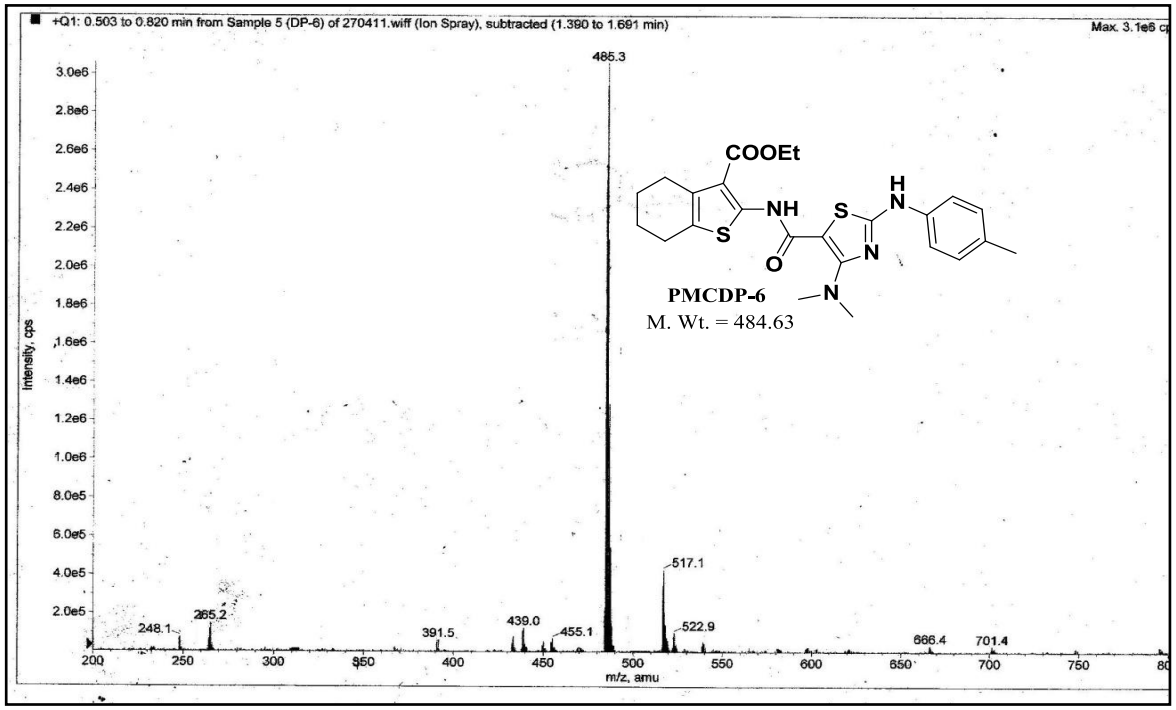
^{1}H NMR spectrum of PMCDP-5



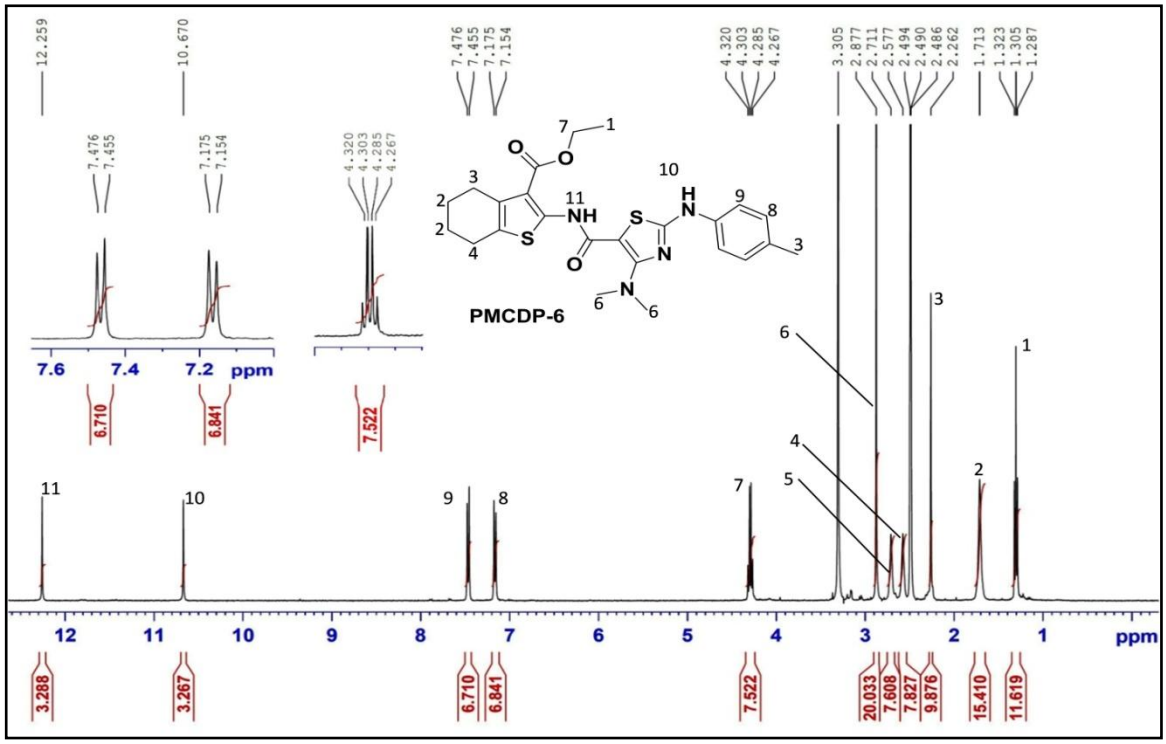
^{13}C NMR spectrum of PMCDP-5



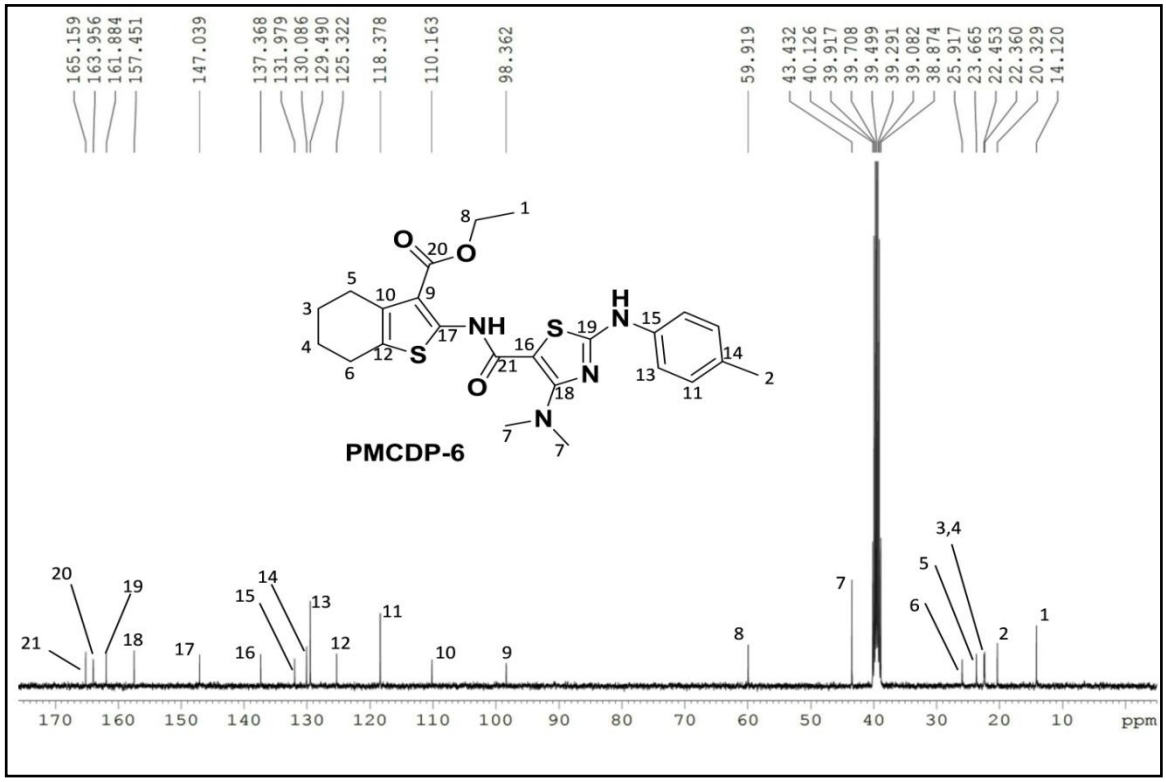
LC-MS spectrum of PMCDP-6



^{1}H NMR spectrum of PMCDP-6



^{13}C NMR spectrum of PMCDP-6



CHAPTER-4

Design, synthesis and biological evaluation of N-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes as adenosine receptor ligands

PAGE NO. 59 TO 82

Chapter 4

Design, synthesis and biological evaluation of *N*-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes as adenosine receptor ligands

4.1 Introduction

In continuation of our work on thiophenyl-thiazole carboxamides from chaper-3, here we have synthesized and evaluated new thiophenyl-thiazole carboxamides as adenosine receptor ligands. In the previous chapter the molecules were potentially active and the biological activity discussion of previous chapter suggested that the activity may be due to different group present on thiazole ring, so here we have modified the molecules to come up with better biological results.

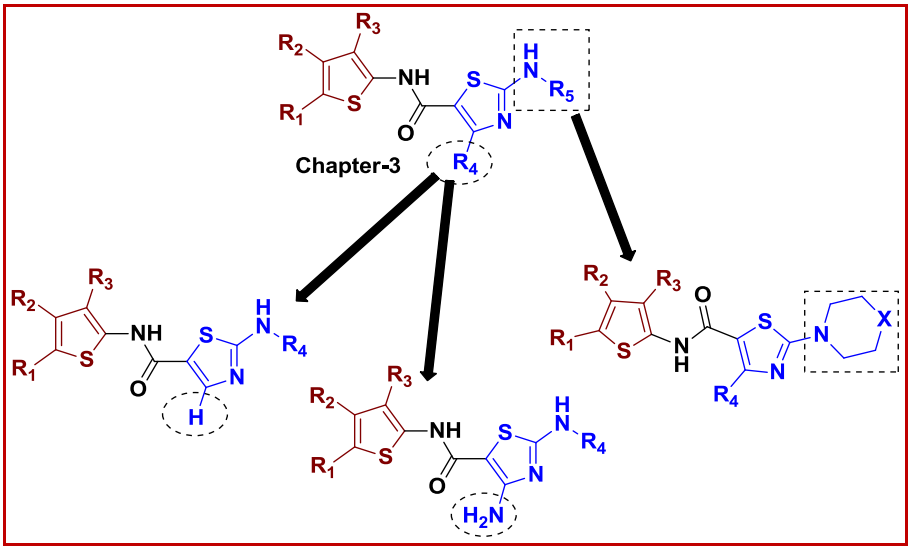


Figure 4.1 Design of molecule *N*-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes

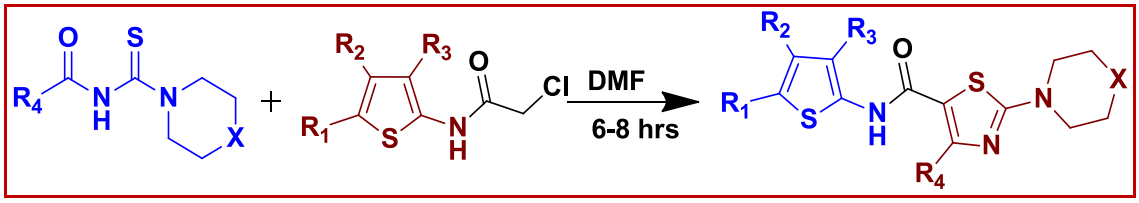
Previous reports from our group suggested that 2-cyclicamino group at the 2nd position of thiazole ring resulted into good adenosine receptor ligands (Inamdar 2007; Pandya 2011). So keeping in mind the previous results, here we have designed, synthesized and tested three different set of thiophenyl-thiazolyl carboxamides. In one set, we have replaced

arylamino group of 2th position of thiazole with *N*-cyclicamino group, in second set we have coupled thiophenes with disubstituted thiazoles and in third set of molecules diamino substituted thiazoles are coupled with thiophenes (Figure 4.1).

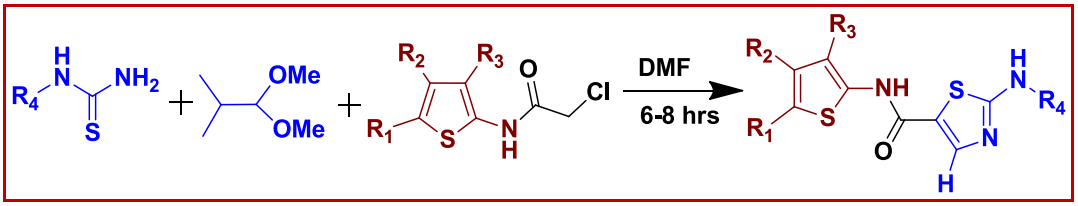
4.2 Present work

Present work describes the design of the three different kind of thiazoles coupled with thiophenes. We were majorly interested in the modification of the thiazole moity, as it is found to be active against adenosine receptors (Cole et al. 2009; Scheiff et al. 2010; Inamdar et al. 2013).

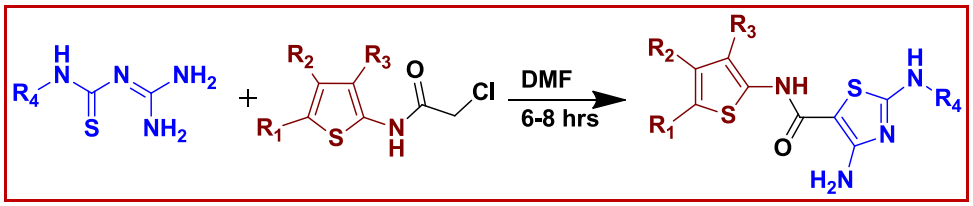
The synthesis of *N*-cyclicaminothiazoles coupled with thiophenes was achieved by reacting various *N*-cyclicamino carbanothioyl arylamides with 2-chloroacetamido thiophenes at room temperature (Scheme 4.1). The *N*-cyclicamino carbanothioyl arylamide was synthesized by the reaction of aroyl isothiocynate with secondary amines. The synthesis of disubstituted thiazoles coupled with thiophenes was achieved by reacting monosubstituted thiourea with *N*,*N*-Dimethyl formamide dimethyl acetal (DMF-DMA) followed by addition of 2-chloroacetamido thiophene (Scheme 4.2). The synthesis of diamino substituted thiazoles coupled with thiophenes was achieved by reacting guanidinothiourea with 2-chloroacetamido thiophene (Scheme 4.3). All the compounds **(PMCDP-n)** were synthesized with good yields and they were confirmed by mass and NMRs.



Scheme 4.1 Synthetic scheme for *N*-cyclicamino thiazoles coupled with thiophenes



Scheme 4.2 Synthetic scheme for disubstituted thiazoles coupled with thiophenes



Scheme 4.3 Synthetic scheme for diamino thiazoles coupled with thiophenes

As an example, the reaction of *N*-phenyl thiourea with DMF-DMA was done to furnish *(E)*-*N*,*N*-dimethyl-*N'*-(phenylcarbamothioyl) formimidamide. This formimidamide intermediate (1 mmol) was further reacted with Ethyl 2-(2-chloroacetamido)-4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (1 mmol) in DMF at room temperature which resulted into **PMCDP-16** with good yields. It was further confirmed by LC-MS (M+), ^{1}H NMR and ^{13}C NMR. The mass spectrum of **PMCDP-16** (Figure 4.2) displayed the molecular ion peak at *m/z* 428.1(M+1) which confirm the molecular weight of the compound 427.5 calculated for $C_{21}H_{21}N_3O_3S_2$.

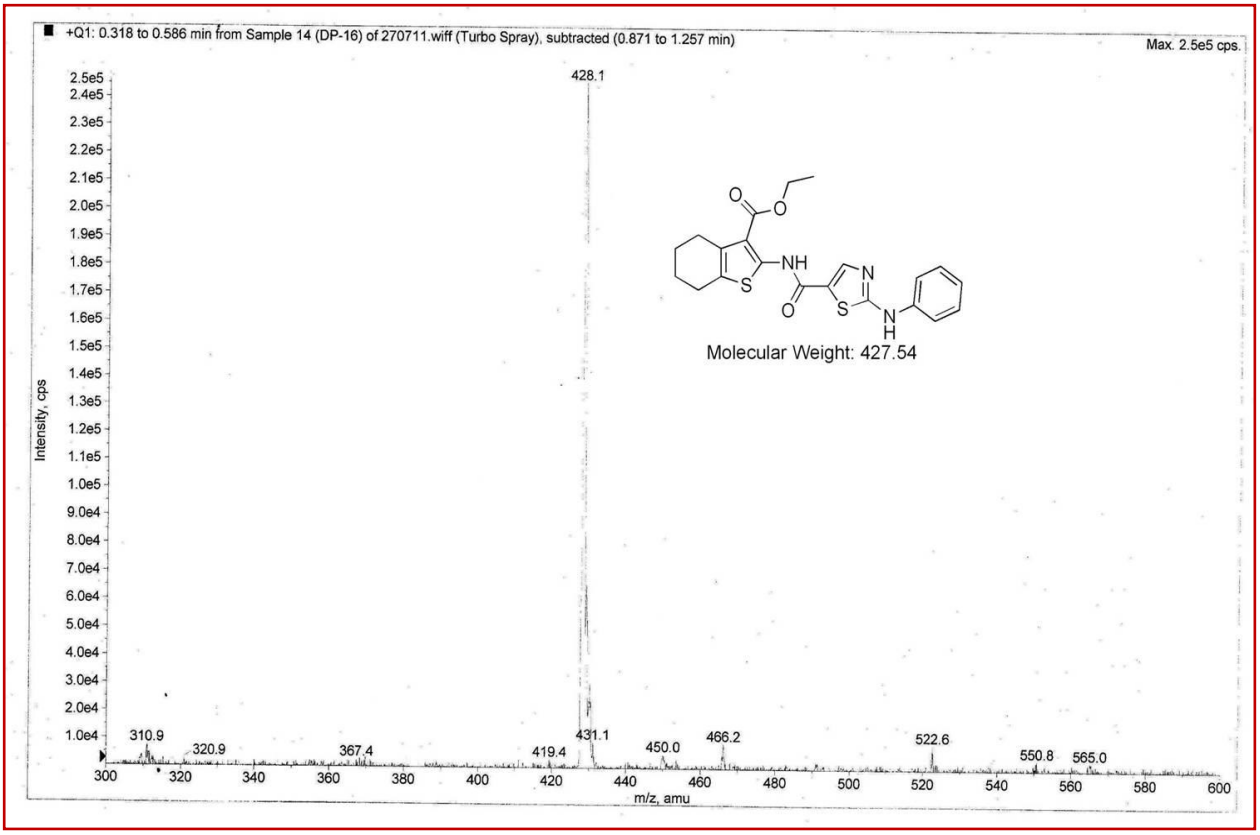


Figure 4.2 LC-MS $(M+1)^+$ of **PMCDP-16**

The structure of the molecule was further confirmed by ^{1}H NMR (500 MHz, $CDCl_3$), which gave the characteristic peak of all the hydrogens present. The amide proton –NH singlet came at 12.02 δ and the thaizole –NH singlet proton came at 8.20 δ. The protons of ester methyl group (-CH_3) was observe at 1.41-1.44 δ and splited into triplet due to presence of adjacent methylene group(–CH_2). The eight protons of four methylene group were found in the range 1.82-2.81 δ. The two protons of ester methylene group were found at 4.37-4.41 with quartet. All the five aromatic protons of phenyl ring were observed from 7.18-7.86 δ and singlet hydrogen of thiazole was observed at 7.97 δ (Figure-4.3).

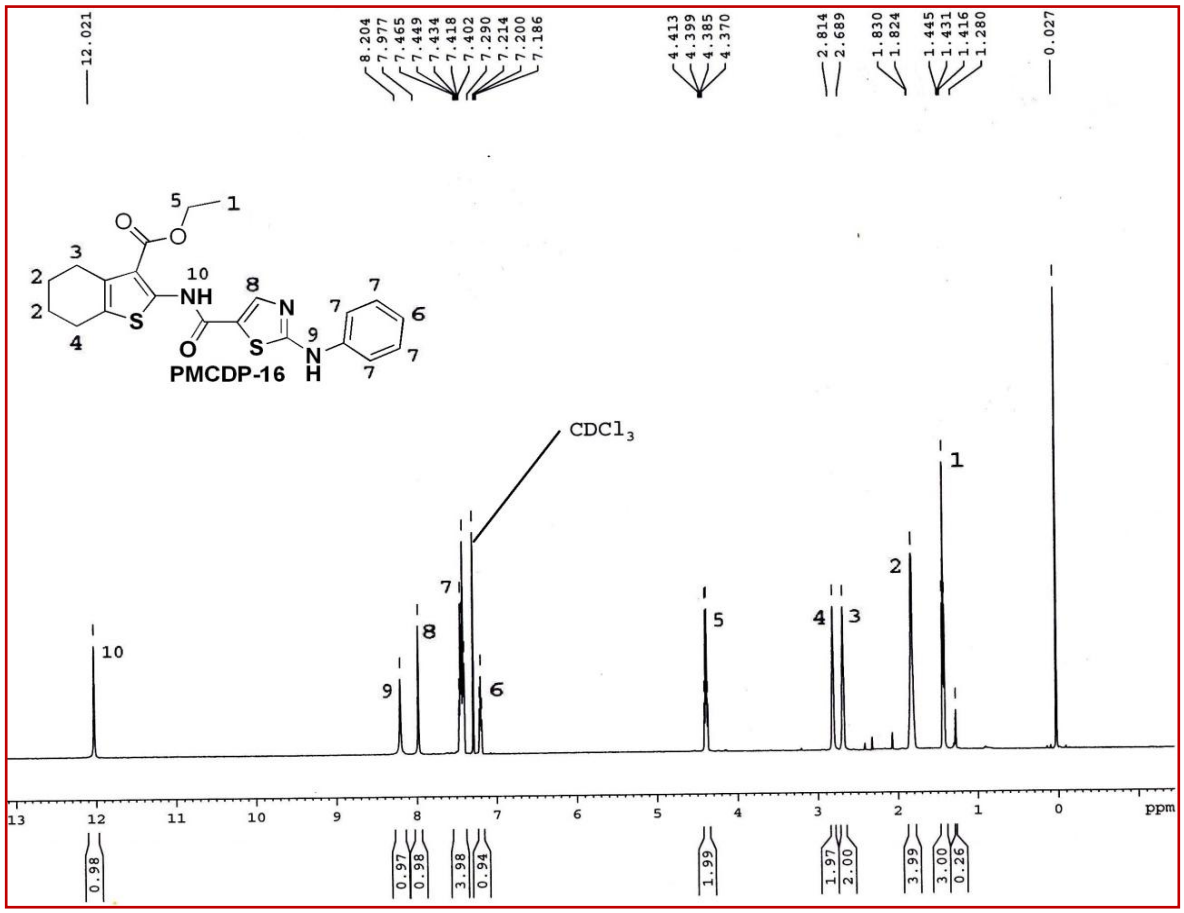


Figure 4.3 ^{1}H NMR of **PMCDP-16**

Further, the structure of the molecule was confirmed by ^{13}C NMR (500 MH_Z in $CDCl_3$). The carbonyl carbon present in the ester group of thiophene is observed most downfield at 166.94 δ. The other carbonyl carbon of amide was observed at 157.48 δ. The carbon of methyl group present on ester of thiophene was observed most upfield at 14.137 δ. The four methylene group carbons of cyclohexane attached to thiophene was observed in the range of 22-26 δ. The methylene group carbon of ester was observed at 60.62. All the aromatic carbon present on phenyl, thiophene and thiazole rings were observed in the range of 98-147 δ (Figure 4.4).

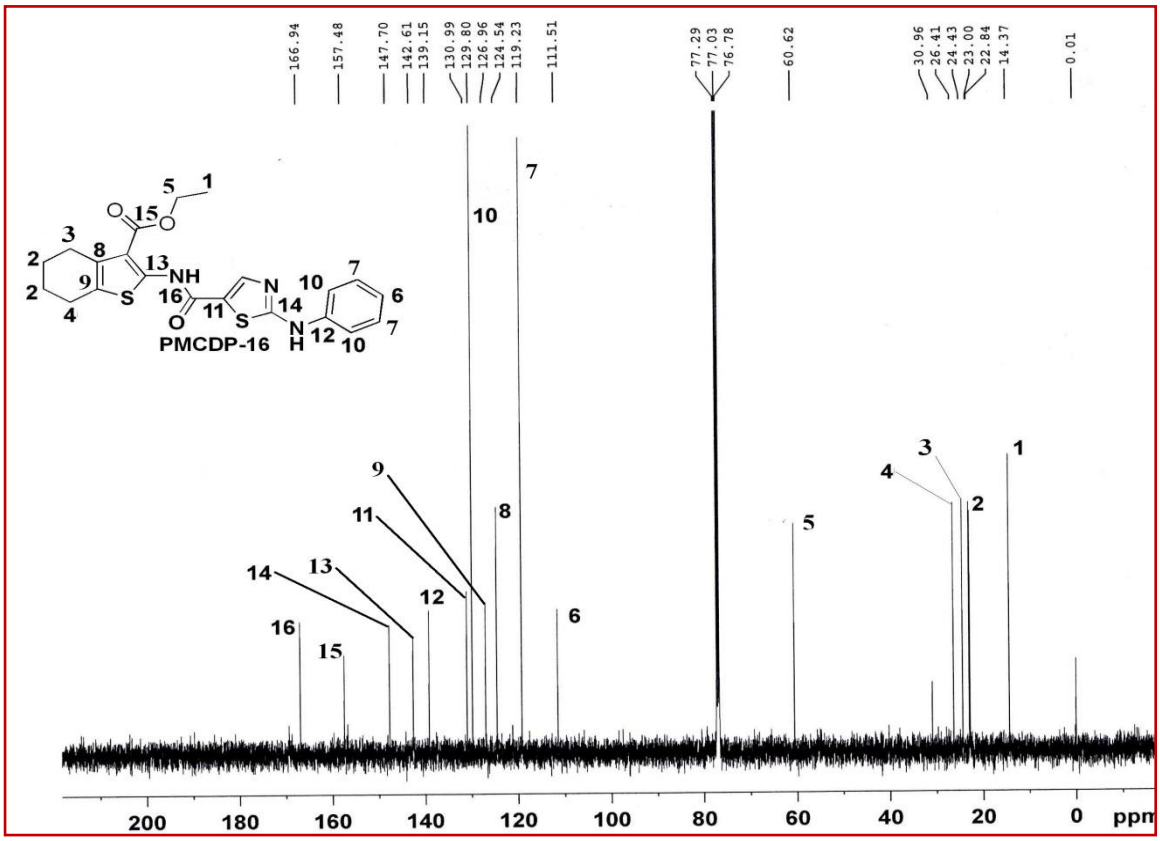
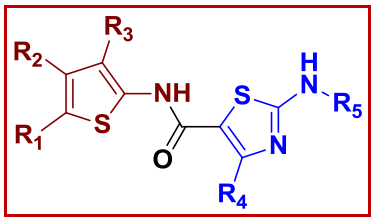


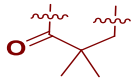
Figure 4.4 ^{13}C NMR of **PMCDP-16**

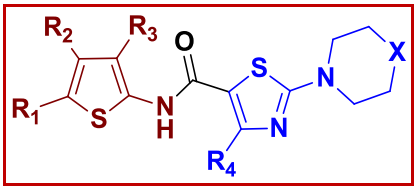
After establishing the synthetic methodology, we synthesized library of compounds with structural divercification (PMCDP-n, Table 4.1).

Table 4.1 Synthesis of *N*-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes



No. 1-10

No	Name	R_1	R_2	R_3	R_4	R_5
1	PMCDP-16	$-(CH_2)_4-$		-COOEt	-H	Ph
2	PMCDP-17	$-(CH_2)_4-$		-COOEt	-H	4-Me Ph
3	PMCDP-18	$-(CH_2)_4-$		-COOEt	-H	4-Cl Ph
4	PMCDP-23	-COOMe	-Me	-COOEt	-H	4-Cl Ph
5	PMCDP-31	$-(CH_2)_4-$		-CN	-H	4-Cl Ph
6	PMCDP-32	-COOMe	-Me	-COOEt	-H	Ph
7	PMCDP-33	$-(CH_2)_4-$		$-CONH_2$	-H	4-Cl Ph
8	PMCDP-36	-COOMe	-Me	-CN	$-NH_2$	4-Me Ph
9	PMCDP-40	-Me	-H	-COOEt	-H	4-F Ph
10	PMCDP-41			$-CONH_2$	$-NH_2$	Ph



No. 11-19

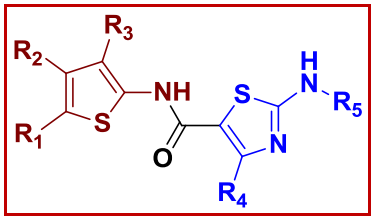
No	Name	R_1	R_2	R_3	R_4	X
11	PMCDP-24	$-(CH_2)_4-$		-COOEt	Ph	O
12	PMCDP-26	$-(CH_2)_4-$		-CN	Ph	N-Me
13	PMCDP-27	$-(CH_2)_4-$		-CN	Furan-2-yl	N-Me

14	PMCDP-28	-COOMe	Me	-COOEt	Furan-2-yl	O
15	PMCDP-29	-$(CH_2)_4$-		-COOEt	Furan-2-yl	O
16	PMCDP-30	-COOMe	Me	-COOEt	Ph	O
17	PMCDP-34	-COOMe	Me	$-CONH_2$	Furan-2-yl	O
18	PMCDP-35	-COOMe	Me	-CN	Furan-2-yl	O
19	PMCDP-37	-COOMe	Me	-CN	Furan-2-yl	N-Me

4.3 Biological results and discussion

The binding affinity of the compounds was evaluated as per the procedure described in the chapter-3. The compounds **PMCDP-24**, **PMCDP-29** and **PMCDP-41** found to show affinity towards A_1, A_{2A} and A_3 adenosine receptors with Ki values in micromolar range. The compound **PMCDP-29** was non-selective with hA_1/hA_3=1.7 and hA_{2A}/hA_3=2.1. The compound **PMCDP-24** was hA_1/hA_3>10 and hA_{2A}/hA_3>10 fold selective and **PMCDP-41** was hA_1/hA_3>2 and hA_{2A}/hA_3>2 fold selective. Presence of phenyl or 2-furyl group on the 4th position of thiazole ring was found to be responsible for good activity for this series of compounds. Whereas presence of hydrogen on the 4th position of thiazole ring was found to be deleterious for the biological activity (Table 4.2).

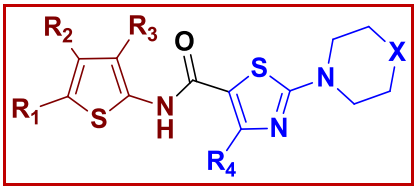
Table 4.2 Binding affinity of synthesized molecules



No. 1 to10

No	Compound	Ki in µM at 95 % Confidence Limits			
		hA_1[a]	hA_{2A}[b]	hA_{2B}[d]	hA_3[c]
1	PMCDP-16	>100	>100	>10	>100
2	PMCDP-17	>10	>10	>10	>3
3	PMCDP-18	>30	>30	>10	>30

4	**PMCDP-23**	>10	>10	>10	>10
5	**PMCDP-31**	>100	>100	>10	>30
6	**PMCDP-32**	>10	>10	>10	>3
7	**PMCDP-33**	N.D.	N.D.	N.D.	N.D.
8	**PMCDP-36**	>10	>10	>10	>10
9	**PMCDP-40**	>30	>100	>10	14.5
10	**PMCDP-41**	>10	>10	>10	**4.9**



No. 11-19

No	Compound	Ki in µM at 95 % Confidence Limits			
		hA_1[a]	hA_{2A}[b]	hA_{2B}[d]	hA_3[c]
11	**PMCDP-24**	>30	>30	>10	**2.9**
12	**PMCDP-26**	>100	>100	>100	>100
13	**PMCDP-27**	>10	>10	>10	>10
14	**PMCDP-28**	>30	18	>10	7.3
15	**PMCDP-29**	**7.2**	**9.1**	>10	**4.3**
16	**PMCDP-30**	>10	>10	>10	>10
17	**PMCDP-34**	>100	>100	>10	>100
18	**PMCDP-35**	>10	>10	>10	>10
19	**PMCDP-37**	>10	8.3	>10	>10

Data are expressed as geometric means with 95% confidence limits.
[a]Displacement of specific [^{3}H]CCPA binding at human A_1 receptors expressed in CHO cells.
[b]Displacement of specific [^{3}H]NECA binding at human A_{2A} receptors expressed in CHO cells.
[c] Displacement of specific [^{3}H]HEMADO binding at human A_3 receptors expressed in CHO cells.
[d]Inhibition of NECA-stimulated adenylyl cyclase activity at human A_{2B} receptors expressed in CHO cells.

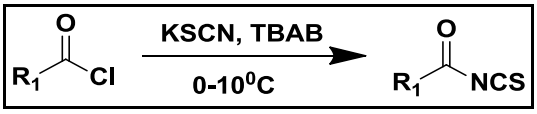
4.4 Conclusion

In conclusion, here we have designed new series of new *N*-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes. Further, the molecules were synthesized and evaluated for their radio ligand binding assay against adenosine receptors. The compounds were active towards adenosine receptors and particularly they were found selectivity against A_3 adenosine receptor with high selectivity. But overall the affinity of the molecules to adenosine receptor was found to be less as compared to affinity of the molecules from chapter-3.

4.5 Experimental

Melting points were recorded on scientific melting point apparatus (Veego; Model: VMP-DS) and are uncorrected. The ^{1}H NMR spectra were recorded on Bruker NMR spectrometer (400 MHz) using TMS as an internal standard and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer at 75 MHz. Proton chemical shifts are expressed in ppm relative to internal tetramethylsilane. Mass spectra were recorded on Perkin Elmer Sciex API 165. TLC was carried out on Merck Kieselgel 60 PF_{254}. IUPAC name of the compounds were generated using Cambridge soft ChemBioDraw ultra 12.0. The preparations of substituted 2-chloroacetamido thiophenes are described in the chaptet-3.

4.5.1. General procedure for the synthesis of Aroyl isothiocyanates



A mixture of aroyl chloride (1 mmole), and tetra butyl ammonium bromide (3 mol %) was stirred at 0-10^{0}C in chloroform. Potassium thiocyanate(1 mmol) solution in water was added drop wise over a 25 minutes and stirring is continued for an additional 2 hours at room temperature. The mixture was then separated and the water layer was extracted with chlorform. The combined organic layer was dried with magnesium sulphate. This solution was filtered and the solvent was evaporated under reduced pressure to yield crude aroyl isothiocyanate as oil. It was stored under nitrogen at 0°C. It was used further without purification.

4.5.1.1. Synthesis of Benzoyl Isothiocyanate

Benzoyl isothiocyanate was synthesized using the procedure as described in 4.5.1 by the reaction of benzoyl chloride and pottasium thiocyanate to afford crude product. It was used further without purification. Rf: 0.82 (Chloroform).

4.5.1.2. Synthesis of Furoyl Isothiocyanate

Furoyl isothiocyanate was synthesized using the procedure as described in 4.5.1 by the reaction of furoyl chloride and pottasium thiocyanate to afford crude product. It was used further without purification. Rf: 0.70 (Chloroform).

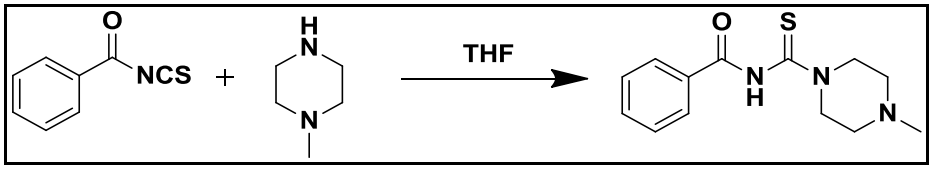
4.5.2. General procedure for the synthesis *N*-Cyclicamino carbanothioyl arylamide

To a stirred solution of aroyl isothiocynate (1 mmol) in THF at room temperature, morpholine (1eq) was added dropwise via syringe. The reaction was stirred for 3h and reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into the cold water to give precipitates of the desired product. The resultant solid was filtered through high vacuum suction pump and then dissolved in chloroform and dried over anhydrous Na_2SO_4. The solvent was removed on rotary evaporator to give desired compounds which was sufficiently pure and directly used for the next step.

4.5.2.1. Synthesis of N-(Morpholine-4-carbonothioyl)benzamide (C1)

The title compound was synthesized using the procedure as described in 4.5.2 by the reaction of benzoyl isothiocyante and morpholine to afford the product. **%Yield:** 58, **LCMS:** (M+1) at 251, **Molecular Formula:** $C_{12}H_{14}N_2O_2S$, **m.p.=** 141-3°C, **Rf:** 0.71 (Tol :ACN :: 7:3).

4.5.2.2. Synthesis of N-(4-Methylpiperazine-1-carbonothioyl)benzamide (C2)

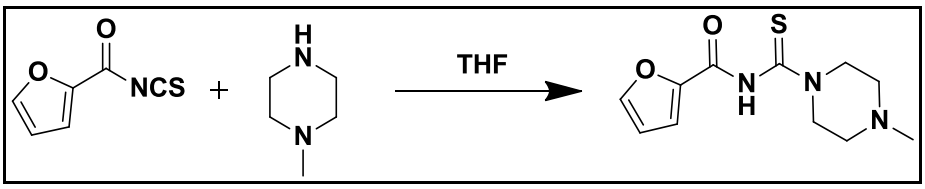


The title compound was synthesized using the procedure as described in 4.5.2 by the reaction of benzoyl isothiocyante and 4-methyl piperazine to afford the product.

4.5.2.3. Synthesis of N-(Morpholine-4-carbonothioyl)furan-2-carboxamide (C3)

The title compound was synthesized using the procedure as described in 4.5.2 by the reaction of furoyl isothiocyante and morpholine to afford the product.

4.5.2.4. Synthesis of N-(4-Methylpiperazine-1-carbonothioyl)furan-2-carboxamide(C4)



The title compound was synthesized using the procedure as described in 4.5.2 by the reaction of benzoyl isothiocyante and 4-methyl piperazine to afford the product.

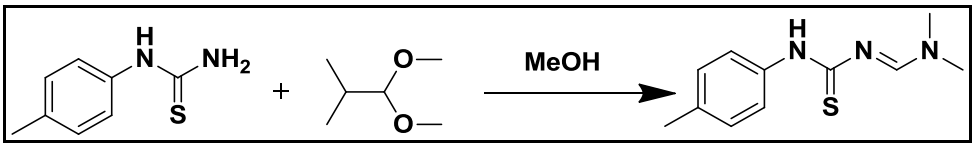
4.5.3. General procedure for the synthesis of Amidinothiourea

To a stirred solution of *N*-aryl thiourea (1 mmol) in methanol at room temperature, *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA, 1.5 mmol) were added drop wise via syringe. The resultant reaction mixture was stirred for 2 hours to give white precipitates of the *N*-monosubstituted thiourea (amidinothiourea). The solid was filtered and washed with methanol to give sufficiently pure product which was directly used for the next step.

4.5.3.1. Synthesis of (E)-N,N-Dimethyl-N'-(phenylcarbamothioyl) formimidamide(C5)

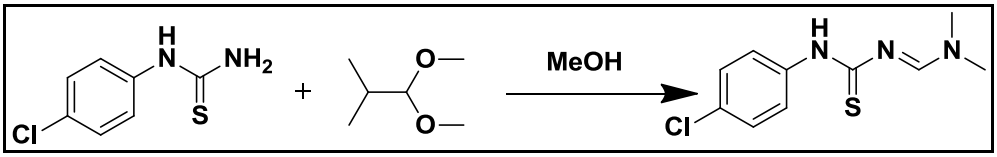
The title compound was synthesized using the procedure as described in 4.5.3 by the reaction of *N*-phenyl thiourea and *N*,*N*-dimethylformamide dimethylacetal(DMF-DMA) to afford the title product.

4.5.3.2. Synthesis of (E)-N,N-Dimethyl-N'-(p-tolylcarbamothioyl) formimidamide(C6)



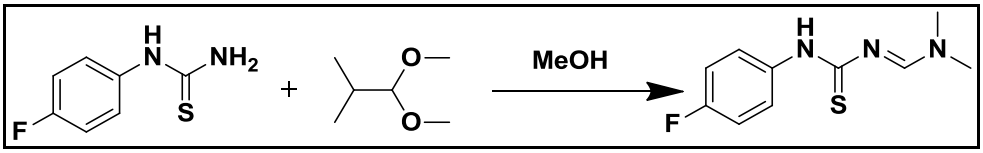
The title compound was synthesized using the procedure as described in 4.5.3 by the reaction of *N*-(p-tolyl) thiourea and *N*,*N*-dimethylformamide dimethylacetal(DMF-DMA) to afford the title product.

4.5.3.3. Synthesis of (E)-N'-((4-Chlorophenyl)carbamothioyl)-N,N-dimethylformimidamide (C7)



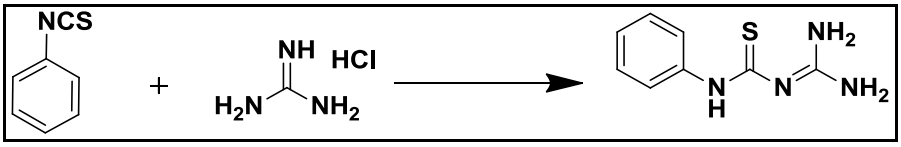
The title compound was synthesized using the procedure as described in 4.5.3 by the reaction of *N*-(4-chloro phenyl) thiourea and *N*,*N*-dimethylformamide dimethylacetal(DMF-DMA) to afford the title product.

4.5.3.4. Synthesis of (E)-N'-((4-Fluorophenyl)carbamothioyl)-N,N-dimethylformimidamide (C8)



The title compound was synthesized using the procedure as described in 4.5.3 by the reaction of *N*-(4-fluoro phenyl) thiourea and *N*,*N*-dimethylformamide dimethylacetal(DMF-DMA) to afford the title product.

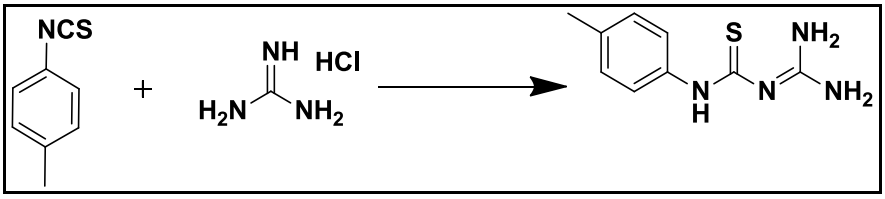
4.5.3.5. Synthesis of 1-Amidino-3-phenyl thiourea (C9)



To a stirred suspension of guanidine hydrochloride (3 mmole) and phenyl isothiocyanate (1 mmole) in THF, was added sodium hydroxide (3 mmole) dissolved in water at 0°C in

10 min. Stirring was continued at ambient temperature for 4 hours. The solid generated during the reaction was filtered with 10 ml water and washed with water and dried to give the title product. TLC : Mobile Phase : Ethylacetate, Rf : 0.63. Mass (LC-MS Found, M+1) : 195

4.5.3.6. Synthesis of 1-Amidino-3(4-methylphenyl)thiourea (C10)



To a stirred suspension of guanidine hydrochloride (3 mmole) and 4-methyl phenyl isothiocyanate (1 mmole) in THF, was added sodium hydroxide (3 mmole) dissolved in water at 0°C in 10 min. Stirring was continued at ambient temperature for 4 hours. The the solid generated during the reaction was filtered with 10 ml water and washed with water and dried to give the title product.

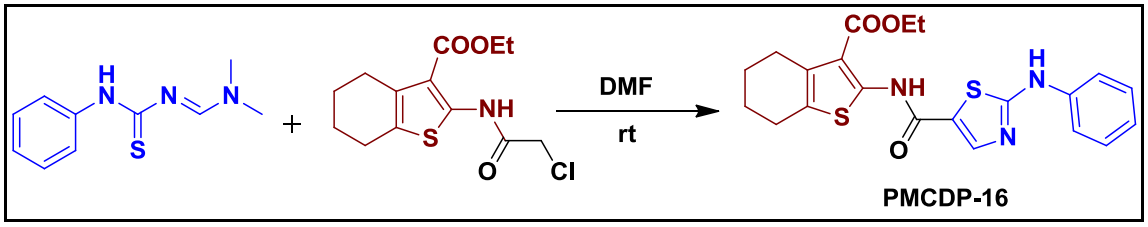
4.5.4. General procedure for synthesis of disubstituted/diamino thiazoles coupled with thiophenes

To a hot air dried round bottomed flask, containing a solution of amidinothiourea (1 mmol) in DMF (5 mL), 2-chloroacetamidothiophene (1 mmol) in DMF (5 mL) was added at ambient temperature and the reaction was further stirred for 6-8 h with stirring. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through Buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-n)** were assigned with the help of NMR and mass spectra.

4.5.5. General procedure for synthesis of *N*-Cycloaminothiazoles coupled with thiophenes

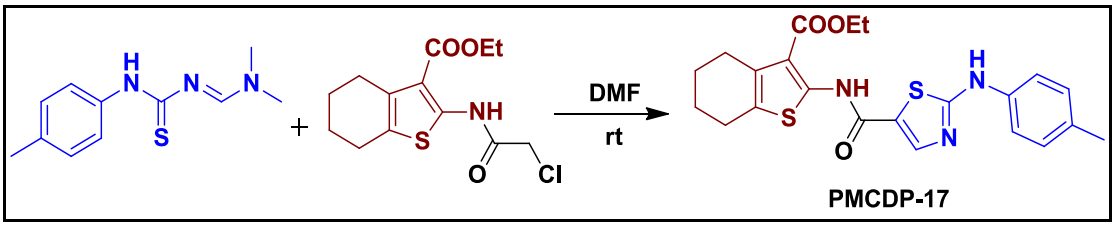
To a hot air dried round bottomed flask, containing a solution of *N*-cyclicamino carbanothioyl arylamides in DMF (5 mL), 2-chloroacetamidothiophene in DMF (5 mL) was added at ambient temperature and the reaction was further stirred for 6-8 h with stirring. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through Buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-n)** were assigned with the help of NMR and mass spectra.

PMCDP-16: Ethyl 2-(2-(phenylamino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



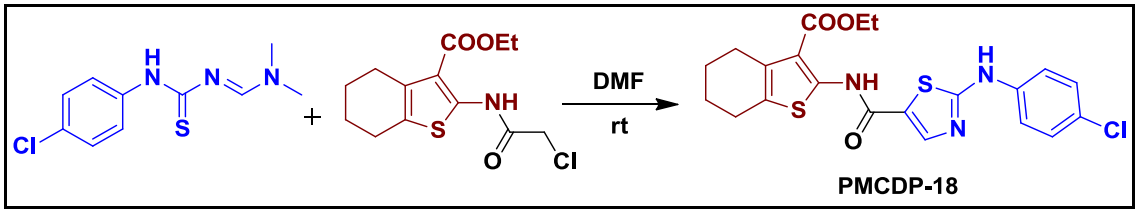
Yield: 63%, Off white solid, mp: 226-229 ^{0}C, **Molecular formula: $C_{21}H_{21}N_3O_3S_2$**, LC-MS calculated: 427.1, found: 428.3(M+1), **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.41(t, 3H), 1.82(d, 4H), 2.69(s, 2H), 2.81(s, 2H), 4.37(q, 2H), 7.18(d, 1H), 7.40-7.46(m, 4H), 7.97(s, 1H), 8.20(s, 1H, -NH), 12.02(s, 1H,-NH). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 14.37, 22.84, 23, 24.43, 26.41, 30.96, 60.62, 111.51, 119.23, 124.54, 126.96, 129.80, 130.99, 139.15, 142.61, 147.70, 157.48, 166.94.

PMCDP-17: Ethyl 2-(2-(p-tolylamino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



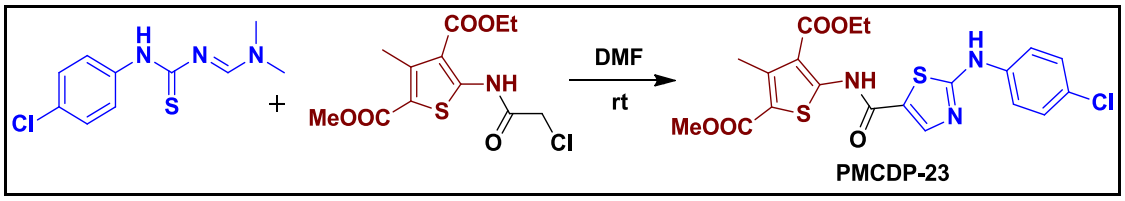
Yield: 67%, Light yellow solid, mp: 235-239 ^{0}C, **Molecular formula: $C_{22}H_{23}N_3O_3S_2$,** LC-MS calculated: 441.3, found: 442.3(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.41(t, 3H), 1.82(s, 4H), 2.38(s, 3H), 2.68(s, 2H), 2.81(s, 2H), 4.36(q, 2H), 7.23(d, 2H), 7.28(d, 2H), 7.92(s, 1H), 7.95(s, 1H, -NH), 11.98(s, 1H, -NH).

PMCDP-18: Ethyl 2-(2-((4-chlorophenyl)amino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



Yield: 67%, Light yellow solid, mp: 230-233 ^{0}C, **Molecular formula: $C_{21}H_{20}ClN_3O_3S_2$,** LC-MS calculated: 461.1, found: 462.3(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.31(t, 3H), 1.72(s, 4H), 2.61(s, 2H), 2.71(s, 2H), 4.28(q, 2H), 7.39(d, 2H), 7.65(d, 2H), 7.92(s, 1H), 10.95(s, 1H, -NH), 11.58(s, 1H, -NH).

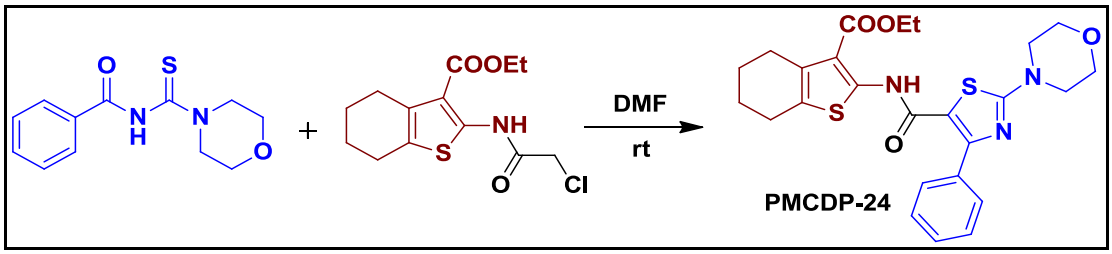
PMCDP-23: 4-Ethyl 2-methyl 5-(2-((4-chlorophenyl)amino)thiazole-5-carboxamido)-3-methylthiophene-2,4-dicarboxylate



Yield: 68%, White solid, mp: >250 ^{0}C, **Molecular formula: $C_{20}H_{18}ClN_3O_5S_2$,** LC-MS calculated: 479.1, found: 480.4(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.35(t, 3H),

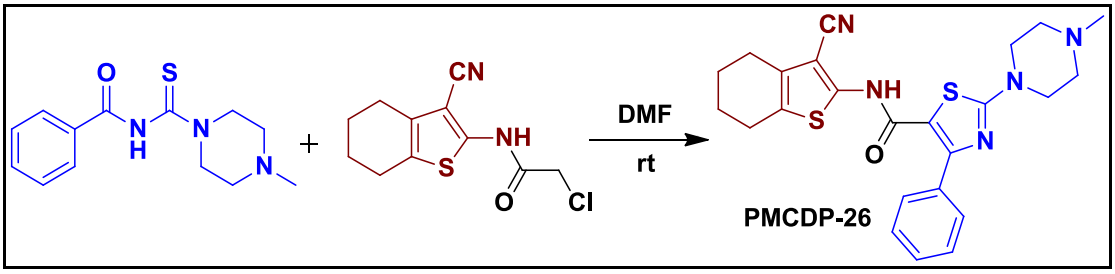
2.67(s, 3H), 3.79(s, 3H), 4.34(q, 2H), 7.39(d, 2H), 7.65(d, 2H), 7.98(s, 1H), 11.03(s, 1H, -NH), 11.73(s, 1H, -NH).

PMCDP-24: Ethyl 2-(2-morpholino-4-phenylthiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



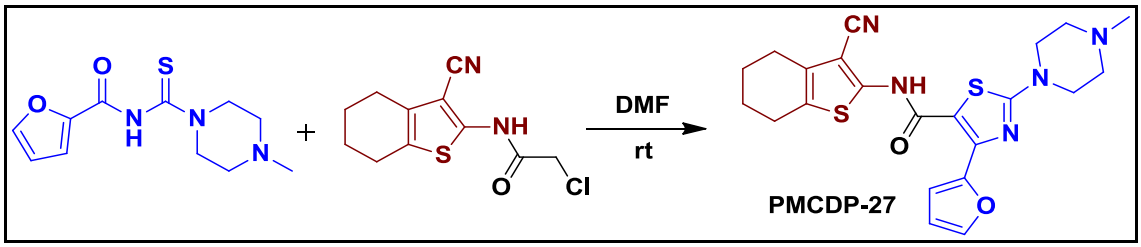
Yield: 74%, Off white solid, mp: 235-237 ^{0}C, **Molecular formula: $C_{25}H_{27}N_3O_4S_2$**, LC-MS calculated: 497.2, found: 498.4(M+1).**^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.25(t, 3H), 1.78(s, 4H), 2.65(s, 2H), 2.72(s, 2H), 3.62(s, 4H), 3.85(s, 4H), 4.06(q, 2H), 7.42(d, 3H), 7.67(d, 2H), 11.20(s, 1H).

PMCDP-26: *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-2-(4-methyl piperazin-1-yl)-4-phenylthiazole-5-carboxamide



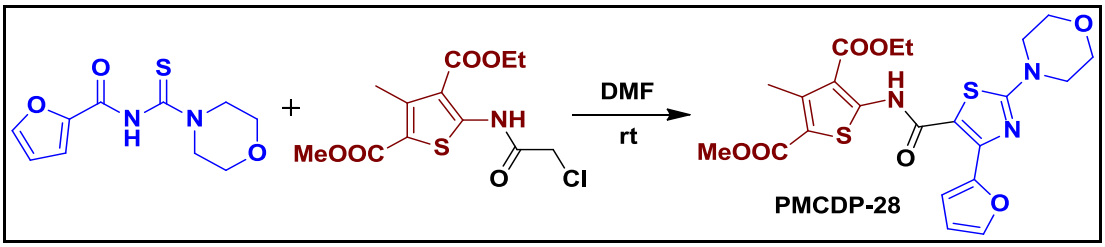
Yield: 72%, Off white solid, mp: 219-222 ^{0}C, **Molecular formula: $C_{24}H_{25}N_5O_1S_2$**, LC-MS calculated: 463.4, found: 464.4(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.74(s, 4H), 2.46(s, 2H), 2.59(s, 2H), 2.85(s, 3H), 3.36(s, 8H), 7.43(s, 3H), 7.62(s, 2H), 10.49(s, 1H), 10.84(s, 1H).

PMCDP-27: *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-4-(furan-2-yl)-2-(4-methylpiperazin-1-yl)thiazole-5-carboxamide



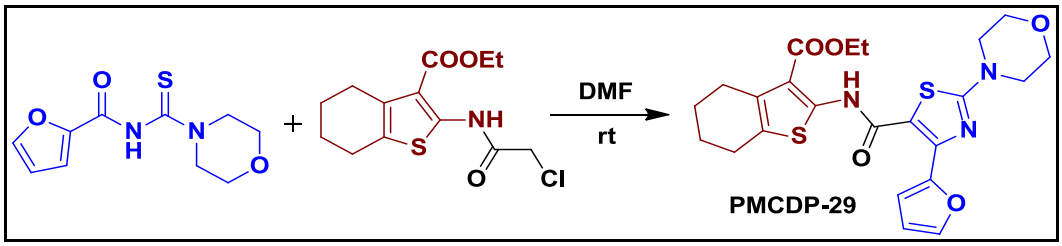
Yield: 69%, Off white solid, mp: >250 ^{0}C, **Molecular formula: $C_{22}H_{23}N_5O_2S_2$**, LC-MS calculated: 453.4, found: 454.4(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.77(s, 4H), 2.67(s, 2H), 2.74(s, 2H), 3.52(t, 4H), 3.71(t, 4H), 6.70(s, 1H), 7.08(d, 1H), 7.85(s, 1H), 11.49(s, 1H, -NH).

PMCDP-28: 4-Ethyl 2-methyl 5-(4-(furan-2-yl)-2-morpholinothiazole-5-carboxamido)-3-methylthiophene-2,4-dicarboxylate



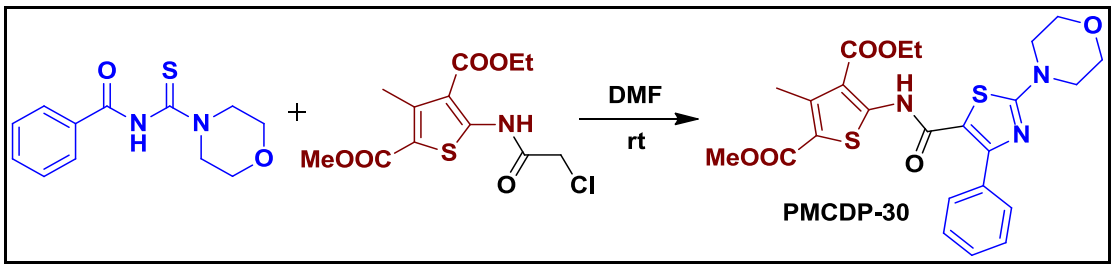
Yield: 59%, Off white solid, mp: 182-185 ^{0}C, **Molecular formula: $C_{22}H_{23}N_3O_7S_2$**, LC-MS calculated: 505.1, found: 506.7(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.41(t, 3H), 2.80(s, 3H), 3.64(s, 4H), 3.87(s, 7H), 6.58(s, 1H), 7.18(s, 1H), 7.73(s, 1H), 12.52(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 14.27, 15.60, 47.93, 51.59, 60.89, 66.07, 111.81, 113.92, 114.63, 115.57, 117.42, 143.24, 144.31, 144.82, 148.86, 152.93, 158.98, 163.60, 165.85, 170.86.

PMCDP-29: Ethyl 2-(4-(furan-2-yl)-2-morpholinothiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



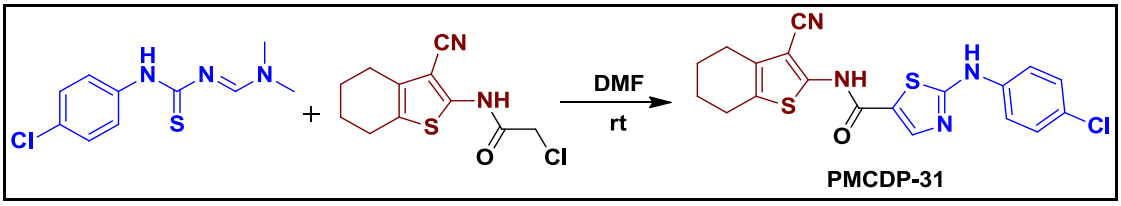
Yield: 59%, White solid, mp: 199-202 ^{0}C, **Molecular formula: $C_{23}H_{25}N_3O_5S_2$,** LC-MS calculated: 487.1, found: 487.5(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.24(t, 3H), 1.73(s, 4H), 2.61(s, 2H), 2.72(s, 2H), 3.52(t, 4H), 3.72(t, 4H), 4.21-4.28(s, 2H), 6.68(q, 1H), 7.10 (d, 1H), 7.86(s, 1H), 11.99(s, 1H, -NH).

PMCDP-30: 4-Ethyl 2-methyl 3-methyl-5-(2-morpholino-4-phenylthiazole-5-carboxamido)thiophene-2,4-dicarboxylate



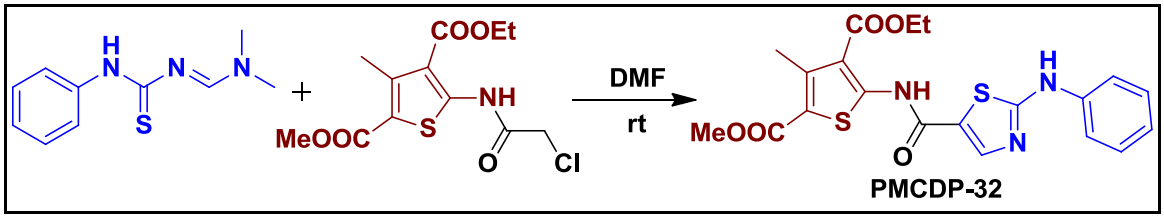
Yield: 59%, White solid, mp: 197-199 ^{0}C, **Molecular formula: $C_{24}H_{25}N_3O_6S_2$,** LC-MS calculated: 515.1, found: 515.6(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.41(t, 3H), 2.80(s, 3H), 3.64(s, 4H), 3.87(s, 7H), 6.58(s, 1H), 7.18(s, 1H), 7.73(s, 1H), 12.52(s, 1H) **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 14.27, 15.60, 47.93, 51.59, 60.89, 66.07, 111.81, 113.92, 114.63, 115.57, 117.42, 143.24, 144.31, 144.82, 148.86, 152.93, 158.98, 163.60, 165.85, 170.86.

PMCDP-31: 2-((4-Chlorophenyl)amino)-N-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*] thiophen-2-yl)thiazole-5-carboxamide



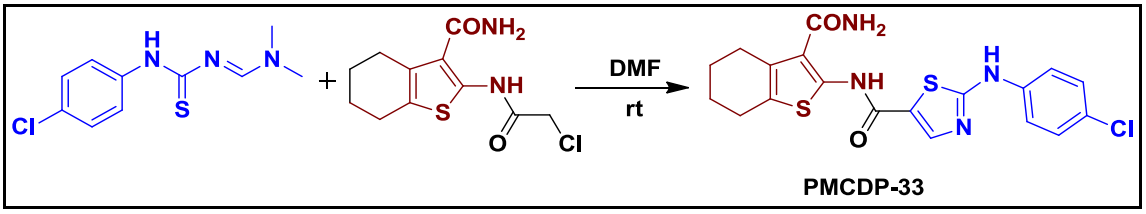
Yield: 60%, White solid, mp: 203-206 ^{0}C, **Molecular formula: $C_{19}H_{15}ClN_4OS_2$,** LC-MS calculated: 414.1, found: 415.6(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.73(s, 4H), 2.60(s, 2H), 2.72(s, 2H), 7.39(d, 2H), 7.65(d, 2H), 7.92(s, 1H), 10.98(s, 1H, -NH), 11.72(s, 1H, -NH).

PMCDP-32: 4-Ethyl 2-methyl 3-methyl-5-(2-(phenylamino)thiazole-5-carboxamido)thiophene-2,4-dicarboxylate



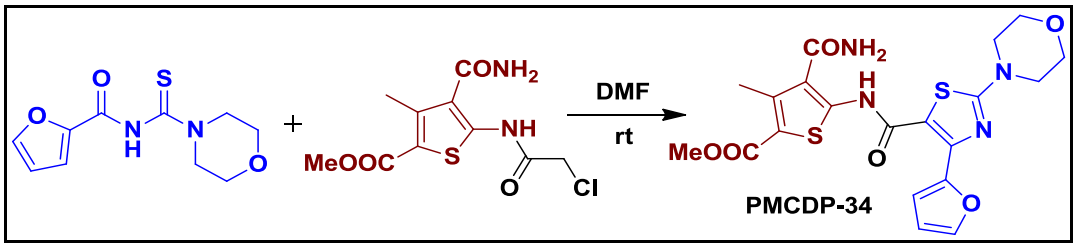
Yield: 57%, White solid, **Molecular formula: $C_{20}H_{19}N_3O_5S_2$,** LC-MS calculated: 445.1, found: 446.4(M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.32(t, 3H), 2.26(s, 3H), 2.90(s, 6H), 3.78(s, 3H), 4.33(q, 2H), 7.19(d, 1H), 7.40-7.46(m, 4H), 7.97(s, 1H), 10.20(s, 1H, -NH), 12.02(s, 1H,-NH).

PMCDP-33: *N*-(3-Carbamoyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-2-((4-chlorophenyl)amino)thiazole-5-carboxamide



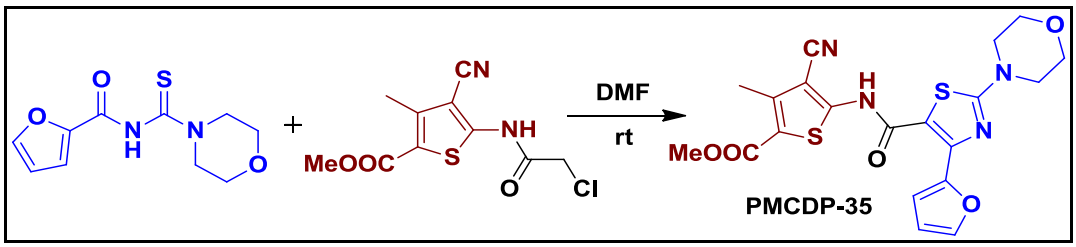
Yield: 54%, White solid, **Molecular formula: $C_{19}H_{17}ClN_4O_2S_2$,** LC-MS calculated: 432.1, found: 433.7(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.71(s, 4H), 2.61(s, 2H), 2.74(s, 2H), 6.39(s, 2H,-NH_2), 7.40(d, 2H), 7.63(d, 2H), 7.92(s, 1H), 10.98(s, 1H, -NH), 11.72(s, 1H, -NH).

PMCDP-34: Methyl 4-carbamoyl-5-(4-(furan-2-yl)-2-morpholinothiazole-5-carboxamido)-3-methylthiophene-2-carboxylate



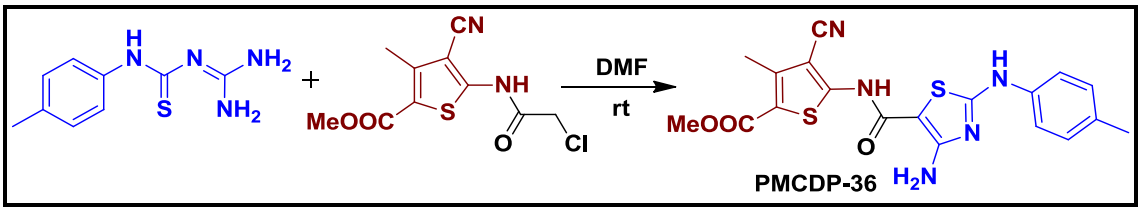
Yield: 50%, White solid, **Molecular formula: $C_{22}H_{22}N_4O_5S_2$**, LC-MS calculated: 486.1, found: 487.7(M+1).

PMCDP-35: Methyl 4-cyano-5-(4-(furan-2-yl)-2-morpholinothiazole-5-carboxamido)-3-methylthiophene-2-carboxylate



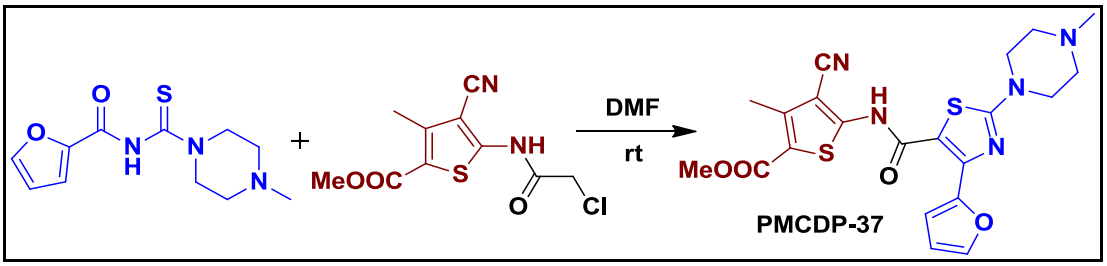
Yield: 53%, off white solid, **Molecular formula: $C_{22}H_{20}N_4O_4S_2$**, LC-MS calculated: 468.1, found: 469.4(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 2.82(s, 3H), 3.65(s, 4H), 3.78(s, 3H), 3.87(s, 4H), 6.58(s, 1H), 7.18(s, 1H), 7.73(s, 1H), 12.52(s, 1H).

PMCDP-36: Methyl 5-(4-amino-2-(p-tolylamino)thiazole-5-carboxamido)-4-cyano-3-methylthiophene-2-carboxylate



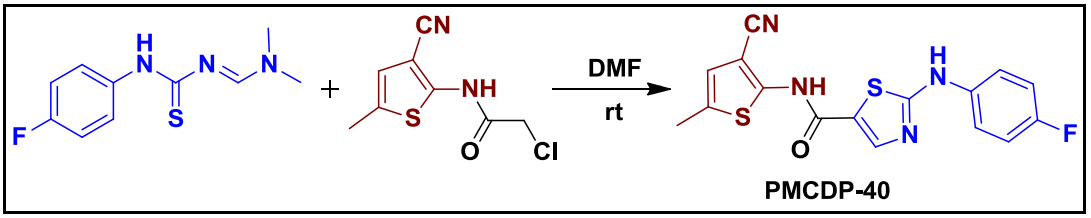
Yield: 58%, Yellow solid, mp: 193-196 ^{0}C, **Molecular formula: $C_{19}H_{17}N_5O_3S_2$**, LC-MS calculated: 427.1, found: 428.7(M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 2.38(s, 3H), 2.80(s, 3H), 3.77(s, 3H), 6.05(s, NH_2), 6.58(s, 1H), 7.37-7.40(d, 2H), 7.65-7.68(d, 2H), 10.98(s, 1H, -NH), 11.70(s, 1H, -NH).

PMCDP-37: Methyl 4-cyano-5-(4-(furan-2-yl)-2-(4-methylpiperazin-1-yl)thiazole-5-carboxamido)-3-methylthiophene-2-carboxylate



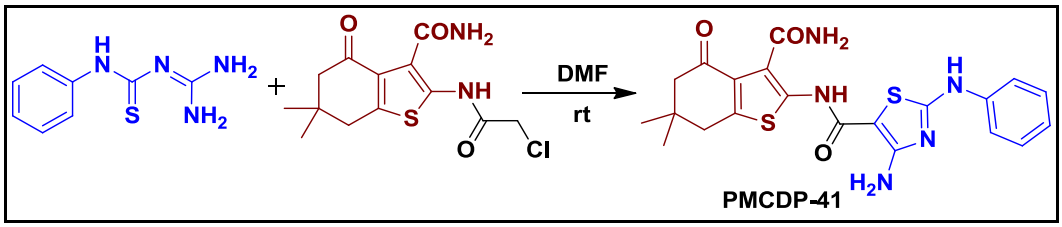
Yield: 55%, Yellow solid, **Molecular formula: $C_{21}H_{21}N_5O_4S_2$**, LC-MS calculated: 471.1, found: 472.6(M+1).

PMCDP-40: *N*-(3-Cyano-5-methylthiophen-2-yl)-2-((4-fluorophenyl)amino) thiazole-5-carboxamide



Yield: 49%, Yellow solid, **Molecular formula: $C_{16}H_{11}FN_4OS_2$**, LC-MS calculated: 358.1, found: 359.6(M+1).

PMCDP-41: 4-Amino-*N*-(3-carbamoyl-6,6-dimethyl-4-oxo-4,5,6,7-tetrahydrobenzo [*b*]thiophen-2-yl)-2-(phenylamino)thiazole-5-carboxamide



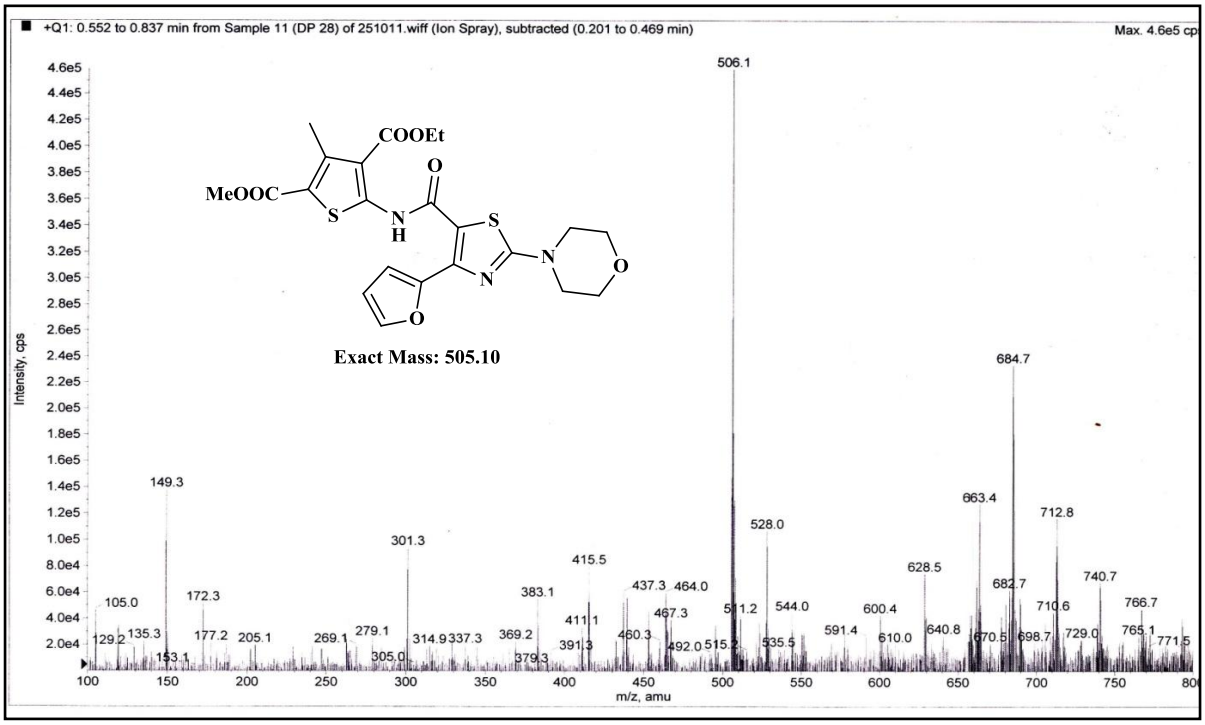
Yield: 49%, Yellow solid, **Molecular formula: $C_{21}H_{21}N_5O_3S_2$**, LC-MS calculated: 455.1, found: 455.6(M+1).

4.5.6. Method used for pharmacological evaluation

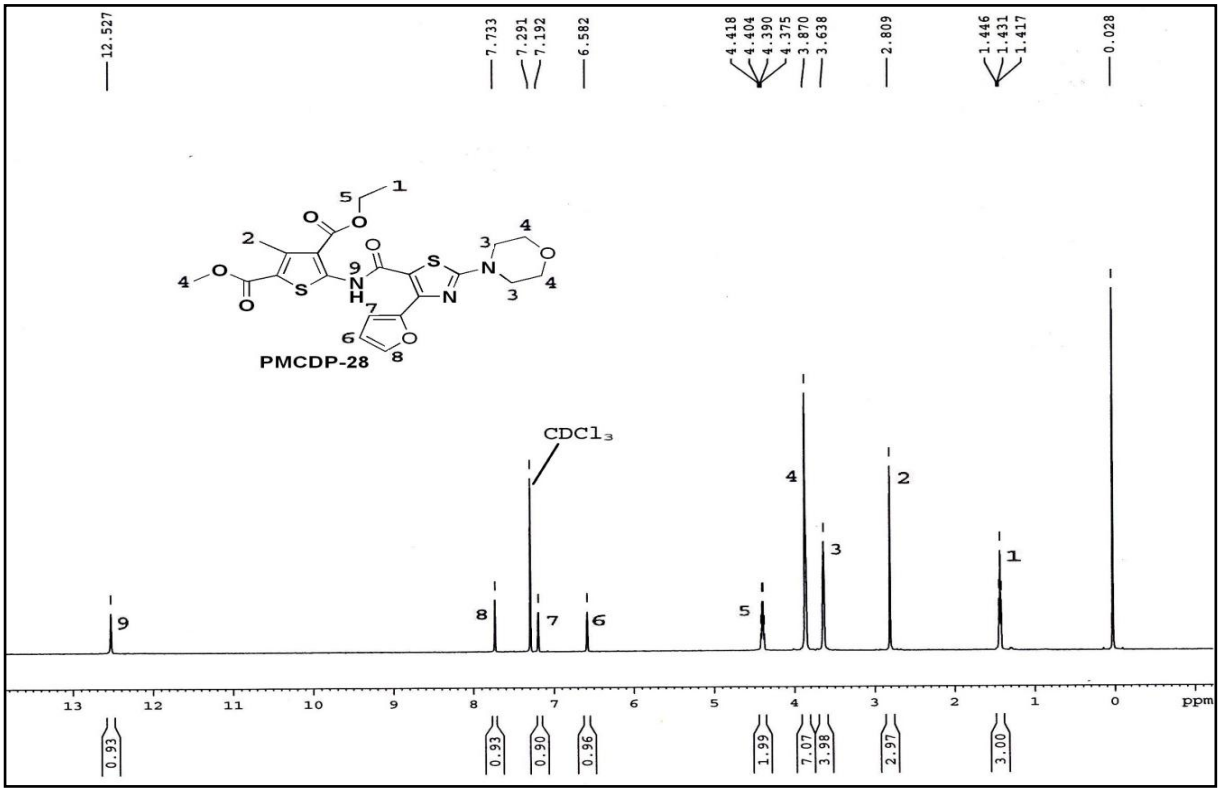
The methods described in the chapter-3 were used for the pharmacological evaluation of the compounds.

Supporting Information
(LC-MS, ^{1}H-NMR, ^{13}C NMR, of representative compounds)

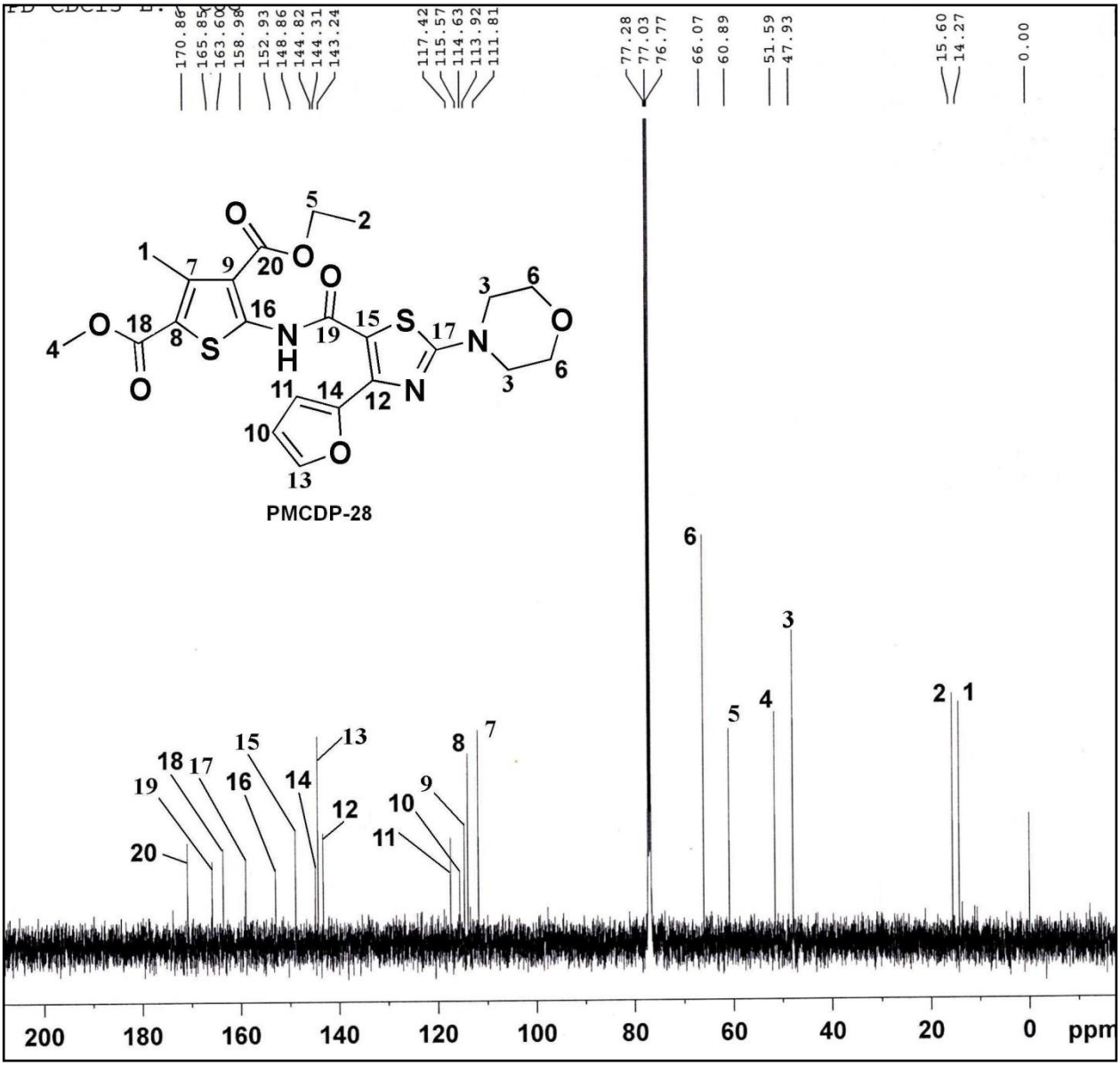
LC-MS spectrum of PMCDP-28



^{1}H spectrum of PMCDP-28



^{13}C spectrum of PMCDP-28



CHAPTER-5

Thiophenyl-thiophene carboxamides: New ligands for adenosine receptors

PAGE NO. 83 TO 103

Chapter 5

Thiophenyl-thiophene carboxamides: New ligands for adenosine receptors

5.1 Introduction

Thiophenes are important class of heterocyclic compounds as they are found to have many applications in pharmaceutical sciences (Gronowitz, 1991), fragrance compounds (Stevens, 1993), important pharmacophores (Sperry & Wright, 2005) and polymers (Roncali, 1992). Further aminothiophene molecules are important structure found to be present in many drugs like Olanzepine and Tinoridine which has been approved for Schizophrenia and anti-inflammatory agents respectively (Figure 5.1A). 2-aminothiophene incorporating compounds have been reported to be modulators of adenosine receptor, especially as allosteric modulators (Göblyös & Ijzerman, 2011) (Figure 5.1B).

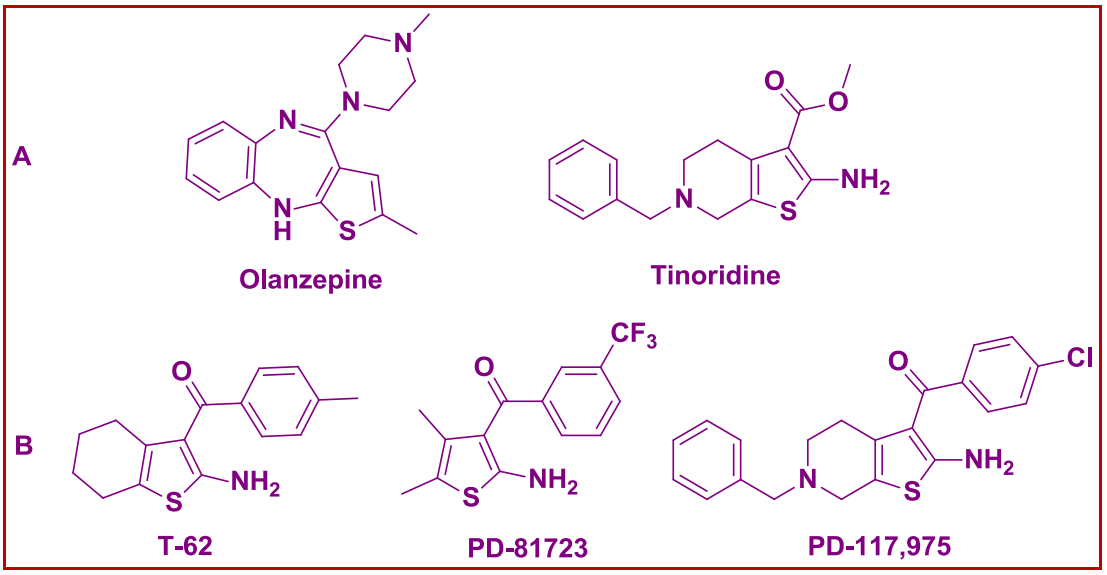


Figure 5.1 (A) Drugs containing 2-aminothiophene structure (B) 2-aminothiophene containing compounds as adenosine receptor modulators

5.2 Present work

As explained earlier thiophene moiety is found to be present in many of the pharmaceutical agents and also shown high affinity towards adenosine receptors (Aurelio et al., 2011; Valant et al., 2012). Many of the thiophene derivatives prepared in our lab like quinzoline-thiophenes, thiophenyl-thiophenes, thiazolyl-thiophenes have been found to be highly active in NF-kB and AP-1 implicated in inflammation and cancer which was further patented (Giordano, Vasu, & Sudarsanam, 2012). Dimerization of receptors either homo or heterodimers for activation have been implicated in various diseases. So combining two pharmacophoric feature in one single molecular framework can even bind to dimerised receptor and may result in distinct biological effect.

So, keeping in mind the importance of thiophene and in continuation of our earlier work, here we have modified the thiophenyl-thiazole carboxamides into thiophenyl-thiophene carboxamides as adenosine receptor ligands (Figure 5.2). As per our findings, this thiophenyl-thiophene carboxamides are new scaffold and it is checked first time in adenosine receptors for their biological activity.

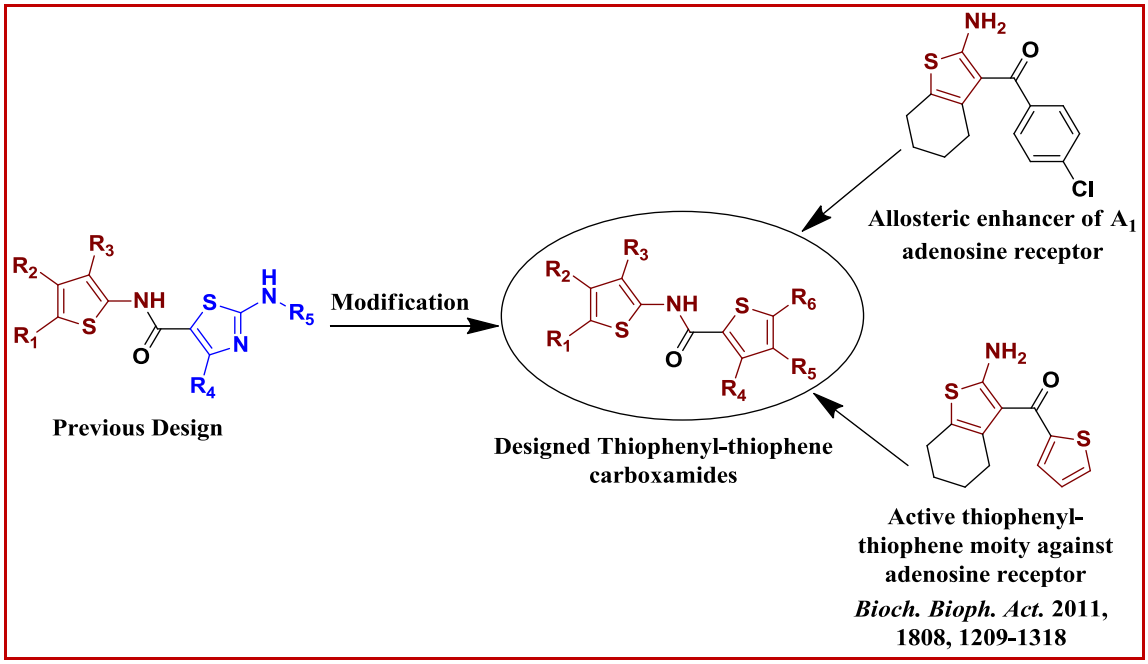
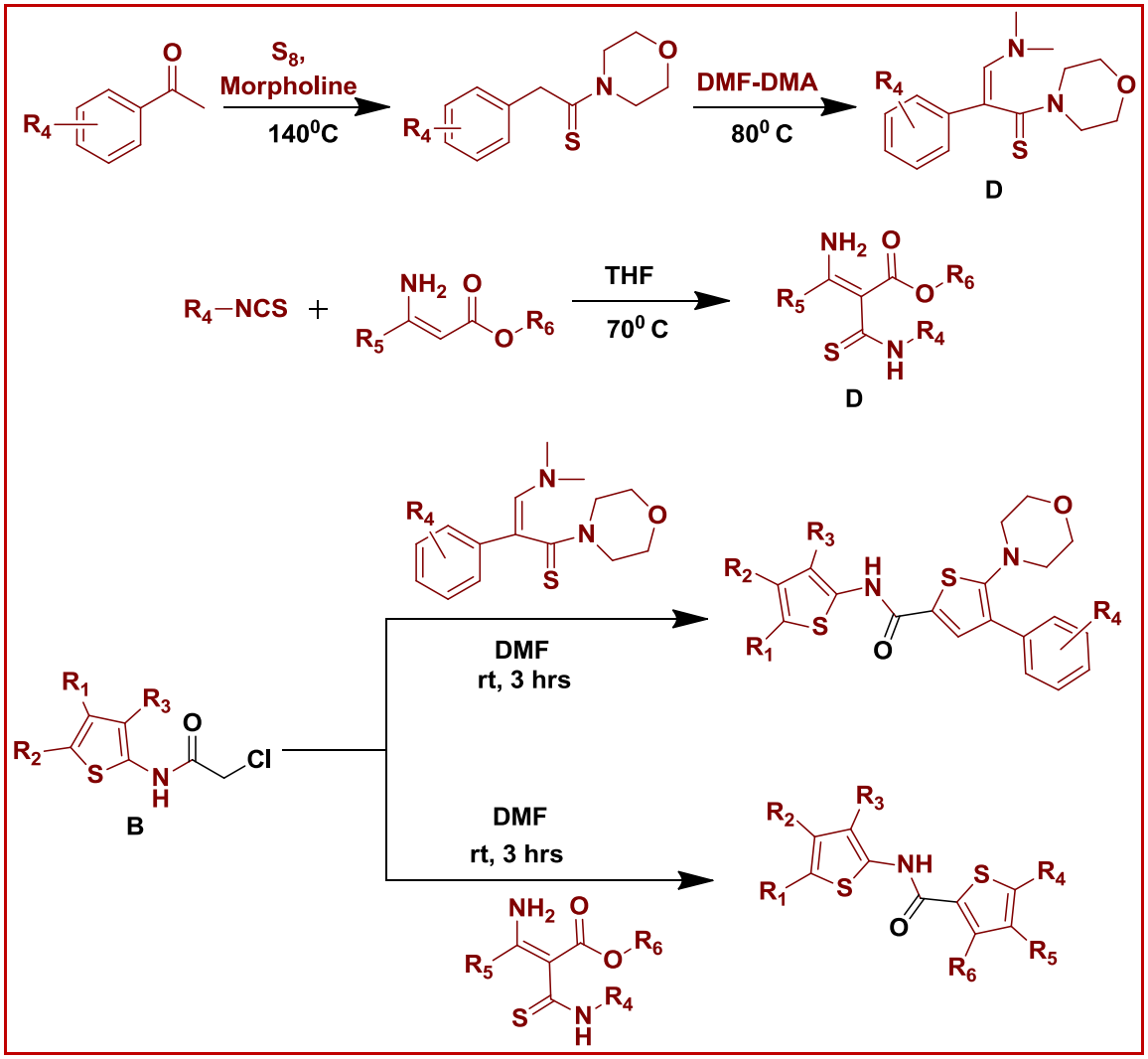


Figure 5.2 Design of thiophenyl-thiophene carboxamides

In the present work we have synthesized two different thiophenes to come up at thiophenyl-thiophene carboxamides. One thiophene formation was carried out from the Willgerodt-kindler intermediate (D), whereas the other thiophene formation was achieved by enamine-isothiocyanate (D) intermediate. These both intermediates were reacted with the 2-chloracetamido thiophene (B) derivatives to achieve the thiophenyl-thiophene carboxamides. All the compounds **(PMCDP-BTP-n)** were synthesized with good yields and they were confirmed by mass and NMRs. The two synthetic schemes are illustrated in Scheme 5.1.



Scheme 5.1 Synthetic Scheme for thiophenyl-thiophene carboxamides

As an example, willgerodt kindler intermediate *(Z)*-3-(dimethylamino)-1-morpholino-2-(pyridin-4-yl)prop-2-ene-1-thione (1 mmol) was reacted with Ethyl 2-(2-chloroacetamido)-4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (1 mmol) in DMF at room temperature which resulted into **PMCDP-BTP-3** with good yields. It was further confirmed by LC-MS (M+1) and ^{1}H NMR. The mass spectrum of **PMCDP-BTP-3** (Figure 5.3) displayed the molecular ion peak at *m/z* 498.3(M+1) which confirm the molecular weight of the compound 497.1 calculated for $C_{25}H_{21}N_3O_4S_2$.

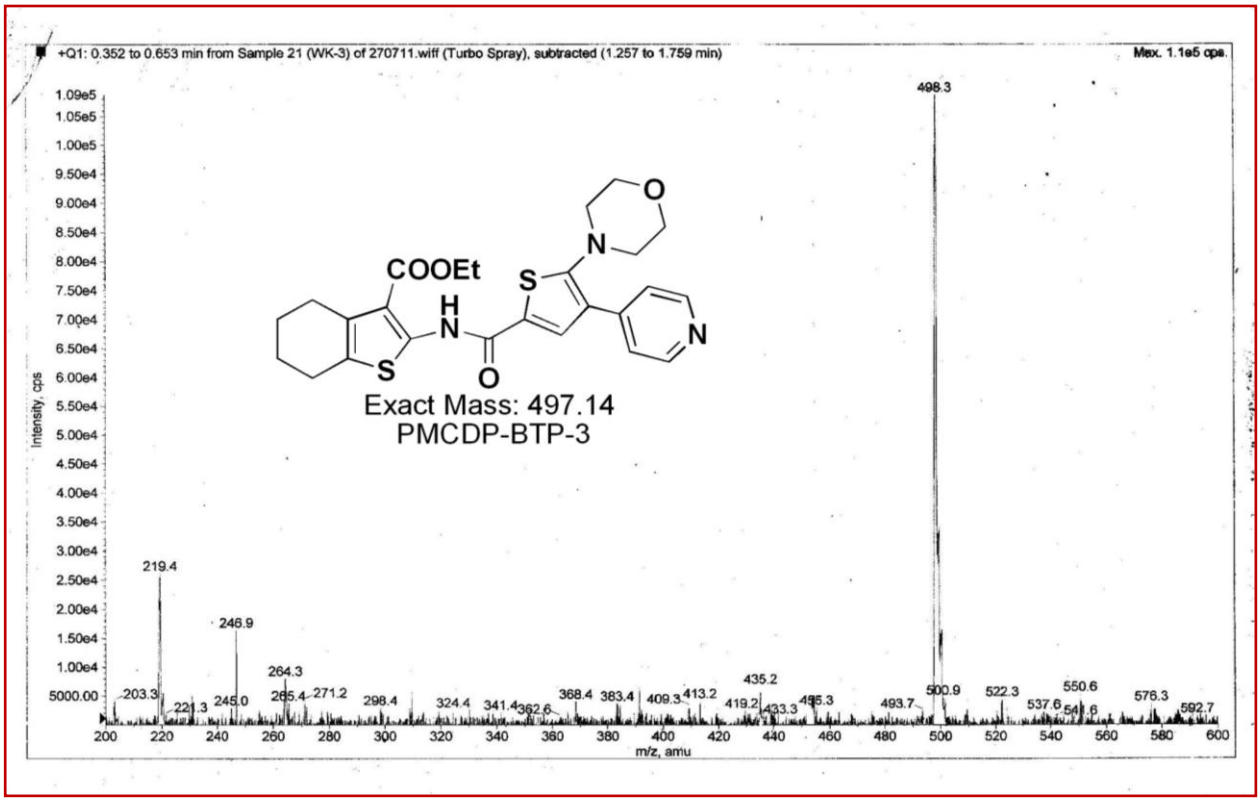


Figure 5.3 LC-MS (M+1) of **PMCDP-BTP-3**

The structure of the molecule was further confirmed by ^{1}H NMR (300 MHz, DMSO-D6), which gave the characteristic peak of all the hydrogen present. The amide proton –NH singlet came at 12.06 δ. The protons of ester methyl group (-CH_3) was observe at 1.38-1.43 δ and splited into triplet due to presence of adjacent methylene group (–CH_2). The sixteen protons of eight methylene group were found in the range 1.80-3.81 δ. The two protons of ester methylene group were found at 4.33-4.41 with quartet. All the five

aromatic protons of phenyl ring were observed from 7.62-8.66 δ and singlet hydrogen of thiophene was observed at 7.70 δ (Figure-5.4).

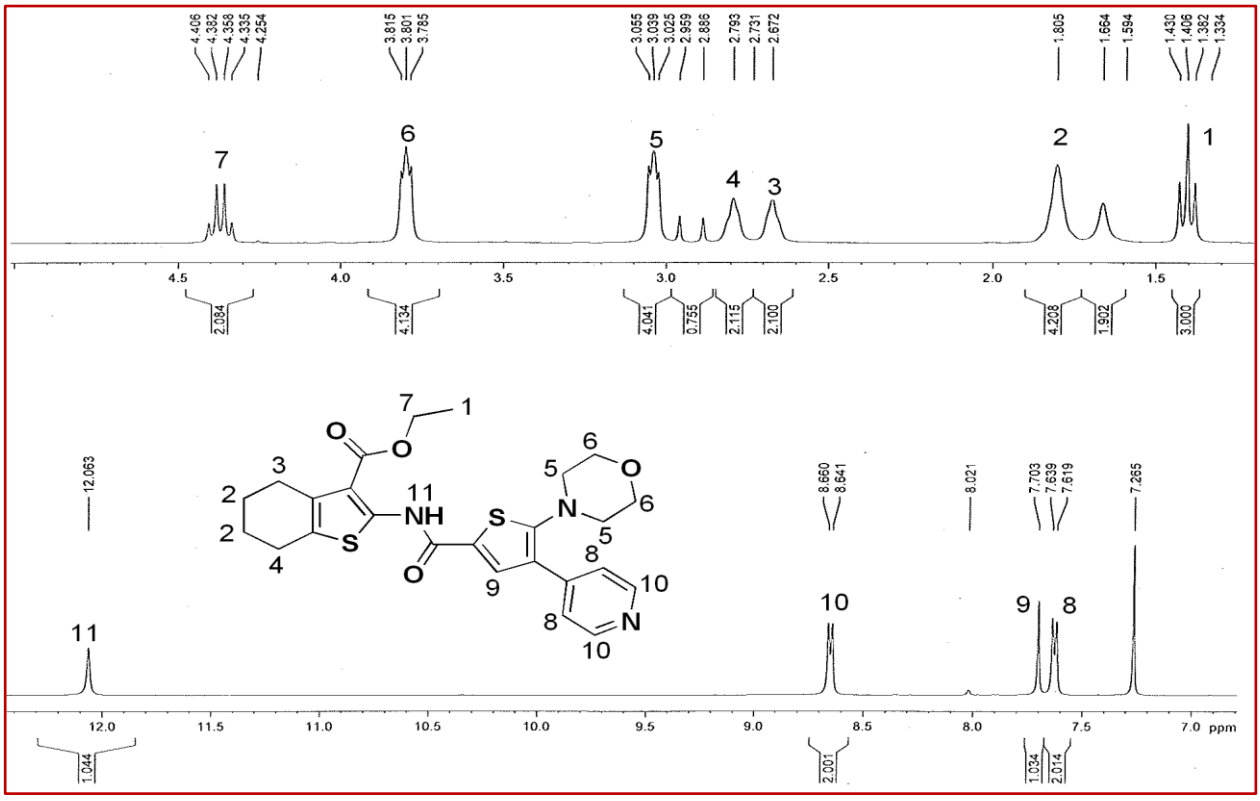
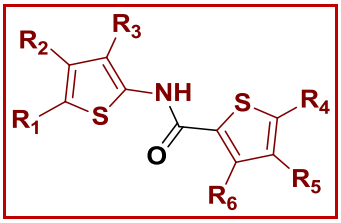


Figure 5.4 ^{1}H NMR spectrum of **PMCDP-BTP-3**

After establishing the synthetic methodology, we library of compounds with structural diversification were synthesized (**PMCDP-BTP-n, Table-5.1**).

Table 5.1 Synthesis of thiophenyl-thiophene carboxamides



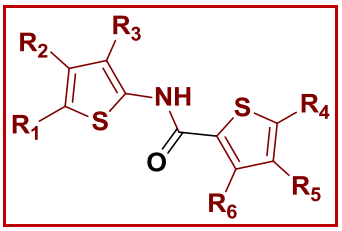
Code	R_1	R_2	R_3	R_4	R_5	R_6
PMCDP-BTP-1	**-$(CH_2)_4$-**		**-COOEt**	**Morpholin-4-yl**	**-Ph**	**-H**
PMCDP-BTP-2	**-$(CH_2)_4$-**		**-$CONH_2$**	**Morpholin-4-yl**	**-Ph**	**-H**

PMCDP-BTP-3	**-$(CH_2)_4$-**		**-COOEt**	**Morpholin-4-yl**	**Pyridin-4-yl**	**-H**
PMCDP-BTP-4	**-$(CH_2)_4$-**		**-CN**	**Morpholin-4-yl**	**-Ph**	**-H**
PMCDP-BTP-5	**-$(CH_2)_4$-**		**-COOEt**	**Morpholin-4-yl**	**p-Cl Ph**	**-H**
PMCDP-BTP-6	**-$(CH_2)_4$-**		**-$CONH_2$**	**Morpholin-4-yl**	**p-Cl Ph**	**-H**
PMCDP-BTP-7	**-COOMe**	**-Me**	**-COOEt**	**Morpholin-4-yl**	**p-Cl Ph**	**-H**
PMCDP-BTP-8	**-COOMe**	**-Me**	**-CN**	**Morpholin-4-yl**	**Pyridin-3-yl**	**-H**
PMCDP-BTP-13	**-COOMe**	**-Me**	**-COOEt**	**-NHPh(4-Me)**	**-COOMe**	**-Me**
PMCDP-BTP-14	**-$(CH_2)_4$-**		**-$CONH_2$**	**-NHCOPh (4-OMe)**	**-COOMe**	**-Me**
PMCDP-BTP-15	**-$(CH_2)_4$-**		**-$CONH_2$**	**-NHPh(4-Me)**	**-COOMe**	**-Me**
PMCDP-BTP-17	**-COOMe**	**-Me**	**-CN**	**Morpholin-4-yl**	**-Ph**	**-H**
PMCDP-BTP-19	**-$(CH_2)_4$-**		**-$CONH_2$**	**-NHPh(4-Me)**	**-COOMe**	**-Me**
PMCDP-BTP-21	**-COOMe**	**-Me**	**-CN**	**-NHCOPh (4-OMe)**	**-COOMe**	**-Me**

5.3 Biological results and discussion

The binding affinity of the synthesized compounds was evaluated as per the procedure described in the chapter-3. The compounds **PMCDP-BTP-4**, **PMCDP-BTP-8, PMCDP-BTP-13** and **PMCDP-BTP-14** found to show affinity towards A_1, A_{2A} and A_3 adenosine receptors with Ki values in micromolar range. The compound **PMCDP-BTP-13** was non-selective with Ki values of 2.5 μM, 1.4 μM and 7.5 μM against adenosine A_1, A_{2A} and A_3 receptors respectively. The compound **PMCDP-BTP-4, PMCDP-BTP-8, PMCDP-BTP-14** was selective against adenosine A_3 receptor with the Ki values 0.9, 10.2 and 21.7 μM respectively. The compounds with the tri-substituted thiophenes coupled with the tetrasubstituted thiophenes were found to be lost in the activity, **PMCDP-BTP-4** being the exception. The compound **PMCDP-BTP-13** was found to inhibit the three receptors because of high substitution on the both of the thiophene rings.

Table 5.2 Binding affinity of synthesized thiophenyl-thiophene carboxamides



No	Compound	Ki in µM at 95 % Confidence Limits			
		hA_1[a]	**hA_{2A}**[b]	**hA_{2B}**[d]	**hA_3**[c]
1	**PMCDP-BTP-1**	>10	>10	>10	>10
2	**PMCDP-BTP-2**	>10	>10	>10	>10
3	**PMCDP-BTP-3**	>10	>10	>10	>10
4	**PMCDP-BTP-4**	>100	>30	>30	**0.9**
5	**PMCDP-BTP-5**	>100	>100	>10	>100
6	**PMCDP-BTP-6**	>100	>100	>10	>100
7	**PMCDP-BTP-7**	>10	>10	>10	>10
8	**PMCDP-BTP-8**	>30	>30	>10	**10.2**
9	**PMCDP-BTP-13**	**2.5**	**1.4**	>10	**7.5**
10	**PMCDP-BTP-14**	>100	>100	>10	**21.7**
11	**PMCDP-BTP-15**	>100	>100	>10	>30
12	**PMCDP-BTP-17**	N.D.	N.D.	N.D.	N.D.
13	**PMCDP-BTP-19**	N.D.	N.D.	N.D.	N.D.
14	PMCDP-BTP-21	N.D.	N.D.	N.D.	N.D.

Data are expressed as geometric means with 95% confidence limits.
[a]Displacement of specific [^{3}H]CCPA binding at human A_1 receptors expressed in CHO cells.
[b]Displacement of specific [^{3}H]NECA binding at human A_{2A} receptors expressed in CHO cells.
[c] Displacement of specific [^{3}H]HEMADO binding at human A_3 receptors expressed in CHO cells.
[d]Inhibition of NECA-stimulated adenylyl cyclase activity at human A_{2B} receptors expressed in CHO cells.

5.4 Conculsion

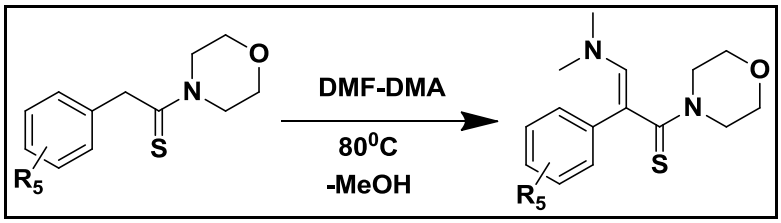
Here we have designed and synthesized new thiophenyl-thiophene carboxamides. Further the molecules were evaluated for their radio ligand binding assay against adenosine receptors. The compounds were active towards adenosine receptors and particularly one molecule PMCDP-BTP-4 was active against A_3 adenosine receptor with high selectivity. Overall the affinity of this series of molecules was similar to the chapter-4 molecules.

5.5 Experimental

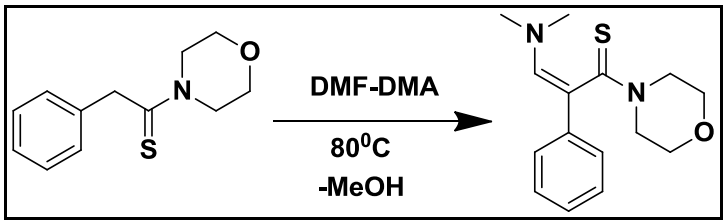
The ^{1}H NMR spectra were recorded on Bruker NMR spectrometer (400 MHz) using TMS as an internal standard and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer at 75 MHz. Proton chemical shifts are expressed in ppm relative to internal tetramethylsilane. Mass spectra were recorded on Perkin Elmer Sciex API 165. TLC was carried out on Merck Kieselgel 60 PF_{254}. IUPAC name of the compounds were generated using Cambridge soft ChemBioDraw ultra 12.0. The preparations of substituted 2-chloroacetamido thiophenes (**B**) are described in the chaptet-3. The preparation of the synthesis of 1-morpholino-2-phenylethanethione has been well documented in the literature (Pillai et al., 2004). The preparation of enamine and isothiocyanate has been also given in the literature (Franklin et al., 2008; Molvi, Vasu, Yerande, Sudarsanam, & Haque, 2007; Pillai et al., 2005).

5.5.1 General procedure for the preparation of substituted 1-Morpholino-2-arylprop-2-ene-1-thione (D)

In a hot air dried flask, 1-morpholino-2-arylethanethione (1.0 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (1.5 mmol) were mixed together and heated to 80–85^{0}C for 6–10 h. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (4:6), showing the consumption of both the starting materials. The crude reaction mixture is distilled off to remove the *in-situ* generated solvent methanol. Then the mixture is treated with the hexane and/or ether to generate the intermediate 1-morpholino-2-arylprop-2-ene-1-thione which was directly taken for the next final step without further purification.

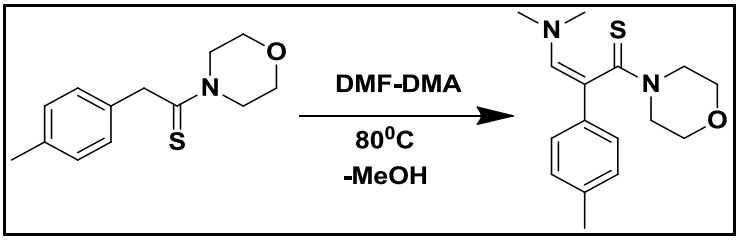


5.5.1.1. Preparation of (Z)-3-(Dimethylamino)-1-morpholino-2-phenylprop-2-ene-1-thione (D1)



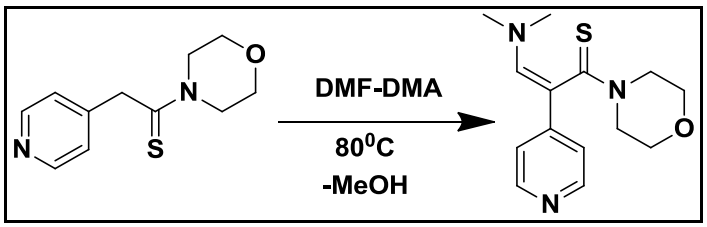
The title compound was synthesized using the procedure as described in 5.5.1 by the reaction of 1-morpholino-2-phenylethanethione and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford the title product.

5.5.1.2. Preparation of (Z)-3-(Dimethylamino)-1-morpholino-2-(p-tolyl)prop-2-ene-1-thione (D2)



The title compound was synthesized using the procedure as described in 5.5.1 by the reaction of 1-morpholino-2-(p-tolyl)ethanethione and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford the title product.

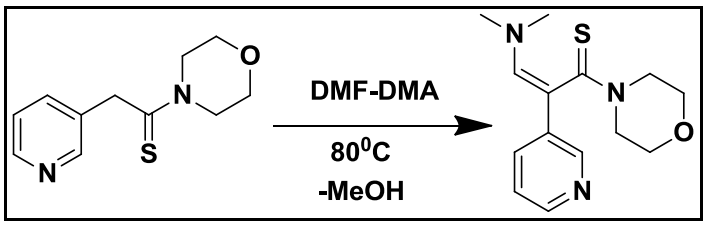
5.5.1.3. Preparation of (Z)-3-(Dimethylamino)-1-morpholino-2-(pyridin-4-yl)prop-2-ene-1-thione (D3)



The title compound was synthesized using the procedure as described in 5.5.1 by the reaction of 1-morpholino-2-(pyridin-4-yl)ethanethione and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford the title product.

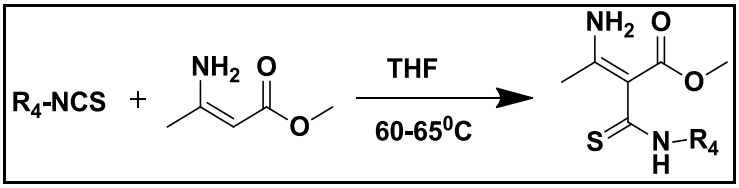
5.5.1.4. Preparation of (Z)-3-(Dimethylamino)-1-morpholino-2-(pyridin-3-yl)prop-2-ene-1-thione (D4)

The title compound was synthesized using the procedure as described in 5.5.1 by the reaction of 1-morpholino-2-(pyridin-3-yl)ethanethione and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford the title product.



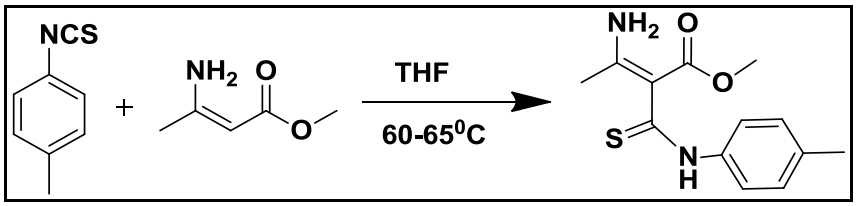
5.5.2 General procedure for the preparation of substituted Methyl 3-amino-2-(arylcarbamothioyl)but-2-enoate (D)

To demonstrate the practical viability of the present protocol, we started the reaction by stirring a solution of aryl/aroyl isothiocyanate (1 mmol) and methyl enamino ester (1 mmol) in a mixture of solvent tetrahydrofuran. The reaction mixture was warmed to 60-65^{0}C and maintained for 5-6 h. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (4:6), showing the consumption of both the starting materials. The crude reaction mixture is distilled off to remove the solvent. Then the mixture is treated with the hexane and/or ether to generate the methyl 3-amino-2-(arylcarbamothioyl) but-2-enoate which was directly taken for the next final step without further purification.



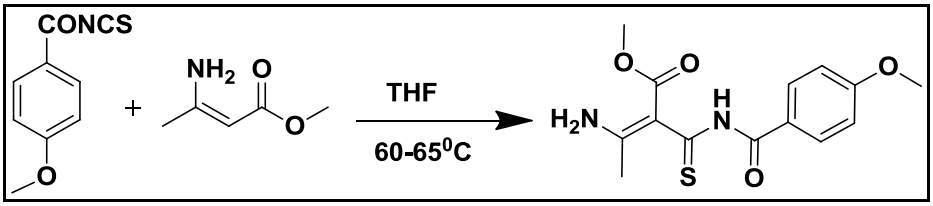
5.5.2.1. Preparation of (E)-Methyl 3-amino-2-(p-tolylcarbamothioyl)but-2-enoate (D5)

The title compound was synthesized using the procedure as described in 5.5.2 by the reaction of 4-methyl phenyl isothiocyanate and *(Z)*-methyl 3-aminobut-2-enoate to afford the title product.



5.5.2.2. Preparation of (E)-Methyl 3-amino-2-((4-methoxybenzoyl) carbamothioyl)but-2-enoate (D6)

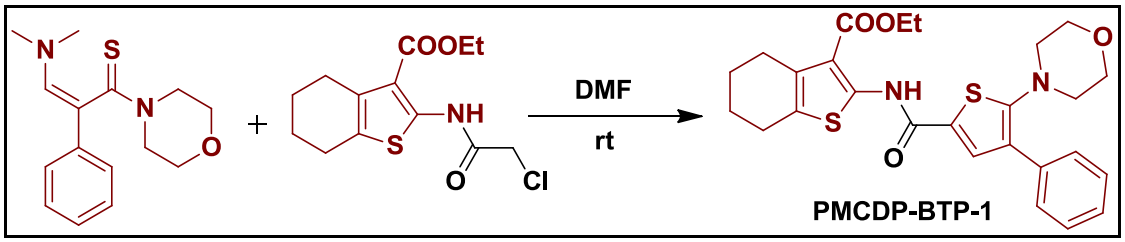
The title compound was synthesized using the procedure as described in 5.5.2 by the reaction of 4-methoxy benzoyl isothiocyanate and *(Z)*-methyl 3-aminobut-2-enoate to afford the title product.



5.5.3 General procedure for the preparation of thiophenyl-thiophene carboxamides

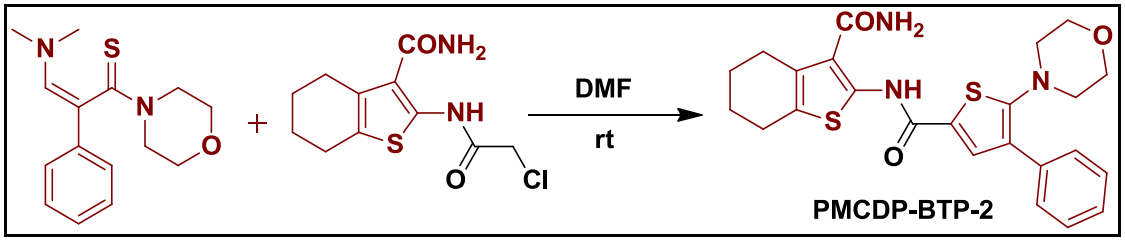
To a hot air dried round bottomed flask, containing a solution of 1-morpholino-2-arylprop-2-ene-1-thione or methyl 3-amino-2-(arylcarbamothioyl)but-2-enoate (1 mmol) in DMF (5 mL), 2-chloroacetamidothiophene (1 mmol) in DMF (5 mL) was added at ambient temperature and the reaction was further stirred for 3-4 h with stirring. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through buchner funnel. These precipitates were dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-BTP-n)** were assigned with the help of NMR and mass spectra.

PMCDP-BTP-1: Ethyl 2-(5-morpholino-4-phenylthiophene-2-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



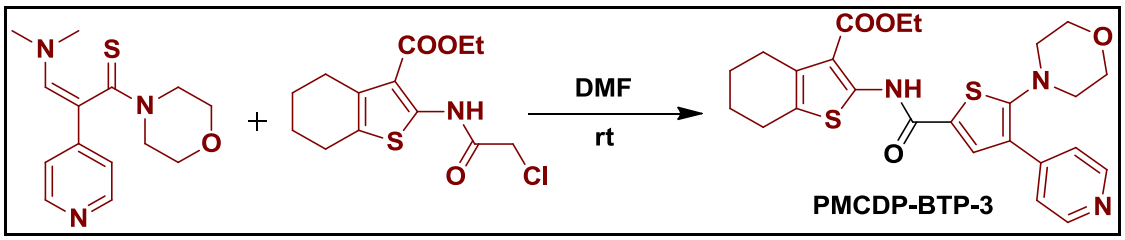
Yield: 63%, White solid, mp: 192-196 ^{0}C, **Molecular formula: $C_{26}H_{28}N_2O_4S_2$**, LC-MS calculated: 496.1, found: 496.7(M+). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.33(t, 3H), 1.76(s, 4H), 2.61(s, 2H), 2.72(s, 2H), 3.02(s, 4H), 4.37(q, 2H), 7.29(t, 1H), 7.40(t, 2H), 7.61(s, 1H), 7.67(d, 2H), 11.94(s, 1H, NH).

PMCDP-BTP-2: 2-(5-Morpholino-4-phenylthiophene-2-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide



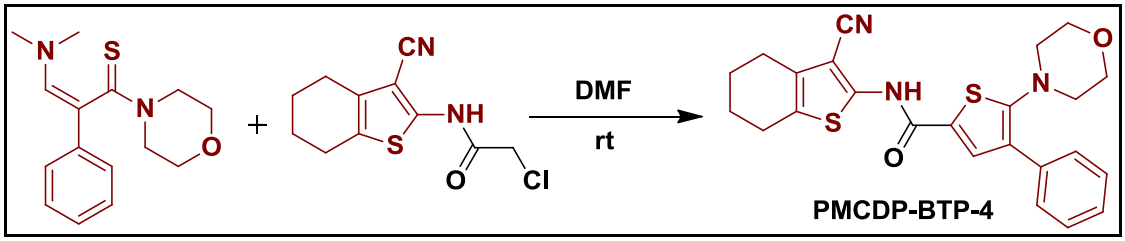
Yield: 60%, White solid, mp : 180-183 ^{0}C, **Molecular formula: $C_{24}H_{25}N_3O_3S_2$**, LC-MS calculated: 467.1, found: 468.5(M+1) **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.71(s, 4H), 2.62(s, 2H), 2.71(s, 2H), 2.91(t, 4H), 3.66(t, 4H), 7.27(t, 1H), 7.42(t, 2H), 7.62(s, 1H), 7.67(d, 2H), 12.77(s, 1H, NH).

PMCDP-BTP-3: Ethyl 2-(5-morpholino-4-(pyridin-4-yl)thiophene-2-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



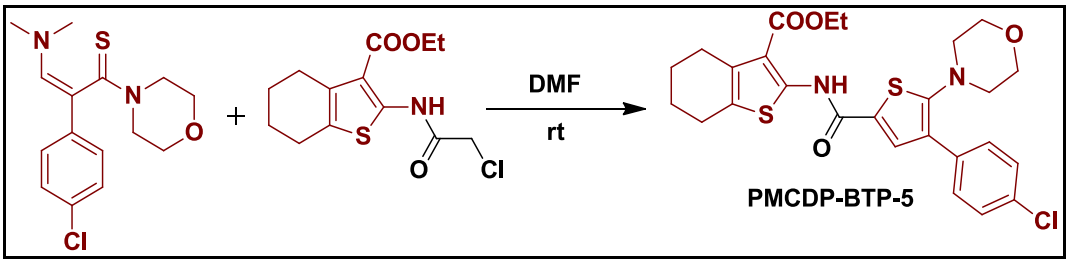
Yield: 50%, light green solid, mp: 177-180 ^{0}C, **Molecular formula: $C_{25}H_{27}N_3O_4S_2$**, LC-MS calculated: 497.1, found: 498.5(M+1) **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.38(t, 3H), 1.80(s, 4H), 2.67(s, 2H), 2.79(s, 2H), 3.02(t, 4H), 3.78(t, 4H), 4.33(q, 2H), 7.61(d, 2H), 7.70(s, 1H), 8.64(d, 2H), 12.06(s, 1H, NH).

PMCDP-BTP-4: *N*-(3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)-5-morpholino-4-phenylthiophene-2-carboxamide



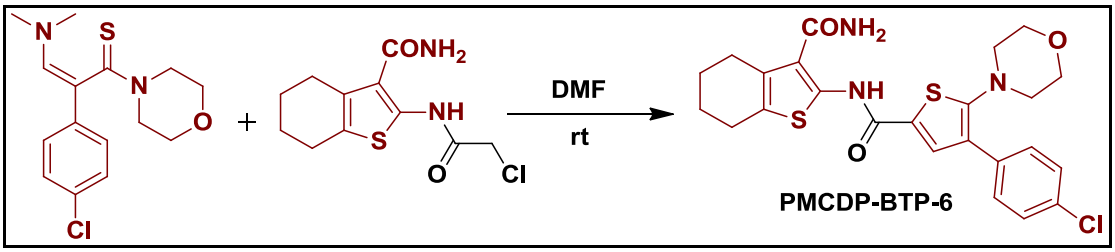
Yield: 59%, light yellow solid, mp: 214-216 ^{0}C, **Molecular formula: $C_{24}H_{23}N_3O_2S_2$**, LC-MS calculated: 449.1, found: 449.8 (M+) **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.82(t, 4H), 2.57 (m, 4H), 3.03(q, 4H), 3.74(q, 4H), 7.27-7.32(m, 1H), 7.38(q, 2H), 7.62(t, 3H), 8.58(s, 1H, NH).

PMCDP-BTP-5: Ethyl 2-(4-(4-chlorophenyl)-5-morpholinothiophene-2-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



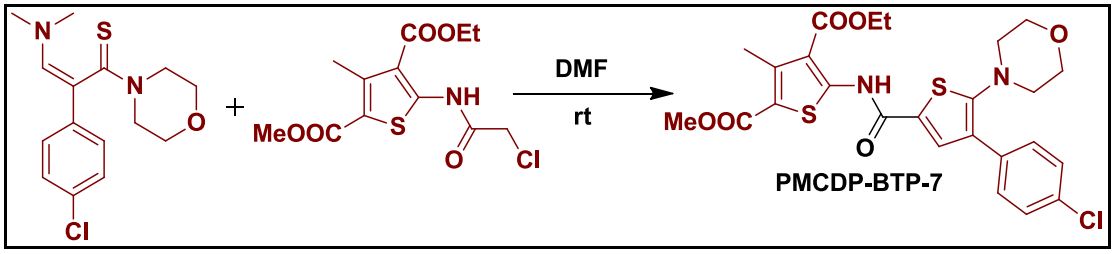
Yield: 59%, light yellow solid, mp: 183-186 ^{0}C, **Molecular formula: $C_{26}H_{27}ClN_2O_4S_2$**, LC-MS calculated: 530.3, found: 531.4 (M+1) **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.37-1.40(t, 3H), 1.79(s, 4H), 2.65(s, 2H), 2.77(s, 2H), 2.99(s, 4H), 4.34(q, 2H), 7.36(d, 2H), 7.61(s, 3H), 11.98(s, 1H, NH). **^{13}C NMR (400 MHz, $CDCl_3$)** δ: 14.38, 22.87, 23.01, 24.43, 26.44, 60.56, 66.35, 111.39, 125.18, 125.95, 126.87, 128.94, 129.03, 130.93, 131.38, 132.87, 133.84, 140.04, 158.23, 159.59, 166.95.

PMCDP-BTP-6: 2-(4-(4-Chlorophenyl)-5-morpholinothiophene-2-carboxamido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide



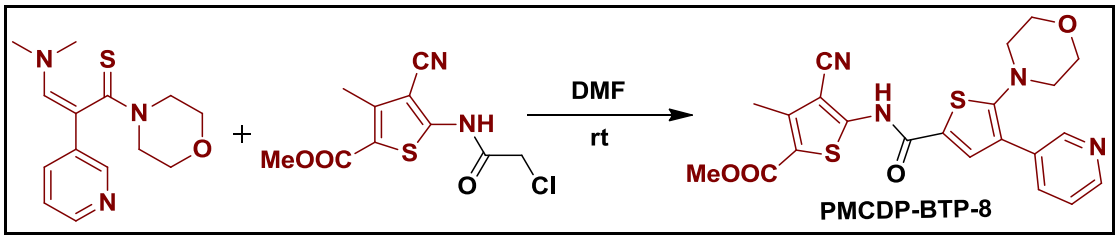
Yield: 73%, light yellow solid, **Molecular formula: $C_{24}H_{24}ClN_3O_3S_2$,** LC-MS calculated: 501.2, found: 502.6 (M+1). **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ: 1.82(s, 2H), 2.55(s, 2H), 2.67(s, 2H), 2.85(s, 2H), 2.97(s, 4H), 3.73(s, 4H), 7.41(s, 2H), 7.59-7.67(m, 4H), 8.03(s, 1H), 8.58(s, 1H).

PMCDP-BTP-7: 4-Ethyl 2-methyl 5-(4-(4-chlorophenyl)-5-morpholinothiophene-2-carboxamido)-3-methylthiophene-2,4-dicarboxylate



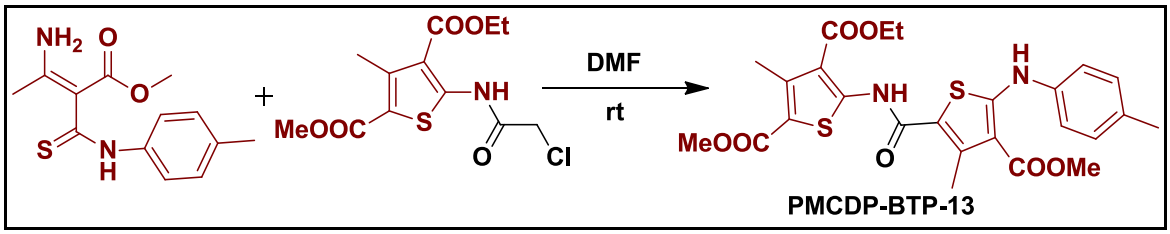
Yield: 73%, white solid, mp: 179-183 ^{0}C **Molecular formula: $C_{25}H_{25}ClN_2O_6S_2$,** LC-MS calculated: 548.2, found: 549.6 (M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.41(t, 3H), 2.74(s, 3H), 3.00(t, 4H), 3.74(t, 4H), 3.84(s, 3H), 4.38(q, 2H), 7.37(d, 2H), 7.59(t, 2H), 12.26(s, 1H, NH). **^{13}C NMR (400 MHz, $CDCl_3$)** δ: 14.25, 15.24, 51.68, 52.23, 61.31, 66.26, 113.71, 117.12, 123.93, 125.82, 129, 132.25, 133.04, 133.67, 145.05, 153.32, 158.76, 160.49, 163.40, 166.83.

PMCDP-BTP-8: Methyl 4-cyano-3-methyl-5-(5-morpholino-4-(pyridin-3-yl)thiophene-2-carboxamido)thiophene-2-carboxylate



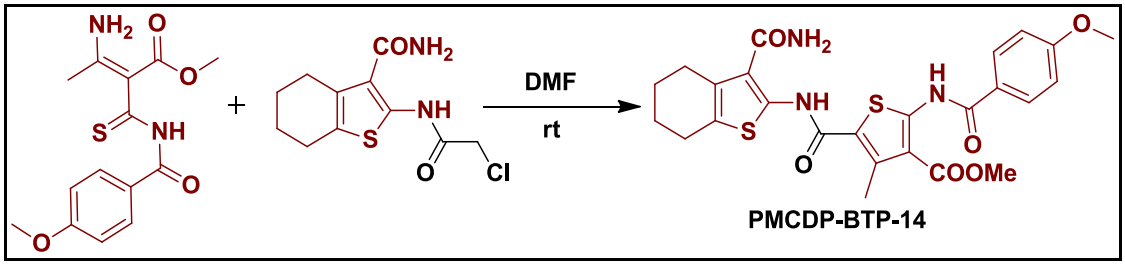
Yield: 50%, light brown solid, mp: 210-213 ^{0}C, **Molecular formula: $C_{22}H_{20}N_4O_4S_2$**, LC-MS calculated: 468.2, found: 469.5 (M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 2.58(s, 3H), 3.01(q, 4H), 3.75(q, 4H), 3.84(s, 3H) 7.39(q, 1H), 8.06(d, 1H), 8.30(s, 1H), 8.51(d, 1H), 8.90(s, 1H), 11.63(s, 1H). **^{13}C NMR (400 MHz, $CDCl_3$)** δ: 14.17, 51.40, 51.94, 65.53, 97.32, 113.69, 117.52, 122.58, 123.24, 123.79, 130.93, 133.55, 134.17, 143.73, 147.72, 148.03, 151.60, 159.41, 161.36, 161.96.

PMCDP-BTP-13: 4-Ethyl 2-methyl 5-(4-(methoxycarbonyl)-3-methyl-5-(p-tolylamino)thiophene-2-carboxamido)-3-methylthiophene-2,4-dicarboxylate



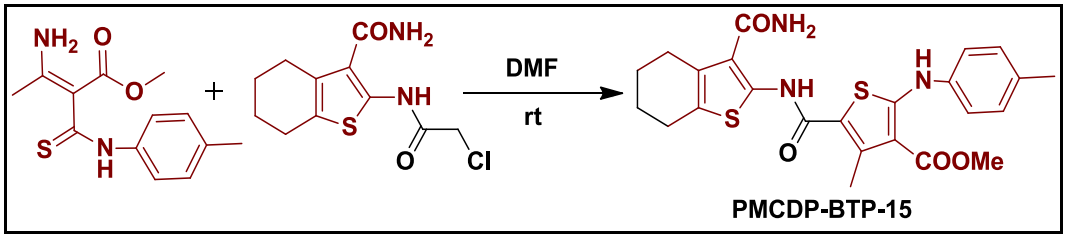
Yield: 59%, light brown solid, mp: 156-158 ^{0}C, **Molecular formula: $C_{24}H_{24}N_2O_7S_2$**, LC-MS calculated: 516.1, found: 517.5 (M+1).

PMCDP-BTP-14: Methyl 2-benzamido-5-((3-carbamoyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)carbamoyl)-4-methylthiophene-3-carboxylate



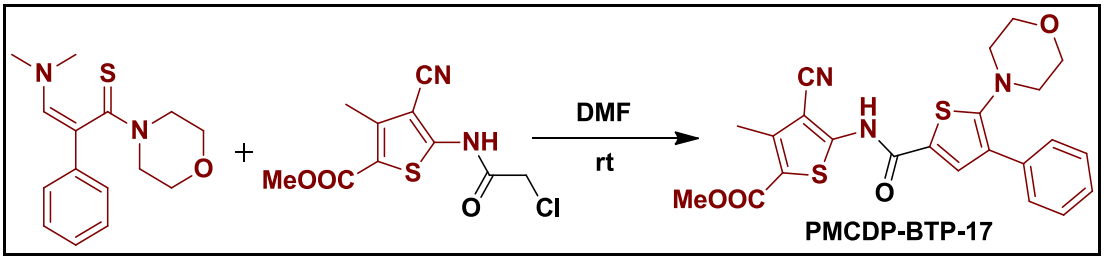
Yield: 65%, off white solid, **Molecular formula: $C_{24}H_{23}N_3O_5S_2$**, LC-MS calculated: 497.1, found: 498.7 (M+1).

PMCDP-BTP-15: Methyl 5-((3-carbamoyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)carbamoyl)-4-methyl-2-(p-tolylamino)thiophene-3-carboxylate



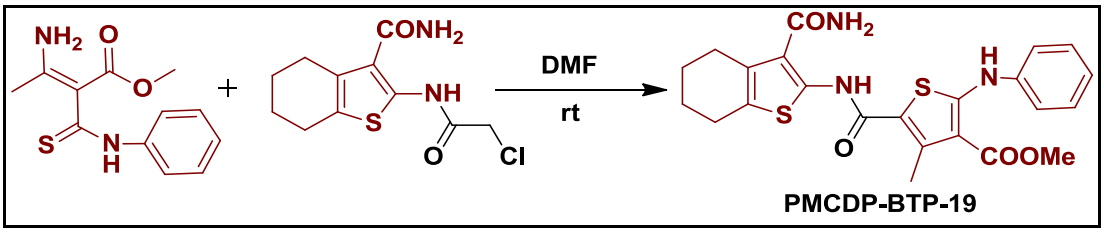
Yield: 62%, light yellow solid, **Molecular formula: $C_{24}H_{25}N_3O_4S_2$**, LC-MS calculated: 483.1, found: 484.6 (M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.78(s, 2H), 2.39(s, 3H), 2.56(s, 2H), 2.62(s, 3H), 2.66(s, 2H), 2.85(s, 2H), 3.89(s, 3H), 7.26(d, 2H), 7.39(d, 2H), 7.77(s, 2H), 11.70(s, 1H).

PMCDP-BTP-17: Methyl 4-cyano-3-methyl-5-(5-morpholino-4-(pyridin-3-yl)thiophene-2-carboxamido)thiophene-2-carboxylate



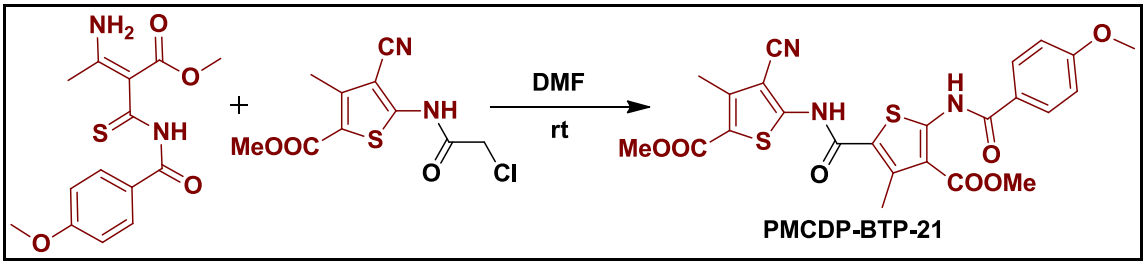
Yield: 62%, light yellow solid, mp: 225-228 ^{0}C, **Molecular formula: $C_{23}H_{21}N_3O_4S_2$**, LC-MS calculated: 467.1, found: 468.4 (M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 2.58(s, 3H), 3.01(q, 4H), 3.75(q, 4H), 3.84(s, 3H), 7.37(d, 2H), 7.59(t, 2H), 7.70 (s, 1H), 11.60(s, 1H).

PMCDP-BTP-19: Methyl 5-((3-carbamoyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamoyl)-4-methyl-2-(phenylamino)thiophene-3-carboxylate



Yield: 65%, White solid, **Molecular formula: $C_{23}H_{23}N_3O_4S_2$**, LC-MS calculated: 469.1, found: 470.4 (M+1). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 1.82(s, 2H), 2.55(s, 2H), 2.60(s, 3H), 2.67(s, 2H), 2.85(s, 2H), 3.89(s, 3H), 7.01-7.03(t, 1H), 7.26(d, 2H), 7.39(d, 2H), 7.77(s, 2H), 11.70(s, 1H).

PMCDP-BTP-21: Methyl 4-cyano-5-(5-(4-methoxybenzamido)-4-(methoxycarbonyl)-3-methylthiophene-2-carboxamido)-3-methylthiophene-2-carboxylate



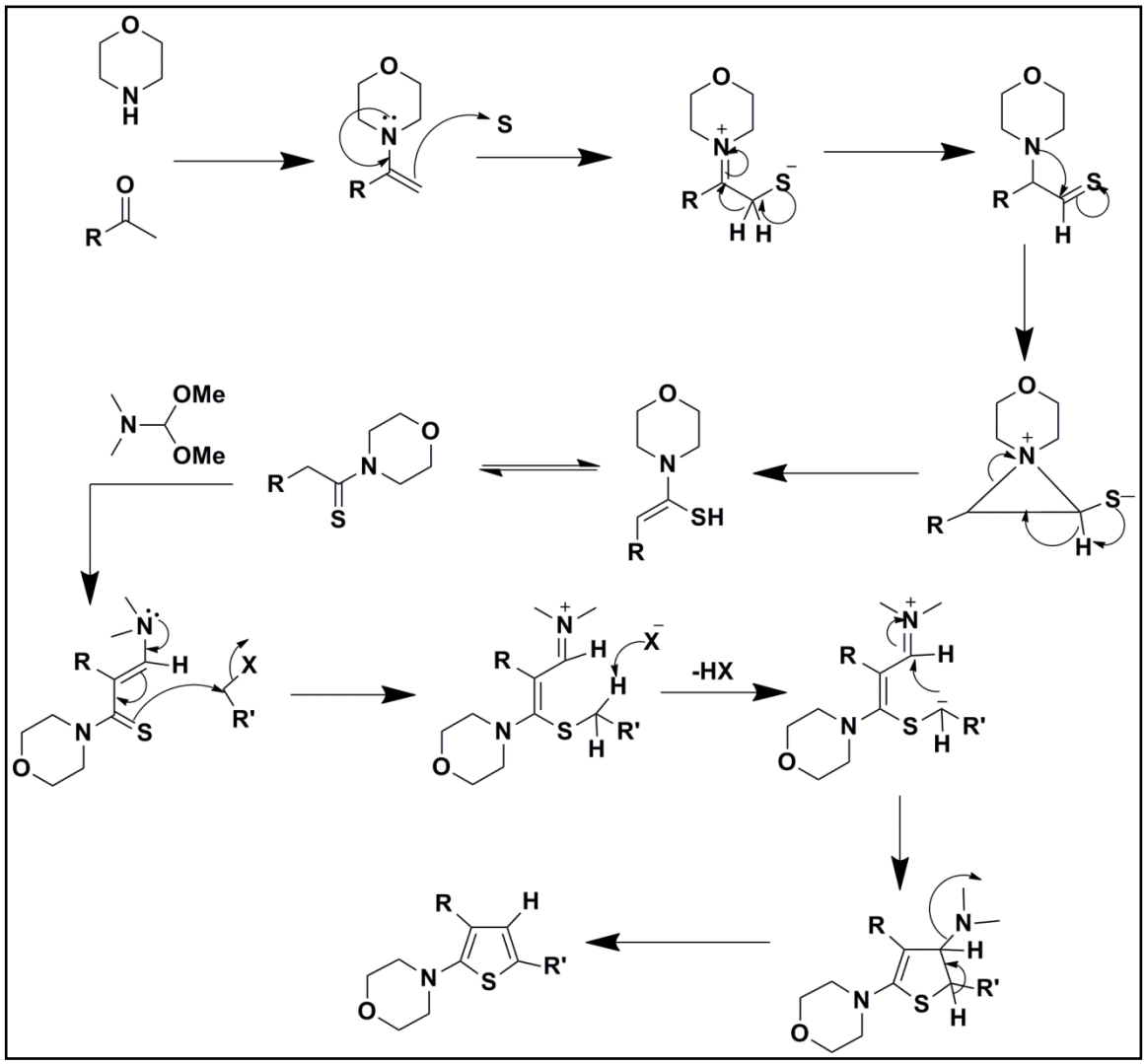
Yield: 65%, White solid, **Molecular formula: $C_{24}H_{21}N_3O_7S_2$**, LC-MS calculated: 469.1, found: 470.4 (M+1).

5.5.4 Method used for pharmacological evaluation

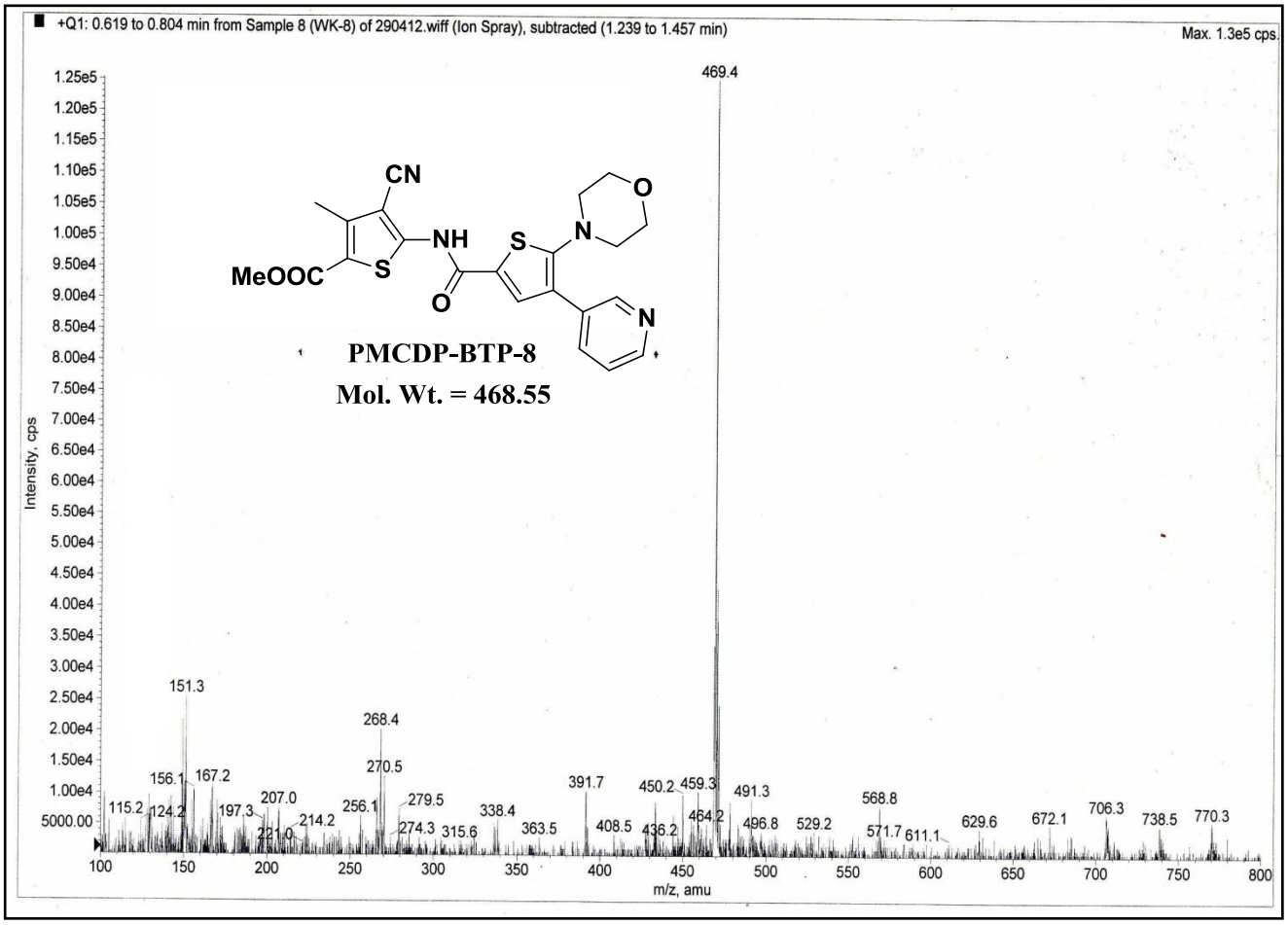
The methods described in the chapter-3 were used for the pharmacological evaluation for the compounds.

Supporting Information
(LC-MS, ^{1}H-NMR, ^{13}C NMR, of representative compounds)

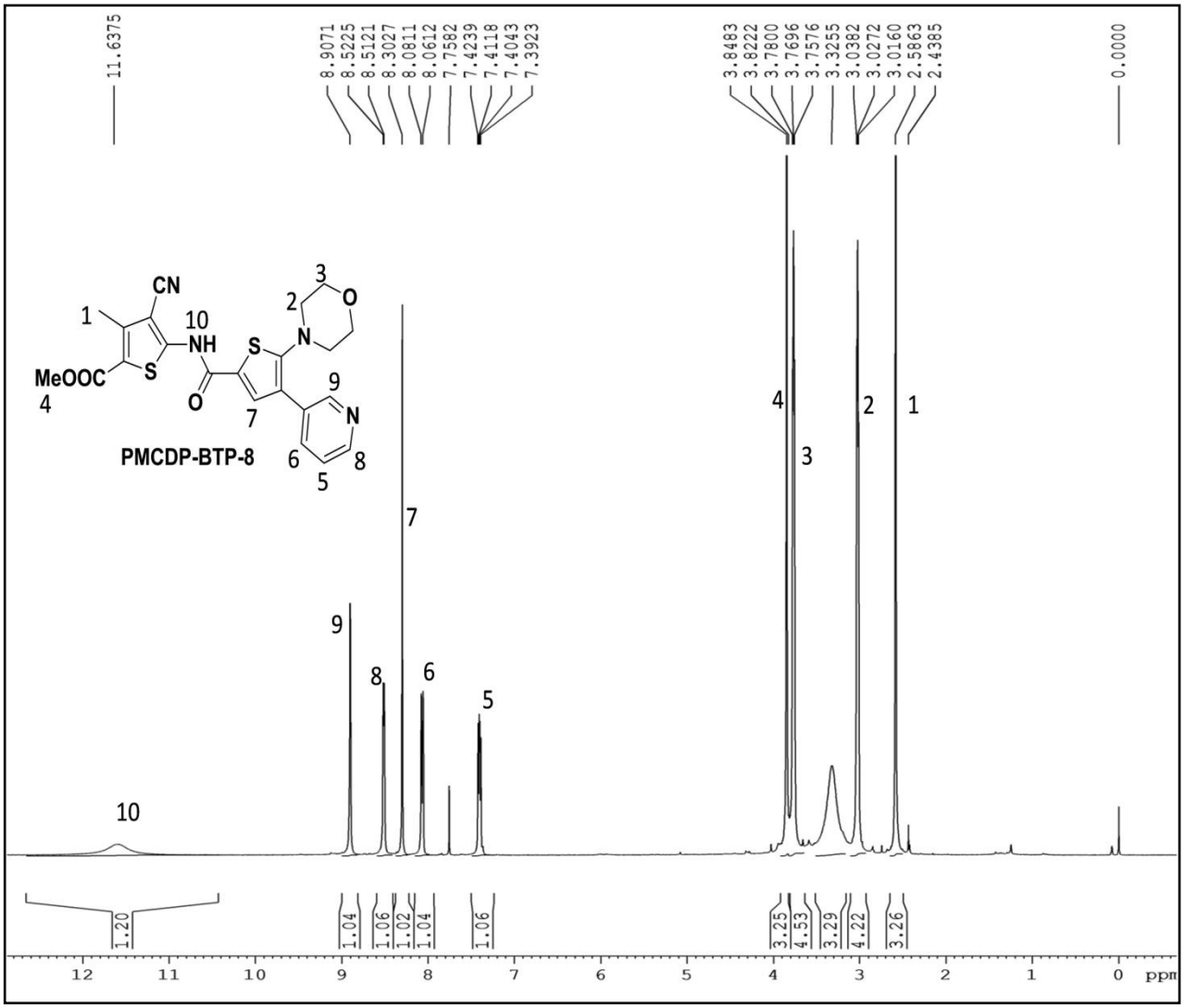
General Reaction Mechanism for the formation of Thiophene via Willgerodt-Kindler Mechanism



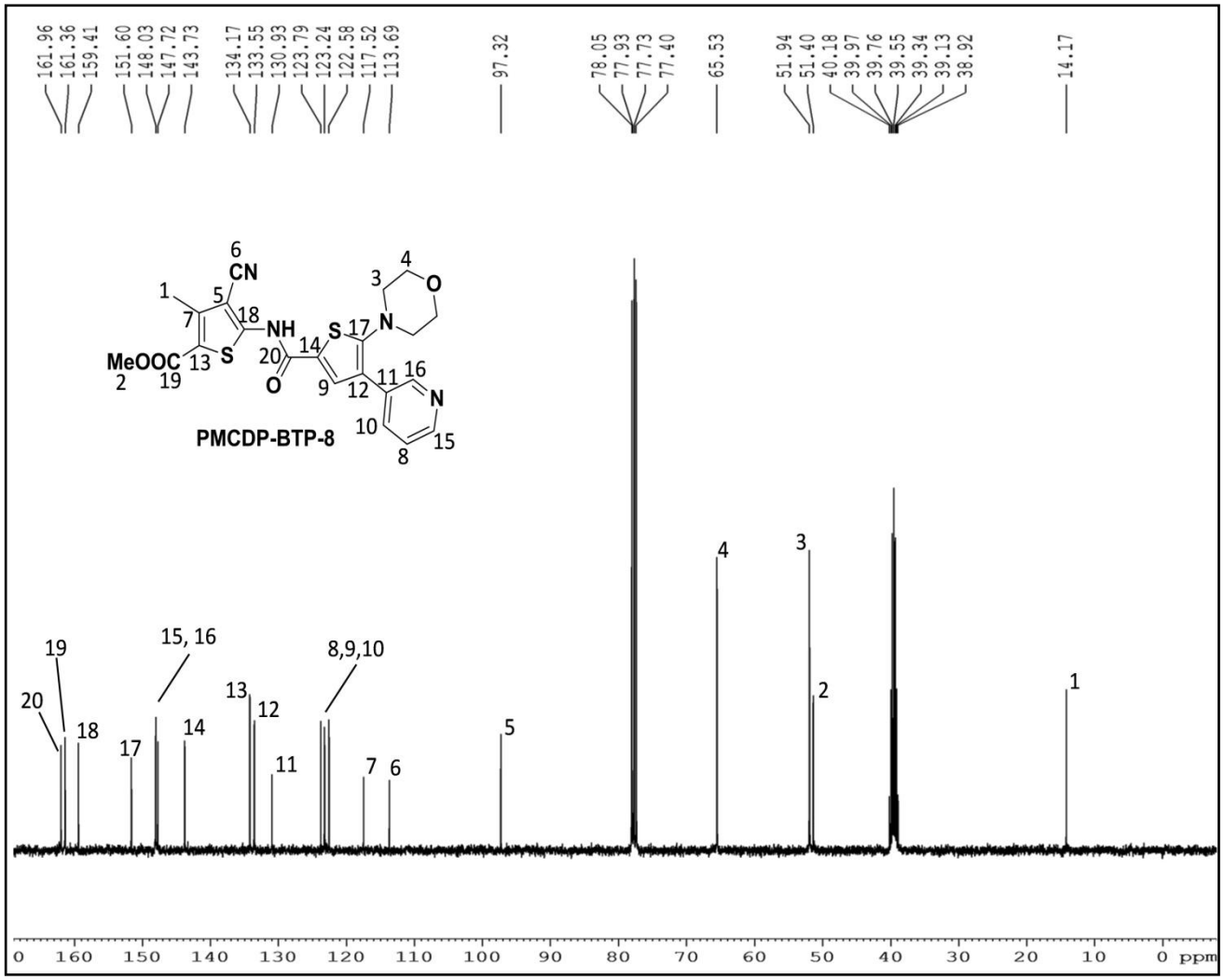
LC-MS spectrum of PMCDP-BTP-8



^{1}H NMR spectrum of PMCDP-BTP-8



^{13}C NMR spectrum of PMCDP-BTP-8



CHAPTER-6

Novel substituted *N*-cyclicamino thiazoles as adenosine receptor ligands

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Chapter 6

Novel substituted *N*-cyclicamino thiazoles as adenosine receptor ligands

6.1 Introduction

In the earlier chapters we had described the design and synthesis of new substituted thiophenyl-thiazole carboxamide and thiophenyl-thiophene carboxamide analogues which showed good affinity to adenosine receptors. To come up to more active molecules, here we have designed new confirmationally restricted thiazoles i.e. substituted *N*-cyclicamino thiazoles. Substituted thiazole moieties have been reported to show affinity towards adenosine receptors. Particularly disubstituted thiazole derivatives reported by Ijherman and co authors showed good affinity to adenosine receptors (van Muijlwijk-Koezen et al. 2001). 2-cyclicaminothiophenes prepared in our lab was active as anti-inflammatory compounds (Pillai et al. 2004). Here we have tried to design similar structural feature with replacement of 2-cycloaminothiophens with 2-cycloaminothiazoles to come up at good adenosine receptor ligands. Not many monocyclic derivatives have been tested against adenosine receptors, so here we have designed new substituted *N*-cyclicamino thiazole derivatives for adenosine receptors (Figure 6.1).

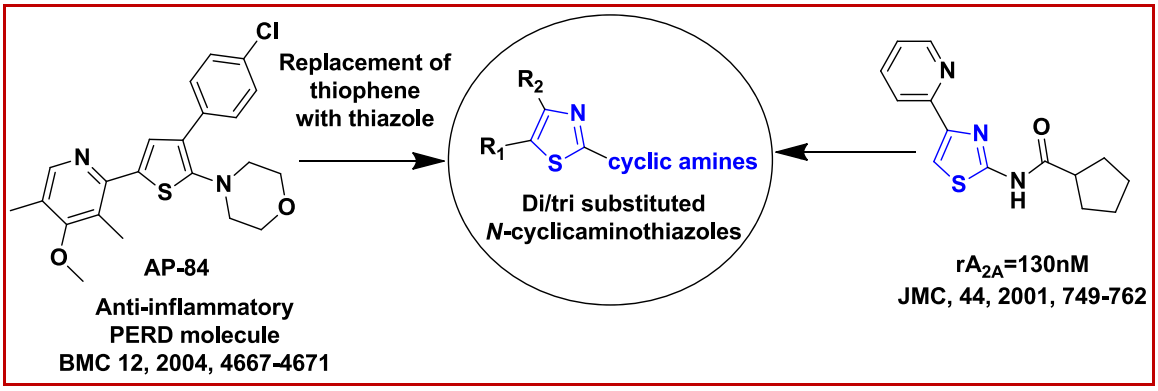
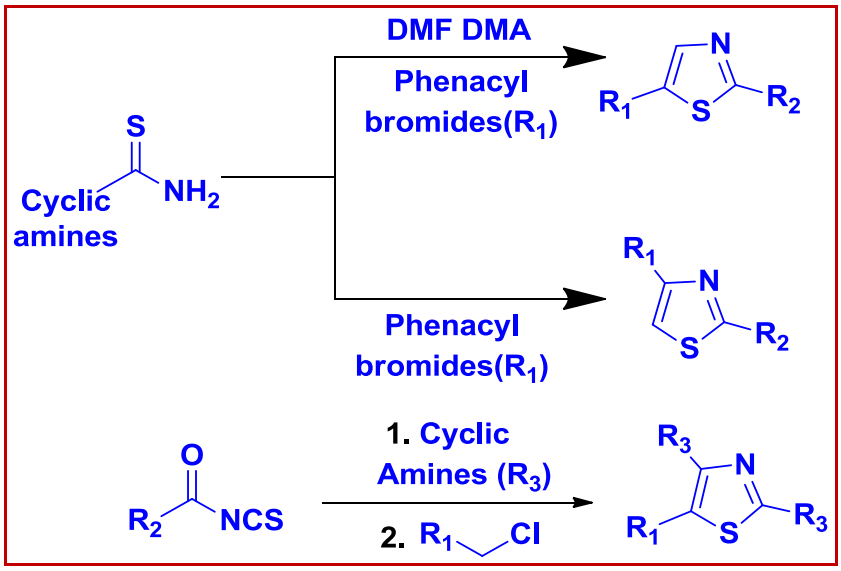


Figure 6.1 Design of substituted *N*-cylicaminothiazoles

6.2 Present Work

The present work describes the synthesis of new 2,4/ 2,5- disubstituted and trisubstituted *N*-cycilcamino thiazoles **(PMCDP-TZAn)**. The synthesis of 2,4-disubstituted thiazoles were achieved by the reaction between various cyclicamino carbothioamide and phenacyl bromide. Synthesis of 2, 5-disubstituted thiazoles was achieved by reaction between cyclicamino carbothioamide, DMF-DMA and phenacyl bromide. And the trisubstituted thiazoles were synthesized by the reaction between *N*-cyclicamino carbanothioyl arylamide and halomethylene compounds. All the structures of the molecules were further confirmed by spectral techniques like Mass and NMR. They were further evaluated for their binding affinity against adenosine receptor. All the three schemes are illustrated in Scheme 6.1.



Scheme 6.1 General synthetic schemes for di/tri substituted *N*-cyclicaminothiazoles

As an example, the reaction of *N*-morpholine thiourea with DMF-DMA was done to furnish *(Z)-N*-((dimethylamino)methylene)morpholine-4-carbothioamide. This carbothioamide (1 mmol) intermediate was further reacted with p-chloro phenacyl bromide (1 mmol) in DMF at room temperature which resulted into **PMCDP-TZA1** with good yields. The structure was further confirmed by LC-MS and ^{1}H NMR.

The mass spectrum of **PMCDP-TZA1** (Figure 6.2) displayed the molecular ion peak at *m/z* 309.4(M+1)+ which confirms the molecular weight of the compound 308.7 calculated for $C_{14}H_{13}ClN_2O_2S$.

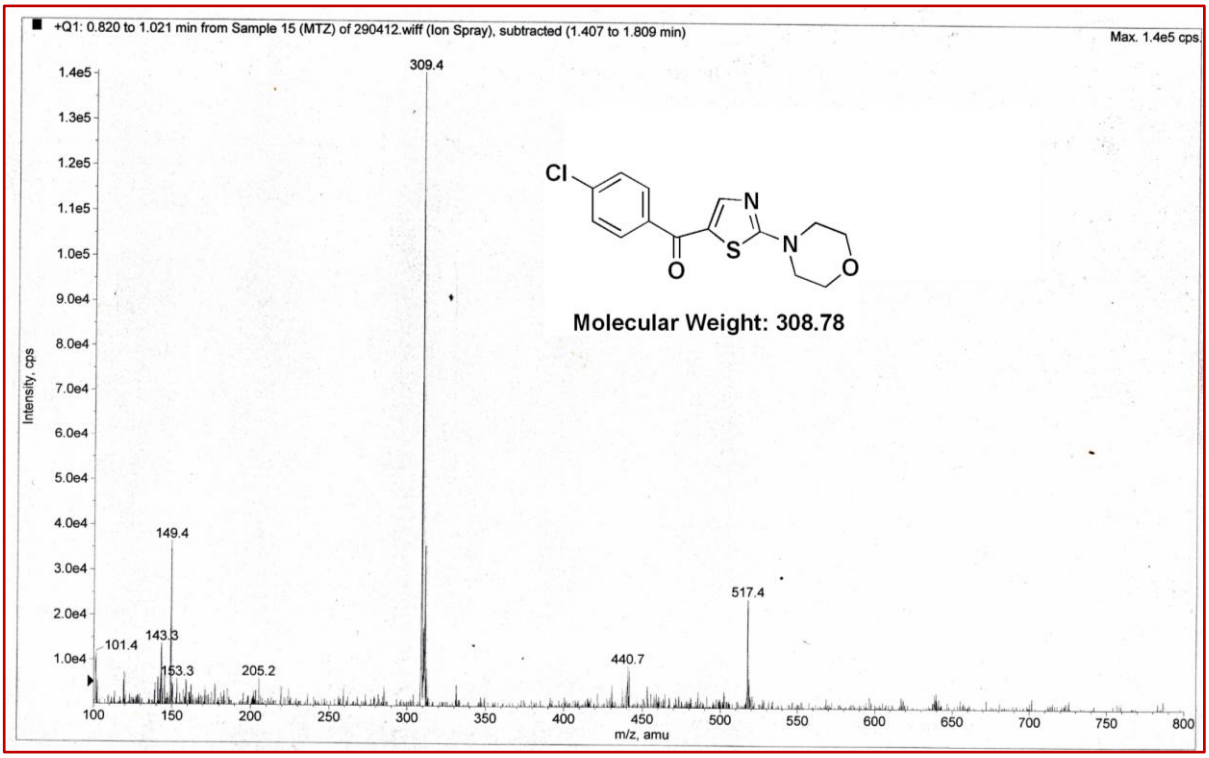


Figure 6.2 LC-MS (M+1) of **PMCDP-TZA1**

The structure of the molecule was further confirmed by ^{1}H NMR (500 MHz, DMSO D6), which gave the characteristic peaks of all the hydrogens present. The eight protons of four methylene groups present in the morpholine were found in the range of 3.60-3.72 δ. The four aromatic protons of the phenyl ring were found in the range of 7.58-7.79 δ with doublets. The single hydrogen of the thiazole moiety was found most downfield as singlet at 7.86 δ (Figure 6.3).

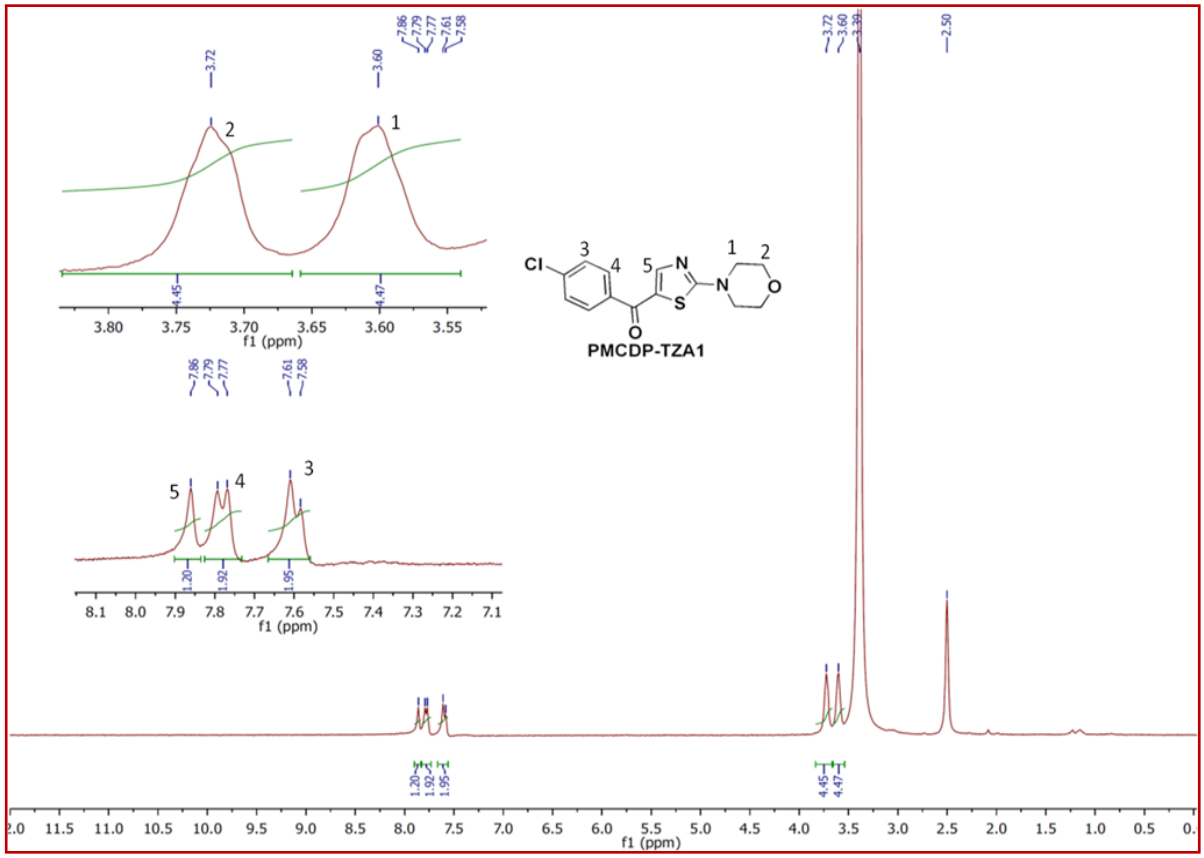
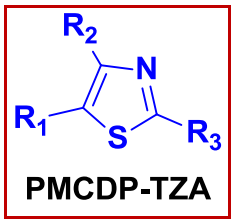


Figure 6.3 ^{1}H NMR spectrum of **PMCDP-TZA1**

After confirmation, library of compounds with structural diversification was synthesized (PMCDP-TZAn, Table 6.1).

Table 6.1 Synthesis of substituted *N*-cyclicaminothiazole derivatives



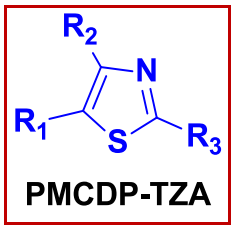
No	Name	R_1	R_2	R_3
1	PMCDP-TZA1	(4-Cl)PhCO	H	Morpholin-4-yl
2	PMCDP-TZA2	(4-Cl)PhCO	H	Pyrrolidin-4-yl
3	PMCDP-TZA3	(4-SO_2Me)PhCO	H	Pyrrolidin-4-yl

4	**PMCDP-TZA5**	**(4-OMe)PhCO**	**H**	**Pyrrolidin-4-yl**
5	**PMCDP-TZA7**	**(4-OMe)PhCO**	**H**	**Morpholin-4-yl**
6	**PMCDP-TZA8**	**H**	**4-ClC_6H_4**	**Morpholin-4-yl**
7	**PMCDP-TZA9**	**Pyridine-4-yl**	**C_6H_5**	**Morpholin-4-yl**
8	**PMCDP-TZA12**	**Pyridine-4-yl**	**Furan-2-yl**	**Morpholin-4-yl**
9	**PMCDP-TZA13**	**Pyridine-4-yl**	**Furan-2-yl**	**2,4 Dichloro phenyl piperazin-4-yl**
10	**PMCDP-TZA14**	**Pyridine-4-yl**	**C_6H_5**	**2,4 Dichloro phenyl piperazin-4-yl**
11	**PMCDP-TZA15**	**H**	**4-MeC_6H_4**	**Morpholin-4-yl**
12	**PMCDP-TZA16**	**Pyridine-4-yl**	**C_6H_5**	**Diethyl amino**
13	**PMCDP-TZA17**	**H**	**Pyridine-2-yl**	**Piperidin-4-yl**

6.3 Biological results and discussion

The binding affinity of the synthesized compounds was evaluated as per the procedure described in the chapter-3. The compounds **PMCDP-TZA9**, **PMCDP-TZA13, PMCDP-TZA14** and **PMCDP-TZA16** were found to show affinity towards A_1, A_{2A} and A_3 adenosine receptors. The compound **PMCDP-TZA14** was selective with affinity for the A_3 subtype (K_i 8.4 μm). The molecule **PMCDP-TZA16** was the most active compound showed less selective due to significant affinity for the A_1, A_{2A} and A_3 subtypes. Particularly trisubstituted thiazoles were found to be active in this series of molecules. Presence of phenyl or 2-furyl group on the 4^{th} position of thiazole ring was found to be responsible for good activity for the compounds. The presence of hydrogen on the 4^{th} position of thiazole ring was found to be deleterious for the activity **(Table 6.2).**

Table 6.2 Binding affinity of synthesized *N*-cyclicaminothiazole derivatives



No	Compound	Ki in µM at 95 % Confidence Limits			
		hA_1[a]	hA_{2A}[b]	hA_{2B}[d]	hA_3[c]
1	**PMCDP-TZA1**	>100	>100	>10	>100
2	**PMCDP-TZA2**	>100	>100	>10	>30
3	**PMCDP-TZA3**	>100	>100	>10	>100
4	**PMCDP-TZA5**	N.D.	N.D.	N.D.	N.D.
5	**PMCDP-TZA7**	N.D.	N.D.	N.D.	N.D.
6	**PMCDP-TZA8**	>100	>100	>20	>100
7	**PMCDP-TZA9**	2.61	5.57	>20	11.8
8	**PMCDP-TZA12**	N.D.	N.D.	N.D.	N.D.
9	**PMCDP-TZA13**	18.2	>100	>20	24.9
10	**PMCDP-TZA14**	>100	>100	>20	8.44
11	**PMCDP-TZA15**	>100	>100	>100	>20
12	**PMCDP-TZA16**	0.836	1.77	>20	1.89
13	**PMCDP-TZA17**	>100	>100	>20	30.2

Data are expressed as geometric means with 95% confidence limits.
[a]Displacement of specific [^{3}H]CCPA binding at human A_1 receptors expressed in CHO cells.
[b]Displacement of specific [^{3}H]NECA binding at human A_{2A} receptors expressed in CHO cells.
[c] Displacement of specific [^{3}H]HEMADO binding at human A_3 receptors expressed in CHO cells.
[d]Inhibition of NECA-stimulated adenylyl cyclase activity at human A_{2B} receptors expressed in CHO cells.

6.4 Conclusion

In conclusion, here we have designed new di and trisubstituted *N*-cyclicaminothiazoles for adenosine receptors. New synthetic methodology was developed for the synthesis of

novel 2,4- and 2,5- disubstituted 2-cyclicaminothiazoles. The structure of all the compounds were confirmed and then investigated for their radio ligand binding assay in adenosine receptors. The compound PMCDP-TZA16 was the most active compound as it showed affinity towards the A_1, A_{2A} and A_3 subtypes in low micromolar range. Overall these series of molecules showed better affinity then the molecules from chapter-4 and chapter-5.

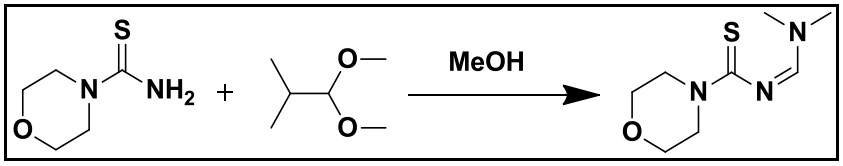
6.5 Experimental

The ^{1}H NMR spectra were recorded on Bruker NMR spectrometer (400 MHz) using TMS as an internal standard and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer at 75 MHz. Proton chemical shifts are expressed in ppm relative to internal tetramethylsilane. Mass spectra were recorded on Perkin Elmer Sciex API 165. TLC was carried out on Merck Kieselgel 60 PF_{254}. IUPAC name of the compounds were generated using Cambridge soft ChemBioDraw ultra 12.0. The synthesis of *N*-cyclicamino carbanothioyl arylamide has been described in the chaper-4.

6.5.1. General procedure for the synthesis of cyclicamino methanethiones

To a stirred solution of cyclic amino carbothioamide (1 mmol) in methanol at room temperature, *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA, 1.2 mmol) was added drop wise via syringe. The reaction mixture is stirred for 2 hours and the progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). The solvent is distilled off to get the reaction mass which is further triturated to get solid compound cyclicamino methanethione (amidinothiourea). The solid was filtered and washed with methanol to give crude product which was directly used for the next step.

6.5.1.1. Synthesis of (Z)-N-((Dimethylamino)methylene)morpholine-4-carbothioamide(E1)



The title compound was synthesized using the procedure as described in 6.5.1 by the reaction of morpholin-4-carbothioamide and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford the title product.

6.5.1.2. Synthesis of (Z)-N-((Dimethylamino)methylene)pyrrolidine-4-carbothioamide(E2)

The title compound was synthesized using the procedure as described in 6.5.1 by the reaction of pyrrolidin-4-carbothioamide and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford the title product.

6.5.1.3. Synthesis of (Z)-N-((Dimethylamino)methylene)piperidine-4-carbothioamide(E3)

The title compound was synthesized using the procedure as described in 6.5.1 by the reaction of pyrrolidin-4-carbothioamide and *N*,*N*-dimethylformamide dimethylacetal (DMF-DMA) to afford the title product.

6.5.2. General procedure for synthesis of 2,5-disubstituted *N*-cyclicamino thiazoles

To a hot air dried round bottomed flask, containing a solution of cyclicamino methanethione (1 mmol) in methanol (2 mL), phenacyl bromide (1 mmol) in methanol (5 mL) was added at ambient temperature and the reaction was further stirred for 4 h with stirring. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through Buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and

dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-TZAn)** were assigned with the help of NMR and mass spectra.

6.5.3. General procedure for synthesis of 2,4-disubstituted *N*-cyclicamino thiazoles

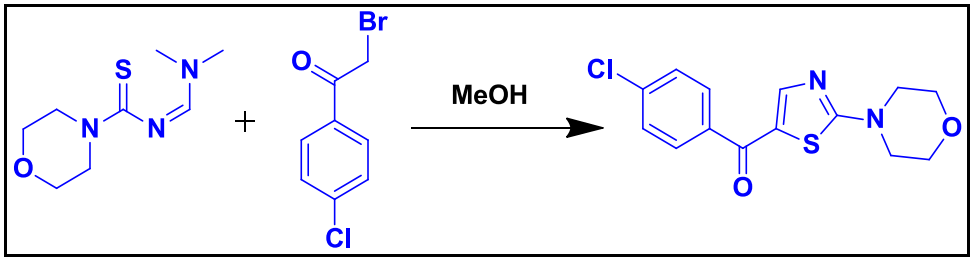
To a hot air dried round bottomed flask, containing a solution of cyclicamino 4-carbothioamide (1 mmol) in methanol (2 mL), phenacyl bromide (1 mmol) in methanol (5 mL) was added and the reaction was further stirred for 4 h at 50^0 C. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through Buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-TZAn)** were assigned with the help of NMR and mass spectra.

6.5.4. General procedure for synthesis of trisubstituted *N*-cyclicamino thiazoles

To a hot air dried round bottomed flask, containing a solution of *N*-cyclicamino carbanothioyl arylamide (1 mmol) in methanol (5 mL), halomethylene compound (1 mmol) in methanol (2 mL) was added. To this solution triethylamine (2 mmol) was added as base and the reaction was further stirred for 4 h at 50^0 C. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through Buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium

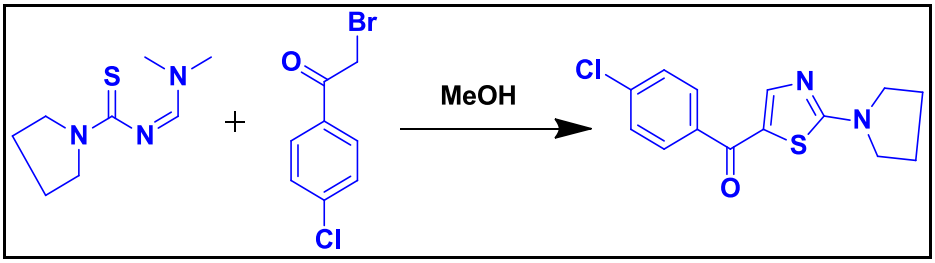
sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-TZAn)** were assigned with the help of NMR and mass spectra.

PMCDP-TZA1: (4-Chlorophenyl)(2-morpholinothiazol-5-yl)methanone



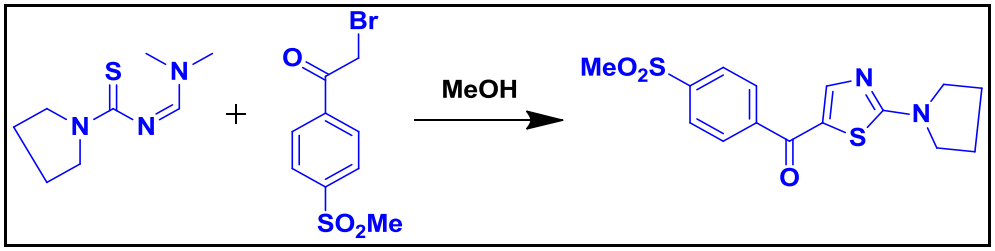
Yield: 81%, light yellow solid, mp: 174-181 ^{0}C, **Molecular formula: $C_{14}H_{13}ClN_2O_2S$**, LC-MS calculated: 308.7, found: 309.4 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.60(s, 4H), 3.72(s, 4H), 7.58 (d, 2H), 7.77 (d, 2H), 7.86(s, 1H).

PMCDP-TZA2: (4-Chlorophenyl) (2-(pyrrolidin-1-yl)thiazol-5-yl)methanone



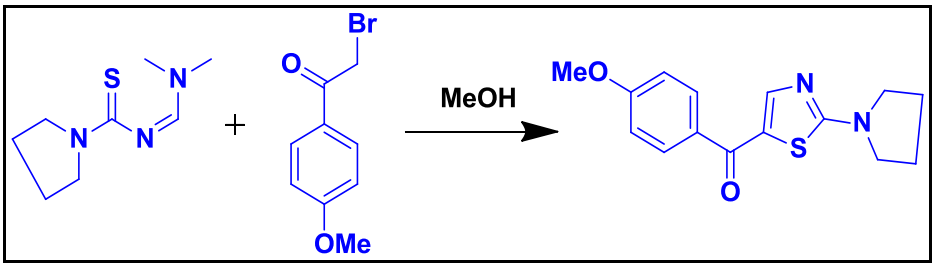
Yield: 80%, white solid, mp: 144-147 ^{0}C, **Molecular formula: $C_{14}H_{13}ClN_2OS$**, LC-MS calculated: 292.1, found: 292.7 (M+). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.14 (t, 2H), 2.01(s, 4H), 3.06(d, 2H), 7.57(d, 2H), 7.74(d, 2H), 7.83(s, 1H).

PMCDP-TZA3: (4-(Methylsulfonyl)phenyl)(2-(pyrrolidin-1-yl)thiazol-5-yl)methanone



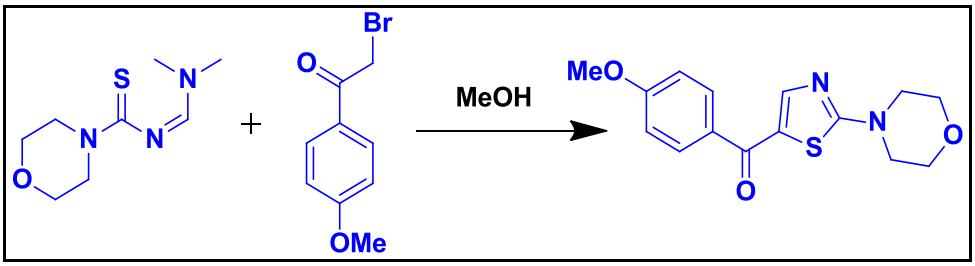
Yield: 79%, white solid, mp: 172-177 ^{0}C, **Molecular formula: $C_{15}H_{16}N_2O_3S_2$**, LC-MS calculated: 336.2, found: 336.9 (M+). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 2.05(s, 4H), 2.5(s, 2H), 3.22(s, 3H), 3.51(s, 2H), 7.77(s, 1H), 7.91(d, 2H), 8.03(d, 2H).

PMCDP-TZA5: (4-Methoxyphenyl) (2-(pyrrolidin-1-yl)thiazol-5-yl)methanone



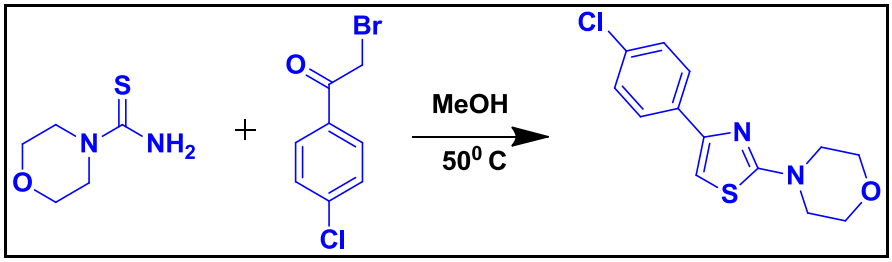
Yield: 73%, white solid, mp: 139-144 ^{0}C, **Molecular formula: $C_{15}H_{16}N_2O_2S$**, LC-MS calculated: 288.4, found: 289.3 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 2.03(s, 4H), 2.25(s, 2H), 3.48(d, 2H), 3.83(s, 3H), 7.01(d, 2H), 7.70(d, 2H), 7.98(s, 1H).

PMCDP-TZA7: (4-Methoxyphenyl)(2-(morpholin-1-yl)thiazol-5-yl)methanone



Yield: 78%, white solid, mp: 144-146 ^{0}C, **Molecular formula: $C_{15}H_{16}N_2O_3S$**, LC-MS calculated: 304.5, found: 305.3 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.56(t, 4H), 3.72(d, 4H), 3.84(s, 3H), 7.02(d, 2H), 7.74(d, 3H).

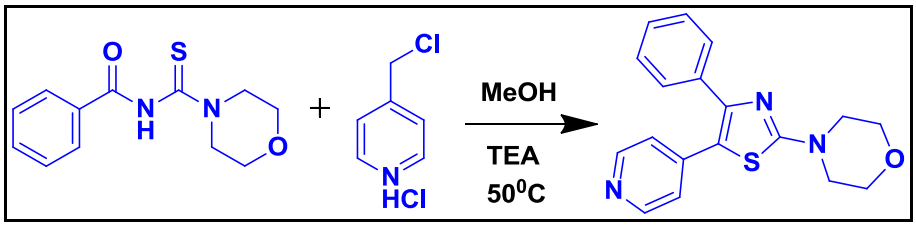
PMCDP-TZA8: 4-(4-(4-Chlorophenyl)thiazol-2-yl)morpholine



Yield: 59%, white solid, mp: 250-253 ^{0}C, **Molecular formula: $C_{13}H_{13}ClN_2OS$**, LC-MS calculated: 280.3, found: 281.5 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.57(s, 4H),

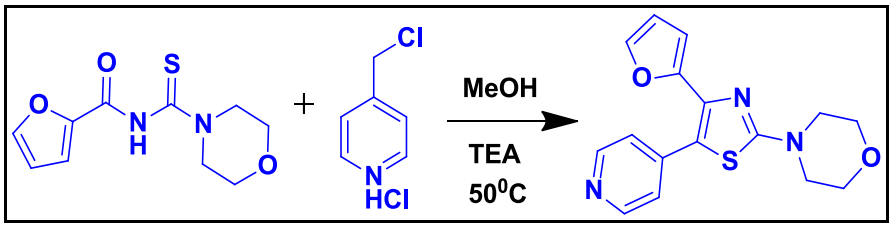
3.65(s, 4H), 7.60(d, 2H), 7.80(d, 2H), 7.90(s, 1H). **^{13}H NMR (400 MHz, DMSO-d_6)** δ: 48.18, 48.64, 65.38, 65.28, 103.19, 128.32, 129.49, 132.23, 133.11, 146.61, 170.66.

PMCDP-TZA9: 4-(4-Phenyl-5-(pyridin-4-yl)thiazol-2-yl)morpholine



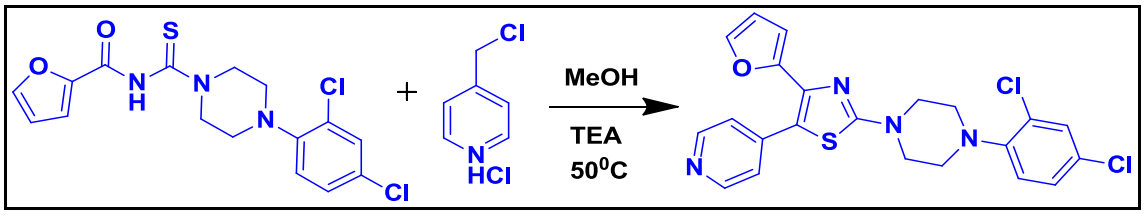
Yield: 75%, light yellow solid, mp: 187-190 ^{0}C, **Molecular formula: $C_{18}H_{17}N_3OS$**, LC-MS calculated: 323.1, found: 324.8 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.48(t, 4H), 3.75(t, 4H), 7.09(q, 2H), 7.32(q, 3H), 7.42(q, 2H), 8.38(q, 2H). **^{13}H NMR (400 MHz, DMSO-d_6)** δ: 47.84, 65.36, 117.42, 122.29, 128.14, 128.20, 128.66, 134.83, 140.02, 149.05, 149.60, 168.82.

PMCDP-TZA12: 4-(4-(Furan-2-yl)-5-(pyridin-4-yl)thiazol-2-yl)morpholine



Yield: 71%, light yellow solid, **Molecular formula: $C_{16}H_{15}N_3O_2S$**, LC-MS calculated: 313.1, found: 314.6 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.49(t, 4H), 3.79(t, 4H), 7.03(t, 1H), 7.33(d, 1H), 7.42(q, 1H), 7.86(q, 2H), 8.38(q, 2H).

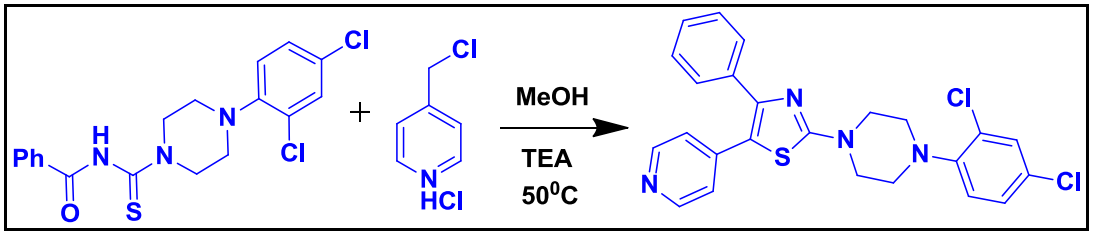
PMCDP-TZA13: 2-(4-(2,4-Dichlorophenyl)piperazin-1-yl)-4-(furan-2-yl)-5-(pyridin-4-yl)thiazole



Yield: 77%, white solid, **Molecular formula: $C_{22}H_{18}Cl_2N_4OS$**, LC-MS calculated: 456.2, found: 457.7 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.12(t, 4H), 3.66(t, 4H), 6.52(q,

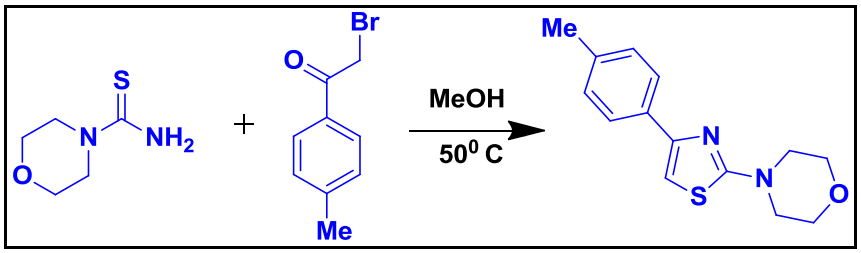
1H), 6.69(q, 1H), 7.17(d, 1H), 7.28(q, 2H), 7.33(q, 1H), 7.49(d, 1H), 7.55(q, 1H), 8.51(q, 2H).

PMCDP-TZA14: 2-(4-(2,4-Dichlorophenyl)piperazin-1-yl)-4-phenyl-5-(pyridin-4-yl)thiazole



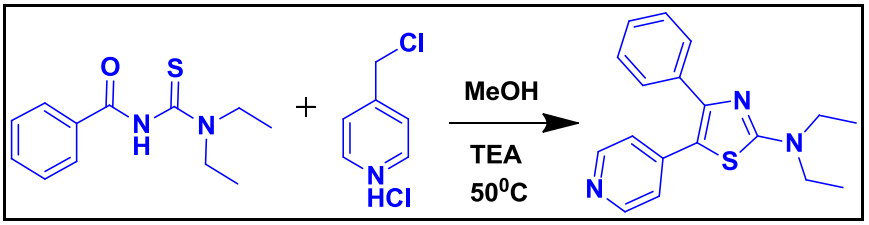
Yield: 82%, white solid, mp: 210-214 ^{0}C **Molecular formula: $C_{24}H_{20}Cl_2N_4S$**, LC-MS calculated: 456.2, found: 457.5 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.10(t, 4H), 3.60(t, 4H), 6.59(d, 1H), 7.17(q, 2H), 7.38(t, 1H), 7.49(t, 2H), 7.77(d, 2H), 7.81(s, 1H), 7.95(2H, d) 8.51(q, 2H).

PMCDP-TZA15: 4-(4-(p-Tolyl)thiazol-2-yl)morpholine



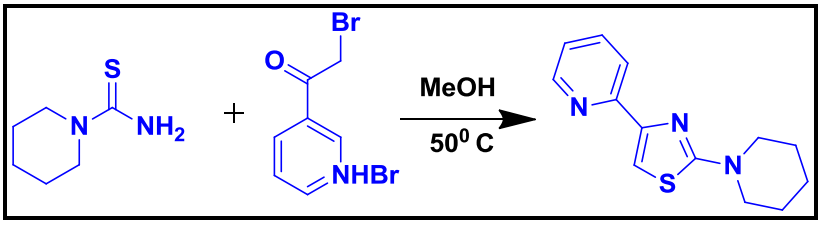
Yield: 65%, off white solid, **Molecular formula: $C_{14}H_{16}N_2OS$**, LC-MS calculated: 260.6, found: 261.5 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 2.32(s, 3H), 3.44(t, 4H), 3.75(t, 4H), 7.06(s, 1H), 7.15(d, 2H), 7.72(d, 2H).

PMCDP-TZA16: *N*,*N*-Diethyl-4-phenyl-5-(pyridin-4-yl)thiazol-2-amine



Yield: 70%, light yellow solid, mp: 145-148 ^{0}C, **Molecular formula: $C_{18}H_{19}N_3S$**, LC-MS calculated: 309.1, found: 309.8 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.52(t, 2H), 3.49(q, 4H), 7.05(q, 2H), 7.33(q, 3H), 7.43-7.45(m, 2H), 8.34(q, 2H).

PMCDP-TZA17: 2-(Piperidin-1-yl)-4-(pyridin-2-yl)thiazole



Yield: 48%, light yellow solid, **Molecular formula: $C_{13}H_{15}N_3S$**, LC-MS calculated: 245.3, found: 246.2 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.52(t, 4H), 1.59(t, 4H), 3.53(t, 4H), 7.82(t, 2H), 8.45(q, 2H).

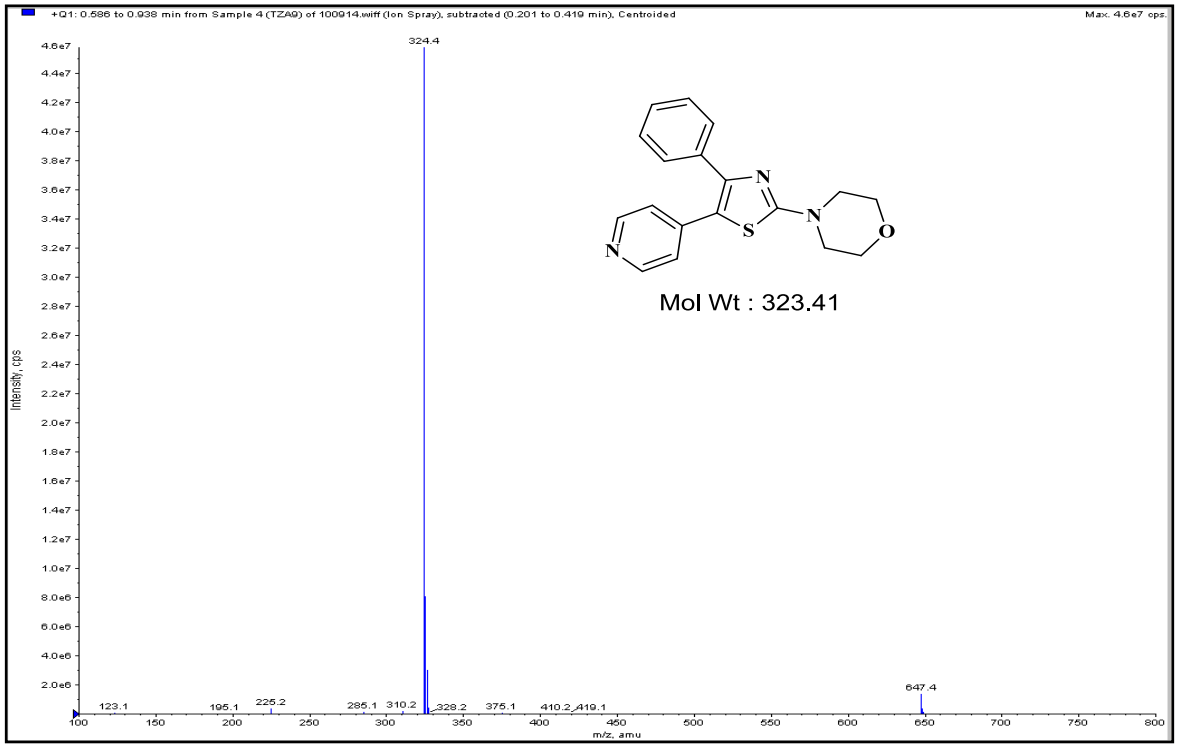
6.5.5. Method used for pharmacological evaluation

The methods described in the chapter-3 were used for the pharmacological evaluation for the compounds.

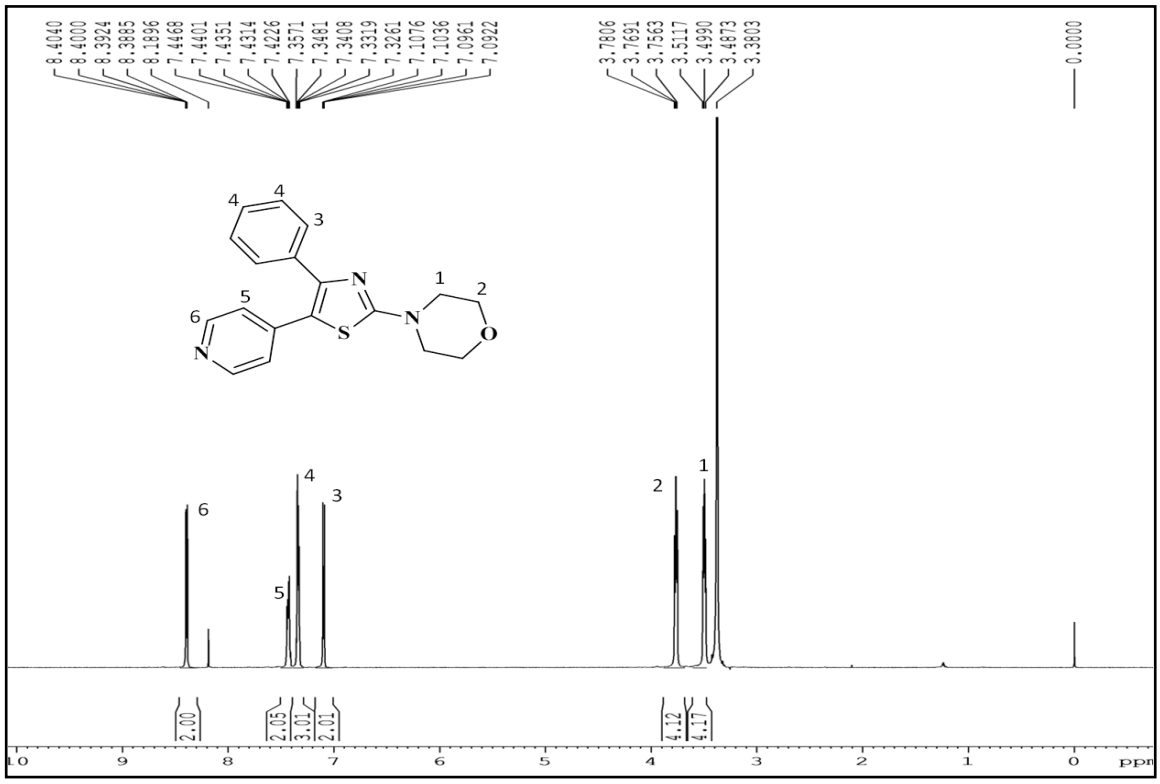
Supporting Information

(LC-MS, ^{1}H-NMR and ^{13}C NMR of representative compounds)

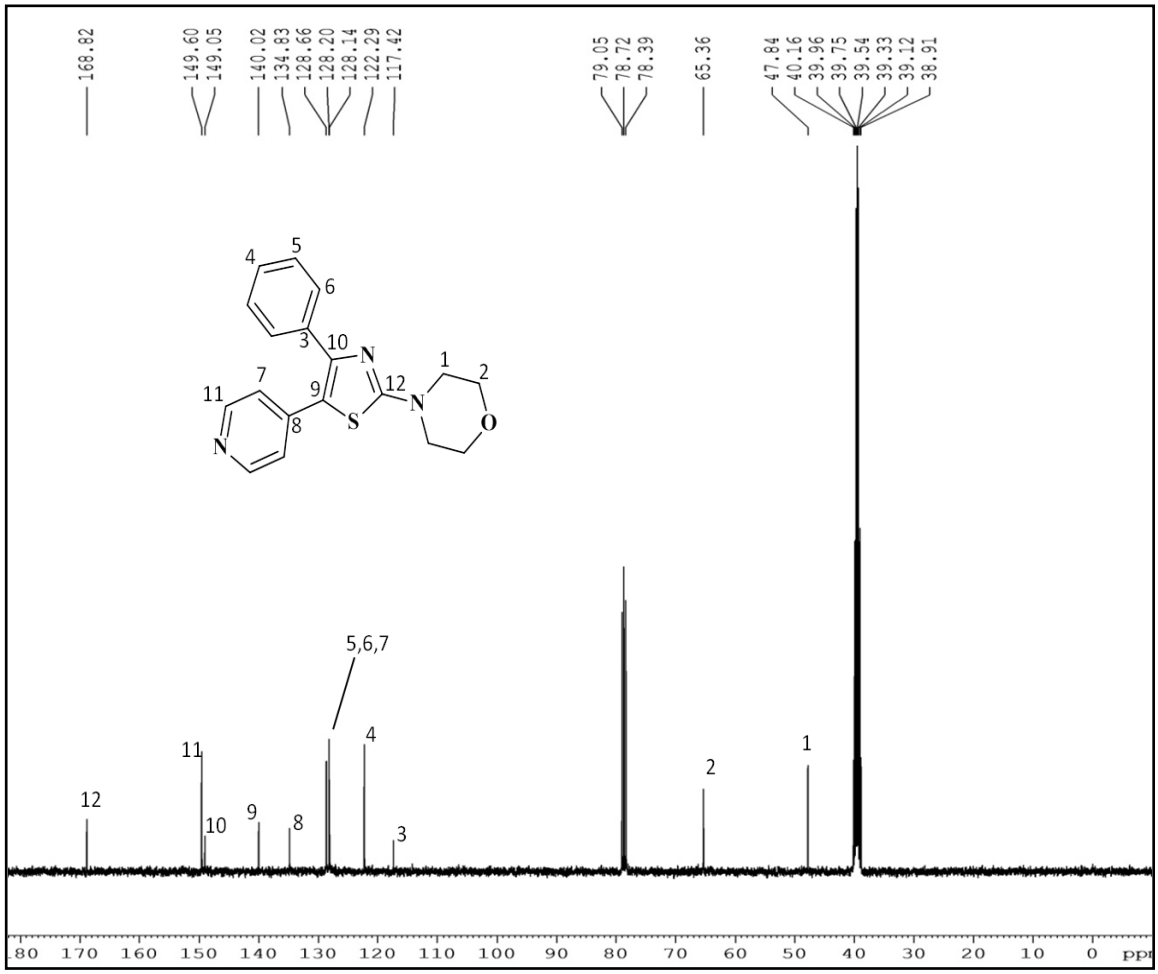
LC-MS spectrum of PMCDP-TZA9



^{1}H spectrum of PMCDP-TZA9



^{13}C spectrum of PMCDP-TZA9



CHAPTER-7

New substituted 2-amino thiazoles as adenosine receptor ligands

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Chapter 7

New substituted 2-amino thiazoles as adenosine receptor ligands

7.1 Introduction

One of these novel classes of monocyclic non-xanthine adenosine receptor antagonists recently developed are thiazole derivatives. Novartis compound QAF-805 with 2-aminothiazole ring couple to pyrazine and imidazole heterocycles had shown high affinity in A_{2B} and A_3 adenosine receptors (Press et al. 2005) and the molecule was further developed in asthma (Müller & Jacobson 2011). Different thiazole derivatives designed and synthesized in our department had shown remarkable affinity and selectivity towards adenosine receptors (Scheiff et al. 2010; Inamdar et al. 2013) (Figure 7.1).

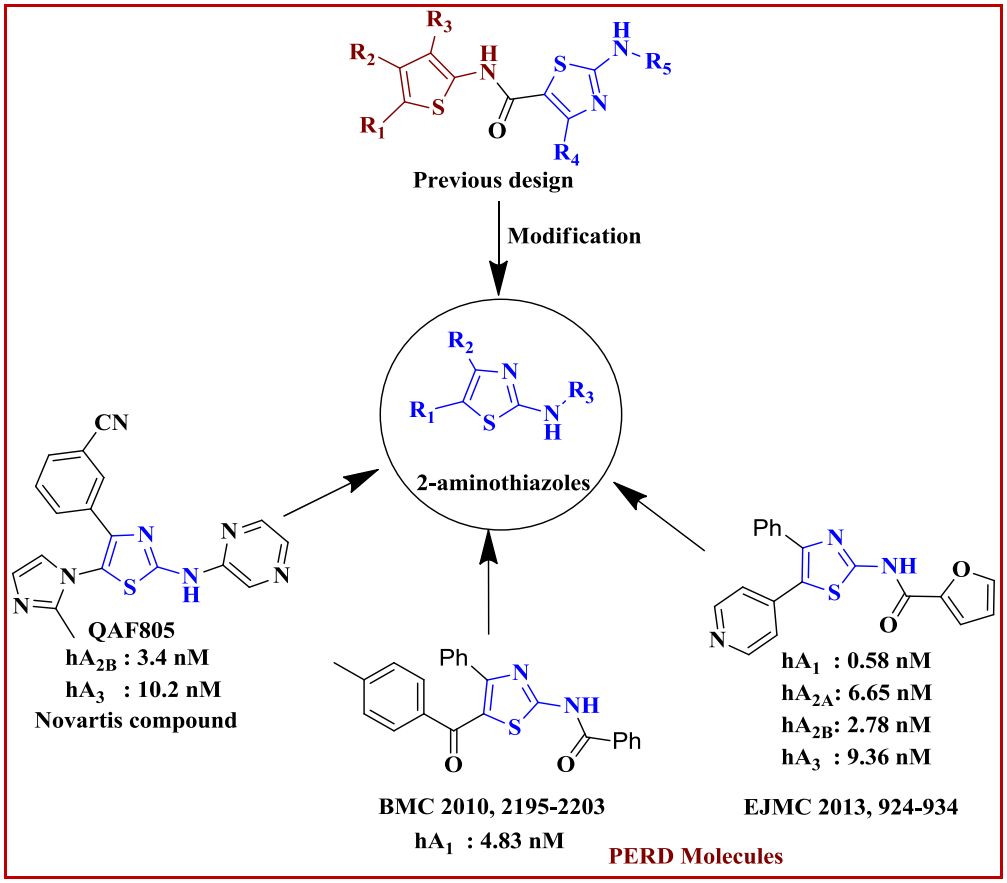
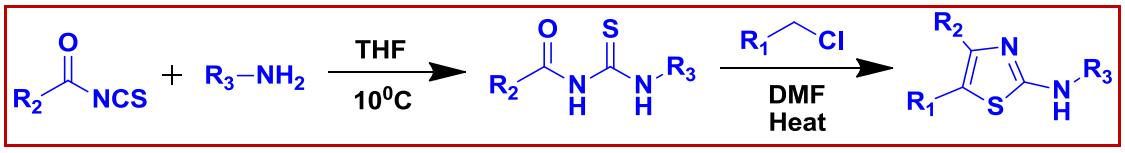


Figure 7.1 Design of substituted 2-aminothiazoles

Various stubstituted thiazoles synthesized in chapter-6, had shown good affinity towards adenosine receptors. So, in continuation of previous series of work, here we have design new series of 2-aminothiazole and coupled it with either heterocyclic derivatives or aryl acid derivatives. Further the affinity of these molecules for the adenosine receptors is examined.

7.2 Present work

In this chapter we have modified our design from substituted thiophenyl-thiophene carboxamide and thiophenyl-thiophene carboxamide to substituted 2-aminothiazole derivatives. The synthesis substituted 2-amino thiazoles was achieved by the reaction between *N*-aroyl-*N'*-aryl/heteroaryl thiourea derivatives and halomethylene compounds. The structures of the molecules (**PMCDP-TZBn**) were further confirmed by spectral techniques like Mass and NMR. They were further evaluated for their binding affinity against adenosine receptor. The most active compounds against the subtypes were further docked into the binding site of the respective subtypes to see the possible interaction between the compound and the receptor. The synthetic scheme is illustrated in scheme 7.1.



Scheme 7.1 Synthetic scheme for substituted 2-aminothiazole

As an example, benzoyl isothiocyanate is reacted with 2-aminopyridine to get the intermediate *N*-(pyridine-2-ylcarbamothioyl)benzamide. This intermediate (1 mmol) was dissolved in DMF and 4-(bromoacetyl)pyridine hydrobromide (1 mmol) in DMF was added to it. The reaction was heated to 50^0C and it was stirred for 3 hrs. The reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured into ice cold water and the resulted precipitate was collected. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compound

PMCDP-TZB2. The structure of the molecule was further confirmed by LC-MS, ^{1}H NMR and ^{13}C NMR.

The mass spectrum of **PMCDP-TZB2** (Figure 7.2) displayed the molecular ion peak at *m/z* 358.7(M+) which confirms the molecular weight of the compound 358.1 calculated for $C_{20}H_{14}N_4OS$.

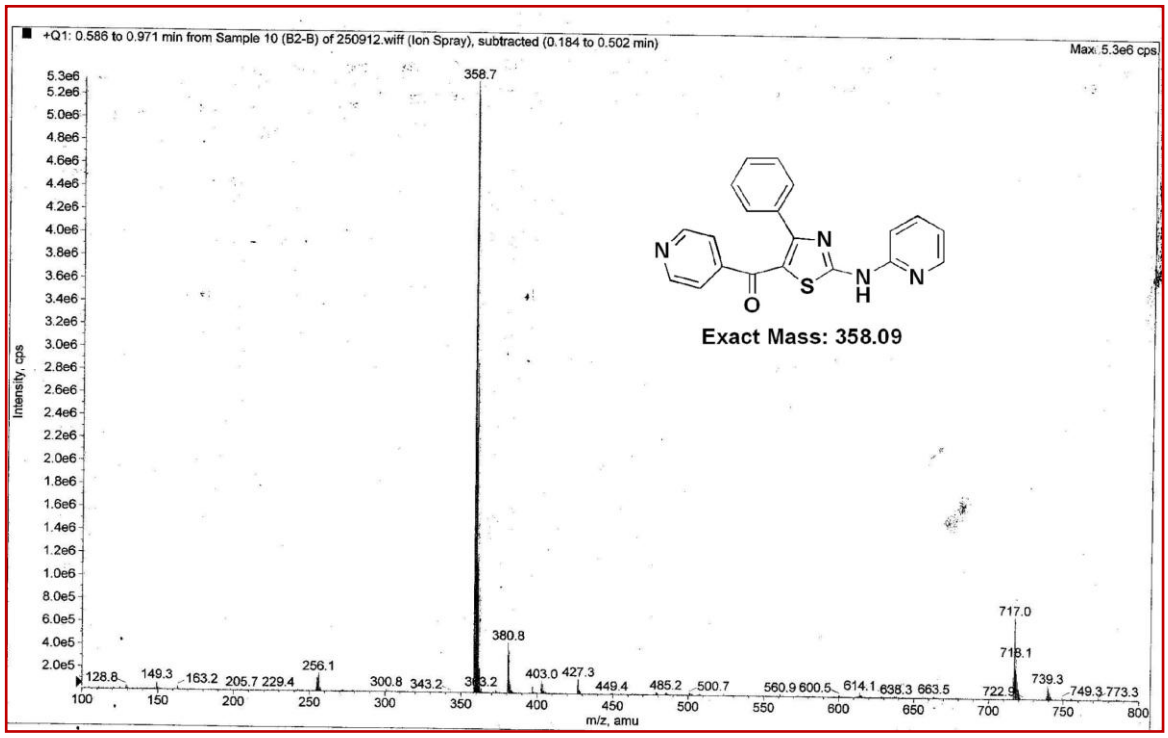


Figure 7.2 LC-MS $(M)^{+}$ of PMCDP-TZB2

Further the structure of the molecule was confirmed by ^{1}H NMR (400 MHz, DMSO-*d6*), and it gave the characteristic peak of all the hydrogens present in the molecule. The single –NH proton was observed at 12.17 as singlet. All the other hydrogens were aromatic and found in the region of 7.04-8.43 (Figure 7.3). Further, the structure was confirmed by ^{13}C NMR (400 MH_Z in DMSO-*d6*). The carbonyl carbon present in the molecule was observed most downfield at 188.4. All the aromatic carbon present on phenyl, thiophene and thiazole rings were observed in the range of 111.5-161.9 δ (Figure 7.4).

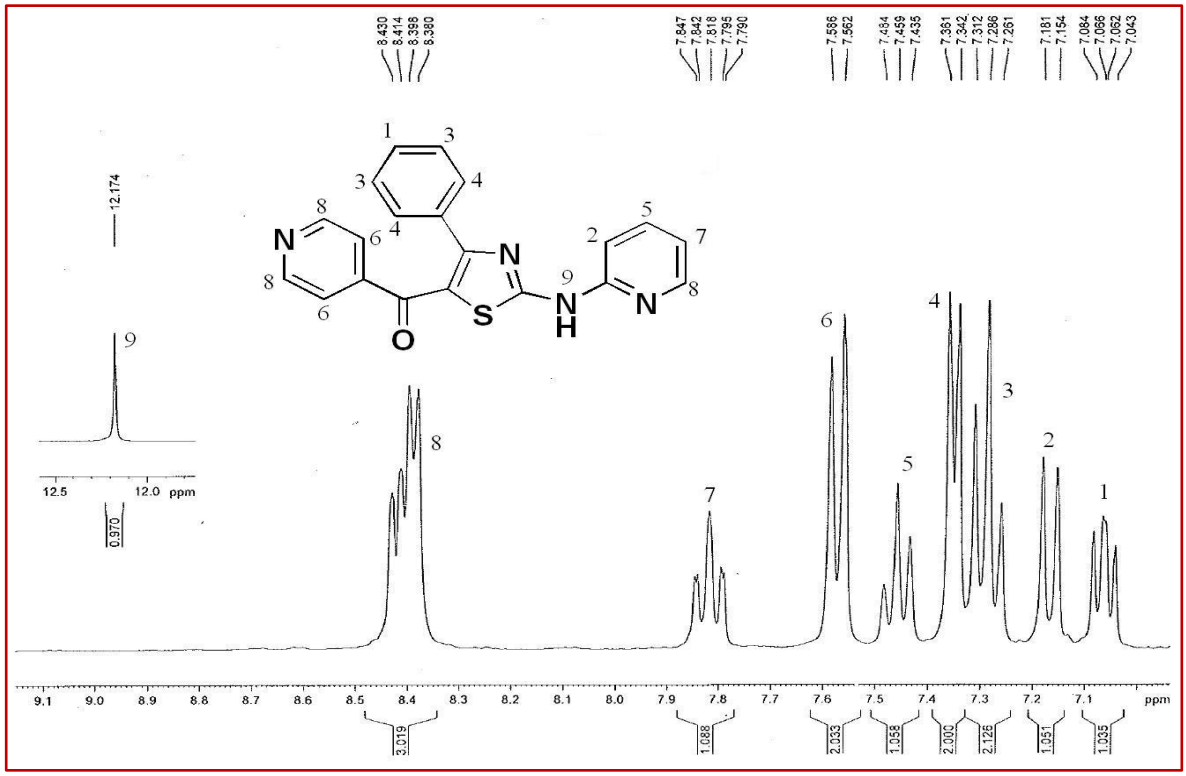


Figure 7.3 ^{1}H NMR spectrum of **PMCDP-TZB2**

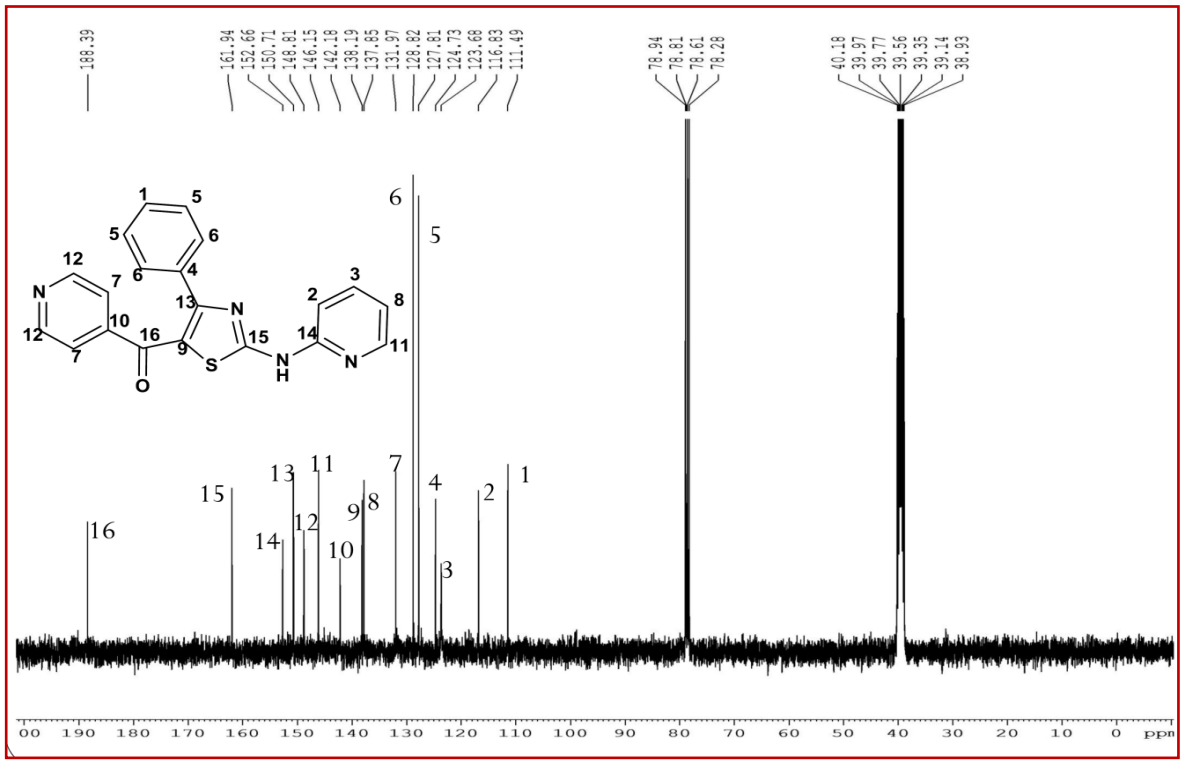
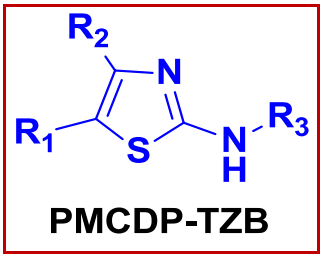


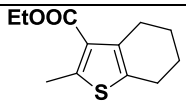
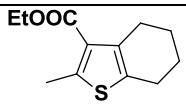
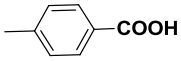
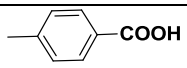
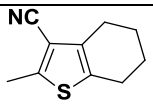
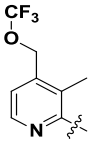
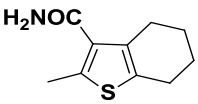
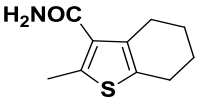
Figure 7.4 ^{13}C NMR spectrum of **PMCDP-TZB2**

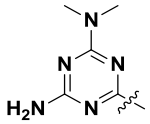
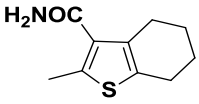
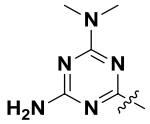
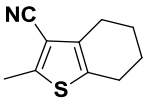
After confirmation, library of compounds with structural diversification were synthesized (PMCDP-TZAn, Table 7.1).

Table 7.1 Synthesis of substituted 2-aminothiazole derivatives



PMCDP-TZB

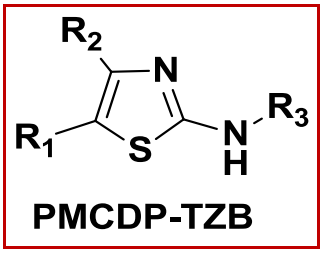
No	Code	R_1	R_2	R_3
1	**PMCDP-TZB2**	4-Pyridoyl	C_6H_5	Pyridin-2-yl
2	**PMCDP-TZB4**	(4-OMe)Ph-CO	Furan-2-yl	
3	**PMCDP-TZB5**	4-Pyridoyl	Furan-2-yl	
4	**PMCDP-TZB7**	(4-Cl)PhCO	4-OMeC_6H_4	
5	**PMCDP-TZB9**	(4-Cl)PhCO	Furan-2-yl	Pyrimidin-2-yl
6	**PMCDP-TZB10**	4-Pyridoyl	4-OMeC_6H_4	
7	**PMCDP-TZB11**	4-Pyridoyl	4-OMeC_6H_4	
8	**PMCDP-TZB12**		Furan-2-yl	Pyrimidin-2-yl
9	**PMCDP-TZB13**	(4-OMe)PhCO	C_6H_5	
10	**PMCDP-TZB14**	Pyridine-4-yl	C_6H_5	
11	**PMCDP-TZB15**	(4-OMe)PhCO	Furan-2-yl	Pyrimidin-2-yl

12	**PMCDP-TZB16**	(4-OMe)PhCO	C_6H_5	Pyrimidin-2-yl
13	**PMCDP-TZB19**		C_6H_5	
14	**PMCDP-TZB20**		4-OMeC_6H_4	
15	**PMCDP-TZB21**	Pyridine-4-yl	C_6H_5	Pyrimidin-2-yl
16	**PMCDP-TZB22**	Pyridine-4-yl	C_6H_5	Pyridin-2-yl

7.3 Biological results and discussion

The new compounds were investigated in radioligand binding studies at human adenosine receptors as per the procedure described in the chapter-3. Interestingly, most of the compounds showed moderate to high affinity with selectivity at adenosine receptors, with K_i values in the low micromolar to nanomaolar range. The affinity and selectivity toward A_1, A_{2A} and A_3 receptors was considerably high compared to A_{2B} receptor. The compounds **PMCDP-TZB2** (K_i value: A_{2A} = 32.9 nM, A_3 = 42.6 nM), **PMCDP-TZB15** (K_i value: A_{2A} = 418 nM, A_3 = 383 nM), and **PMCDP-TZB22** (K_i value: A_1 = 4.54, A_{2A} = 52.5, A_{2B} = 27.5, A_3 = 1.92), are nonselective compounds with high affinity. For the hA_1 adenosine receptor, compounds **PMCDP-TZB14** and **PMCDP-TZB21** showed high affinity (K_i: 361 nM and 20.2 nM respectively) with high selectivity (>15 folds and >10 folds respectively). For the hA_{2A} adenosine receptor, compound **PMCDP-TZB9** showed high affinity (K_i: 169 nM) with selectivity (>7 folds). For the hA_3 adenosine receptor, compounds **PMCDP-TZB4, PMCDP-TZB7,** and **PMCDP-TZB16** showed high affinity (K_i: 390 nM, 34.7 nM and 28.2 nM respectively) with high selectivity (>160 folds, >98 folds and >45 folds respectively). All the binding affinity is given in the Table 7.2.

Table 7.2 Binding affinity of synthesized substituted 2-aminothiazoles



PMCDP-TZB

No	Compound	Ki in µM at 95 % Confidence Limits			
		A_1	A_{2A}	A_{2B}	A_3
1	**PMCDP-TZB2**	416	**32.9**	>10,000	**42.6**
2	**PMCDP-TZB4**	>30	5700	>10,000	390
3	**PMCDP-TZB5**	>30	21,700	>10,000	7,080
4	**PMCDP-TZB7**	5,790	3,430	>10,000	**34.7**
5	**PMCDP-TZB9**	>30,000	169	>10,000	1100
6	**PMCDP-TZB10**	28,900	6,020	>10,000	1,180
7	**PMCDP-TZB11**	16,800	24,500	>10,000	2,550
8	**PMCDP-TZB12**	>10,000	>10,000	>10,000	>10,000
9	**PMCDP-TZB13**	2,700	6,160	>10,000	2,570
10	**PMCDP-TZB14**	361	7,270	>10,000	5,350
11	**PMCDP-TZB15**	10,100	418	>10,000	383
12	**PMCDP-TZB16**	1260	2110	>10,000	**28.2**
13	**PMCDP-TZB19**	>10,000	2320	>10,000	2190
14	**PMCDP-TZB20**	>10,000	>10,000	>10,000	2030
15	**PMCDP-TZB21**	**20.2**	1230	218	393
16	**PMCDP-TZB22**	**4.54**	**52.5**	**27.5**	**1.92**

Data are expressed as geometric means with 95% confidence limits.
[a]Displacement of specific [^{3}H]CCPA binding at human A_1 receptors expressed in CHO cells.
[b]Displacement of specific [^{3}H]NECA binding at human A_{2A} receptors expressed in CHO cells.
[c] Displacement of specific [^{3}H]HEMADO binding at human A_3 receptors expressed in CHO cells.
[d]Inhibition of NECA-stimulated adenylyl cyclase activity at human A_{2B} receptors expressed in CHO cells.

7.4 Molecular docking study

The molecular docking study of the most active molecules **PMCDP-TZB2** and **PMCDP-TZB22** was carried out with the A_{2A} and A_3 adenosine receptors respectively in SurflexDock, SYBYL X-2.0. The molecule PMCDP-TZB2 was docked into the binding pocket of A_{2A} adenosine receptor (PDB ID: 3EML). The bound ligand was removed from the receptor and PMCDP-TZB2 was docked at the same site. The molecule was found to interact with ASN253 and TYR271 by H-bonding interaction. The nitrogen of 4-pyridoyl ring was interacted with hydrogen of hydroxyl group of TYR271 and the nitrogen of 2-pyridyl present on thiazole interacted with amido hydrogen of ASN253. The docking CSCORE was found to be 5 (Figure 7.5). The molecule **PMCDP-TZB22** was docked into the binding pocket of A_3 adenosine receptor (PDB ID: 1OEA). The active site was defined by automatic method of SurflexDock and the ligand PMCDP-22 was docked into it. The molecule was found to interact with ASN250 by H-bonding interaction. The nitrogen present on thiazole ring was found to interact with the amido group hydrogens of ASN250 with the CSCORE of 4 (Figure 7.6).

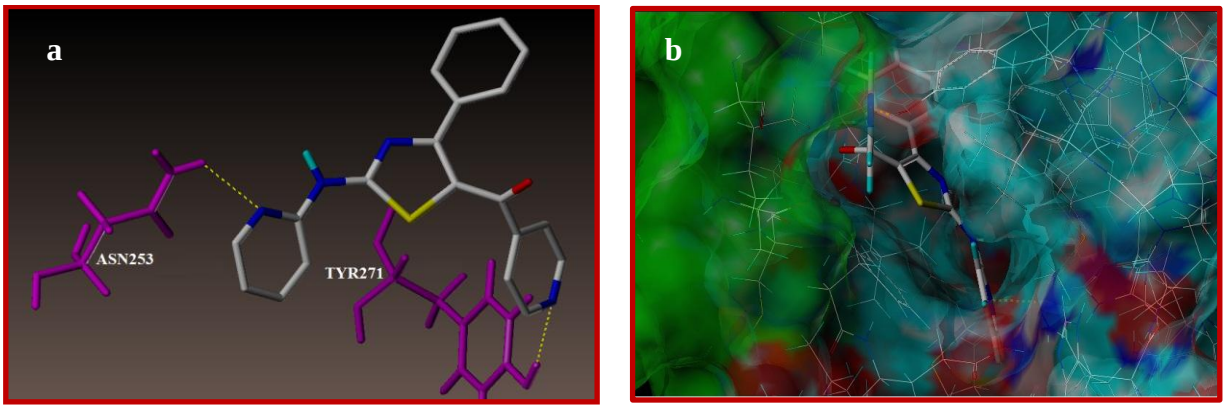


Figure 7.5(a) Hydrogen interaction of PMCDP-TZB2 with A_{2A} adenosine receptor (b) PMCDP-TZB2 in binding pocket of adenosine A_{2A} receptor

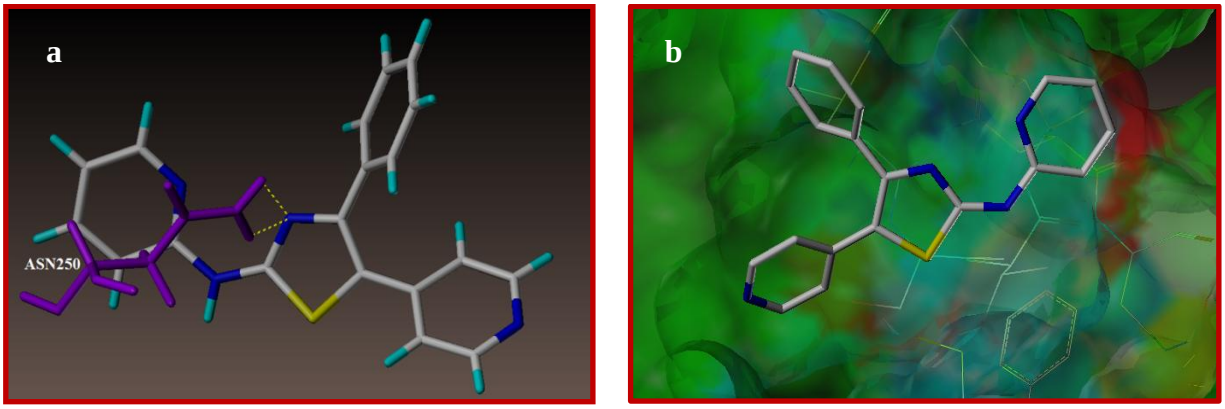


Figure 7.6(a) Hydrogen interaction of PMCDP-TZB22 with A_3 adenosine receptor (b) PMCDP-TZB22 in binding pocket of adenosine A_3 receptor

7.5 Conclusion

In conclusion, here we have designed new series of 2-aminothiazoles. The compounds were synthesized and investigated for their radio ligand binding assay against adenosine receptors. Particularly this series of compounds were found to show high affinity with selectivity towards adenosine receptors in nanomoar range. And the affinity of this series of molecules was highest as compared to the molecules from previous chapters. Further molecular docking study of the most active compounds (towards A_{2A} and A_3 receptor) was carried out in SurflexDock, SYBYL-X 2.0.

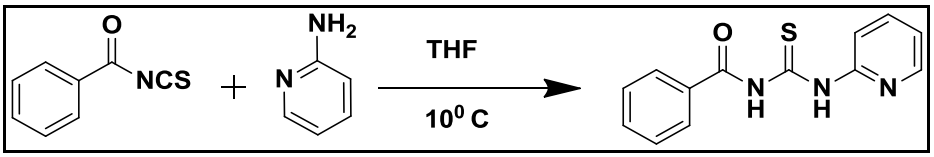
7.6 Experimental

The ^{1}H NMR spectra were recorded on Bruker NMR spectrometer (400 MHz) using TMS as an internal standard and ^{13}C NMR spectra were recorded on Bruker NMR spectrometer at 75 MHz. Proton chemical shifts are expressed in ppm relative to internal tetramethylsilane. Mass spectra were recorded on Perkin Elmer Sciex API 165. TLC was carried out on Merck Kieselgel 60 PF_{254}. IUPAC name of the compounds were generated using Cambridge soft ChemBioDraw ultra 12.0. The molecular docking study was carried out in the SurflexDock tool available in software SYBYL X-2.0. The synthesis of 6-(chloromethyl)-N^2,N^2-dimethyl-1,3,5-triazine-2,4-diamine is well documented in literature (Inamdar et al. 2013).

7.6.1 General procedure for synthesis for *N*-aroyl-*N'*-aryl/heteroaryl thiourea derivatives

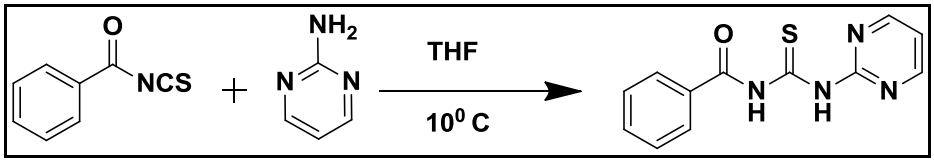
To a stirred solution of aroyl isothiocynate (1 mmol) in THF at room temperature, aryl or heteroaryl amine dissolved in THF (1 mmol) was added drop wise. The reaction was stirred for 3h and monitored by TLC. After completion of the reaction, the reaction mixture was poured into the cold water to give precipitates of the desired product. The resultant solid was filtered through high vacuum suction pump and then dissolved in chloroform and dried over anhydrous Na_2SO_4 to remove traces of water. Removal of solvent on rotary evaporator afforded desired compounds after triturating with hexane.

7.6.1.1. Synthesis of N-(Pyridin-2-ylcarbamothioyl)benzamide (F1)



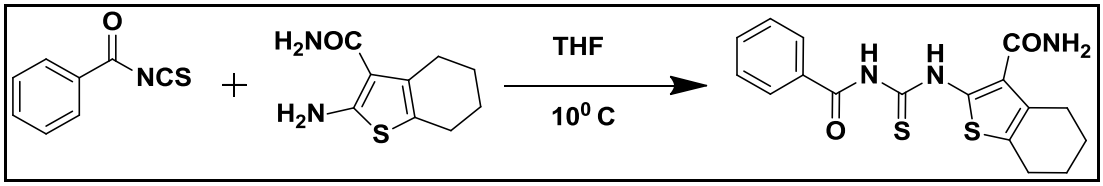
The title compound was synthesized using the procedure as described in 7.6.1 by the reaction of benzoyl isothiocyante and 2-aminopyridine to afford the product. **Yield:** 70 %, **LC-MS found:** (M+1) at 258, **Molecular Formula:** $C_{13}H_{11}N_3OS$

7.6.1.2. Synthesis of N-(Pyrimidin-2-yl carbamothioyl)benzamide (F2)



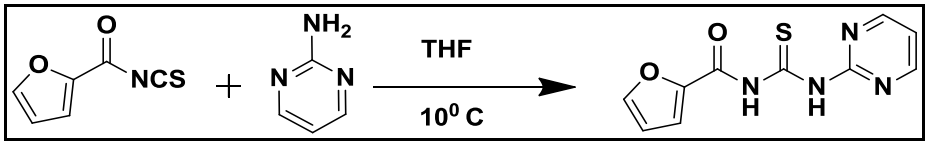
The title compound was synthesized using the procedure as described in 7.6.1 by the reaction of benzoyl isothiocyanate and 2-aminopyrimdine to afford the product. **Yield:** 66 %, **LC-MS found:** (M+1) at 259, **Molecular Formula:** $C_{12}H_{10}N_4OS$.

7.6.1.3. Synthesis of 2-(3-Benzoylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (F3)



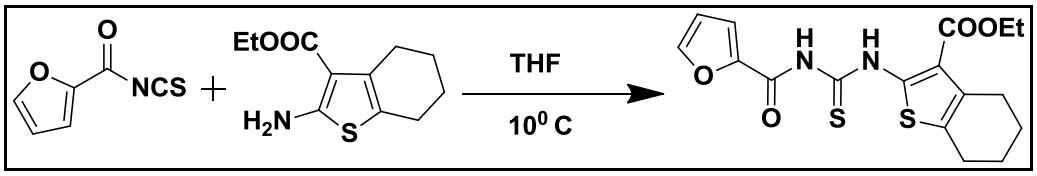
The title compound was synthesized using the procedure as described in 7.6.1 by the reaction of benzoyl isothiocyante and 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide to afford the product. **Yield:** 75 %, **LC-MS Found:** (M+1) at 360, **Molecular Formula:** $C_{17}H_{17}N_3O_2S_2$

7.6.1.4. Synthesis of N-(Pyrimidin-2-yl carbamothioyl)furan-2-carboxamide (F4)



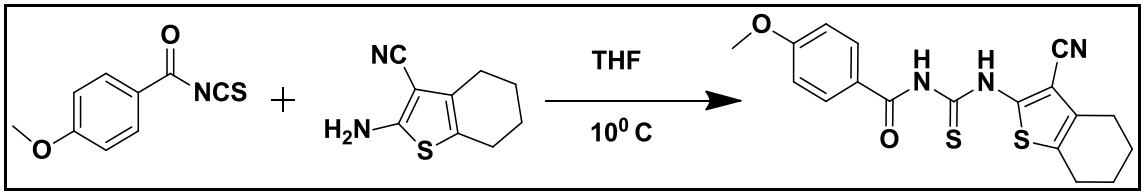
The title compound was synthesized using the procedure as described in 7.6.1 by the reaction of furoyl isothiocyante and 2-aminopyrimidin to afford the product. **Yield:** 59 %, **LC-MS Found:** (M+) at 248, **Molecular Formula:** $C_{10}H_8N_4O_2S$

7.6.1.5. Synthesis of Ethyl 2-(3-(furan-2-carbonyl)thioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (F5)



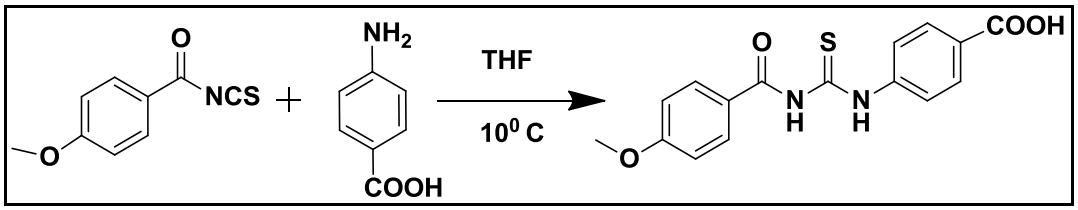
The title compound was synthesized using the procedure as described in 7.6.1 by the reaction of furoyl isothiocyante and ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylateto afford the product. **Yield:** 55 %, **LC-MS Found:** (M+) at 378.2, **Molecular Formula:** $C_{17}H_{18}N_2O_4S_2$

7.6.1.6. Synthesis of N-((3-Cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)carbamothioyl)-4-methoxybenzamide (F6)



The title compound was synthesized using the procedure as described in 7.6.1 by the reaction of 4-methoxy benzoyl isothiocyante and 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide to afford the product. **Yield:** 67 %, **LC-MS Found:** (M+) at 371, **Molecular Formula:** $C_{18}H_{17}N_3O_2S_2$

7.6.1.7. Synthesis of 4-(3-(4-Methoxybenzoyl)thioureido)benzoic acid (F7)

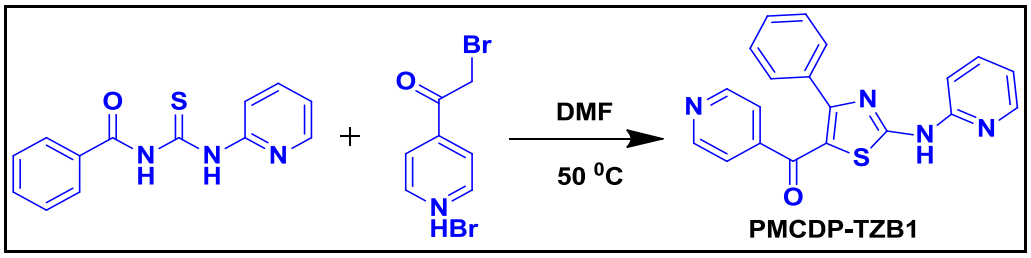


The title compound was synthesized using the procedure as described in 7.6.1 by the reaction of 4-methoxy benzoyl isothiocyante and 4-aminobenzoic acid to afford the product. **Yield:** 58 %, **LC-MS Found:** (M+) at 330, **Molecular Formula:** $C_{16}H_{14}N_2O_4S$

7.6.2 General procedure for the synthesis substituted of 2-aminothiazoles

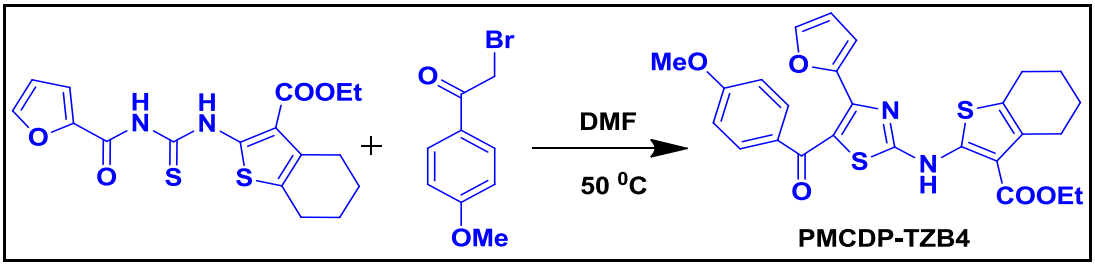
To a hot air dried round bottomed flask, containing a solution of *N*-aroyl-*N'*-aryl/heteroaryl thiourea (1 mmol) in DMF (5 mL), substituted phenacyl bromide (1 mmol) or helomethylene compound in DMF (2 mL) was added and the reaction was further stirred for 4 h at 50^0 C. (3 mmol of TEA was added in case of use of starting material like 4-(bromoacetyl)pyridine hydrobromide or 4-chloromethyl pyridine hydrochloride) Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-TZBn)** were assigned with the help of NMR and mass spectra.

PMCDP-TZB2: (4-Phenyl-2-(pyridin-2-ylamino)thiazol-5-yl) (pyridin-4-yl)methanone



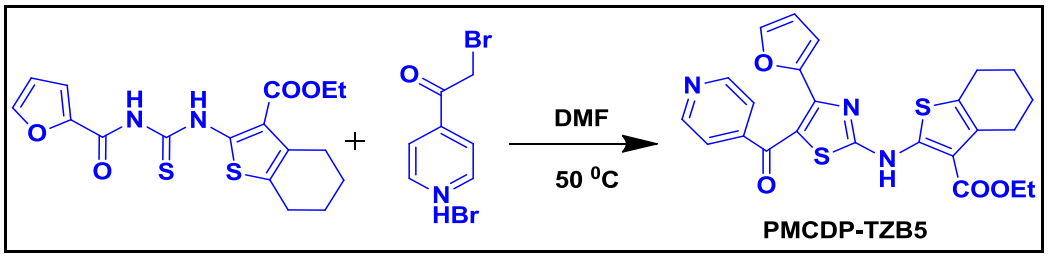
Yield: 59%, Yellow solid, mp: 160-164 ^{0}C, **Molecular formula: $C_{20}H_{14}N_4OS$**, LC-MS calculated: 358.3, found: 359.4 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 7.04(q, 1H), 7.15(d, 1H), 7.26(t, 2H), 7.34(d, 2H), 7.43(t, 1H), 7.56(d, 2H), 7.79-7.84(m, 1H) 8.38(q, 3H), 12.172(s, 1H). **^{13}C NMR (400 MHz, DMSO-d_6)** δ: 111.49, 116.83, 123.68, 124.73, 127.81, 128.82, 131.97, 137.85, 138.19, 142.18, 146.15, 148.81, 150.71, 152.66, 161.94, 188.39.

PMCDP-TZB4: Ethyl 2-((4-(furan-2-yl)-5-(4-methoxybenzoyl)thiazol-2-yl)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



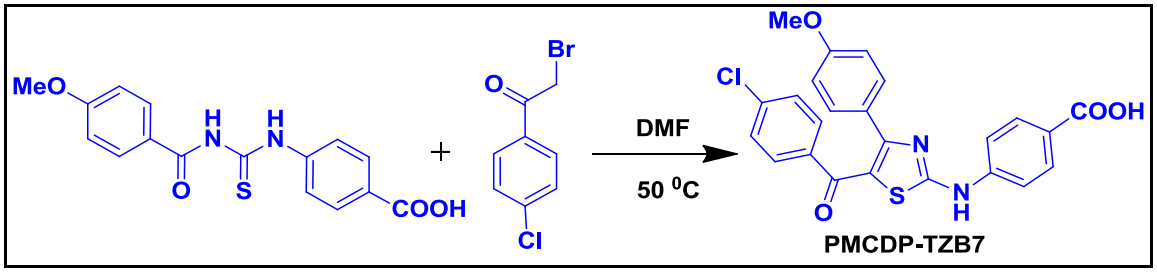
Yield: 63%, light yellow solid, **Molecular formula: $C_{26}H_{24}N_2O_5S_2$**, LC-MS calculated: 508.3, found: 509.5 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.16(q, 3H), 1.71(d, 4H), 2.44(d, 2H), 2.65(d, 2H), 3.72(s, 3H), 4.01(t, 2H), 6.61(q, 1H), 6.86(d, 2H), 6.95(d 1H), 7.59(d, 2H), 7.89(s, 1H), 12.72(s, 1H).

PMCDP-TZB5: Ethyl 2-((4-(furan-2-yl)-5-isonicotinoylthiazol-2-yl)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate



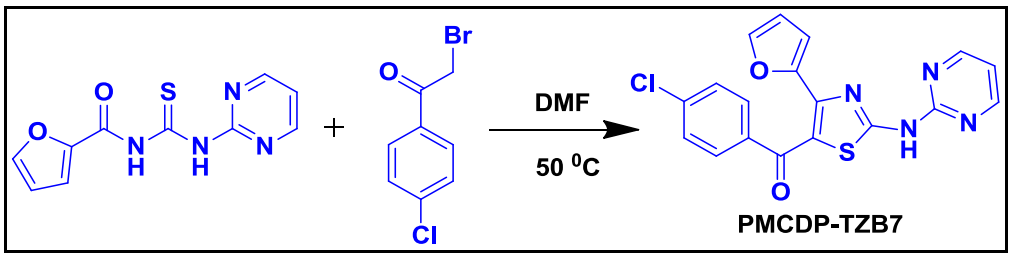
Yield: 53%, off white solid, **Molecular formula: $C_{24}H_{21}N_3O_4S_2$**, LC-MS calculated: 479.1, found: 479.7 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.16(q, 3H), 1.73(t, 4H), 2.61(t, 2H), 2.79(t, 2H), 4.08(q, 2H), 6.59(q, 1H), 6.97(q, 1H), 7.65(q, 2H), 7.86(s, 1H), 8.27(d, 1H), 8.55(q, 2H).

PMCDP-TZB7: 4-((5-(4-Chlorobenzoyl)-4-(4-methoxyphenyl)thiazol-2-yl)amino) benzoic acid



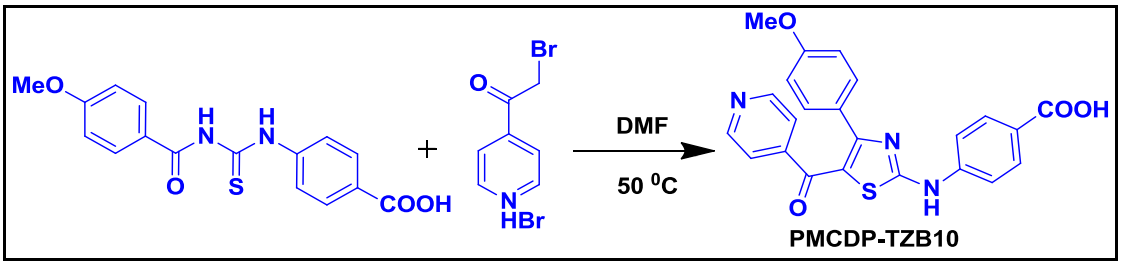
Yield: 55%, light yellow solid, mp: 154-158 ^{0}C, **Molecular formula: $C_{24}H_{27}ClN_2O_4S$**, LC-MS calculated: 464.4, found: 463.5 (M-1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.16(q, 3H), 1.73(t, 4H), 2.61(t, 2H), 2.79(t, 2H), 4.08(q, 2H), 6.59(q, 1H), 6.97(q, 1H), 7.65(q, 2H), 7.86(s, 1H), 8.27(d, 1H), 8.55(q, 2H).

PMCDP-TZB9: (4-Chlorophenyl)(4-(furan-2-yl)-2-(pyrimidin-2-ylamino)thiazol-5-yl)methanone



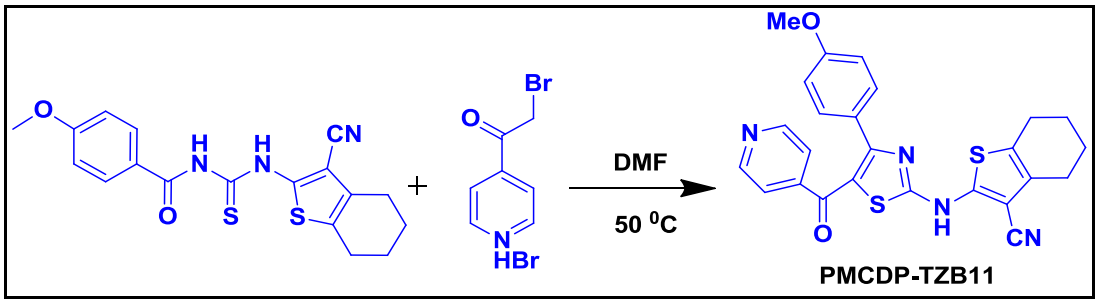
Yield: 57%, yellow solid, mp: 170-174 ^{0}C, **Molecular formula: $C_{18}H_{11}ClN_4O_2S$**, LC-MS calculated: 382.5, found: 383.4 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 5.82(s, 1H), 6.30(s, 1H), 7.10(d, 2H), 7.62(d, 2H), 8.16(d, 2H), 8.81(s, 2H), 12.68(s, 1H).

PMCDP-TZB10: 4-((5-Isonicotinoyl-4-(4-methoxyphenyl)thiazol-2-yl)amino)benzoic acid



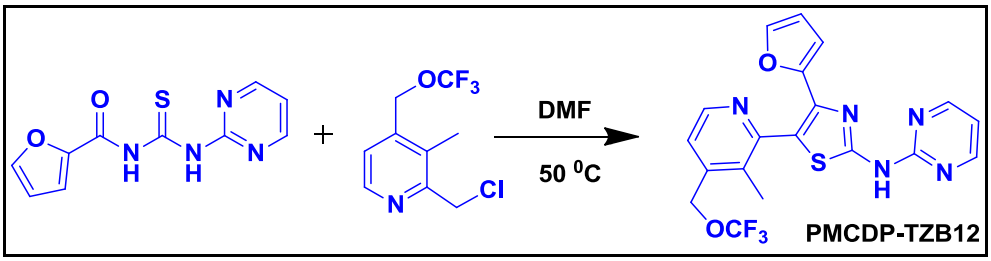
Yield: 55%, light yellow solid, mp: 140-144 ^{0}C, **Molecular formula: $C_{23}H_{17}N_3O_4S$**, LC-MS calculated: 431.6, found: 430.6 (M-1).

PMCDP-TZB11: 2-((5-Isonicotinoyl-4-(4-methoxyphenyl)thiazol-2-yl)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile



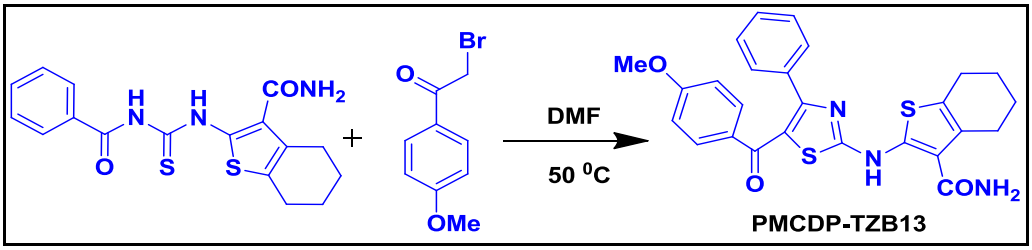
Yield: 55%, off white solid, mp: 17-174 ^{0}C, **Molecular formula: $C_{25}H_{20}N_4O_2S_2$**, LC-MS calculated: 472.1, found: 472.8 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.80(s, 3H), 2.53(s, 4H), 3.78(s, 4H), 4.01(t, 2H), 6.79(d, 2H), 7.38(d, 2H), 7.59(d, 2H), 8.42(d, 2H), 12.19(s, 1H).

PMCDP-TZB12: (4-(Furan-2-yl)-5-(3-methyl-4-((trifluoromethoxy)methyl)pyridin-2-yl)-*N*-(pyrimidin-2-yl)thiazol-2-amine



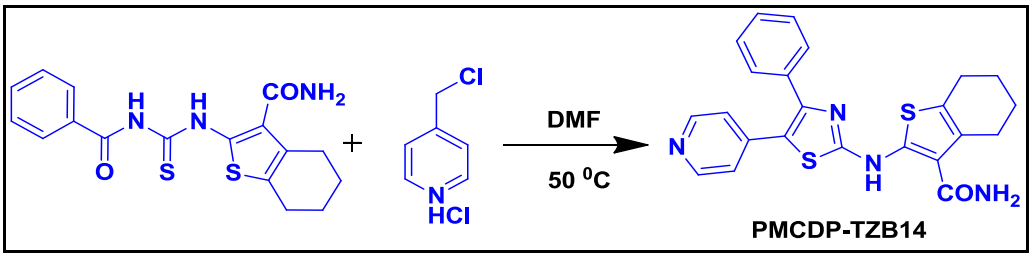
Yield: 66%, yellow solid, mp: 233-236 ^{0}C, **Molecular formula: $C_{19}H_{14}F_3N_5O_2S$**, LC-MS calculated: 433.1, found: 433.8 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.90(s, 3H), 4.86 (q, 2H), 6.41(d, 1H), 6.45(q, 1H), 7.01(t, 1H), 7.15(d, 1H), 7.49(s, 1H), 8.41(d, 1H), 8.61(d, 2H) 12.72(s, 1H).

PMCDP-TZB13: 2-((5-(4-Methoxybenzoyl)-4-phenylthiazol-2-yl)amino)- 4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carboxamide



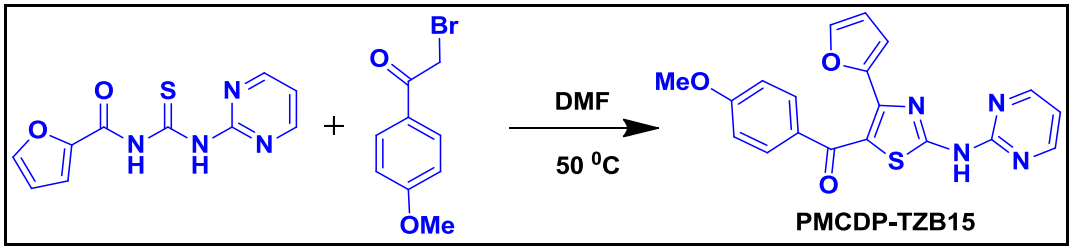
Yield: 62%, White solid, **Molecular formula: $C_{25}H_{20}N_4O_2S_2$**, LC-MS calculated: 489.7, found: 490.3 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.71(s, 4H), 2.57(s, 2H), 2.65(s, 2H), 3.83(s, 3H), 4.89(q, 2H), 7.18(S, 1H), 7.41(d, 2H), 7.51(d, 2H), 7.80(m, 4H), 12.72(s, 1H).

PMCDP-TZB14: 2-((4-Phenyl-5-(pyridin-4-yl)thiazol-2-yl)amino)- 4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene-3-carboxamide



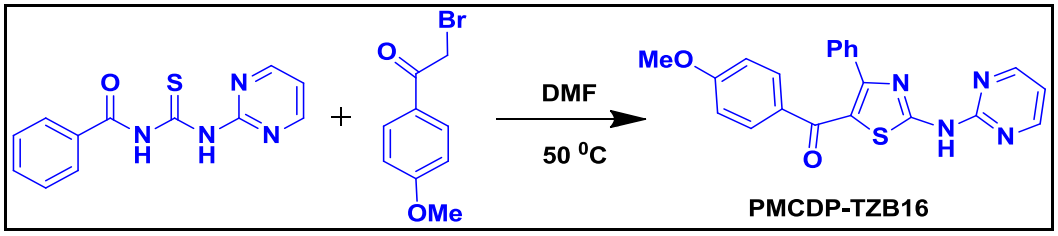
Yield: 57%, yellow solid, **Molecular formula: $C_{23}H_{20}N_4OS_2$**, LC-MS calculated: 432.5, found: 433.2 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.76(s, 4H), 2.65(m, 4H), 7.39-7.41(m, 3H), 7.50-7.53(m, 3H), 7.95(d, 1H), 8.49(d, 2H) 11.54(d, 1H), 11.82(s, 1H), 14.40(s, 1H).

PMCDP-TZB15: (4-(Furan-2-yl)-2-(pyrimidin-2-yl amino) thiazol-5-yl) (4-methoxyphenyl)methanone



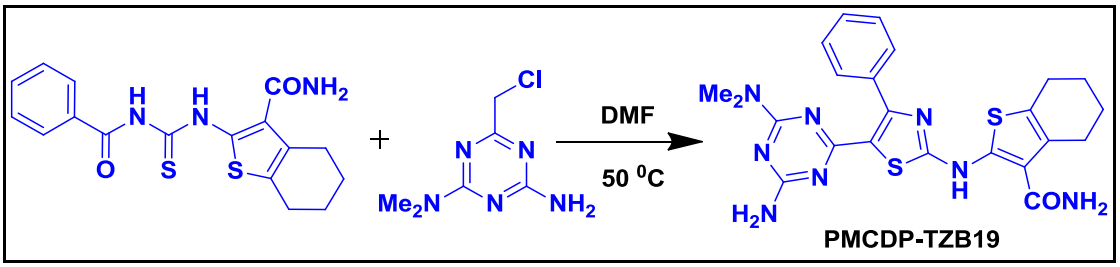
Yield: 65%, light yellow solid, mp: 212-215 ^{0}C, **Molecular formula: $C_{19}H_{14}N_4O_3S$**, LC-MS calculated: 378.4, found: 379.2 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.86(s, 3H), 5.85(s, 1H), 7.03(d, 2H), 7.31(s, 1H), 7.62(d, 2H), 8.16(d, 2H), 8.81(s, 2H), 12.75(s, 1H).

PMCDP-TZB16: (4-Methoxyphenyl) (4-phenyl-2-(pyrimidin-2-ylamino)thiazol-5-yl)methanone



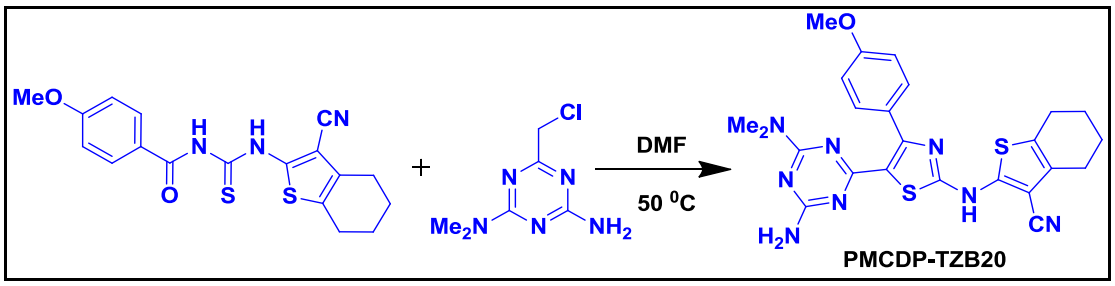
Yield: 68%, Light yellow solid, mp: 200-203 ^{0}C, **Molecular formula: $C_{21}H_{16}N_4O_2S$**, LC-MS calculated: 388.4, found: 389.3 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 3.86(s, 3H), 6.94(s, 1H), 6.99(d, 2H), 7.31(m, 3H), 7.78(m, 4H), 8.66 (d, 2H), 12.90(s, 1H).

PMCDP-TZB19: 2-((5-(4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl)-4-phenylthiazol-2-yl)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide



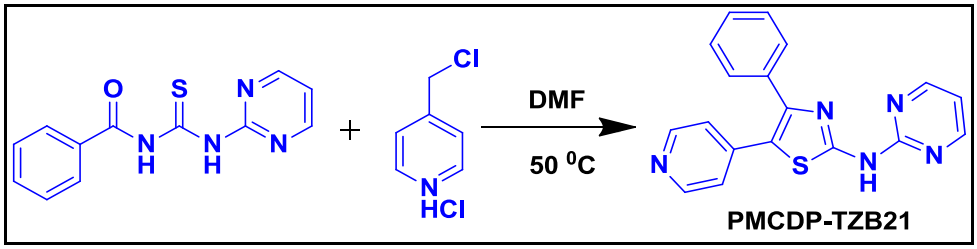
Yield: 60%, yellow solid, mp: 137-141^{0}C, **Molecular formula: $C_{23}H_{24}N_8OS_2$**, LC-MS calculated: 492.5, found: 493.4 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 1.90(s, 3H), 4.86(q, 2H), 6.41 (d, 1H), 6.45(q, 1H), 7.01(t, 1H), 7.15(d, 1H), 7.49 (s, 1H), 8.41(d, 1H), 8.61(d, 2H) 12.72(s, 1H).

PMCDP-TZB20: 2-((5-(4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl)-4-(4-methoxyphenyl)thiazol-2-yl)amino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile



Yield: 62%, bright yellow solid, mp: 148-152 ^{0}C, **Molecular formula: $C_{23}H_{24}N_8OS_2$**, LC-MS calculated: 504.5, found: 505.2 (M+1).

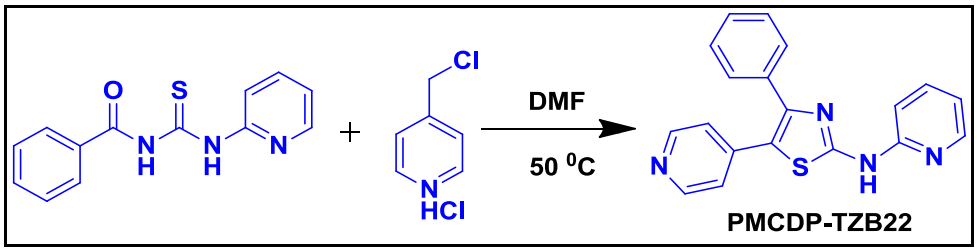
PMCDP-TZB21: 4-Phenyl-5-(pyridin-4-yl)-*N*-(pyrimidin-2-yl)thiazol-2-amine



Yield: 81%, light yellow solid, mp: 175-180 ^{0}C, Molecular **formula: $C_{18}H_{13}N_5S$**, LC-MS calculated: 331.2, found: 332.5 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 6.41(d, 1H),

6.45(s, 1H), 7.01(t, 1H), 7.28(t, 1H), 7.57(m, 3H), 7.99(q, 2H), 8.77(d, 2H) 12.07(s, 1H), 13.67(s, 1H).

PMCDP-TZB22: 4-Phenyl-5-(pyridin-4-yl)-*N*-(pyridin-2-yl)thiazol-2-amine



Yield: 81%, light yellow solid, mp: >250^{0}C, **Molecular formula: $C_{18}H_{13}N_5S$**, LC-MS calculated: 331.2, found: 332.5 (M+1). **^{1}H NMR (400 MHz, DMSO-d_6)** δ: 7.06(q, 1H), 7.15(d, 1H), 7.30(t, 2H), 7.38(d, 2H), 7.43(t, 1H), 7.56(d, 2H), 7.80(t, 1H) 8.38(q, 3H), 12.20 (s, 1H).

7.6.3 Protocol for molecular docking study

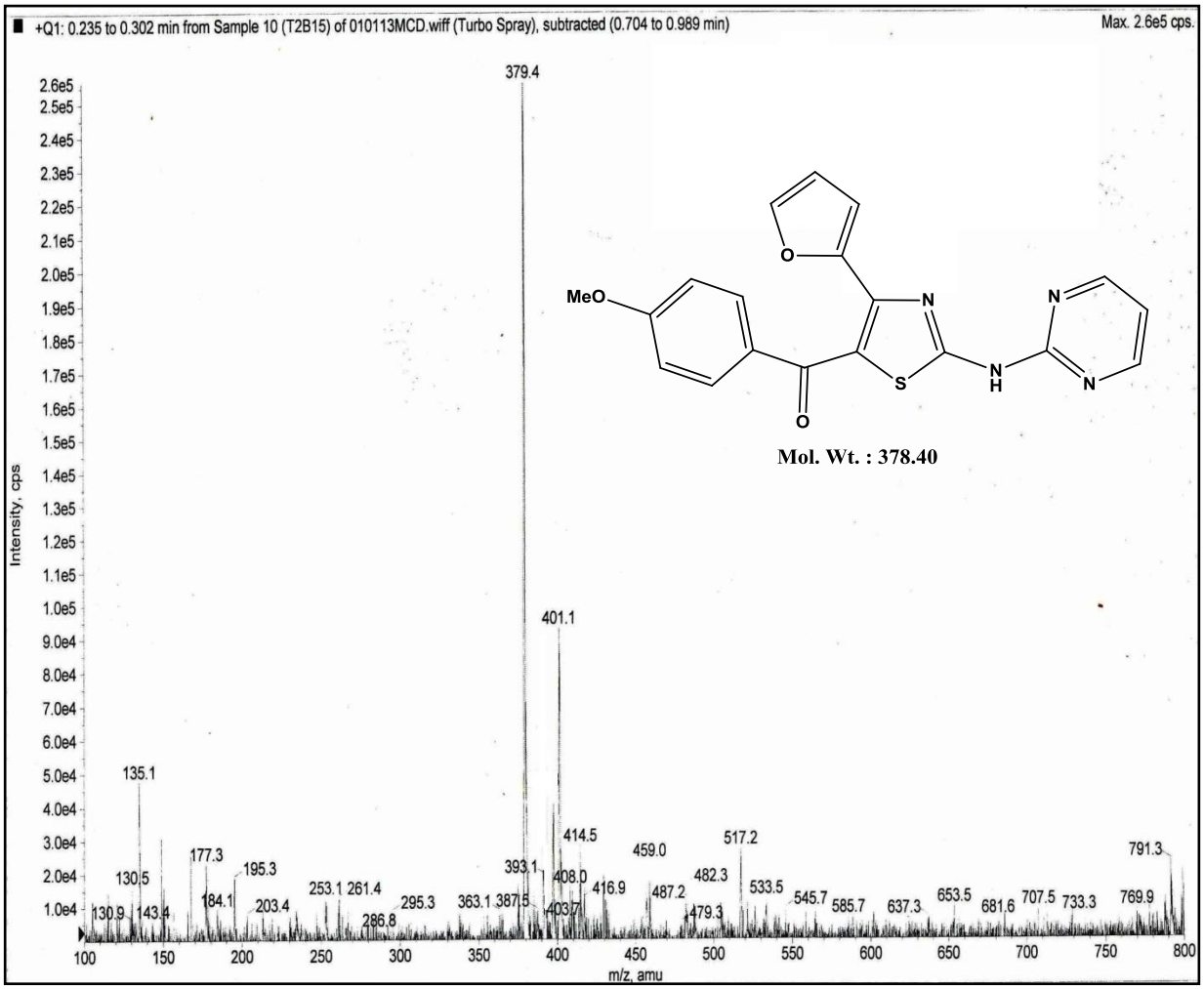
The molecular docking study of our the two most active molecules PMCDP-TZB2 and PMCDP-TZB22 was carried out with A_{2A} and A_3 adenosine receptors respectively in SuflexDock tool available in the SYBYL X-2.0 (Sybyl-X 2.0, Tripos International, A Certara company). The ligands were drawn using the SKETCH module available in the SYBYL X-2.0. The molecules were given Gasteigere-Huckel charges and minimized to a minimum energy conformation. The x-ray crystallographic A_{2A} adenosine receptor was downloaded from protein data bank (PDB ID: 3EML). The A_{2A} adenosine receptor was already having a bound ligand, so that ligand was extracted and that site was taken as binding site. During the protein preparation water molecule was removed, hydrogen atoms, atom types were fixed charges were added and staged minimization was done. The protomol generation gave the binding site of protein and the flexible docking of PMCDP-TZB2 with A_{2A} adenosine receptor was facilitated through the SuflexDock, SYBYL X-2.0. For the docking of PMCDP-TZB22 with A_3 adenosine receptor, the homology modeled receptor (PDB ID: 1EAO) was downloaded from protein data bank. The protein and ligands were prepared explained as earlier.

7.6.4 Method used for pharmacological evaluation

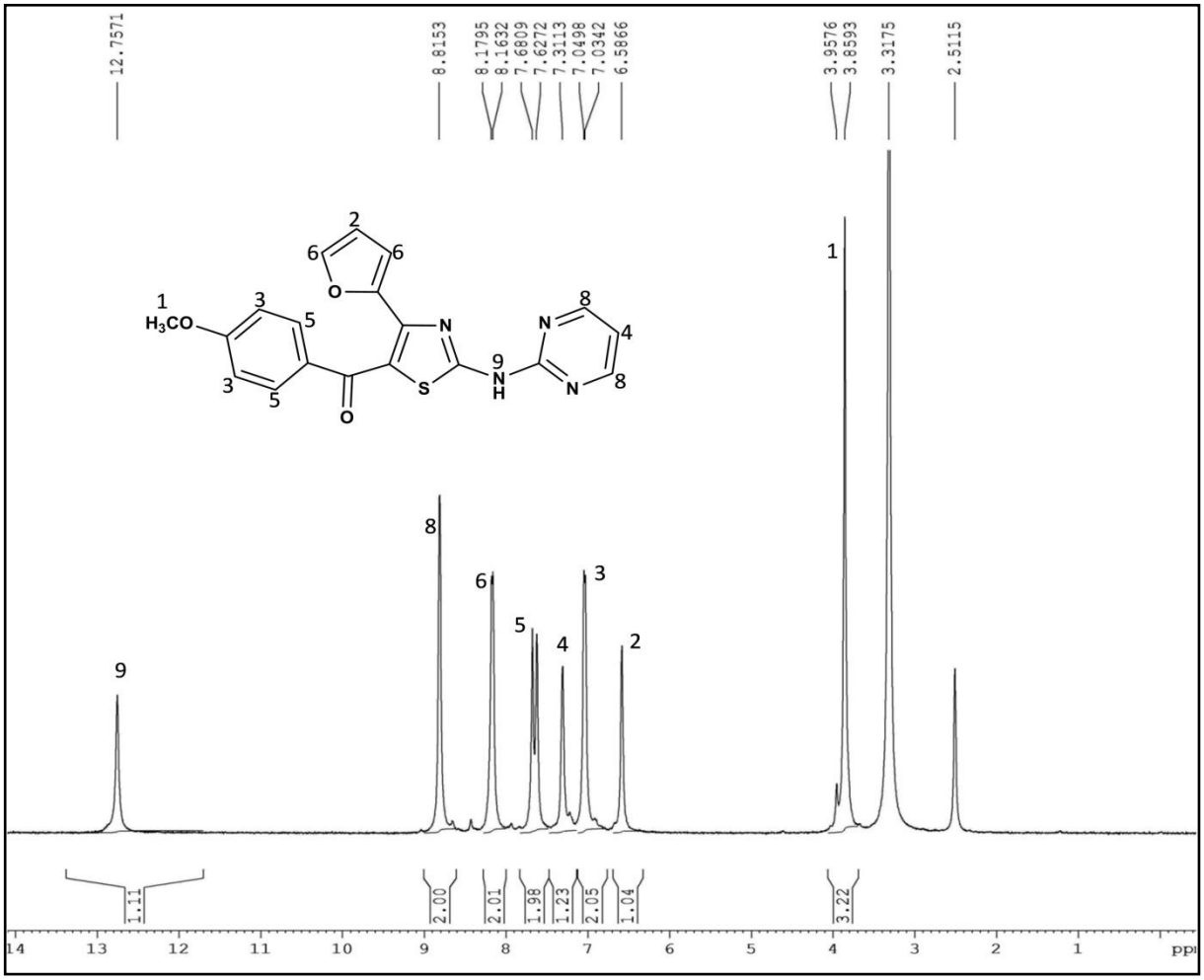
The methods described in the chapter-3 were used for the pharmacological evaluation for the compounds.

Supporting Information
(LC-MS and ^{1}H-NMR of representative compounds)

LC-MS spectrum of PMCDP-TZB15



^{1}H spectrum of PMCDP-TZB15



CHAPTER-8

3D-QSAR study of thiazole derivatives as adenosine receptor antagonists

PAGE NO. 143 TO 160

Chapter 8

3D-QSAR study of thaizole derivatives as adenosine receptor antagonists

8.1 Introduction

The understanding of how a molecular structure brings about a particular effect in a biological system is a key to unlock the relationship between molecule and biological system. Development of these relationships has proved to be the foundation for the development of predictive models. If we take a series of chemicals and attempt to form a *quantitative relationship* between the biological effects (i.e. the *activity*) and the chemistry (i.e. the *structure*) of each of the chemicals, then we are able to form a *quantitative structure–activity relationship* or QSAR. The purpose of QSAR studies is (a) To predict biological activity and physico-chemical properties by rational means and (b) To comprehend and rationalize the mechanisms of action within a series of chemicals. Three-dimensional quantitative structure–activity relationship (3D-QSAR) techniques are the most prominent computational means to support chemistry within drug design projects where no three-dimensional structure of the macromolecular target is available. The primary aim of these techniques is to establish a correlation of biological activities of a series of structurally and biologically characterized compounds with the spatial fingerprints of numerous field properties of each molecule like as steric and electrostatic interactions. The number of 3D-QSAR studies has exponentially increased over the last decade, since a variety of methods are commercially available in user-friendly, graphically guided software. 3D-QSAR correlates spatially localized features across a chemical series with biological activity. As the descriptors used to represent chemical structure usually encode location dependent structural characteristics that account for activity, the molecular structures need to be aligned across the series. Descriptor types distinguish the two primary types of 3D-QSAR methods: lattice-based descriptors and surface based descriptors. Among the lattice-based methods, CoMFA is by far the most

studied and applied 3D-QSAR method (Cramer et al. 1988). In principle, 3D QSAR is more powerful than classical QSAR, because:

- 3D structures are considered instead of only 2D structures
- More heterogeneous sets of compounds can be included than in classic QSAR
- Molecular fields are calculated instead of just substituent constants
- Contour maps show the effect of certain chemical properties in certain regions.

8.2 Present work

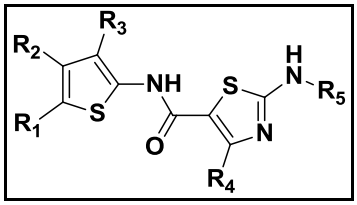
In the present work we have performed COMFA studies on the all the molecules which have shown activity towards adenosine receptor subtypes from chapter-3, chapter-4, chapter-6 and chapter-7. Three different set of molecules with biological activities against A_1, A_{2A} and A_3 and were taken and the COMFA study was performed. The statistical results and contour maps generated through 3D QSAR study were analyzed.

8.3 Material and Method

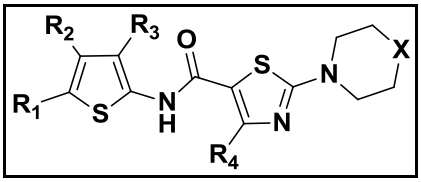
8.3.1 Data set

The study involved the three different set of molecules active against A_1, A_{2A} and A_3 adenosine receptor subtypes. The data set of (i) 18 active compounds against A_1 subtype (ii) 18 active compounds against A_{2A} subtype and (iii) 26 active compounds against A_3 subtype was taken for the study. The affinity (K_i) was transformed to negative logarithmic units marked as pK_i in the CoMFA. The data set was divided into training set to generate the 3D-QSAR models and test set to evaluate the predictive ability of the developed models. (Table 8.1-8.3)

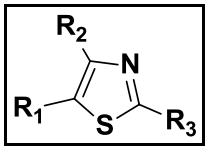
Table 8.1 Structures and biological activity of A_1 adenosine receptor antagonists used for 3D QSAR study



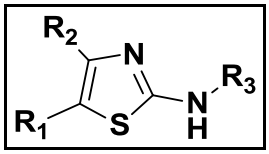
No	Code	R_1	R_2	R_3	R_4	R_5	PK_i (A_1)
1	PMCDP-5	-$(CH_2)_4$-		-COOEt	4-MeC_6H_4	C_6H_5	5.11
2	PMCDP-6	-$(CH_2)_4$-		-COOEt	-$N(Me)_2$	4-MeC_6H_4	4.63
3	PMCDP-12	-$(CH_2)_4$-		-$CONH_2$	4-MeC_6H_4	C_6H_5	5.63
4	PMCDP-21	-COOMe	Me	-COOEt	-$N(Me)_2$	4-$OMeC_6H_4$	4.85
5	PMCDP-25	-COOMe	Me	-COOEt	-$N(Me)_2$	4-MeC_6H_4	4.72



No	Code	R_1	R_2	R_3	R_4	X	PK_i (A_1)
6	PMCDP-29	-$(CH_2)_4$-		-COOEt	Furan-2-yl	O	5.14



No	Code	R_1	R_2	R_3	PK_i (A_1)
7	PMCDP-TZA9	Pyridine-4-yl	C_6H_5	Morpholin-4-yl	5.58
8	PMCDP-TZA13	Pyridine-4-yl	Furan-2-yl	2,4-Dichloro phenyl piperazin-4-yl	4.73
9	PMCDP-TZA16	Pyridine-4-yl	C_6H_5	Diethyl amino	6.07



No	Code	R_1	R_2	R_3	PK_i (A_1)
10	PMCDP-TZB2	4-Pyridoyl	C_6H_5	Pyridin-2-yl	6.38

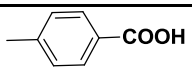
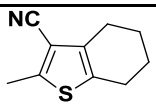
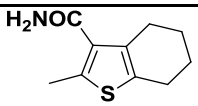
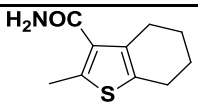
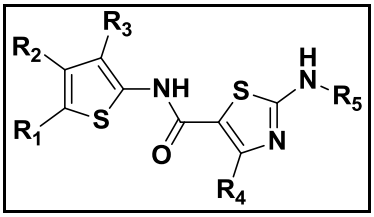
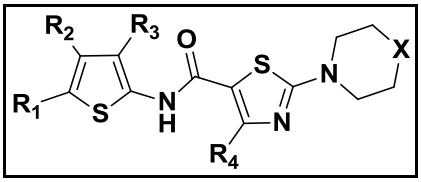
11	PMCDP-TZB7	(4-Cl)PhCO	4-OMeC$_6$H$_4$		5.23
12	PMCDP-TZB11	4-Pyridoyl	4-OMeC$_6$H$_4$		4.77
13	PMCDP-TZB13	(4-OMe)PhCO	C$_6$H$_5$		5.56
14	PMCDP-TZB14	Pyridine-4-yl	C$_6$H$_5$		6.44
15	PMCDP-TZB15	(4-OMe)PhCO	Furan-2-yl	Pyrimidin-2-yl	4.99
16	PMCDP-TZB16	(4-OMe)PhCO	C$_6$H$_5$	Pyrimidin-2-yl	5.90
17	PMCDP-TZB21	Pyridine-4-yl	C$_6$H$_5$	Pyrimidin-2-yl	7.70
18	PMCDP-TZB22	Pyridine-4-yl	C$_6$H$_5$	Pyridin-2-yl	8.34

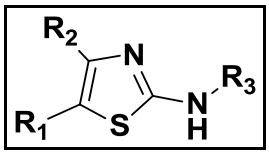
Table 8.2 Structures and biological activity of A_{2A} adenosine receptor antagonists used for 3D QSAR study



No	Code	R_1	R_2	R_3	R_4	R_5	PK_i (A_{2A})
1	PMCDP-5	-(CH$_2$)$_4$-		-COOEt	4-MeC$_6$H$_4$	C$_6$H$_4$	4.54
2	PMCDP-6	-(CH$_2$)$_4$-		-COOEt	-N(Me)$_2$	4-MeC$_6$H$_4$	4.74
3	PMCDP-11	-(CH$_2$)$_4$-		-CONH$_2$	-N(Me)$_2$	4-MeC$_6$H$_4$	4.65
4	PMCDP-20	-(CH$_2$)$_4$-		-CONH$_2$	4-MeC$_6$H$_4$	C$_6$H$_5$	5.25
5	PMCDP-21	-COOMe	Me	-COOEt	-N(Me)$_2$	4-OMeC$_6$H$_4$	5.02
6	PMCDP-25	-COOMe	Me	-COOEt	-N(Me)$_2$	4-MeC$_6$H$_4$	4.76



No	Code	R_1	R_2	R_3	R_4	X	PK_i (A_{2A})
7	PMCDP-28	-COOMe	Me	-COOEt	Furan-2-yl	O	4.74
8	PMCDP-29	$-(CH_2)_4-$		-COOEt	Furan-2-yl	O	5.04
9	PMCDP-37	-COOMe	Me	-CN	Furan-2-yl	N-Me	5.08



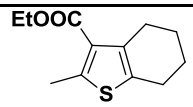
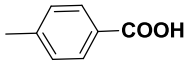
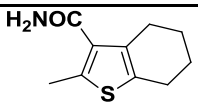
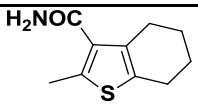
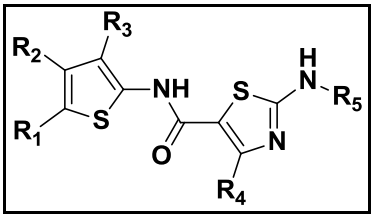
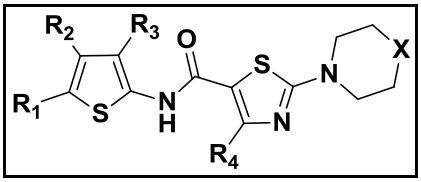
No	Code	R_1	R_2	R_3	PK_i (A_{2A})
10	PMCDP-TZB2	4-Pyridoyl	C_6H_5	Pyridin-2-yl	7.48
11	PMCDP-TZB4	(4-OMe)PhCO	Furan-2-yl		5.24
12	PMCDP-TZB9	(4-Cl)PhCO	4-OMeC_6H_4		6.77
13	PMCDP-TZB13	(4-OMe)PhCO	C_6H_5		5.21
14	PMCDP-TZB14	Pyridine-4-yl	C_6H_5		5.14
15	PMCDP-TZB15	(4-OMe)PhCO	Furan-2-yl	Pyrimidin-2-yl	6.38
16	PMCDP-TZB16	(4-OMe)PhCO	C_6H_5	Pyrimidin-2-yl	5.68
17	PMCDP-TZB21	Pyridine-4-yl	C_6H_5	Pyrimidin-2-yl	5.91
18	PMCDP-TZB22	Pyridine-4-yl	C_6H_5	Pyridin-2-yl	7.28

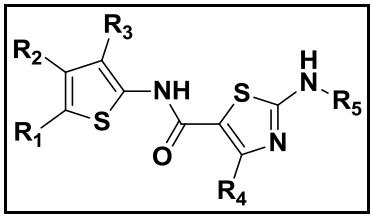
Table 8.3 Structures and biological activity of A_3 adenosine receptor antagonists used for 3D QSAR study

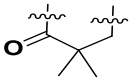


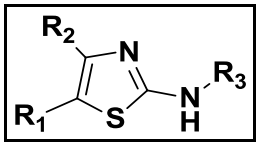
No	Code	R_1	R_2	R_3	R_4	R_5	PK_i (A_3)
1	PMCDP-2	-$(CH_2)_4$-		-COOEt	Me	4-MeC_6H_4	5.08
2	PMCDP-3	-$(CH_2)_4$-		-COOEt	P-MeC_6H_4	4-MeC_6H_4	5.66
3	PMCDP-4	-$(CH_2)_4$-		-COOEt	P-MeC_6H_4	4-$OMeC_6H_4$	6.28
4	PMCDP-5	-$(CH_2)_4$-		-COOEt	4-MeC_6H_4	C_6H_5	5.39
5	PMCDP-6	-$(CH_2)_4$-		-COOEt	-$N(Me)_2$	4-MeC_6H_4	5.33
6	PMCDP-8	-$(CH_2)_4$-		-$CONH_2$	4-MeC_6H_4	C_6H_5	4.45
7	PMCDP-11	-COOMe	Me	-COOEt	-$N(Me)_2$	4-$OMeC_6H_4$	5.15
8	PMCDP-14	-$(CH_2)_4$		-CN	4-MeC_6H_4	4-$OMeC_6H_4$	5.02
9	PMCDP-19	-$(CH_2)_4$-		-$CONH_2$	4-MeC_6H_4	P-MeC_6H_4	6.48
10	PMCDP-20	-$(CH_2)_4$-		-CN	Me	P-MeC_6H_4	5.41
11	PMCDP-25	-COOMe	Me	-COOEt	-$N(Me)_2$	4-MeC_6H_4	5.41

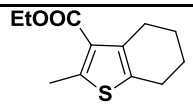
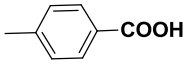
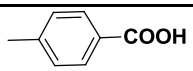
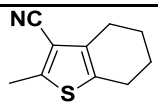
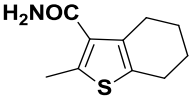
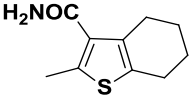


No	Code	R_1	R_2	R_3	R_4	X	PK_i (A_3)
12	PMCDP-24	-$(CH_2)_4$-		-COOEt	C_6H_5	O	5.54
13	PMCDP-28	-COOMe	Me	-COOEt	Furan-2-yl	O	5.14
14	PMCDP-29	-$(CH_2)_4$-		-COOEt	Furan-2-yl	O	5.37



No	Code	R_1	R_2	R_3	R_4	R_5	PK_i (A_3)
15	PMCDP-41			-$CONH_2$	-NH_2	C_6H_5	5.31



No	Code	R_1	R_2	R_3	PK_i (A_3)
16	PMCDP-TZB2	4-Pyridoyl	C_6H_5	Pyridin-2-yl	7.38
17	PMCDP-TZB4	(p-OMe)PhCO	Furan-2-yl		6.41
18	PMCDP-TZB7	(4-Cl)PhCO	4-$OMeC_6H_4$		7.47
19	PMCDP-TZB9	(4-Cl)PhCO	4-$OMeC_6H_4$		5.96
20	PMCDP-TZB11	4-Pyridoyl	4-$OMeC_6H_4$		5.59
21	PMCDP-TZB13	(4-OMe)PhCO	C_6H_5		5.59
22	PMCDP-TZB14	Pyridine-4-yl	C_6H_5		5.27
23	PMCDP-TZB15	(4-OMe)PhCO	Furan-2-yl	Pyrimidin-2-yl	6.42
24	PMCDP-TZB16	(4-OMe)PhCO	C_6H_5	Pyrimidin-2-yl	7.55
25	PMCDP-TZB21	Pyridine-4-yl	C_6H_5	Pyrimidin-2-yl	6.41
26	PMCDP-TZB22	Pyridine-4-yl	C_6H_5	Pyridin-2-yl	8.72

8.3.2 Molecular modeling and Alignment

All the molecules were drawn using the SKETCH module available in SYBYL X-2.0 and the staticstical anyalysis was performed in the QSAR module of SYBYL X-2.0 (Sybyl-X 2.0, Tripos International, A Certara company). Energy minimization of the molecular structure was performed using the Powell gradient algorithm with the Tripos force field (Clark et al. 1989) and Gasteiger–Huckel charge (Gasteiger & Marsili 1980). The lowest energy conformation was considered to perform 3D-QSAR calculations. Here the most potent compound (i) PMCDP-TZB22 for A_1 adenosine receptor (ii) PMCDP-TZB2 for A_{2A} adenosine receptor (iii) PMCDP-TZB22 for A_3 adenosine receptor were chosen as the template for alignment. The reference atoms for (i) A_1 adenosine receptor antagonist shown in figure 1a (ii) A_{2A} adenosine receptor antagonist shown in figure 2a (iii) A_3 adenosine receptor antagonist shown in figure 3a were chosen for alignment of the compounds. Each compound was aligned to the template using the Align Database function due to its easy implementation and effectiveness. The core structure taken for alignment and the aligned compounds for the three different databases are displayed in for Figure 8.1, Figure 8.2 and Figure 8.3.

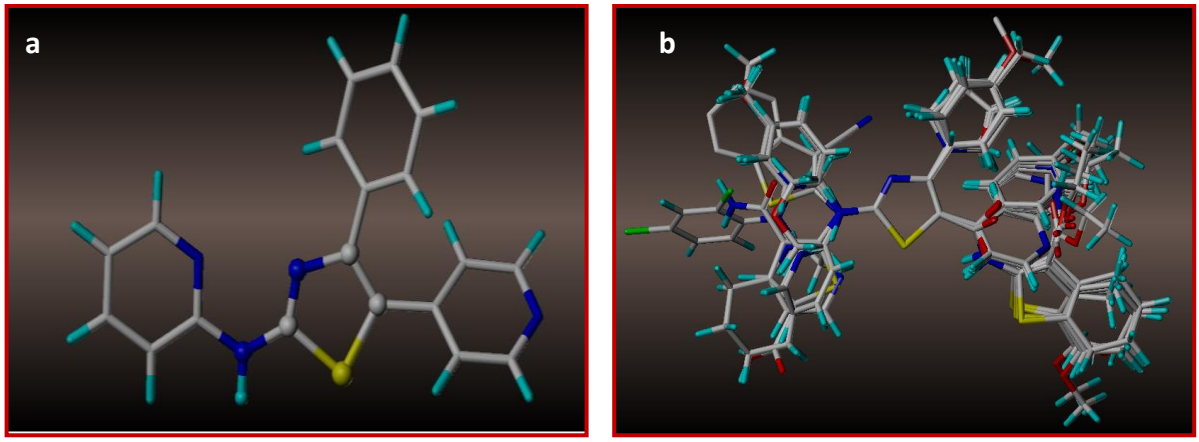


Figure 8.1 Alignment of all the active molecules in training and test sets (a) scaffold of compound PMCDP-TZB22, the atoms used for alignment are depicted as balls (b) 3D-view of aligned molecules of A_1 adenosine receptor antagonists

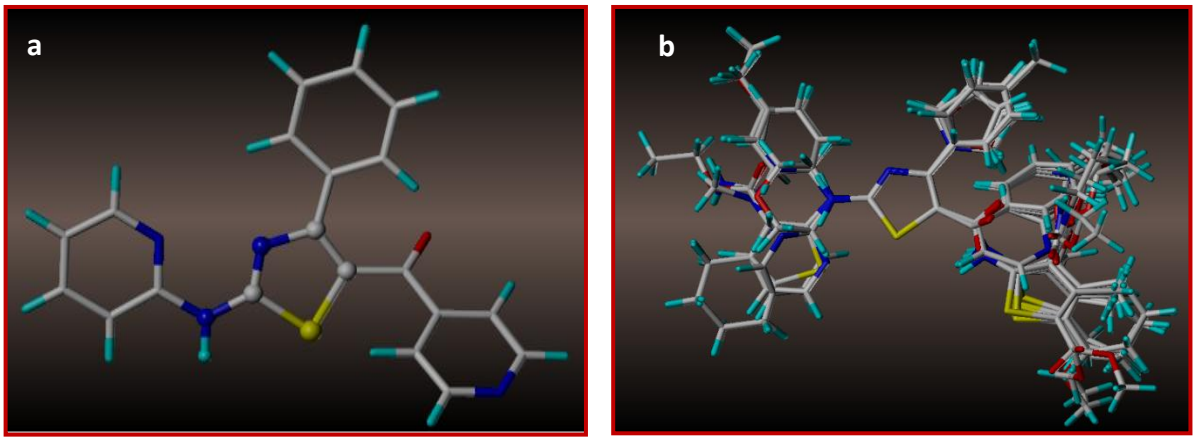


Figure 8.2 Alignment of all the molecules in training and test sets (a) scaffold of compound PMCDP-TZB2, the atoms used for alignment are depicted as balls (b) 3D-view of aligned molecules of A_{2A} adenosine receptor antagonists

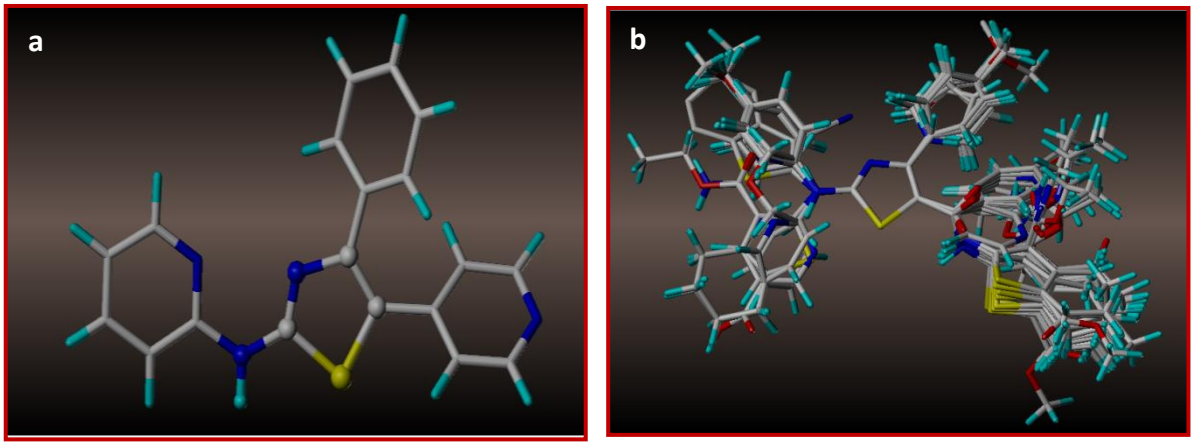


Figure 8.3 Alignment of all the molecules in training and test sets (a) scaffold of compound PMCDP-TZB22, the atoms used for alignment are depicted as balls (b) 3D-view of aligned molecules of A_3 adenosine receptor antagonists

8.3.3 CoMFA

The CoMFA descriptors, steric (Lennard–Jones 6–12 potential) and electrostatic (Coulomb potential) fields energies between the probe and the test molecule at each lattice intersection were calculated with a grid step size of 2 Å using sp^3 C^+ as probe atom. The CoMFA fields generated automatically were scaled by the CoMFA-STD method with default energy of 30 kcal/mol.

8.3.4 Regression

The partial least squares (PLS) (Ståhle & Wold 1987) method implemented in the QSAR module of SYBYL was used to construct and validate the models. The CoMFA descriptors derived above and pK_i values were used as dependent variable in PLS regression analysis. The performance of models was calculated using the leave-one-out (LOO) cross-validation method (Wold 1978; Cramer et al. 1988). The cross-validated coefficient, q^2, was evaluated as:

$$q^2 = 1 - \frac{\sum_{i=1}^{n} \left(Y_{\text{predicted}} - Y_{\text{observed}}\right)^2}{\sum_{i=1}^{n} \left(Y_{\text{observed}} - Y_{\text{mean}}\right)^2}$$

where $Y_{predicted}$, $Y_{observed}$, and Y_{mean} are predicted, observed, and mean values of the target property (pK_i), respectively. The optimal number of components (ONC) equal to that yielding the highest cross-validated q^2 was used to generate the final PLS regression (non-cross-validated conventional analysis) models. The conventional correlation coefficient r^2 and its standard error of estimate (SEE) were subsequently computed for the final PLS models. CoMFA coefficient maps were generated by interpolation of the pairwise products between the PLS coefficients and the standard deviations of the corresponding CoMFA descriptor values.

8.4 Result and Discussion

8.4.1 CoMFA Statistical results

CoMFA models were developed for three different set of compounds and all the models gave statistically significant results. The statistical results for CoMFA models are summarized in table 4. The best CoMFA model gave (1) the cross-validated q^2 of 0.554 with an optimal number of components (ONC) of 3 and non-cross-validated correlation coefficient r^2 of 0.977 for data set of A_1 adenosine receptor antagonists, (2) the cross-validated q^2 of 0.448 with an optimal number of components (ONC) of 3 and non-cross-validated correlation coefficient r^2 of 0.96 for data set of A_{2A} adenosine receptor antagonists and (3) the cross-validated q^2 of 0.502 with an optimal number of components

(ONC) of 6 and non-cross-validated correlation coefficient r^2 of 0.982 for data set of A_3 adenosine receptor antagonists (Table 8.4).

Further to test the predictive ability of the model, the three CoMFA models were selected for the prediction of the biological activity for the same three set of molecules. These results are a clear indication that our models have good predictive ability. Table 8.5-8.7 shows the observed and predicted biological activity for all the three data sets. These models were further taken for the generation of contour maps and analysis of biological activity of the compounds. The plots of the predicted versus observed pK_i values for the three models are shown in Figure 8.5-8.7.

Table 8.4 Summary of CoMFA results for all the three datasets

Adenosine receptor		**A_1**	**A_{2A}**	**A_3**
No. of Molecules	**Training Set**	12	13	21
	Test Set	5	5	5
q^{2a}		0.554	0.448	0.502
r^{2b}		0.977	0.96	0.982
SEE[c]		0.264	0.22	0.165
ONC[d]		3	3	6

[a]Cross-validated correlation coefficient after leave-one-out procedure.
[b]Optimal number of principal components.
[c]Correlation coefficient.
[d]Standard error of estimate.

Table 8.5 Observed and predicted activities for training and test sets compounds by 3D QSAR models for A_1 adenosine receptor antagonists

No	**Code**	**Observed PK_i (A_1)**	**Predicted PK_i (A_1)**	**Residue**
1	PMCDP-5	5.11	5.12	0.01
2	PMCDP-6	4.74	4.74	0
3	PMCDP-12	5.64	5.10	-0.54
4	PMCDP-21	4.86	4.75	-0.11
5	PMCDP-25	4.72	4.73	0.01

6	PMCDP-29	5.14	4.22	-0.92
7	PMCDP-TZA9	5.58	6.05	0.47
8	PMCDP-TZA13	4.74	4.51	-0.23
9	PMCDP-TZA16	6.08	6.46	0.38
10	PMCDP-TZB2	6.38	6.34	-0.04
11	PMCDP-TZB7	5.24	5.18	-0.06
12	PMCDP-TZB11	4.77	4.78	0.01
13	PMCDP-TZB13	5.57	5.50	-0.07
14	PMCDP-TZB14	6.44	6.35	-0.09
15	PMCDP-TZB15	5.00	5.66	0.66
16	PMCDP-TZB16	5.90	6.09	0.19
17	PMCDP-TZB21	7.70	7.68	-0.02
18	PMCDP-TZB22	8.35	8.18	-0.17

Table 8.6 Observed and predicted activities for training and test sets compounds by 3D QSAR models for A_{2A} adenosine receptor antagonists

No	**Code**	**Observed PK_i (A_{2A})**	**Predicted PK_i (A_{2A})**	**Residue**
1	PMCDP-5	4.54	4.44	-0.1
2	PMCDP-6	4.74	4.81	0.07
3	PMCDP-11	4.65	5.06	0.41
4	PMCDP-20	5.25	5.22	-0.03
5	PMCDP-21	5.02	4.99	-0.03
6	PMCDP-25	5.58	4.55	-1.03
7	PMCDP-28	4.74	4.64	-0.1
8	PMCDP-29	5.04	5.18	0.14
9	PMCDP-37	5.08	5	-0.08
10	PMCDP-TZB2	7.48	7.21	-0.27
11	PMCDP-TZB4	5.24	5.21	-0.03
12	PMCDP-TZB9	6.77	6.86	0.09
13	PMCDP-TZB13	5.21	5.14	-0.07
14	PMCDP-TZB14	5.14	5.41	0.27
15	PMCDP-TZB15	6.38	6.62	0.24
16	PMCDP-TZB16	6.68	6.76	0.08
17	PMCDP-TZB21	5.91	6.32	0.41
18	PMCDP-TZB22	7.28	6.97	-0.31

Table 8.7 Observed and predicted activities for training and test sets compounds by 3D QSAR models for A_3 adenosine receptor antagonists

No	Code	Observed PK_i (A_3)	Predicted PK_i (A_3)	Residue
1	PMCDP-2	5.08	5.02	-0.06
2	PMCDP-3	5.65	5.66	0.01
3	PMCDP-4	6.28	6.28	0
4	PMCDP-5	5.39	5.38	-0.01
5	PMCDP-6	5.33	5.33	0
6	PMCDP-8	4.45	4.45	0
7	PMCDP-11	5.15	5.14	-0.01
8	PMCDP-14	5.02	5.02	0
9	PMCDP-19	6.48	6.59	0.11
10	PMCDP-20	5.41	5.41	0
11	PMCDP-25	5.41	5.41	0
12	PMCDP-24	5.54	5.53	-0.01
13	PMCDP-28	5.14	5.13	-0.01
14	PMCDP-29	5.37	5.36	-0.01
15	PMCDP-41	5.31	5.31	0
16	PMCDP-TZB2	7.38	7.37	-0.01
17	PMCDP-TZB4	6.41	6.41	0
18	PMCDP-TZB7	7.47	7.47	0
19	PMCDP-TZB9	5.96	5.96	0
20	PMCDP-TZB11	5.59	5.6	0.01
21	PMCDP-TZB13	5.59	5.6	0.01
22	PMCDP-TZB14	5.27	5.27	0
23	PMCDP-TZB15	6.42	6.42	0
24	PMCDP-TZB16	7.55	7.55	0
25	PMCDP-TZB21	6.41	6.41	0
26	PMCDP-TZB22	8.72	8.72	0

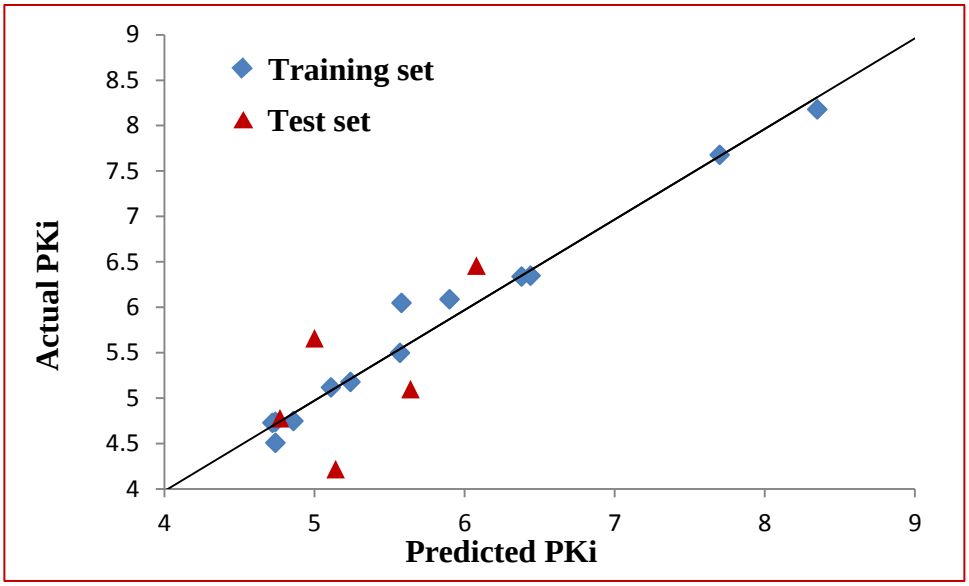


Figure 8.4 Plot of the predicted versus observed pK_i values for all the molecules based on CoMFA (q^2 = 0.554, r^2 = 0.977) model of A_1 adenosine receptor antagonists

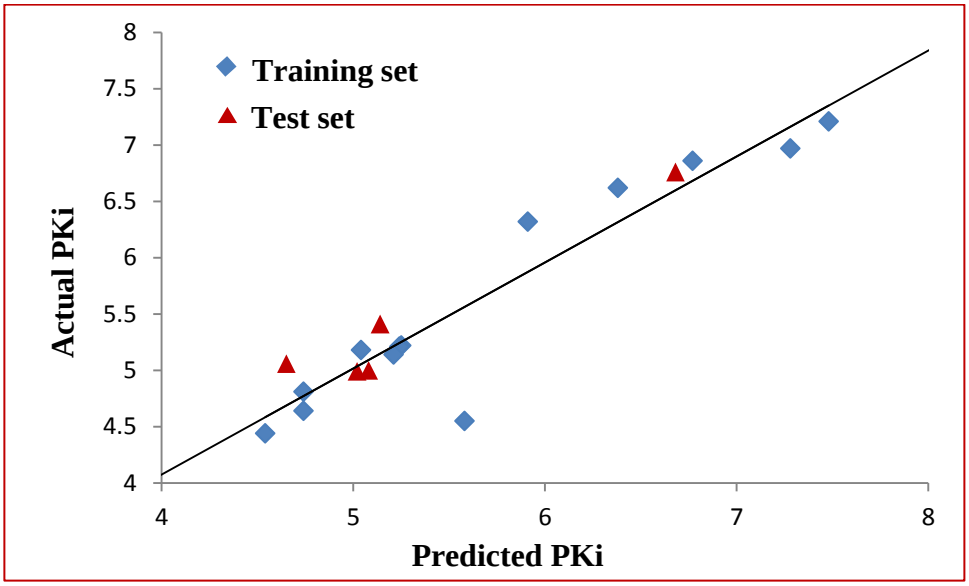


Figure 8.5 Plot of the predicted versus observed pK_i values for all the molecules based on CoMFA (q^2 = 0.448, r^2 = 0.96) model of A_{2A} adenosine receptor antagonists

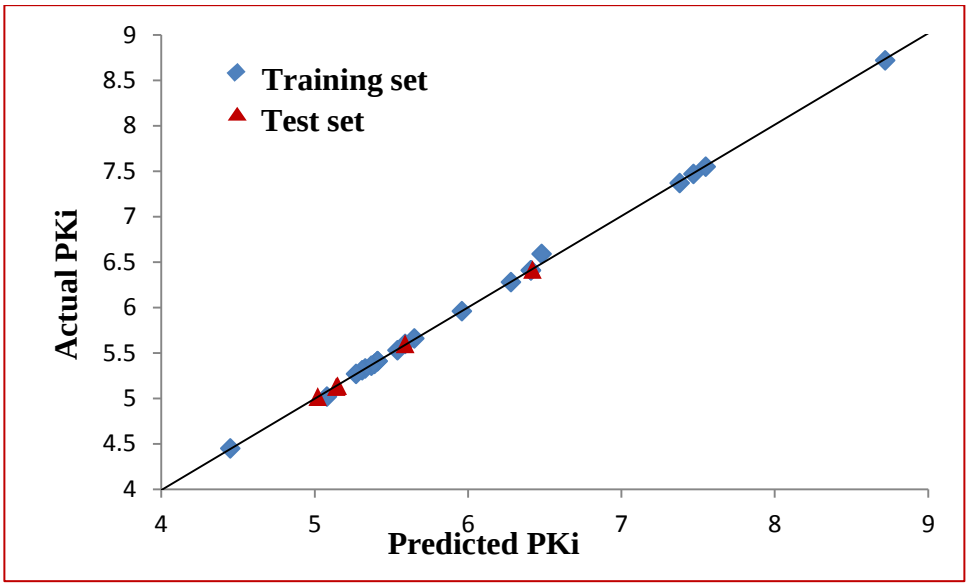


Figure 8.6 Plot of the predicted versus observed pK_i values for all the molecules based on CoMFA (q^2 = 0.502, r^2 = 0.982) model of A_3 adenosine receptor antagonists

8.4.2 CoMFA contour maps

The steric and electrostatic contour maps of the best CoMFA model are shown in Figure 7-9. Compounds PMCDP-TZB2 was used as reference for the contour maps of A_1 and A_3 adenosine receptor whereas PMCDP-TZB22 was used as a reference in case of contour maps of A_{2A} adenosine receptor. The green contours represent regions where bulky substituents would increase the activity, while the yellow contours represent regions where steric bulky group would be unfavourable. Furthermore, the blue and red contours depict the position where positively charged groups and negatively charged groups would be favorable, respectively.

- *Analysis of CoMFA contour maps of A_1 adenosine receptor antagonists*

In the CoMFA steric contour map (Fig. 7A), the steric favorable green contour on pyridine-2-yl at 2nd position of thiazole ring indicates that bulky group in this region is important for activity. The two steric unfavorable yellow contours near the on the both side of thiazole ring suggest that further substitution on these two pyridine rings may decrease activity. Moreover, in the CoMFA electrostatic contour map (Fig. 7B), a red

contour is majorly distributed on pyridine-2-yl at 2nd position of thiazole ring where electronegative groups are favourable. These findings suggest that groups groups like -NO_2, -Cl or –CH_3 can increase the activity near red area.

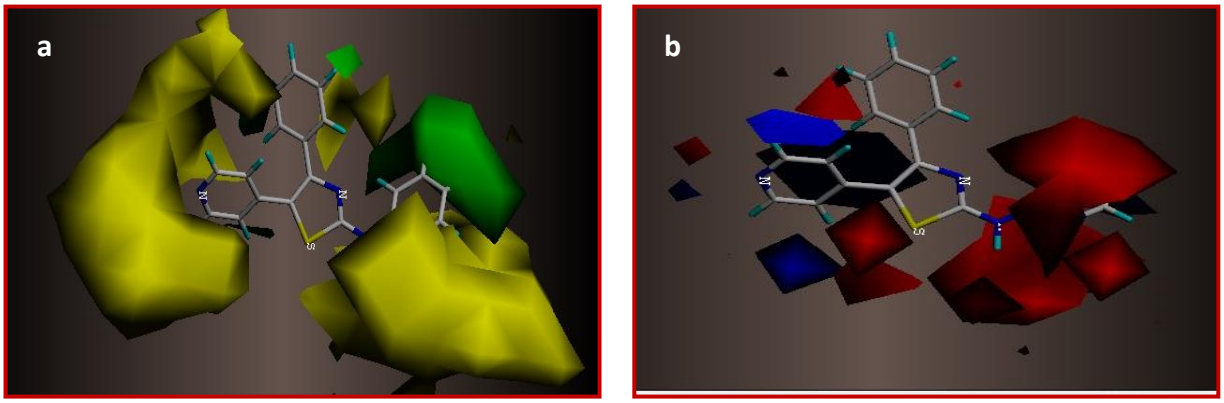


Figure 8.7 (a) Steric contour maps around PMCDP-TZB22 (b) Electrostatic contour maps around PMCDP-TZB22

- *Analysis of CoMFA contour maps of A_{2A} adenosine receptor antagonists*

In the CoMFA steric contour map (Fig. 8A), the steric favorable green contour on phenyl ring at 4th position of thiazole ring indicates that bulky group in this region is important for activity. The green contour map is also present just under the secondary amino (–NH) at 2nd position of thiazole ring suggesting that bulk is important for the biological activity. In the electrostatic contour map (Fig. 8B), on the both side of thiazole ring suggested that electropositive groups are favoured. Red contour is present just under the thiazole moiety where electronegative groups are favourable. The groups -OH and -NH_2 can increase the activity near blue area and the groups -NO_2, -Cl or –CH_3 can increase the activity near red area.

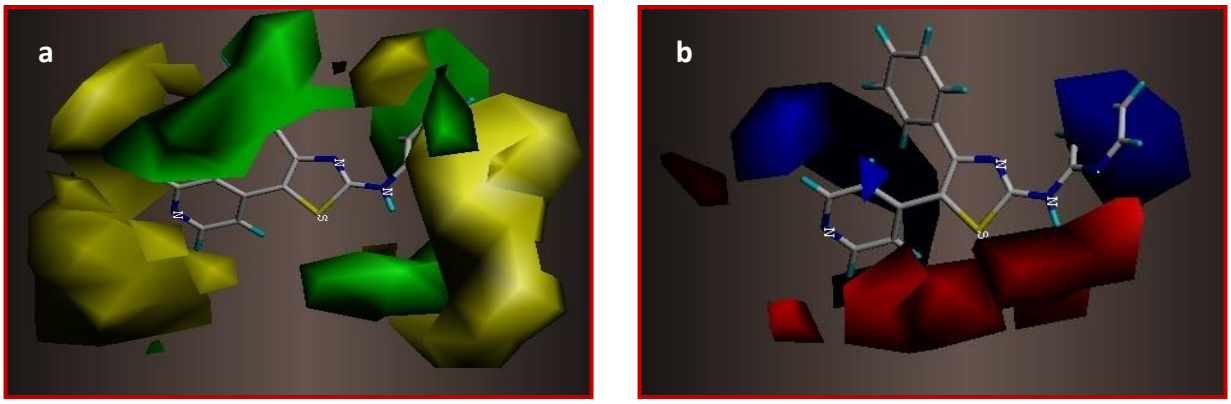


Figure 8.8 (a) Steric contour maps around PMCDP-TZB2 (b) Electrostatic contour maps around PMCDP-TZB2

- *Analysis of CoMFA contour maps of A_3 adenosine receptor antagonists*

In the CoMFA steric contour map (Fig. 9A), the steric favorable green contour on phenyl ring at 4th position of thiazole ring indicates that bulky group in this region is important for activity. The green contour map is also present just under the para position of aminopyridine ring of thiazole moiety where more bulk can increase activity. The yellow contour present at the 4th position of the pyridine-4-yl indicates that no further bulk is required. In the electrostatic contour map (Fig. 9B), majorly blue contour is present on the both pyridine ring of the thiazole moiety suggesting that electropositive groups can favour the activity. Presence of groups like -OH and -NH_2 can increase the activity near blue area.

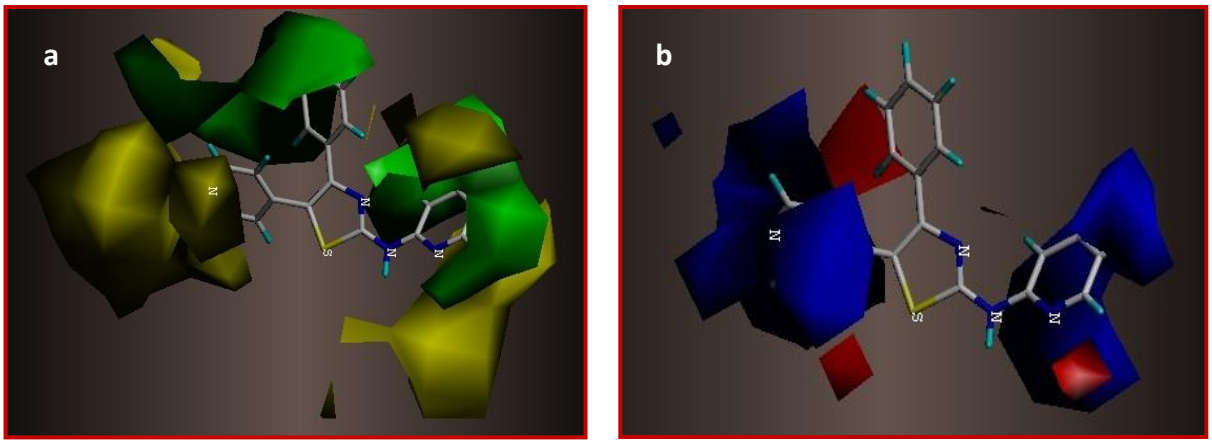


Figure 8.9 (a) Steric contour maps around PMCDP-TZB22(b) Electrostatic contour maps around PMCDP-TZB22

8.5 Conclusion

The CoMFA 3D-QSAR models for three set of A_1, A_{2A} and A_3 receptor antagonists were developed. The best CoMFA models gave the cross-validated q^2 values of 0.554, 0.448 and 0.502 and the non-crossvalidated r^2 values of 0.977, 0.96 and 0.982 for A_1, A_{2A} and A_3 adenosine receptor respectively. The CoMFA model showed better predictive ability for the activity. The CoMFA contour maps can help to understand the structure–activity relationship and identify structural features influencing the biological activity. Furthermore, the satisfactory predictive ability of 3D-QSAR models observed for the same set of compounds indicates that these models could be successfully used for predicting activity of the antagonists and can guide the further modification of these compounds.

CHAPTER-9

Novel synthetic methodology: Development of new 2-amino/cyclicamino 1,3,4-thiadiazines

PAGE NO. 161 TO 169

Chapter 9

Novel synthetic methodology: Development of new 2-amino/cyclicamino 1,3,4-thiadiazines

9.1 Introduction

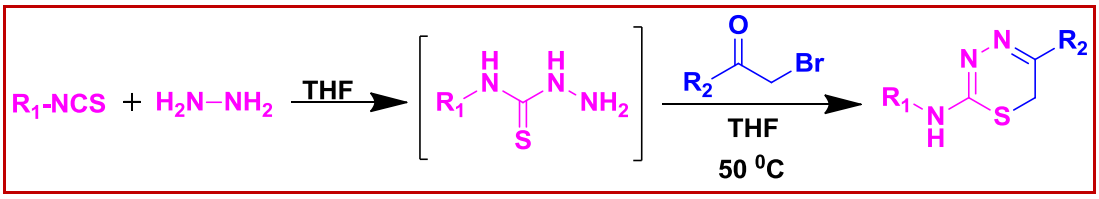
With the interest in the development of the biologically important heterocyclic compound, herein we have developed novel synthetic methodology for new 2-amino/cyclicamino 1,3,5-thiadiazines. The thiadiazines are important heterocyclic compounds and molecules bearing the 1,3,4-thiadiazine core have received attention due to their therapeutic activities (Schröder et al. 2001; Karegoudar et al. 2008; el-Shehry et al. 2010). Literature survey shows that the most common route for the synthesis of 1,3,4-thiadiazine derivatives is via the condensation of thiosemicarbazides and α-haloketones (Kilburn et al. 2002; Moghimi et al. 2013). In addition, the diversity of the possible thiadiazines from this method is limited due to the use of only two starting materials.

9.2 Present work

As part of development of structurally and biologically novel heterocycles, herein we report series of novel 2-amino/cyclicamino 1,3,4 thiadiazines from substituted 4-aryl thiosemicarbazide and 4-cyclicamino thiosemicarbazides.

9.2.1 Synthesis of substituted 2-amino 1,3,4- thiadiazine

The synthesis of 2-amino 1,3,4- thiadiazine involves reaction of substituted isothiocyanate with hydrazine in THF which gives substituted thiosemicarbazide intermediate. This thiosemicarbazide intermediate on further reaction with various α-haloketones followed by cyclization and dehydration at 50 ^{0}C gives substituted 2-amino 1,3,4 thiadiazines (Scheme 9.1). Later this synthetic methodology was modified to the sequential one-pot reaction for the preparation of the substituted 2-amino 1,3,4-thiadiazines by reaction the three starting materials in the sequence of isothiocyanate, hydrazine and phenacyl bromide in one flask to give the desired product.



Scheme 9.1 Synthetic scheme for substituted 2-amino 1,3,4-thiadiazine

As an example, the reaction of p-tolyl isothiocyanate (1 mmol) was reacted with hydrazine (1.2 mmol) in THF to furnish 4-tolyl thiosemicarbazide. This thiosemicarbazide intermediate (1 mmol) was directly reacted with 4-chlorophenyacyl bromide (1 mmol) in THF at 50 ^{0}C which resulted into 5-(4-chlorophenyl)-*N*-(*p*-tolyl)-6H-1,3,4-thiadiazin-2-amine (**PMCDP-TD1)** with good yield. The mass spectrum of **PMCDP-TD1** (Figure 9.1) displayed the molecular ion peak at *m/z* 316.2(M+1) which confirms the molecular weight of the compound 315.06 calculated for $C_{16}H_{14}ClN_3S$.

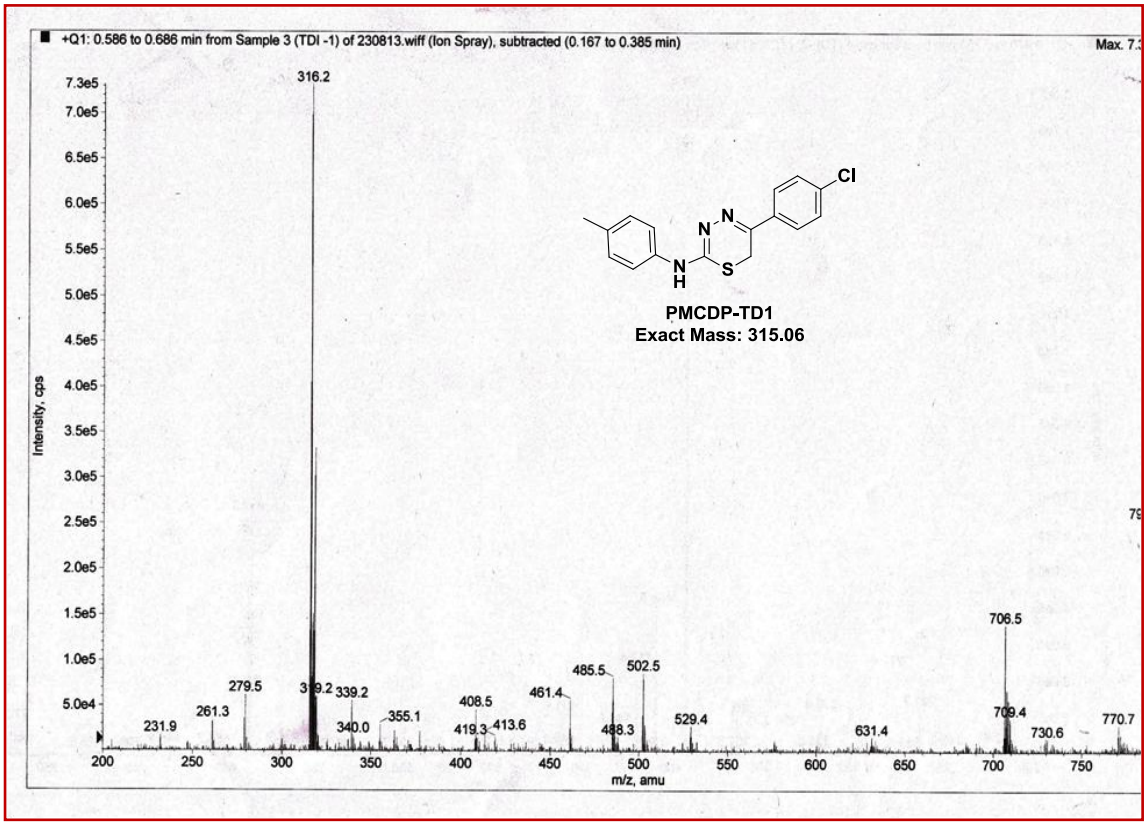
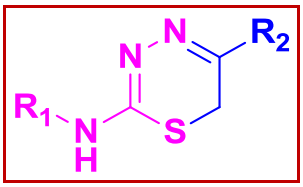


Figure 9.1 LC-MS spectrum of PMCDP-TD1

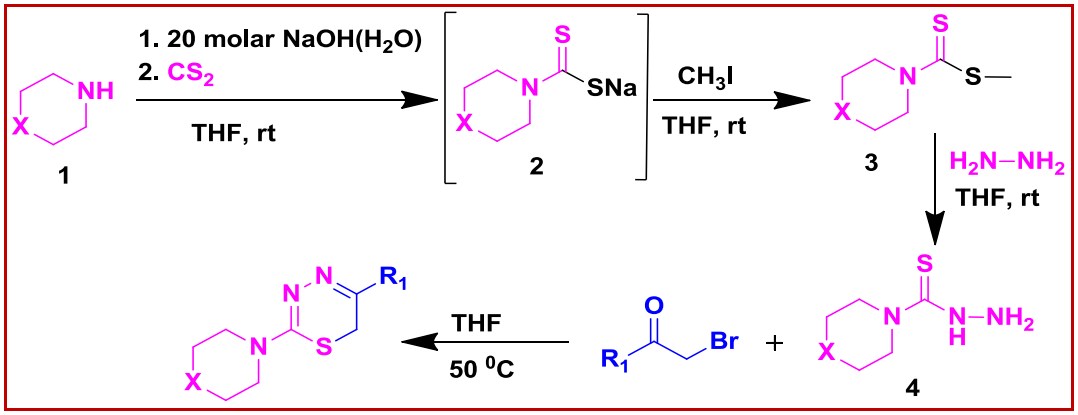
Table 9.1 Synthesis of 2-amino 1,3,4-thiadiazine derivatives



No.	Compound	R_1	R_2
1	PMCDP-TD1	4-MeC_6H_4	4-ClC_6H_4
2	PMCDP-TD4	4-MeC_6H_4	4-MeC_6H_4
3	PMCDP-TD5	4-ClC_6H_4	4-ClC_6H_4
4	PMCDP-TD6	-H	4-ClC_6H_4

9.2.2 Synthesis of substituted 2-cyclicamino 1,3,4- thiadiazine

The synthesis of 2-cyclicamino 1,3,4-thiadiazine derivatives was started by the reaction of cyclic amine with 20 molar solution of sodium hydroxide and carbon disulphide in THF at 0-5 ^{0}C. After 30 minutes methyl iodide was added dropwise at 0-5 ^{0}C to this solution. Stirring was continued for 2 hrs and the mixture was poured into water. The precipitates of the product intermediate carbodithioate (**3**) was filtered, washed with hexane and dried for further reaction. The intermediate **3** was further dissolved in THF and to this solution hydrazine hydrate was added and the reaction was stirred for 8 hrs at room temperature to give 4-cyclicamino thiosemicarbazide **(4).** This 4-cyclicamino thiosemicarbazide on further reaction with various α-haloketones followed by cyclization and dehydration at 50 ^{0}C gives substituted 2-cyclicamino 1,3,4 thiadiazines (**5**) (Schme 9.2).



Scheme 9.2. Synthetic scheme for substituted 2-cyclicamino 1,3,4-thiadiazine

As an example, the reaction of 4-(2,4-dichlorophenyl)piperazine-1-carbothiohydrazide (1 mmol) was reacted with 4-methoxy phenacyl bromide (1 mmol) in THF at 50 ^{0}C to give 2-(4-(2,4-dichlorophenyl)piperazin-1-yl)-5-(p-tolyl)-6H-1,3,4-thiadiazine (**PMCDP-TD9)** with good yields. The mass spectrum of **PMCDP-TD9** (Figure-9.2) displayed the molecular ion peak at *m/z* 419.1(M+1) which confirms the molecular weight of the compound 418.06 calculated for $C_{20}H_{20}Cl_2N_4S$.

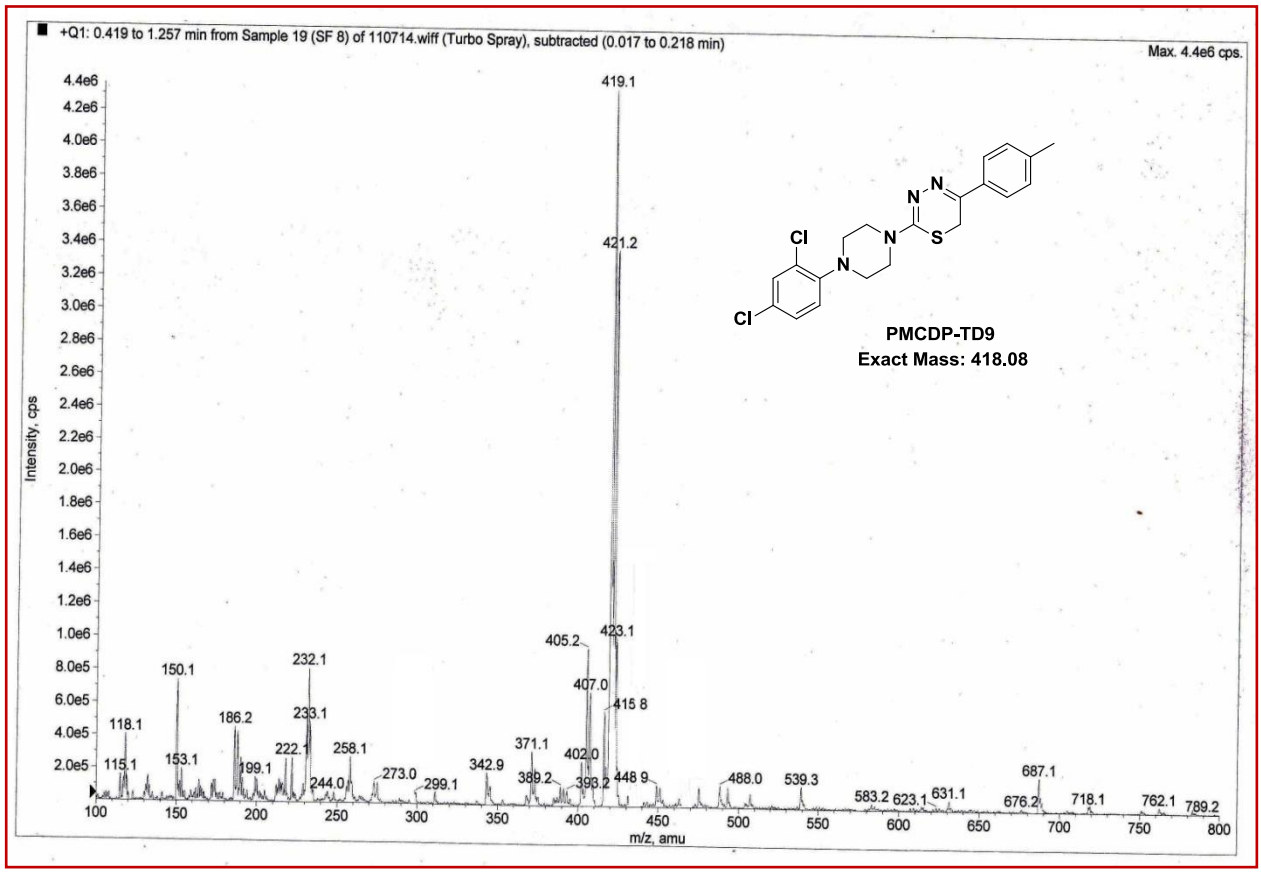
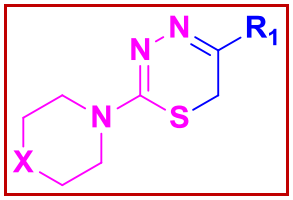


Figure 9.2 LC-MS spectrum of PMCDP-TD9

Table 9.2- Synthesis of 2-cyclicamino 1,3,4-thiadiazine derivatives



No.	Code	R_1	X
1	PMCDP-TD9	4-MeC_6H_4	2,4 dichloro phenyl piperazin-4-yl
2	PMCDP-TD10	4-MeC_6H_4	O

9.3 Conclusion

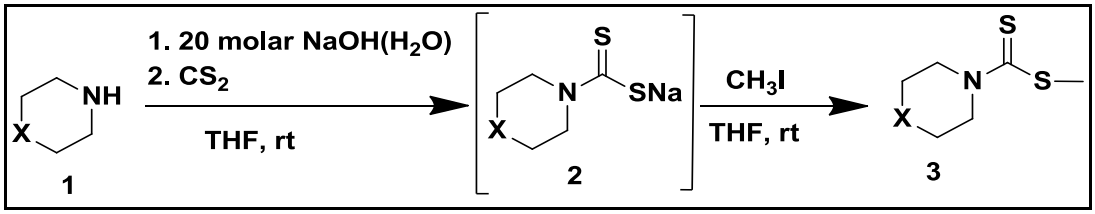
In conclusion, we have developed a novel synthesis of 2-amino 1,3,4-thiadiazine and 2-cyclicamino 1,3,4-thiadiazine using substituted thiosemicarbazide and α-haloketones in good to excellent yields. The synthesis of 2-amino 1,3,4-thiadiazine was developed as one-pot synthesis. The advantage of this method is its facile conditions and the product can be isolated with good purity. This approach could be useful for the generation of compound libraries of thiadiazine scaffold with diverse substitutions.

9.4 Experimental

Mass spectra were recorded on Perkin Elmer Sciex API 165. TLC was carried out on Merck Kieselgel 60 PF_{254}. IUPAC name of the compounds were generated using Cambridge soft ChemBioDraw ultra 12.0.

9.4.1 General procedure for the synthesis of substituted 4-cyclicamino carbodithioate

To a stirred solution of cyclic amine (1 mmol) in THF at room temperature, aqueous 20 molar sodium hydroxide and carbon disulphide (1.3 mmol) were added at 0-5 ^{0}C. After 30 minutes, methyl iodide (1.3 mmol) was added dropwise at 0-5 ^{0}C to this solution. Stirring was continued for 2 hrs and the mixture was poured into water. The precipitates of the product intermediate 4-cyclicamino carbodithioate (**3**) was filtered, washed with hexane and dried for further reaction.



9.4.1.1 Synthesis of methyl 4-(2,4-dichlorophenyl)piperazine-1-carbodithioate

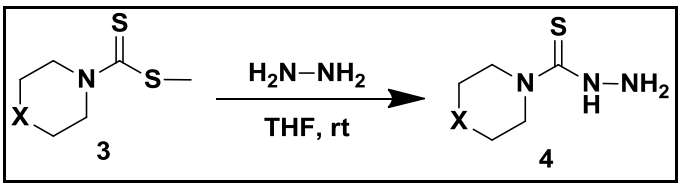
4-(2,4-dichlorophenyl)piperazine-1-carbodithioate was synthesized using the procedure described in 9.4.1 by the reaction of 1-(2,4-dichlorophenyl)piperazine, sodium hydroxide and methyl iodide to afford the crude product which was directly taken for the next step.

9.4.1.2 Synthesis of methyl morpholin-4-carbodithioate

Morpholin-4-carbodithioate was synthesized using the procedure described in 9.4.1 by the reaction of morpholine, sodium hydroxide and methyl iodide to afford the crude product which was directly taken for the next step.

9.4.2 General procedure for the synthesis of substituted 4-cyclicamino thiosemicarbazide

The 4-cyclicamino carbodithioate (**3)** was dissolved in THF and to this solution hydrazine hydrate was added and the reaction was stirred for 8 hrs at room temperature to give substituted 4-cyclicamino thiosemicarbazide **(4).**



9.4.2.1 Synthesis of 4-(2,4-Dichlorophenyl)piperazine-1-carbothiohydrazide

4-(2,4-dichlorophenyl)piperazine-1-carbothihydrazide was synthesized using the procedure described in 9.4.2 by the reaction of 4-(2,4-dichlorophenyl)piperazine-1-carbodithioate and hydrazine hydrate.

9.4.2.2 Synthesis of Morpholine-4-carbothiohydrazide

Morpholine-4-carbothiohydrazide was synthesized using the procedure described in 9.4.2 by the reaction of morpholin-4-carbodithioate and hydrazine hydrate.

9.5 General procedure for the synthesis of substituted 2-amino 1,3,4- thiadiazine

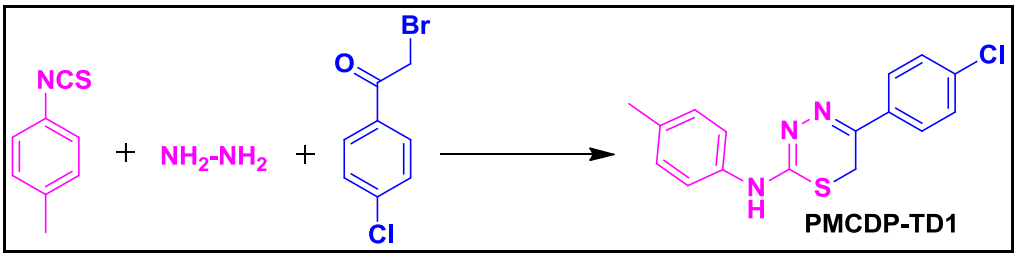
To a hot air dried round bottomed flask, containing a solution of isothiocyanate (1 mmol) in THF (5 mL), hydrazine (1.2 mmol) was added at 20–25 ^{0}C and the solution was stirred for 2–3 h. To the above solution, phenacyl bromide (1 mmol) in THF (2 mL) was added

at ambient temperature and the reaction was further stirred for 6-8 h at 50 ^{0}C with stirring. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice cold saturated solution of sodium bicarbonate in water. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through Buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-TDn)** were assigned with the help mass spectra.

9.6 General procedure for the synthesis of substituted 2-cyclicamino 1,3,4- thiadiazine

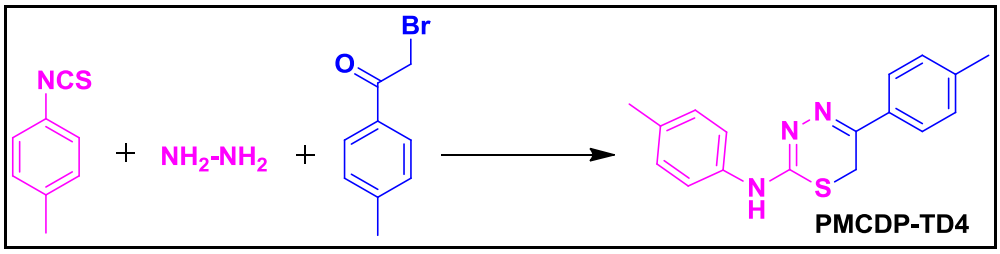
To a hot air dried round bottomed flask, containing a solution of 4-cyclicamino thiosemicarbazide (1 mmol) in THF (5 mL), phenacyl bromide (1 mmol) in THF (2 mL) was added at ambient temperature and the reaction was further stirred for 6-8 h at 50 ^{0}C with stirring. Progress of the reaction was monitored by TLC using ethyl acetate/hexane (2:8). After the completion of reaction, the reaction mixture was poured into ice cold saturated solution of sodium bicarbonate in water. Upon stirring, precipitate was observed (in the case of no precipitation, reaction mass was extracted with either dichloromethane or ethyl acetate) which were collected through buchner funnel. These precipitates were then dissolved in either dichloromethane or ethyl acetate and dried over anhydrous sodium sulfate and concentrated under reduced pressure to furnish crude compound which were further treated with diethyl ether and/or hexane to produce the pure solid compounds. The structures of compounds **(PMCDP-TDn)** were assigned with the help mass spectra.

PMCDP-TD1: 5-(4-Chlorophenyl)-*N*-(p-tolyl)-6H-1,3,4-thiadiazin-2-amine



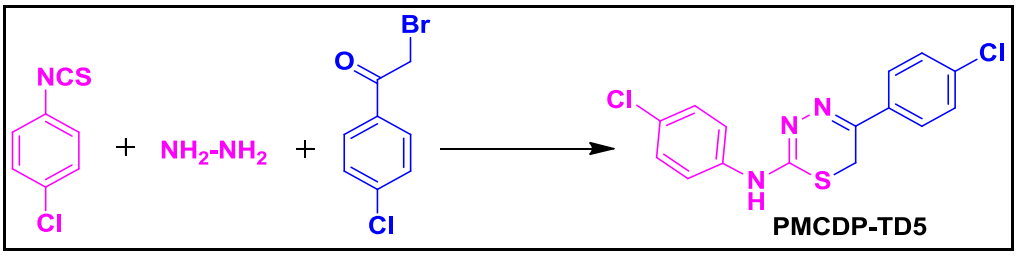
Yield: 72%, light yellow solid, **Molecular formula: $C_{23}H_{14}ClN_3S$**, LC-MS calculated: 315.6, found: 316.2(M+1).

PMCDP-TD4: *N*,5-Di-*p*-tolyl-6*H*-1,3,4-thiadiazin-2-amine



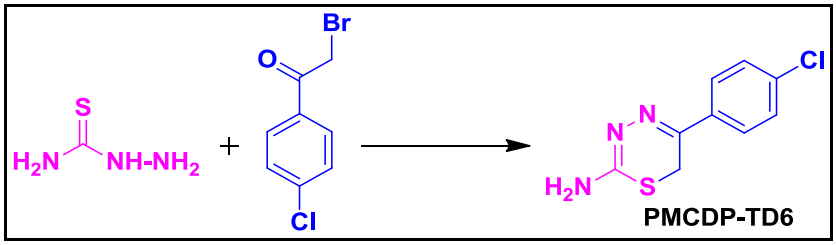
Yield: 67%, light yellow solid, **Molecular formula: $C_{17}H_{17}N_3S$**, LC-MS calculated: 295.3, found: 296.2(M+1).

PMCDP-TD5: *N*,5-Bis(4-chlorophenyl)-6*H*-1,3,4-thiadiazin-2-amine



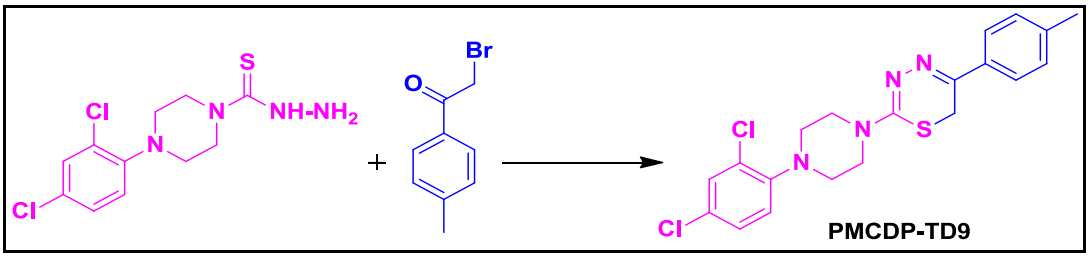
Yield: 73%, light yellow solid, **Molecular formula: $C_{15}H_{11}Cl_2N_3S$**, LC-MS calculated: 335.3, found: 336.1(M+1).

PMCDP-TD6: 5-(4-Chlorophenyl)-6H-1,3,4-thiadiazin-2-amine



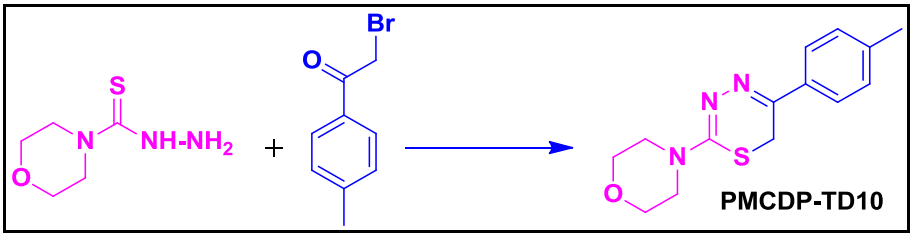
Yield: 68%, light yellow solid, **Molecular formula: $C_9H_8ClN_3S$**, LC-MS calculated: 225.1, found: 225.7(M+).

PMCDP-TD9: 2-(4-(2,4-Dichlorophenyl)piperazin-1-yl)-5-(*p*-tolyl)-6*H*-1,3,4-thiadiazine



Yield: 69%, off white solid, **Molecular formula: $C_{20}H_{20}Cl_2N_4S$**, LC-MS calculated: 418.06, found: 419.1(M+1).

PMCDP-TD10: 4-(5-(p-tolyl)-6*H*-1,3,4-thiadiazin-2-yl)morpholine



Yield: 59%, light yellow solid, **Molecular formula: $C_{14}H_{17}N_3OS$**, LC-MS calculated: 275.1, found: 275.7(M+1).

CHAPTER-10

Summary

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Chapter-10

Summary

Chapter-1 deals with the introduction of related to adenosine, adenosine receptors and various classes of adenosine receptor ligands. The adenosine receptors have been a well-explored field of research over the years. The therapeutic potential that is on offer by being able to manipulate these receptors is immense because the distribution of the receptors is so wide-spread in the physiological system. As there is great and extensive roles of adenosine receptor subtypes in both physiological and pathophysiological events, these receptors are becoming important drug targets in the treatment of a variety of diseases like cardiovascular disorders (A_1 adenosine receptor antagonist), Parkinson disease (A_{2A} adenosine receptor antagonist), inflammation (A_{2B}/A_3 adenosine receptor antagonist) etc. With all the studies it has emerged that adenosine receptors can be safely targeted by various ligands and various highly specific agonists and antagonists of adenosine receptors can be generated. As a result, increasing numbers of clinical trials testing of novel molecules in various indications have been initiated during the past decade.

Chapter-2 deals with rationale behind the molecules designed in the work. Fragment based drug design approach was used for the design of molecule. Considering the good affinity of the two fragments 2-aminothiazole and 2-aminothiophene towards adenosine receptors, they were clubbed into one molecule for the design of the molecule. Further modification of the design was done to come up at good adenosine receptor antagonists. Novel synthetic methodology was also developed for the synthesis of new 2-amino 1,3,4-thiadiazines and 2-cyclicamino 1,3,4-thiadiazines.

Chapter-3 deals with the validation study of the designed thiophenyl-thiazole carboxamides by docking study. After docking validation, series of thiophenyl-thiazole carboxamides were synthesized and the synthetic methodology was further converted modified into one-pot synthetic methodology. Library of molecules were synthesized and all the molecules were tested into radio ligand binding assay for adenosine receptors.

Most of the molecules were found to show affinity towards adenosine receptors particularly towards A_3 adenosine receptor. Particularly the molecules **PMCDP-3, PMCDP-4, PMCDP-12 and PMCDP-19** showed good affinity towards A_1, A_{2A} and A_3 adenosine receptor subtypes. From this the molecules **PMCDP-3, PMCDP-4 and PMCDP-19** were selective towards A_3 adenosine receptor (Figure 10.1).

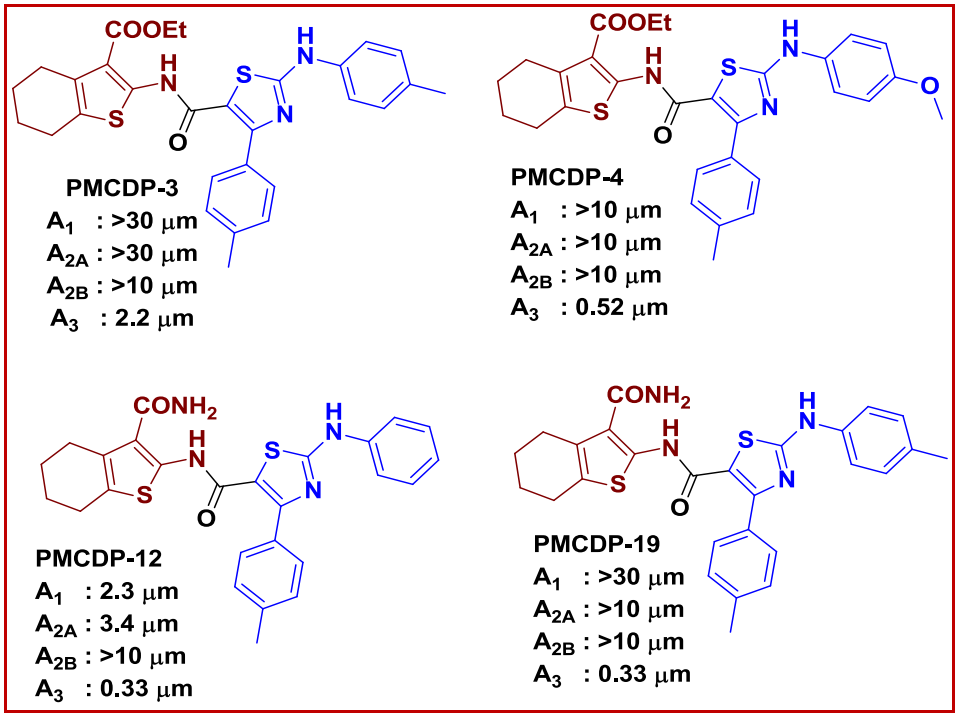

Figure 10.1: Active and selective adenosine receptor thiophenyl-thiazole carboxamides

In the **chapter 4** we have modified the previous series of molecule in to *N*-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes. Library of compounds were synthesized and all the molecules were tested into radio ligand binding assay for adenosine receptors. The molecules were found to show moderate to good activity. The compounds **PMCDP-24**, **PMCDP-29** and **PMCDP-41** found to show affinity towards A_1, A_{2A} and A_3 adenosine receptors. **PMCDP-29** was nonselective whereas **PMCDP-24** and **PMCDP-41** were selective towards A_3 adenosine receptor. From SAR it was found that the molecules with 4^{th} position phenyl or furyl group were found to be active where as the molecules with 4^{th} position hydrogen was found to deleterious in the activity against adenosine receptors (Figure 10.2).

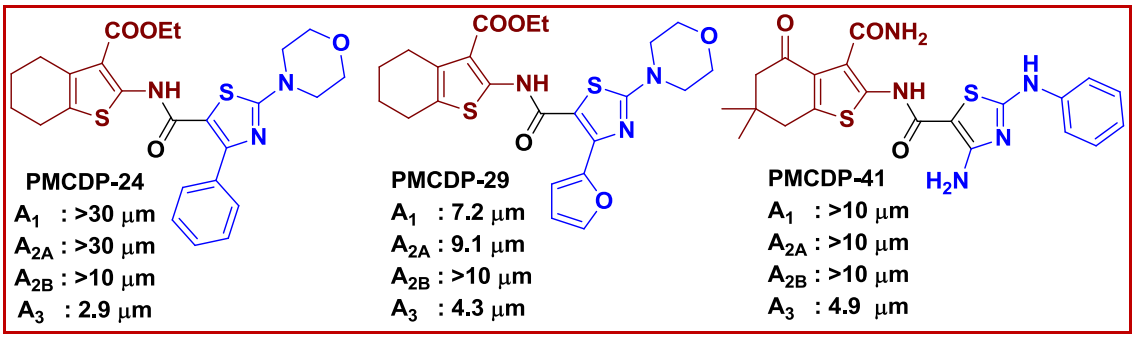

Figure 10.2 : Active and selective *N*-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes against adenosine receptora

In the **Chapter-5** we modified our design from thiophenyl-thiazole carboxamides into thiophenyl-thiophene carboxamides for adenosine receptors. The substituted 2-chloroacetamido thiophene derivatives were coupled with wilgerodt kindler substituted 1-morpholino-2-arylprop-2-ene-1-thione adducts to generate library of thiophenyl-thiophene carboxamide derivatives. The compounds were characterized and they were screened for radio ligand binding assay for adenosine receptors. Particularly the compounds **PMCDP-BTP-4**, **PMCDP-BTP-8** and **PMCDP-BTP-13** found to show affinity towards A_1, A_{2A} and A_3 adenosine receptors. The compound **PMCDP-BTP-13** was non selective whereas the compounds **PMCDP-BTP-4** and **PMCDP-BTP-8** were selective towards A_3 adenosine receptor (Figure 10.3).

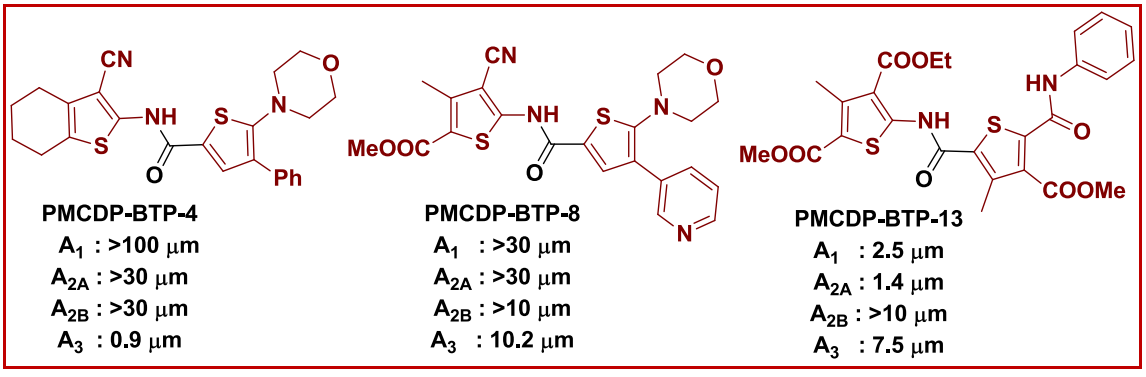

Figure 10.3 : Active and selective thiophenyl-thiophene carboxamides against adenosine receptor

Chapter-6 discusses the synthesis novel di/tri substituted *N*-cyclicamino thiazoles and their biological activity against adenosine receptor. Here we designed new *N*-cyclicamino

thiazole based on our previous active trisubstituted *N*-cyclicamino thiophenes. The new disubstituted thaizoles were synthesized by new route of synthesis. These molecules were further confirmed by various spectral techniques and they were further evaluated for their binding affinity against adenosine receptor. The molecules **PMCDP-TZA9 PMCDP-TZA14** and **PMCDP-TZA16** were found to be active. Here the molecule **PMCDP-TZA14** was the most selective molecule against A_3 adenosine receptor, where as **PMCDP-TZA16** was the most active compound with good affinity towards A_1, A_{2A} and A_3 adenosine receptors (Figure 10.4).

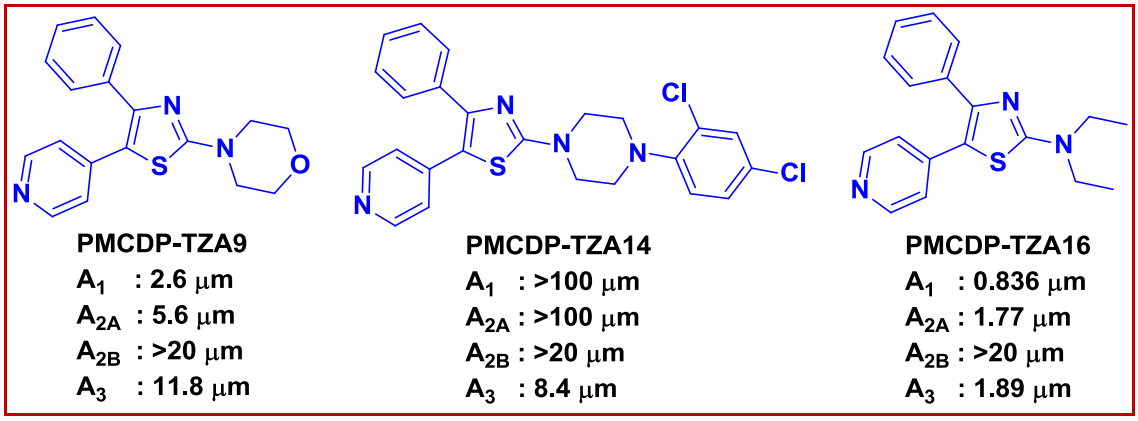

Figure 10.4 : Active and selective *N*-cyclicamino thiazoles against adenosine receptor

In the **Chapter-7** we discussed the design, synthesis and biological activity of new substituted 2-aminothiazoles for adenosine receptors. Based on novartis molecule and our published active molecules against adenosine receptors here we have synthesized new substituted 2-aminothiazoles. The molecules were further confirmed by various spectral techniques and they were further evaluated for their binding affinity against adenosine receptor. This series of molecules were found to be high affinity and selectivity towards adenosine receptors. Particularly the molecules **PMCDP-TZB2, PMCDP-TZB7, PMCDP-TZB16, PMCDP-TZB21** and **PMCDP-TZB22** were active in low nanomolar range. The molecules **PMCDP-TZB2** and **PMCDP-TZB22** were nonselective whereas the molecules **PMCDP-TZB7** and **PMCDP-TZB16** were selective in A_3; **PMCDP-TZB21** was selective in A_1 adenosine receptor. Docking study of the two most active molecules **PMCDP-TZB2** and **PMCDP-TZB22** was carried out with A_{2A} and A_3

adenosine receptors respectively to see the possible interaction and the analysis of the docking justified the biological activity of the two molecules (Figure 10.5).

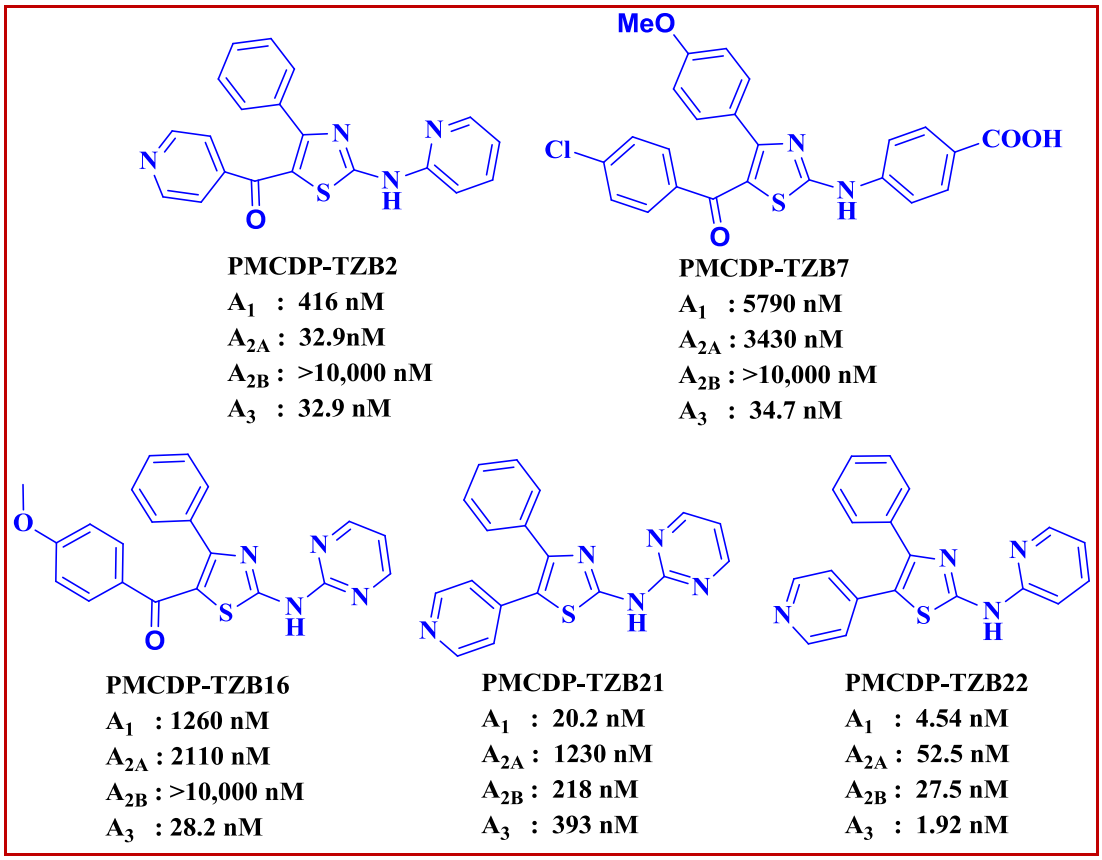

Figure 10.5 : Active and selective *2*-amino thiazoles against adenosine receptor

The **Chapter-8** deals with the 3D QSAR study of the active molecules against the A_1, A_{2A} and A_3 receptor subtypes. The active molecules were taken from the chapter-3, 4, 6 and 7 and aminothiazole was taken as core for alignment. CoMFA was performed and all the statistical parameters were found to be within the range for good QSAR model. The q^2 and r^2 for a) the A_1: 0.55 & 0.98 respectively, b) the A_{2A}: 0.45 & 0.96 respectively and c) the A_3: 0.50 & 0.98 respectively. The CoMFA study generated steric and electrostatic contour maps which were further analyzed.

Chapter-9 discusses the novel synthetic methodology for the synthesis of new 1,3,4-thiadiazines. Here we report novel synthetic methodology has been developed for the synthesis of substituted 2-amino 1,3,4-thiadiazines and 2-cycloamino 1,3,4-

thiadiazines from various substituted thiosemicarbazide derivatives and phenacyl bromides (Figure 10.6).

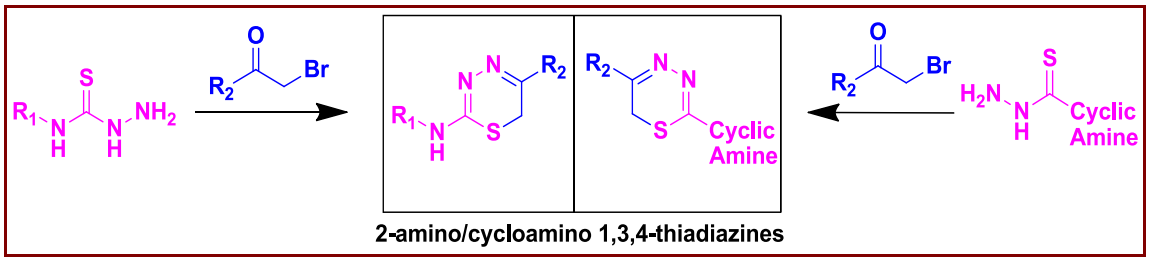

Figure 10.6 : Novel synthetic methodology for the 2-amino/cyclicamino thiadiazines

Overall conclusion of the present work:

- In search of potential antagonist for adenosine receptors here we have adopted a fragment based drug design approach for the design of the new compounds. The designed molecule was further validated with the molecular docking study.
- Synthetic methodology was developed and library of compounds with structural diversification was synthesized from various starting materials. Further all the molecules were characterized by spectral techniques like NMR and Mass.
- All the molecules were screened for their binding affinity to the adenosine receptor subtypes and the molecules were found to be selective and active. Particularly 2-aminothiazoles series were active in low nanomolar range.
- 3D QSAR study of adenosine receptor ligands shown a statistically significant result which is helpful for further modification.
- In conclusion here we are proposing the pharmacophore for lead optimization (Figure 10.7). This suggested that near A, a lipophilic feature with hydrogen-bond donor and acceptor groups like nitrogen heterocycle with an amino function is favourable for better activity. Near B, a bulky and highly lipophilic pi excessive feature such as phenyl ring is favourable. And near C, a lipophilic feature with hydrogen-bond donor and acceptor groups like nitrogen heterocycle with an amino function is favoured.

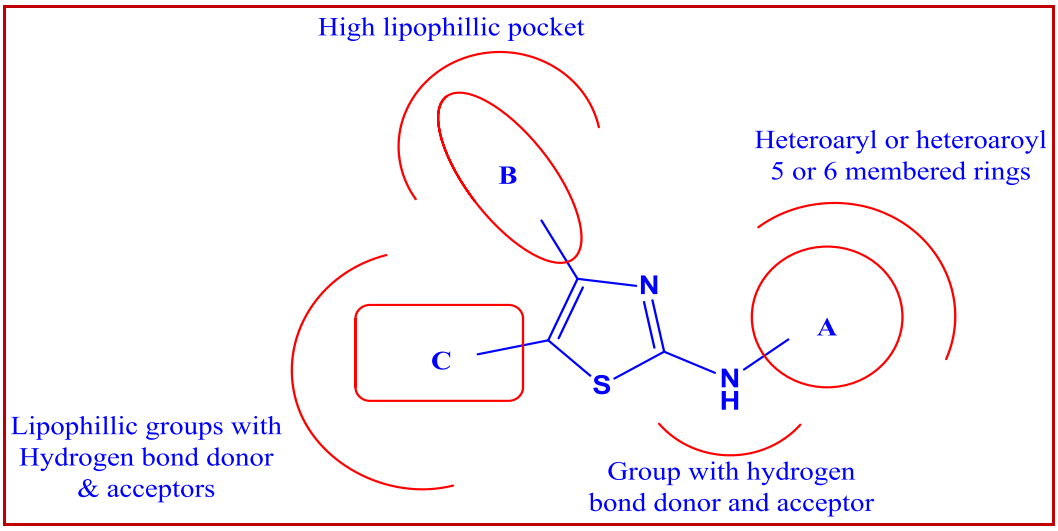

Figure 10.7: Proposed pharmacophore for lead optimization

Future Prospects

- The molecules developed here are the new chemical entity (NCEs) for adenosine receptors.
- Selective and active adenosine receptor antagonist can screened *in vivo* in various diseases like like asthma, parkinson's disease, alzheimer's disease, cancer and cardiovascular diseases.
- The molecules can be chemically manipulated to make novel compounds which can be further checked for various biological activities.
- The novel molecules are also having importance as novel synthons.

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2. HITESH B. JALANI, AMIT N. PANDYA, **DHAIVAT H PANDYA,** JAYESH A. SHARMA, V. SUDARSANAM, KAMALA K VASU. An efficient one-pot synthesis of functionally diverse 2-aminothiazoles from isothiocyanates, amidines/guanidines and halomethylenes. *Tet Lett.* 2013, 54, 5403-5406.
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Patents

1. Novel 2-substituted thiazole compounds. Indian patent application no. 2294/MUM/2014.
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Posters

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Novel thiazole–thiophene conjugates as adenosine receptor antagonists: Synthesis, biological evaluation and docking studies ☆

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ABSTRACT

Here we report novel thiazole–thiophene conjugates as adenosine receptor antagonists. All the molecules were evaluated for their binding affinity for adenosine receptors. Most of the molecules were found to interact with the A_1, A_{2A} and A_3 adenosine receptor subtypes with good affinity values. The most potent and selective compound **8n** showed an A_3 K_i value of 0.33 μM with selectivity ratios of >90 versus the A_1 and >30 versus the A_2 subtypes. For compound **8n** docking studies into the binding site of the A_3 adenosine receptor are provided to visualize its binding mode.

Adenosine regulates a variety of cellular functions through interaction with the four G-protein coupled receptor (GPCR) subtypes A_1, A_{2A}, A_{2B} and A_3. The A_1 and A_3 adenosine receptors couple to G_i and thereby inhibit adenylyl cyclase (AC) with a consequent decrease of cellular cAMP levels. The A_{2A} and A_{2B} receptor couple to G_s and mediate a stimulation of AC resulting in enhanced intracellular cAMP levels.[1,2] Adenosine receptor subtypes are a target of great interest because of their pathological involvement in numerous diseases. Activation of adenosine receptors is beneficial in many conditions like epilepsy, pain, cancer, etc. whereas inhibition of adenosine receptors is helpful in Parkinson's disease, Alzheimer's disease, asthma, diabetes and cancer.[1–3]

Development of adenosine receptor antagonists is a major focus of medicinal chemistry since several decades. Many selective antagonists have been developed so far and are helpful in pathological conditions like renal failure (A_1), Parkinson's disease (A_{2A}), diabetes (A_{2B}), asthma and chronic obstructive pulmonary disease COPD (A_{2B}/A_3), glaucoma and cancer (A_3). The traditional xanthine derivatives like caffeine and theophylline are naturally occurring nonselective antagonists. Selective antagonists have been developed by modifying the xanthine or adenine scaffolds, or heterocyclic moieties with mono-, bi- or tricyclic ring systems.[4] Several of them are in preclinical or clinical trials and the selective A_{2A} receptor antagonist Istradefylline got approval in Japan for the treatment of Parkinson's disease (Fig. 1). The major problem associated with xanthine derivatives is related to pharmacokinetic issues. Consequently, there is a need for new non-xanthine molecules. Structures incorporating thiazole, thiophene and benzothiazinones have been developed as selective adenosine receptor antagonists.[5–7]

As published in many reports, aminothiazoles and aminothiophenes are two important structural elements in adenosine receptor antagonists. Such 2-aminothiophene substituents are also found in modulation of adenosine receptors.[8] Based on similar observations, Aurelio et al. published 2-aminothiophene derivatives as adenosine receptor modulators.[9] Aminothiazole compounds as adenosine receptor antagonists have been reported from our group to show high affinity and selectivity.[5,7] So keeping in mind the importance of both moieties, that is, thiophene and thiazole, here we have designed novel thiazole–thiophene conjugates with an amide spacer (Fig. 2).

The synthesis of thiazole–thiophenes (**8a–8r**, Table 1) was carried out by a four-step reaction. The respective starting derivatives of 2-chloroacetamidothiophene and amidinothiourea were prepared separately. The reaction between 2-chloroacetamidothiophenes (**4**) and amidinothioureas (**7**) was carried out to get the final thiazole–thiophene conjugate compounds (Scheme 1).

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An efficient one-pot synthesis of functionally diverse 2-aminothiazoles from isothiocyanates, amidines/guanidines and halomethylenes ☆

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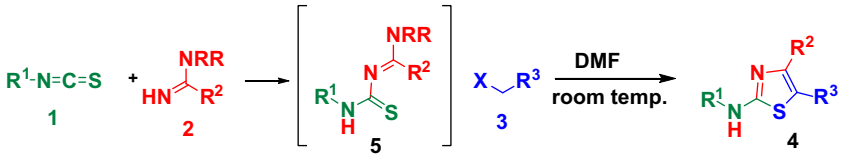
ABSTRACT

An efficient one-pot method for the synthesis of 2-aminothiazoles using simple starting materials like isothiocyanates, amidines/guanidines and various halomethylenes is reported. The synthesis of 2-aminothiazoles involves reactions such as nucleophilic addition, S-alkylation and intramolecular nucleophilic substitution in which amines departs as the leaving group.

2-Aminothiazole is an important and classic heterocyclic scaffold used in the drug discovery programs. The broad spectrum biological activities exhibited by this structure include anticancer,[1] antiviral,[2] antibacterial,[3] antiprion[4] and psychotropic activities[5] that assign it as an indispensible heterocyclic feature in drug design. In addition to this, recently, our group has successfully employed 2-aminothiazole scaffolds in the design of anti-inflammatory agents[6] as well as adenosine receptor antagonist.[7] Recently, 2-aminothiazole analogues have been identified as druglike candidates in the treatment of diabetes[8] and *Mycobacterium tuberculosis*.[9] Apart from biological properties, films of conjugated polyaminothioazole have recently been demonstrated to have electrochemical properties with high thermal stability.[10] All the above described biological and physico-chemical properties of aminothioazole are probably due to its small ring structure with π excessive and π deficient properties, due to nitrogen atom behaving as hydrogen bond acceptor site.

In view of diverse biological and physico-chemical properties by 2-aminothiazoles scaffold, many synthetic protocols have been reported for their synthesis, which includes Hantzsch's cyclocondensation of thiourea with α-haloketones/α-tosylketone[11] and the reactions of α-thiocyanate carbonyl compounds with aromatic or aliphatic amine hydrochlorides.[12] 2-Aminothiazoles are also synthesized by one-pot reaction of enolizable ketones with a mix-



Scheme 1. One-pot synthesis of 2-aminothiazoles.

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CoMFA and CoMSIA 3D QSAR Models for a Series of Some Condensed Thieno[2,3-*d*]pyrimidin-4(3*H*)-ones with Antihistaminic (H_1) Activity

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Abstract: Comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) studies were carried out for a series of thienopyrimidines, novel Histamine H_1 receptor antagonists. Various models were generated. The best predictive CoMFA model gave significant correlation coefficients (cross-validated r^2 (q^2) = 0.514, non-cross-validated r^2 = 0.925), showing the influence of steric and electrostatic fields. Likewise, the best predictive CoMSIA model gave cross-validated r^2 (q^2) = 0.541, non-cross-validated r^2 = 0.862, eliciting the influence of steric, electrostatic, hydrophobic and hydrogen bond acceptor fields. The generated models were externally validated and well correlated with calculated (predicted) and experimental inhibitory concentration (IC_{50}) values, using test sets. The analysis of the contour maps of both CoMFA and CoMSIA models offer important structural insight for designing novel and more active Histamine H_1 receptor antagonists prior to their synthesis.

Keywords: CoMFA, CoMSIA, Thienopyrimidine derivatives, 3D QSAR.

1. INTRODUCTION

Histamine (2-[imidazol-4-yl]-ethylamine), a biological amine mediator exerts a wide range of effects over various biological process, viz. smooth muscle contraction, in inflammation, gastric acid secretion, as a neurotransmitter in central nervous system etc. Targeting its receptors has been a well known therapeutic strategy for over 60 years. Human histamine H_1 receptor (HHR_1), one of the G protein-coupled receptors (GPCRs) known for their constitutive activation in the absence of agonist binding, is involved in allergic reactions like rhinitis, urticaria, hay fever and asthma. Various Histaminic inverse agonists, also called antagonists inhibit this constitutive activity of GPCRs [1].

Three dimensional quantitative structure-activity relationships (3D QSAR) have been employed since early 1970s as useful tools to correlate data set information and to predict the physiochemical and pharmacological properties of untested molecules [2-4]. Compounds containing thienopyrimidine nucleus represents a very important chemical class of medicinal compounds due to their wide range of pharmacological properties, including antiallergic, anti-inflammatory, analgesic, antispasmodic, antibacterial, antifungal etc [5].

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As part of our research program aimed at investigating the 3D QSAR study of a series of thienopyrimidines, we have employed the comparative molecular field analysis (CoMFA) and the comparative molecular similarity indices analysis (CoMSIA) methods. The widely used CoMFA method is based on the assumption that the interactions between a receptor and its ligands are primarily non covalent in nature and conformation dependent [6, 7]. The CoMFA method calculates the energies of Steric and Electrostatic interactions between the 3D structures of a series of compounds and the probe atom kept at the various intersections of a regular 3D-lattice according to Lennard–Jones and Coulombic potentials respectively, and hence correlating the differences in those fields to biological activity. The resulting energies derived from these two potential functions can then be contoured to give a quantitative spatial description of the molecular properties. Partial least squares (PLS) analysis, with a cross validation procedure, was employed to select relevant components from the large set of CoMFA data to build up the best QSAR model. CoMSIA approach, calculated property fields based on similarity indices of drug molecules that have been brought into a common alignment [8, 9]. The fields of different physicochemical properties used a Gaussian type function for the distance dependence between the molecular atoms and the probe atom in order to avoid some of the inherent deficiencies arising from the Lennard–Jones and Coulomb potential functional forms. CoMSIA is applied to gain insight into how Steric, Electrostatic, Hydrophobic and Hydrogen bonding interactions influence the activity. The results from these studies will be helpful for better understanding of the molecular mecha-

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A concise, greener, solvent-free novel one-pot synthesis of trisubstituted thiophenes ☆

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ABSTRACT

Herein, we report a concise, greener, solvent-free, and novel one pot method for the synthesis of 2-morpholino-3-aryl-5-aroyl thiophenes using 1-morpholino-2-arylethanethione, *N,N′*-dimethyl formamide dimethyl acetal, and various phenacyl bromides. The driving force for this reaction is the removal of *N,N′*-dimethylamine from 3-(dimethylamino)-1-morpholino-2-arylprop-2-ene-1-thione resulting in various trisubstituted thiophenes (2-morpholino-3-aryl-5-aroyl thiophenes).

Syntheses of small molecules, especially five-membered ring heterocycles have always attracted the scientific community, because of their vivid therapeutic importance. In addition to this, small heterocyclic scaffolds are present in more than 50% of pharmaceutical substances and allow various interactions with the biological targets due to the presence of side chains or various polar bonds, which are not accessible in carbocyclic scaffolds. It is also revealed that the diversity of synthetic methods used by the pharmaceutical industries to generate heterocycles containing products is based on only five-membered aromatic heterocycles representing more than top 200 best selling drugs.[1]

Thiophene is an important structural motif in medicinal chemistry and it is considered as a classical bioisostere for the benzene ring and due to its small ring structure it is found in many therapeutically active substances (Fig. 1) such as Raloxifene, Olanzepine, Clopidogrel, Tiamenidine, Tiaprofenic acid, Suprofen, Raniliate strontium, potent PI3K inhibitors etc. One of the common methods for the synthesis of 2-morpholino thiophene is the Willgerodt–Kindler[2] reaction of acetophenone, sulfur, and morpholine which gives the intermediate 1-morpholino-2-arylethanethione. This 1-morpholino-2-arylethanthione intermediate on reaction with morpholine and triethyl orthoformate gives the dimorpholide compounds which on reaction with various aryl, aroyl halides result in 2-morpholino thiophenes.[3,4] In addition to this, recently, Huang et al. developed a multi-step synthesis of 2-morpholino thiophene compound known for its PI3K activity.[5] It has also been observed that the 2-aminothiophene compounds are well documented in the literature,[6,7] but the most popular Gewald method,[8] involves the multicomponent condensation of carbonyl compounds, cyanoacetates or malanonitrile, and elemental sulfur. The above mentioned processes are associated with drawbacks such as multistep reactions (except Gewald), harsh reaction conditions, complex and tedious experimental procedures, and lower yields. After a careful literature search, we realized that there is still a need to develop efficient and concise methods for the synthesis of thiophenes.

In continuation of our work on the synthesis of various bioactive heterocyclic compounds using *N,N′*-dimethyl formamide dimethyl acetal (DMF–DMA),[9,10] we were interested to explore 1-morpholino-2-arylethanethione intermediate resulting from the Willgerodt–Kindler reaction of various acetophenones, sulfur, and morpholine. We envisaged that this intermediate could be useful for the synthesis of thiophenes. We have developed an efficient, concise, greener, and novel sequential one-pot method for the synthesis of trisubstituted thiophenes from 1-morpholino-2-arylethanethione, DMF–DMA, and various phenacyl bromides resulting in various 2-morpholino-3-aryl-5-aroyl (trisubstituted) thiophenes. This method neither requires any reagent for the cyclization nor the solvents (Scheme 1) which is reported herein. To the best of our knowledge, the formation of 2-morpholino-3-aryl-5-aroyl (trisubstituted) thiophenes using *N,N′*-dimethyl formamide dimethyl acetal (DMF–DMA) has not been reported so far.

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An efficient, greener, and solvent-free one-pot multicomponent synthesis of 3-substituted quinazolin-4(3*H*)ones and thienopyrimidin-4(3*H*)ones ☆

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ABSTRACT

Herein, we report an efficient, greener, and solvent-free novel method for the synthesis of 3-substituted quinazolin-4(3*H*)ones and thienopyrimidin-4(3*H*)ones in a one-pot sequence using methyl anthranilate or 2-aminothiophene-3-carboxylate with *N*,*N*′-dimethyl formamide dimethyl acetal and various anilines. The driving force for this reaction is the removal of *N*,*N*′-dimethylamine by various anilines resulting in 3-substituted quinazolin-4(3*H*)ones and thienopyrimidin-4(3*H*)ones.

Lots of efforts have been directed toward the design and applications of multicomponent reactions[1] because MCRs are powerful tools for the construction of organic molecules allowing the formation of several bonds in just a single reaction. In addition to this, carrying out such multicomponent reactions with non hazardous and environment friendly reagents could provide an interesting platform to the scientific community over the conventional approaches. To minimize the use of hazardous reagents, emphasis should be given to the development of greener approaches.

Quinazolin-4(3*H*)one is an important class of heterocycles which possess diverse range of biological properties such as antimalarial,[2] anticonvulsant,[3] antibacterial,[4] antidiabetic,[5] and anticancer activities.[6] Diverse range of the pharmacological activities of quinazolin-4(3*H*)one derivatives have tempted considerable interest for the synthesis of quinazolin-4(3*H*)one using versatile and greener methods. The most common method for the synthesis of 3-substituted quinazolin-4(3*H*)one involves the reaction of anthranilic acid with DMF and $POCl_3$, reported by Perumal et al.[7] Other methods for the synthesis of 3-substituted quinazolin-4(3*H*)ones are from anthranilic acid derivatives.[8–17] Recently, quinazolin-4(3*H*)ones were prepared using silica sulfuric acid,[18] PCl_3,[19] $Zn/HCOONH_4$ under microwave irradiation,[20] $LiNO_3$,[21] and HATU.[22] However, some of these methods are associated with drawbacks such as multistep reactions, costly reagents, harsh reaction conditions, complex and tedious experimental procedures, and low yields.

Considering the above facts, there is still need to develop efficient, greener, and economical methods for the synthesis of quinazolin-4(3*H*)ones. In addition to this, a majority of the condensed pyrimidin-4(3*H*)one heterocyclic compounds have been reported from 2-aminobenzoic acid.[8] Earlier reports on the synthesis of 3-substituted quinazolin-4(3*H*)ones incorporate anthranilic acid as starting material, which has electron rich carboxylate resonance structure thus requires various lewis acid catalysts in order to become electron deficient for the cyclization. In case of methyl anthranilate, the carbonyl group being electrophilic in nature, allows the nucleophilic attack facile for the cyclization without use of any catalysts. To the best of our knowledge, the formation of 3-substituted quinazolin-4(3*H*)ones from methyl anthranilate has not been reported so far.

In continuation to our work on the synthesis of biologically important heterocycles such as quinazolin-4(3*H*)ones,[23] we were interested to investigate the formamidine intermediate resulting from the reaction of methyl anthranilate and *N*,*N*′-dimethyl formamide dimethyl acetal (DMF–DMA) can further converted to quinazolin-4(3*H*)ones with the help of various amines. The synthesis of formamidine using either amines or thioureas with DMF–DMA is well documented in the literatures.[24] This formamidine structure has tempted us to utilize it in the synthesis of quinazolin-4(3*H*)ones by heating it with various amines to elicit the 3-substituted quinazolin-4(3*H*)ones. Herein, we report an efficient, greener, sequential one-pot method for the synthesis of 3-susbstitued quinazolin-4(3*H*)ones from methyl anthranilate, DMF–DMA, and various

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An efficient synthesis of 2-aminopyrroles from enaminone–amidine adduct and phenacyl/benzyl/heteroalkyl-halides

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ABSTRACT

Herein, we report an efficient and facile synthesis of substituted 2-aminopyrroles from the reaction of enaminone–amidine adduct and various phenacyl, benzyl, or heteroalkyl halides in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in good to excellent yields. The reaction proceeds through an intramolecular 5-*exo* trig cyclization resulting into diversely substituted 2-aminopyrroles.

Many natural and biologically active compounds like hemes, bile pigments, chlorophyll etc. have pyrrole as the basic scaffold which is also present in pharmaceuticals[1] and compounds of material sciences.[2] 2-Aminopyrroles are part of many different bioactive compounds with reported bioactivities like, IL-6 production inhibition, IkB kinase-β inhibition, intergrin antagonists, signals transduction modulators, antitumor, and antibacterial agents[3] are a few of them. Despite the large number of methods for the synthesis of pyrroles reported so far, it is still challenging to prepare 2-aminopyrroles with various substituents directly from readily available building blocks. Several methods were reported for the synthesis of 2-aminopyrroles by Domling,[4] Zhu,[5] Nair[6] and Shaabani[7] using multi-component reactions of acidic nitriles or isocyanides as starting materials. 2-Aminopyrroles are not readily available precursors; in general, such species are difficult to make and are notoriously prone to decomposition if the adjacent carbon attached to the carbon of the amino group is not bearing the electron withdrawing groups. To the best of our knowledge, there are no simple and convenient methods for the synthesis of substituted 2-aminopyrroles using the readily available and simple starting materials like enaminone–amidine adduct and phenacyl/benzyl/hetero-alkyl halides.

Enaminones (enamines) are versatile and readily available intermediates and their chemistry has received considerable attention in recent years.[8] As far as the chemical reactivity of enamines is concerned, they can react with both electrophiles as well as nucleophiles.[9,10] It is known that the electron rich double bond of enamino-ketones has stronger tendency to react as a nucleophile toward the electron deficient species. The existing methods for the synthesis of pyrroles from enamines and carbonyl compounds are facilitated by oxidizing agents for cyclization and results in pyrroles without amine function.[11]

The focus of our group is to develop new synthetic methods for the small heterocyclic compounds particularly bearing the amino group within the heterocycles. We have developed the synthesis of 2-aminothiophene,[12] 2-aminothiazole[13] and 2-aminoimidazole[14] using different adducts of isothiocyanates with enamines or amidines. We have been working on the reaction of enamines/enaminones with different isothiocyanates to produce the enaminone–isothiocyanate adducts which are useful intermediates for the synthesis of substituted 2-aminothiophenes.[15] Herein, our interest was to check the reactions of enaminones with various electrophiles, in particular their reactions with cyanamide to get the enaminone–amidine adduct.

We reasoned that, we can use the nucleophilic nature of the enaminone by reacting them with electrophilic cyanamide (carbodimide) in the presence of a mild acid to produce the enaminone–amidine adduct. Further reaction of this adduct with active methylene halides could give the desired pyrroles. Herein, we report a novel synthesis of 2-aminopyrroles (**3** and **5**) by the reaction of enaminone–amidine adduct **1** with various phenacyl bromides **2**, benzyl and heteroalkyl halides **4** in the presence of DBU in good to excellent yield (Scheme 1).

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ORIGINAL ARTICLE

Stability-indicating assay method for determination of actarit, its process related impurities and degradation products: Insight into stability profile and degradation pathways ☆

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Abstract The stability of the drug actarit was studied under different stress conditions like hydrolysis (acid, alkaline and neutral), oxidation, photolysis and thermal degradation as recommended by International Conference on Harmonization (ICH) guidelines. Drug was found to be unstable in acidic, basic and photolytic conditions and produced a common degradation product while oxidative stress condition produced three additional degradation products. Drug was impassive to neutral hydrolysis, dry thermal and accelerated stability conditions. Degradation products were identified, isolated and characterized by different spectroscopic analyses. Drug and the degradation products were synthesized by a new route using green chemistry. The chromatographic separation of the drug and its impurities was achieved in a phenomenex luna C18 column employing a step gradient elution by high performance liquid chromatography coupled to photodiode array and mass spectrometry detectors (HPLC–PDA–MS). A specific and sensitive stability-indicating assay method for the simultaneous determination of the drug actarit, its process related impurities and degradation products was developed and validated.

1. Introduction

Actarit is an orally active immunomodulator used in the treatment of rheumatoid arthritis. It suppresses secondary inflammation by activation of Lyt-2+ cells and shows prophylactic and therapeutic effects on secondary inflammation in adjuvant arthritis in rats [1,2]. It prevents the progression of articular lesions [3] and also curtails type II and type IV allergic reactions in mice [4–6].

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