Synthesis, Characterization and Bioevaluation of New Dihydropyrimidine-2-thiones; Phenazone/Ferrocene-thioureas; Phenazone benzamides; 2-Aroyl iminothiazolines and Synthesis of a Tropane Auxiliary for \( \alpha \)-Alkylation of Aldehydes

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By

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Department of Chemistry Quaid-i-Azam University Islamabad 2016
Dedicated
To

My Loving Mother

Her support, encouragement, and Constant love have sustained Me throughout my life &

To all my respected teachers especially Prof. Dr. Aamer Saeed for imparting me knowledge
All praises be Allah, Who bestowed the humans with intelligence, knowledge, and sight to observe, mind to think and judge. Peace and blessing of Allah upon The Holy Prophet Muhammad ﷺ: One of His sayings is: Seek knowledge from cradle to grave.

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Abstract
The work presented in this thesis describes the synthesis, characterization and bioevaluation of different classes of compounds, i.e., 3,4-dihydropyrimidine-2-thiones, thioureas, benzamides and 2-aryliminothiazolines. In some cases, docking and computational studies have also been carried out. In addition, a new multistep synthetic methodology of tropane auxiliary which is used as a stereo-directing element to achieve α-alkylation of aldehydes has been developed.

A series of fourteen 3,4-dihydropyrimidine-2-thiones (248a-n) were synthesized by a green protocol, and their structures were characterized by spectroanalytical data. The compounds were obtained in high yields by efficient annulation of mesityl oxide (4-methyl-pent-3-en-2-one) with anilines in the presence of potassium thiocyanate. The reaction is essentially metal-catalyst- and solvent-free, as mesityl oxide itself is the solvent as well as the reactant.

The compounds were tested for their ability to inhibit the lymphoid tyrosine phosphatase PTPN22, and 5 of the 14 compounds exhibited IC_{50} values in the mid micro-molar range, with the most potent hit being the compound 248d, having the methoxy substituent at the 2-position of the phenyl ring with an IC_{50} value $18 \pm 1 \mu M$, and the second most potent compound 248c with an IC_{50} value of $45 \pm 3 \mu M$, having methyl substituents at both 2- and 4- position of the phenyl ring.

A series of twelve new aryl thiourea derivatives of 4-aminophenazone (252a-l) has been synthesized. The 4-aminophenazone which is also called as ‘4-aminoantipyrine’ or ‘ampyrene’ belongs to a class of non-steroidal anti-inflammatory drugs (NSAID’s) responsible for a broad spectrum of medicinal and therapeutic applications. So, based on the biomedical importance of this drug, the newly-synthesized aryl thiourea derivatives of 4-aminophenazone were screened in vitro against alkaline phosphatase enzyme found in the intestine of calf as well as also evaluated for their antioxidant and cytotoxic potential.

Among the tested compounds, the 2-methyl derivative 252b of the series was found to be the most potent compound showing greater inhibition potential against alkaline phosphatase, besides, displaying greater antioxidant potential. The 3-nitro member 252i of the series came out to be the most active member while screening the synthesized series for their cytotoxic potential using brine shrimp assay. Apart from these bioassays, kinetic analysis of the most potent member of the series on basis of IC_{50} value 252c was performed in order to find the mechanism of enzyme inhibition. The results suggested that the compound 1-(1,5dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)3-m-tolylthiourea 252c is a non-competitive inhibitor of calf intestinal alkaline phosphatase, i.e., it lowers the enzyme concentration by showing non-competitive binding mode with enzyme.

The synthesis of a series of different substituted N-(2,3-dimethyl-5-oxo-1-phenyl-2,5dihydro-1H-pyrazol-4-yl)benzamides was carried out by making use of 4aminophenazone, a compound of great interest in medicinal chemistry. These compounds possess potential biological applications and were screened against human recombinant alkaline phosphatase including human tissue-nonspecific alkaline phosphatase (h-TNAP), tissue specific human intestinal alkaline phosphatase (h-IAP), human placental alkaline phosphatase (h-PLAP) and human germ cell alkaline phosphatase (h-GCAP). These compounds were also tested for their inhibitory potential against recombinant human and rat ecto-5'-nucleotidases (h-e5-NT & r-e5NT, respectively). All benzamide derivatives inhibited APs to a lesser degree than
The reported compounds are of considerable interest for further applications in the field of medicinal chemistry as these compounds have potential to bind nucleotide protein targets. An efficient synthesis of new aroyl thiourea derivatives of ferrocene (261a-q) was also accomplished. Ferrocene substituted benzoyl chloride was reacted with potassium thiocyanate to give the corresponding isothiocyanate intermediate which on reaction with different substituted anilines, afforded thiourea derivatives in good yields. The synthesized series of compounds were evaluated for their in vivo locomotor activity that was carried out inside the mice using diazepam as standard drug. Pharmacokinetic parameters such as absorption, distribution metabolism, LD50 and other characteristics were determined for active members of the synthesized series, i.e., 261d (4-methoxy substituted) and 261g (3-chloro substituted). These compounds were also screened in silico for their pharmacokinetic profiling using Admet-SAR software.

Synthesis of 2-imino-1,3-thiazolines was accomplished in two steps. In the first step, isomeric chloro benzoyl thiourea derivatives (265a-c) were synthesized via reaction between in situ formed isomeric chloro benzoyl isothiocyanates with aqueous ammonia. Structure of one of the crystalline isomeric, i.e., 2-chlorobenzoyl thiourea derivative 265a was determined through single crystal X-ray crystallography and its vibrational properties were determined as well. The conformational analysis of the crystal structure 265a revealed that a local planar structure is preferred with opposite orientation between the C=O and C=S (preference of S-conformation over U-conformation), thus, forming a pseudo six-membered ring structure that promotes a C=O…N intramolecular hydrogen bond.

The second step involved the heterocyclization reaction between different cyclizing agents such as alpha halo carbonyl compounds and diethyl oxalate with synthesized isomeric chloro benzoyl thiourea derivatives to form a series of novel 2-imino-1,3thiazoline derivatives (266, 267, 268, 269a-c). The synthesized derivatives were screened for their antileishmanial activity using Amphotericin B as reference standard. The screening results showed that the derivative 266c having chloro group at para position to be most potent and active member of the series.

An efficient multistep strategy was devised for the synthesis of racemic 1-methyl tropane auxiliary (±)-270. The alkylation potential of the aldenamine (±)-292 derived from racemic tropane auxiliary has been tested in order to exploit the 5-membered ring’s effect on facial selectivity. The dr of α-alkylated diastereomeric iminium ions was determined for the racemic system from 1H-NMR analysis. The experimental findings indicated a dr of 64:36 which are in close agreement with the computationally-determined results (dr of 68:32). It was further concluded from the collaborative computational study and laboratory experimental findings that the presence of 5-membered ring has caused a surprising change in the precise ground state structural orientation in the enamine species. The enamine exocyclic double bond was indicated as leaning more over the face of 6-membered ring (fig. 39) than the 5-membered pyrrolidine ring, thus, directing alkylation towards the face of 5-membered ring, i.e., preference for Si compared to Re addition by ~2 kJ mol⁻¹. All the synthesized compounds were characterized on the basis of their physiochemical parameters and spectral data.
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157 pyrazol-4-yl)

157-158 pyrazol-4-yl)

158 pyrazol-4-yl)

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Heterocyclic chemistry is an integral part of the chemical sciences and constitutes a considerable part of the modern researches that are being carried out throughout the chemical community. The study of heterocyclic chemistry has gained importance from both theoretical and practical point of view and it displays an important role in the design and discovery of new physiologically/pharmacologically-active compounds.¹ In this perspective, heterocyclic scaffolds with well-defined bioactivity profile have fueled intense academic and industrial research in recent years.
1. Pyrimidinethiones

Due to recent developments in medicinal chemistry, the chemistry of pyrimidine has got an immense importance.¹ The possible reason for their activity is attributed to the pyrimidine base which is found in thymine, cytosine and uracil that are the main building blocks of nucleic acids, i.e., the DNA and RNA. Pyrimidinethione is an important class of pyrimidine which is also named as mercapto or thioxopyrimidine. Pyrimidinethione derivatives are of substantial interest to synthetic-biochemists.² Depending on the position of thione group, the pyrimidinethiones exist in three possible structures named as (a) 2-pyrimidinethione (b) 4-pyrimidinethione and (c) 2,4- pyrimidinethione as shown in fig.1.

![Fig. 1 Three possible structures of pyrimidinethione](image)

1.1 Biological activities

Following activities have been shown by various pyrimidinethione derivatives:

a. Anti-HIV activity

Hetero atoms-substituted dihydropyrimidines compound branched ester sec-butyl (1) alkylthio group active against (e.g., and (2) have been HIV.³  

![Chemical structures](image)

b. Anti-tumor activity

After the discovery of first Biginelli anticancer pyrimidinethione compound, a series of further new anticancer compounds possessing the same functionality have been synthesized like monastral (3). In an attempt, the furyl derivative (4) was found to be five times more potent than the monastral.⁴
c. Anti-microbial activity

Apart from other bioapplications, the Biginelli compounds multifunctionalized with isoxazole amines, i.e., 1-aryl-4-methyl-3,6-bis-(5-methylisoxazole-3-yl)-2-thioxo2,3,6,10b-tetrahydro-1H-pyrimido[5,4c]quinolone-5-ones (5) showed anti-microbial activity.\(^5\)

\[
\begin{array}{c}
\text{R=CsH}_5, \text{4-CH}_3\text{CsH}_4, \text{4-CH}_3\text{OCsH}_4, \text{4-BrCsH}_4
\end{array}
\]

\[5\]

d. Analgesic activity

The synthesized 2-methylthio-1,4-dihydropyrimidine derivatives (6) have been shown to be responsible for analgesic activity. The activity exhibited by the title compound derivatives is mainly due to the inhibition of peripheral pain mechanism.\(^6\)

\[
\begin{array}{c}
\text{CH}^3
\end{array}
\]

\[6\]

e. As neoplasm inhibitors

Compound (7) is found to be effective neoplasm inhibitor \textit{in vivo} tests in mice.\(^1\)
f. As herbicides

Phenoxyopyrimidinecarboxylate (8) appears to be a better herbicide against *Podosphaera lucotricha* on apples.  

![Chemical structure of Phenoxyopyrimidinecarboxylate (8)](image)

8

---

g. Anti-bacterial activity

*In vitro* antibacterial activity against *Salmonella* spp., *St. albus*, and *B. subtilis* have been demonstrated by the pyrimidine derivatives (9).  

![Chemical structure of the pyrimidine derivatives (9)](image)

9

Screening of 2-benzylthio-4-substituted amino-6-methylpyrimidines (10) against selected bacteria, *Bacillus subtilis* and *Neisseria* manifested moderate activity.  

![Chemical structure of 2-benzylthio-4-substituted amino-6-methylpyrimidines (10)](image)
R=\text{C}_6\text{H}_5, 4-\text{CH}_3\text{C}_6\text{H}_5, 4-\text{Br-C}_6\text{H}_4

10

h. Anti-oxidant and anti-filarial agent

The following pyrimidine derivative (11) has furnished evidence for anti-oxidant as well as anti-filarial activity. \(^7\)

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\text{F}};
\node (B) at (0,-1) {\text{H}_3\text{C-}};
\node (C) at (1,-1) {\text{N}};
\node (D) at (1,-2) {\text{H}};
\node (E) at (1,-2.5) {\text{S}};
\node (F) at (2,-2.5) {\text{C}_9\text{H}_{11}};
\node (G) at (2.5,-1) {\text{N}};
\node (H) at (2.5,-2) {\text{C}_6\text{H}};
\node (I) at (2.5,-2.5) {\text{C}_6\text{H}_4};
\end{tikzpicture}
\end{center}

11

i. Vasorelaxant activity

It was reported by Atwal \textit{et al}. that 3-substituted-1,4-dihydropyrimidines (12) are responsible for possessing antihypertensive activity. It was found that the activity depends on the size of C5 ester group, isopropyl ester being the best. A variety of substituents (carbamate, sulfonyl, alkyl) can be tolerated at N3. \(^8\)

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\text{R}};
\node (B) at (0,-1) {\text{OOCR}};
\node (C) at (1,-1) {\text{H}_3\text{C-}};
\node (D) at (1,-2) {\text{N}};
\node (E) at (1,-2.5) {\text{H}};
\end{tikzpicture}
\end{center}

12

j. As adenosine A1 receptor antagonist

Sally Ann \textit{et al}.\(^9\) synthesized 4,6-bis[(R-carbamoylethyl)thio]-1-phenylpyrazolo[3,4d] pyrimidine (13) and it was identified as a novel adenosine A1 receptor antagonist, and results in antagonizing adenosine stimulated cyclic adenosine monophosphate generation in guinea pig brain slices. \(^10\)

\begin{center}
\begin{tikzpicture}
\node (A) at (0,0) {\text{HO}};
\node (B) at (0,-1) {\text{EtOOC-}};
\node (C) at (1,-1) {\text{H}_3\text{C-}};
\node (D) at (1,-2) {\text{NH}};
\node (E) at (1,-2.5) {\text{H}};
\node (F) at (2,-2) {\text{N}};
\node (G) at (2,-2.5) {\text{S}};
\end{tikzpicture}
\end{center}
k. ATPase inhibitor

\[ \text{N' - alkylation of 3,4-dihydropyrimidine-2(1H)-ones was effected which revealed that the} \]
\[ \text{parent compound shows better ATPase activity than its N'-alkylated product} \]

(14).  

\[
\begin{array}{c}
\text{H} \\
\text{N} \\
\text{S} \\
\text{N} \\
\text{H} \\
\text{OEt}
\end{array}
\]

l. Ca\(^{2+}\)-channel blockers

The reported pyrimidine thione derivative (15) was tested on dogs and rats and it exhibited efficient inhibitory effect on isolated smooth muscles by stopping their depolarized induced contractions.  

\[
\begin{array}{c}
\text{O} \\
\text{CF}_3 \\
\text{N} \\
\text{S} \\
\text{H} \\
\text{F}
\end{array}
\]

m. DNA photocleavage activity

1-(Substituted)-4, 6,6,trimethyl-3,4-dihydropyrimidine-2(1H)thiones (16) were shown to have photosensitivity besides having significant intersystem crossing ability.  

\[
\begin{array}{c}
\text{NH} \\
\text{N} \\
\text{S} \\
\text{R}
\end{array}
\]

1.2 Synthetic routes towards pyrimidinethione derivatives
a. Biginelli reaction

Under acidic conditions, the reaction between an aldehyde (17), β-ketoester (18), and urea (19) gives rise to the formation of dihydropyrimidine (20) (Scheme 1). This reaction was first reported by Pietro Biginelli in 1893. The reaction was, thus, named as Biginelli reaction, and this one-pot strategy resulted in the generation of compounds with diverse pharmacological activities, such as calcium channel modulation, mitotic kinesin Eg5 inhibition, antiviral and antibacterial activities.\textsuperscript{12} Since the original reaction pathway suffers from poor yields and a limited substrate scope, the recent discovery of dihydropyrimidine’s biological activity has paved way to renewed exploration of the reaction conditions, thus revealing a variety of compatible solvents, acid catalysis, and an expanded substrate scope. Over the past 115 years, the gradual amendments in the Biginelli reaction coupled with the bio evaluation of these compounds has provided an entrance into the relatively unexplored dihydropyrimidine compounds. The general synthetic scheme of Biginelli reaction is shown in scheme 1.

\[
\begin{align*}
  & \text{R}_1 \text{H} + \text{R}_2 \text{O}_2 \text{C} + \text{R}_3 \to \text{O} \text{O} \text{X} \\
  & \text{NH} \text{NNHR}_4 \rightarrow \text{R}_3 \text{N} \text{X} \\
  & \text{H}_2 \text{N}-\text{NH}-\text{H}_2
\end{align*}
\]

\textbf{Scheme 1}

b. From chalcones

The reaction of thiourea (21) with chalcones (22) affords the formation of pyrimidine-2-thione derivatives (23) shown in scheme 2.\textsuperscript{1,14}

\[
\begin{align*}
  & \text{O} \text{O} \text{Ar}_1 \to \text{NH} \text{CSNH}_2 + \text{Ar} \\
  & \text{Ar}
\end{align*}
\]
c. From enamines
Reaction between 2-amino-1-cyanopropene (24) with thiourea (25) resulted in formation of thiopyrimidine (26) as given in scheme 3.  

\[
\begin{align*}
\text{HCN} & \quad \text{NH}_2\text{CSNH}_2 \\
\text{H}_2\text{CNH}_2 & \quad \text{NH}_2\text{CSNH}_2 \\
\text{24} & \quad \text{25} & \quad \text{26}
\end{align*}
\]

\[
\text{Scheme 3}
\]

d. From dicarbonyl compounds
Treatment of diaroylmethane derivatives (27) with thiourea (28) (scheme 4) furnished pyrimidine-2-thione derivatives (29).  

\[
\begin{align*}
\text{Ar} & \quad \text{Ph}^R \\
\text{O} & \quad \text{O} & \quad \text{NH}_2\text{CSNHR} \\
\text{27} & \quad \text{28} & \quad \text{29}
\end{align*}
\]

\[
\text{Scheme 4}
\]
e. From arylidenes
Reaction between β-arylidene malononitrile (30) and thiourea (31) provided a 4amino-6-aryl-5-cyanopyrimidine-2-(3H)-thiones (32) (scheme 5).  

\[
\begin{align*}
\text{Ar} & \quad \text{Ar}_1 \\
a. \quad 2,4-(\text{CH}_3)_2\text{C}_6\text{H}_3 & \quad a. \quad 4-\text{ClC}_6\text{H}_4 \\
b. \quad 3,4-(\text{CH}_3)_2\text{C}_6\text{H}_3 & \quad b. \quad \text{C}_6\text{H}_5 \\
c. \quad \text{C}_6\text{H}_5 &
\end{align*}
\]

\[
\text{Scheme 2}
\]
f. From isothiocyanate derivatives

Isothiocyanate derivatives are employed for the synthesis of 2- and 4-pyrimidinethiones (scheme 6). Thus, the reaction between 4-isothiocyanatobutan-2-one (33) and 4-methyl-2-nitroaniline (34) yields 1-(2-nitro-4-methylphenyl)-6-hydroxy-6-methyl-1,4,5,6-tetrahydropyrimidine-2(3H)thione (35).

\[
\text{Scheme 5}
\]

\[
\text{Scheme 6}
\]

g. Pericyclic reaction with acyclic enamines

Reversed polarization in 2-trimethylsilylthio-1,3-diene (36) proceeds to pericyclic reaction with acyclic enamines (37), hence, resulting in formation of pyrimidinethione (38) (scheme 7).
h. Bronsted-acid catalyzed reaction under solvent-free conditions

An efficient methodology using solvent-free conditions for the synthesis of 2,4dihydropyrimidine-2(1H)-(thio)ones (42) (scheme 8) via condensation of ethyl acetoacetate (39), urea or thiourea (40) and aldehyde (41), while employing novel Bronsted acidic ionic liquid [Btto][p-TSA] for the first time, gave good yields of product compared to the classical Biginelli reaction.\(^\text{19}\)

\[
\begin{align*}
&\text{R}_2\text{OC} \\
&\text{H}_3\text{C} \big| \big| \text{R}_2 + \text{H}_2\text{N} \\
&\text{NH}_2 + \text{R}_1 \big| \text{H} \\
&\quad \quad \quad \xrightarrow{5\text{mol} \% \text{[Btto][p-TSA]}} \\
&\text{H}_3\text{C} \quad \text{N} \quad \text{X} \\
\end{align*}
\]

Scheme 8

i. Ultrasound-assisted synthesis

Due to the advantages associated with ultrasonic-assisted synthesis\(^\text{20}\) (in terms of yield and reaction time) in comparison to the conventional synthetic methods, synthesis of pyrimidine-2-thione (45) derivatives was achieved by the reaction of chalcones (43) with thiourea (44) as shown in scheme 9.

\[
\begin{align*}
&\text{aryl} \big| \big| \text{CH}_3 \\
&\text{X} \quad \text{H}_2\text{NNH}_2 \\
&\quad \quad \quad \quad \xrightarrow{\text{KOH}} \\
&\text{aryl} \big| \big| \text{NH}-\text{NH} \quad \text{S} \\
&\text{X} \quad \text{H}_2\text{NNH}_2 \\
\end{align*}
\]

Scheme 9

\[X = a=2- \quad \text{CH}_3 \quad b=3- \quad \text{CH}_3 \quad c=4- \quad \text{CH}_3 \quad d=2- \quad \text{OCH}_3 \quad e=4- \quad \text{OCH}_3\]
j. Synthesis of N-Mannich bases of 3,4-dihydropyrimidine-2(1H)-thiones (45) Shah et al. while reporting the synthesis of 3,4-dihydropyrimidine-2(1H)-thiones (49) via Mannich reaction between heterocyclic secondary amino compounds (46), produced DHPMs (47) and formaldehyde (48) as presented in scheme 10.  

\[
\begin{align*}
\text{R =} & \text{a=Benzimidazol} \\
& \text{b=Benzotriazole} \\
& \text{c=Pthalimide}
\end{align*}
\]

Scheme 10

k. Microwave assisted synthesis

Adithya et al. reported the microwave-assisted synthesis of 1-substituted-4-(6- substituted-2-hydroxyquinoline-3-yl)-5-acetyl/carboxyethyl-6-methyl-pyrimidine-2 one/thiones (53) via the reaction between 6-substituted-2-hydroxyquinoline-3-carbaldehyde (50), ethylacetoacetate/acetlyacetone (52) and urea/thiourea/phenylthiourea (51) under acidic conditions as shown in scheme 11.  

\[
\begin{align*}
\text{R =} & \text{a=H, CH}_3, \text{OCH}_3 \\
& \text{b=CH}_3, \text{OC}_2\text{H}_5 \\
& \text{c=H, Ph X=O, S}
\end{align*}
\]

Scheme 11
2. 4-Aminophenazone

4-Aminophenazone (54) is one of the antipyrine derivatives which was first synthesized by Knorr in 1833 and ever since the antipyrine derivatives (APDs) have been studied. It has got resemblance to N-substituted amides due to the presence of N-phenyl group and a -CH₂ group on either side of a polar carbonyl compound. Due to large dipole moment (5.48 D) and strong basic character of the carbonyl group in 4aminoantipyrine it behaves as a potential donor group. It has also got an additional potential coordination site in the amino nitrogen and thus due to this, it is worthwhile to study its complexes.

2.1 IUPAC name

4-Amino-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one

2.2 Other names

Other trivial names used for 4-aminophenazone are: a. 4-Aminoantipyrine

b. Ampyrone

2.3 Medicinal uses

The medicinal uses of 4-aminophenazone are: analgesic, antipyretic and antiinflammatory, however, it is also responsible for platelet inhibitory property.

2.4 Therapeutic uses

The therapeutic applications of 4-aminophenazone involve the treatment of chronic arthritis, soft tissue disorders, besides, its utility in the treatment of neuralgia, rheumatism and similar painful conditions.

One of the important aspects about 4-aminophenazone is that it is a non-steroidal antiinflammatory drug (NSAID).
2.5 Uses of pyrazolone ring containing drugs

In pharmaceutical industry, the pyrazolone ring-containing drugs have gained importance on account of their biological applications. For example, the pyrazolones, viz. phenazone, metamizole, ampyrone and prophyphenazone (fig. 2) are useful antipyretic and analgesic drugs. Besides this the pyrazolones also possess antimicrobial, antifungal, antidepressant and antifilarial activities. They also find applications in the extraction and separation of various metal ions in addition to, being used as precursors for dyes, pigments, pesticides and chelating agents. Some representative members of pyrazolone ring-containing drugs are shown in fig. 2.  

![Fig. 2 Members of pyrazolone ring-containing NSAIDs](image)

2.6 Non-steroidal anti-inflammatory drugs (NSAIDs)

The non-steroidal anti-inflammatory drugs, usually abbreviated as NSAIDs, are also termed as non-steroidal anti-inflammatory agents/analgesics (NSAIAs) or nonsteroidal anti-inflammatory medicines (NSAIMs). These are drugs with analgesic and antipyretic effects and, in higher doses, also have anti-inflammatory effects. These drugs are also a source of managing inflammation conditions.

For distinguishing these drugs from steroids, the term ‘non-steroidal’ is used, which shows similar eicosanoid-depressing, anti-inflammatory action apart from displaying broad range of other effects. As analgesics, NSAIDs are unusual in that they are nonnarcotic.

Aspirin (55), ibuprofen (56), and naproxen (57) are the most prominent members of this group of drugs, these are available over the counter in many areas and a marked increase in their use has occurred since their release primarily among patients at low risk from adverse effects of NSAIDs.  

---

52
2.7 Naturally-occurring NSAID

Phenolic compounds obtained from the extract of extra virgin olive oil have gained considerable attention. One of the component of the extract, named as (-)-oleocanthal (58), possesses similar potency as the NSAID ibuprofen, and functions as an inhibitor of the COX-1 and COX-2 enzymes.

2.8 Mechanism of action

By inhibiting production of certain enzymes such as cyclooxygenases (COX), which participate in the production of prostaglandins, NSAIDs reduce pain and inflammation. Prostaglandins are responsible for a number of functions in the body including:

- Causing pain when they come in contact with certain nerve fibers
- Helping in the protection of the stomach lining against acid and digestive enzymes
- Participating in both blood flow and blood clotting regulation

However the inhibition of the COX enzyme is also responsible for many of the side effects shown by NSAIDs.

2.9 Types of NSAIDs

On the basis of their selectivity as an anti-inflammatory drug, NSAIDs are divided into two types:
a. Non-selective NSAIDs

These are responsible for inhibition of the enzymes that are found in the stomach, blood platelets, and blood vessels (COX-1) as well as the enzymes found at sites of inflammation (COX-2) to a similar degree. Aspirin, ibuprofen, naproxen and diclofenac come in the category of non-selective NSAIDs.

b. Selective NSAIDs

These are also termed as COX-2 inhibitors and inhibit the COX enzyme found at the sites of inflammation (COX-2) more than the type of enzyme normally found in the stomach, blood platelets, and blood vessels (COX-1). Celecoxib comes in this category.

2.10 Medicinal uses of NSAIDs

NSAIDs are generally indicated for the symptomatic relief of the following conditions and diseases:

a. For the treatment of Alzheimer’s disease

NSAIDs prove to be more effective in attenuating the symptoms of Alzheimer’s disease even more than other steroids. Some of their ester derivatives also help in improved central nervous system bioavailability in the treatment of this disease. 28-29

b. As analgesics

NSAIDs are also used by post-CABG (coronary artery bypass surgery) patients to alleviate the pain 30 whereas non-steroidal anti-inflammatory drugs, paracetamol, and diclofenac-paracetamol combinations appear equally safe in the management of musculoskeletal pain. 31

c. As anti-cancer drugs

They are used for cancer treatment since 30 NO-donating NSAIDs inhibit colon cancer cell growth.

d. As anti-inflammatory agents

Indomethacin, ibuprofen, naproxen, the ester derivatives of NSAIDs are very potent anti-inflammatory drugs. 32 Similarly, hybrid NO-NSAID prodrugs are seen to show good anti-inflammatory activities with reduced gastric ulcerogenicity. 33

2.11 Adverse effects of NSAIDs
a. Ulceration

Sometimes NSAIDs can also induce small bowel ulcers that may lead to acute bleeding, perforation or chronic scarring responsible for diaphragm-like structures. Concomitant use of NSAIDs may cause gastro-intestinal bleeding.

b. Tonsillectomy

An increased risk of post tonsillectomy hemorrhage with the use of aspirin has been shown while no significant risk of bleeding is encountered in case of non-aspirin NSAIDs in meta-analysis.

c. Gastroesophageal reflux disease

NSAIDs are responsible for GERD, especially in females, alcohol and tobacco users, and patients with asthma, hernia, or obesity.

d. DNA damage

In vitro DNA damage can be introduced upon irradiation by phototoxic non-steroidal anti-inflammatory drugs (NSAIDs).

e. Amnesia

Memory deterioration in elders is found as a result of over dosage of NSAIDs.

2.12 Classification of NSAID drugs

The NSAIDs can be classified on the basis of their chemical structure. The chemical classification is given in Table 1.

Table. 1 Classification of NSAIDs on the basis of chemical structure

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>NSAID types</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyrazolones</td>
<td>Metamizole, Phenazone, Aminopyrine, Prophyphenazine, Phenylbutazone</td>
</tr>
<tr>
<td>#</td>
<td>Class</td>
<td>Examples</td>
</tr>
<tr>
<td>----</td>
<td>-----------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>2</td>
<td>Carboxylic acids</td>
<td>Aspirin, Choline, Salicylate, Diflunisol</td>
</tr>
<tr>
<td>3</td>
<td>Acetic acid</td>
<td>Indomethacin, Diclofenac, Etodaloc</td>
</tr>
<tr>
<td>4</td>
<td>Propionic acid</td>
<td>Brufen, Flurbiprofen, Fenbrufen</td>
</tr>
<tr>
<td>5</td>
<td>Fenamic acid</td>
<td>Piroxicam, Phenylbutazone, Azapropazone</td>
</tr>
<tr>
<td>6</td>
<td>Non-acidic compounds</td>
<td>Nabumetone</td>
</tr>
<tr>
<td>7</td>
<td>COX-2 inhibitors</td>
<td>Rofecoxib, Celecoxib</td>
</tr>
</tbody>
</table>

NSAIDs like indomethacin display excellent anti-inflammatory properties but they are usually associated with side-effects on gastro-intestinal system whereas propionic acid derived NSAIDs have less likelihood of causing side-effects and are generally tolerated.

2.13 Applications of some specific derivatives of 4-aminophenazone

a. Anti-inflammatory and anthelmintic activity

Mohanram et al., reported the synthesis of 4-aminoantipyrine derivatives (59) via a three-component Betti reaction. The synthesis involved the condensation of aromatic aldehyde, 4-aminoantipyrine, and 8-hydroxyquinoline in the presence of fluorite as catalyst in a simple one-step protocol. The synthesized derivatives showed potent anti-inflammatory and anthelmintic activities as a result of their screening using diclofenac and albendazole as reference drugs.
b. Electrochemical applications

Harikumaran et al., studied out the physio-chemical properties of oxomolybdenum (V) complexes (60) of an azo dye derived from 4-amino-2,3-dimethyl-1-phenylpyrazone-5-one which indicated that the ligand acted as the neutral bidentate ligand and displayed distorted octahedral geometry. 40

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{N} \\
\text{CH}_3 & \quad \text{N} \\
\text{OCH}_3 & \quad \text{OH} \\
\text{X} & \quad \text{Cl} \\
\text{Cl} & \quad \text{Cl} \\
\text{X} & \quad \text{Cl}, \text{NO}_3, \text{ClO}_4 \\
(\text{II}) & \\
\end{align*}
\]

60

c. Analgesic activity

Fadda et al., synthesized a series of new enaminonitrile derivatives of antipyrine (61) as potential analgesic agents. 41

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{N} \\
\text{N} & \quad \text{NH}_2 \\
\text{R} & \quad \text{NH}_2 \\
\text{HC} & \quad \text{N} \\
\text{O} & \quad \text{N} \\
\end{align*}
\]

61

d. Optical properties

The antipyrine-derived ligands formed via condensation reaction between 4aminoantipyrine and 4-aminophenol or 4-aminobenzoic acid in ethanolic solution resulted in the formation of the 4-(4-amino-1,5-dimethyl-2-phenyl-1,2-dihydropyrazole-3-ylideneamino)-phenol [L1] (62) and 4-(4-amino-1,5-dimethyl-2-phenyl1,2-dihydro-pyrazole-3-ylideneamino)-benzoic acid [L2] (63). 42 The results of the physiochemical studies suggested that these compounds are suitable candidates for application in organic electronics.

\[
\text{COOH}
\]
e. Antibacterial activity

The antibacterial activity \(^{43}\) of metal complexes \((64)\) derived from Schiff bases of 4aminoantipyrine was checked by screening these complexes against the bacterial species \(S.\) \(aureus\), \(E.\) \(coli\) and \(P.\) \(vulgaris\). The comparative study of MIC values of the ligands and their complexes revealed that the complexes exhibited higher antimicrobial activity and this increased inhibitory activity was explained on the basis of Overtone’s concept \(^{44}\) and Tweedy’s chelation theory. \(^{45}\)

f. Molluscidal activity

The newly-synthesized pyrazole derivatives \((65)\) reported by Fadda et al., have been shown to possess molluscicidal activity to Biomphalaria alexandrina snails. \(^{46}\)
g. Pyrazolinone analgesics prevent the antiplatelet effect of aspirin and preserve human platelet thromboxane synthesis

Holfeld et al., 4-methylantipyrine (MAA) as the active metabolite of dipyrone which largely attenuates or even completely abolishes the inhibition of arachidonic acid-induced platelet aggregation, thromboxane formation and P-selection expression by aspirin. Other pyrazolinones depicted similar sort of results including the conventional NSAIDs ibuprofen and naproxen. It was also demonstrated in their study that MAA attenuates the effect of aspirin on COX activity of platelet microsomes, thus, suggesting a competition with aspirin at the COX-1 enzyme. The docking studies also confirmed these results and revealed that MAA forms a strong hydrogen bond with serine 530 within COX-1, thereby preventing enzyme acetylation by aspirin. 47

The crystal structure of COX-1 and the binding mode of COX-1 monomer with MAA is shown in fig.3. 47

![Fig.3 Crystal structure and binding mode of COX-1 monomer with MAA](image)

h. Antifungal activity

Bondock et al., carried out the synthesized a variety of novel 4-hetarylpyrazoles (66) and furo[2,3-c]pyrazole (67) derivatives and screened them against Fusarium oxysporum and Botrytis fabae. The results suggested that these compounds possess potent antifungal activity against the screened species. 48
i. Preparation of chromatography spray reagents
4-Aminoantipyrine (4-AA) is used as chromatography spray reagent for detection of phenols by forming derivatives of 4-aminoantipyrineas. These methods involve a well-known Emersion reaction between phenols and 4-AA with potassium hexacyanoferrate (III) as the oxidizing agent and also include separation of dyes with HPLC in the visible range.\textsuperscript{49}

j. 4-aminoantipyrine as corrosion inhibitor for zinc in phosphoric acid
Vashi \textit{et al.}, studied the effect of concentrations of acid (phosphoric acid) and temperature on corrosion of zinc metal along with the inhibitory effect of 4aminoantipyrine against corrosion by using polarization method.\textsuperscript{50} The results of their studies suggested that the corrosion rate increases with increase in concentration of acid and temperature whereas the inhibition efficiency (IE) of 4-AA increases with increase in its concentration and decreases with increase in acid concentration.\textsuperscript{51}

k. As substrate for measuring enzyme activity
The 4-aminophenazone has been converted to amino acid-substituted amide \textbf{(68)} which is used as substrate for measuring enzyme activity.\textsuperscript{38}
3. Thioureas
Organosulfur compounds with the formula SC(NR₂)₂ are termed as thioureas. They have got structure resemblance to ureas except that the oxygen atom has been replaced by sulfur atom, however, these display significant difference in their properties. The general structure (69) of thiourea is shown in fig. 4.

![Fig. 4 General structure of thiourea](image)

Thioureas are versatile building blocks for the synthesis of a variety of heterocyclic systems such as 2-imino-1,3-thiazolines, pyrimidine-2-thiones, thiohydantoins, iminothiazolidines, and others.

3.1 Application of thiourea derivatives
Thioureas exhibit broad spectrum applications some of which are described below.

3.1.1 Applications in agriculture

In the field of agriculture, thioureas find a variety of applications. They are utilized as herbicide and fungicide. They are also used to control insect growth, effect plant growth and seed germination.

a. Effect on seed germination and plant growth

The germination and growth of seed is effected by many organic compounds, e.g., the elongation of roots of linseed is decreased up to 50% by the compound (70) at conc. 0.18 μL. The effect shown by this compound is similar to that of trifluralin.

![Fig. 70](image)

b. Insect growth regulators (IGR)

IGRs are the chemical substances which control the population of insects by inhibiting their life cycle e.g., the insect of rice crop named *Planthooper Nilparvata lugens* stål destroys the
crop by transmitting viral diseases which causes sucking of cell sap. The thiourea derivative (71), which is environment friendly as it does not destroy beneficial insects, controls the growth of these insects by destroying nymph at a conc. less than 1 ppm.  

\[
\text{O} \quad \begin{array}{c}
\text{S} \quad \text{CH(CH}_3)_2 \\
\end{array} \\
\text{71}
\]

\[
\text{O} \quad \begin{array}{c}
\text{S} \quad \text{CH(CH}_3)_2 \\
\end{array} \\
\text{71}
\]

c. Antifungal activity
Fungicides are the chemicals or biological organisms which are used to kill fungus or fungal spores. In agriculture, fungicides have got importance since fungi cause serious damage to crops. Complexes of (72) and (73) show antifungal activity against *Saccharomyces cerevisiae* and *Penicillium digitatum*.\(^6^0\) Antifungal and antiviral activity of curative rates is demonstrated by derivatives of (74).\(^6^1\)

\[
\begin{array}{c}
\text{O} \quad \begin{array}{c}
\text{S} \\
\end{array} \\
\text{72}
\end{array}
\]

\[
\begin{array}{c}
\text{O} \quad \begin{array}{c}
\text{S} \\
\end{array} \\
\text{73}
\end{array}
\]

\[
\begin{array}{c}
\text{R}_1 \quad \text{N} \\
\text{Cl} \\
\text{O} \\
\text{N} \\
\text{S} \\
\text{R}_2
\end{array}
\]

\[
\text{74}
\]

3.1.2 Medicinal applications of thiourea derivatives
The applications of thioureas in the field of medicine cannot be ignored. They find use in all types of medicine.

a. As anti-hypertensive agents
The following disubstituted thiourea (75) shows anti-hypertensive activity.\(^6^2\)

\[
\begin{array}{c}
\text{Cl} \quad \text{S} \\
\text{NH}_2 \\
\text{Cl}
\end{array}
\]

\[
\text{75}
\]

b. As anti-inflammatory agents
The reaction of phenylisothiocyanates with iminothiazolines yield thioureas (76) which manifest anti-inflammatory property.\(^6^3\)
c. As antioxidants
Antioxidants are the compounds that prevent the oxidation of other substances. These reactions result in free radicals due to transfer of hydrogen and electrons, thus, destroying cells. Compound (77) is an example of such antioxidants with excellent efficiency.

![Chemical structure of antioxidant](image)

\[ \text{76} \]

\[ \text{77} \]

d. Anti-bacterial activity
Some 1-aryloxy-3-aryl thioureas (78) have great potential to act as antibacterial agents against \textit{E. coli}.

![Chemical structure of anti-bacterial agent](image)

\[ \text{78} \]

e. For treatment of co-infections
Patients suffering from HIV are at greater risk of catching TB and other infections as well. So, there was a need for development of a drug that can cure both diseases simultaneously. Thiourea derivatives represent promising agents in this regard. For example, compounds (79) and (80) are used for treatment of TB as well as HIV.
f. As anti-thyroid drugs
Anti-thyroid drugs are used to treat goiter caused by hyperthyroidism. Example of such drug is compound (81).  

![Chemical structure](image)

\[ \text{81} \]

g. As anti-epileptic drugs
Derivatives of compound (82) are potent anti-convulsant with 50% effect dose of 1.72 mg/kg.  

![Chemical structure](image)

\[ \text{82} \]

h. As anticancer drugs
Many thioureas are employed as cancer therapeutics e.g., (83) which is utilized for treatment of lung cancer and several of them are in clinical trials.  

![Chemical structure](image)

\[ \text{83} \]

3.1.3 General methods for the synthesis of thioureas
A variety of approaches has been quoted in literature for the synthesis of thioureas; some of which are described below.

a. From thiophosgene
Thioureas can be produced by condensing (84) and (85) with (86) in the presence of pyridine as given in scheme 12.
Scheme 12

This resulted in the formation of a mixture of thioureas (87, 88, and 89) which were separated by chromatography.  

b. From cyanamids

N, N′-disubstituted thioureas (92) are formed as a result of reaction of cyanamids (90) with LiAlHSH (91) using 1N HCl solution in diethylether as indicated in scheme 13.

Scheme 13

where R₁=R₂=alkyl

c. From carbon disulfide

Symmetrical and unsymmetrical thioureas (95) (scheme 14) can be synthesized by the reaction of amine (93) with carbon disulfide (94) in acidic conditions, the reactive intermediate formed in this reaction was amino dithiol derivative instead of isothiocyanate.

Scheme 14

Where R₁=R₂=R₃=alkyl or aryl
d. From isonicotinoyl isothiocyanate
Aydin et al., reported the synthesis of a new heterocyclic compound named as N-[2,5-dimethyl-3-oxo-1-phenyl-2,3-dihydro-1H-pyrazol-4yl]carbamothioyl]isonicotinamide (98) from isonicotinoyl isothiocyanate (96) and 4aminoantipyrine (97) in acetonitrile solution as produced in scheme 15. 73

![Scheme 15](image)

e. Microwave assisted synthesis
Kawle et al., reported the microwave-assisted synthesis of 1-acridin-9-yl-3-aryltioureas (101) as a result of condensation reaction between 9-amino acridine hydrochloride (99) and phenyl isothiocyanate (100) in basic medium for 2 minutes as presented in scheme 16. 74

![Scheme 16](image)
f. Green synthesis of thioureas under solvent free conditions
An efficient and convenient method for the synthesis of bifunctional - N-substitutedN′-aryl carbonyl thioureas under solvent free conditions has been reported by Bagheri et al.\textsuperscript{75} This method involved reaction between ammonium thiocyanate (102) and acid chloride (103) without solvent to yield corresponding isothiocyanate which, on reaction with aryl diamines (104), produced aryl dicarbonyl thioureas (105) in good yield as signified in scheme 17.

\[
\begin{align*}
2\text{SCN}^+ + \text{Ar-NH}_2\text{-NH}_2 & \rightarrow \text{Ar-N\dot{-}N\dot{-}H}^+ \\
\text{Ar} & \rightarrow \text{Ar-N\dot{-}N\dot{-}H}^+ \\
\end{align*}
\]

Scheme 17

\[\text{102} + \text{103} + \text{104} \rightarrow \text{105}\]

g. Phase transfer-catalyzed synthesis in presence of ultrasonic radiation
Ke et al., carried out the synthesis of a series of new N′-(4,6-disubstituted-pyrimidin2-yl)-N-(5-aryl-2-furoyl) thiourea derivatives (106) using PEG-400 as solid liquid phase transfer catalyst under ultrasonic irradiation as highlighted in scheme 18.\textsuperscript{76}

\[
\begin{align*}
\text{R-NH}_2 & \rightarrow \text{R-N\dot{-}N\dot{-}H}^+ \\
\text{R} & \rightarrow \text{R-N\dot{-}N\dot{-}H}^+ \\
\text{COOH} & \rightarrow \text{COOH} \\
\text{SOCl}_2 & \rightarrow \text{SOCl}_2 \\
\text{CuCl}_2 & \rightarrow \text{CuCl}_2 \\
\end{align*}
\]

Scheme 18

\[\text{106}\]
4. Amides
Any member of either of two classes of nitrogen containing compounds related to ammonia or amines and containing a carbonyl group is termed as amide. The first class of amides termed as covalent amides is formed when the hydroxyl group of acid is replaced by amino group \((-\text{NR}_2\), in which \(R\) consists of a hydrogen atom or an organic combining group, e.g., methyl). Amides synthesized from carboxylic acid are termed as carboxamides. These are solids except for its simplest member, named formamide, which is a liquid. These are good solvents with high boiling points and are nonconductors of electricity. Covalent amides don’t exist in nature but the long chain polymers with peptide bonds constituting the protein and peptides in living systems are amide linkages. Urea is another example of amide having two amino groups. Ionic amides constitute the second class of amides which are made by reaction between a covalent amide, an amine or ammonia and a reactive metal (e.g., sodium) and are strongly alkaline.

4.1 Classification
Similar to amines, amides can be classified depending on the number of hydrogen atoms substituted in the ammonia molecule as primary (107), secondary (108), or tertiary amides (109) as denoted in fig. 5. Amide having \(\text{NH}_2\) group is a primary amide, the one containing the \(\text{NH}\) group is a secondary amide, and the \(-\text{N}\)-containing amide is termed as tertiary amide.
4.2 Reactivity of amines and amides

Although amino group is present in both amines and amides (NH₂, NH, or N), yet the latter are much weaker bases and much stronger acids than the former.

4.3 Amides of carboxylic acid

The bond formation between carbonyl carbon and nitrogen in an amide is called amide bond. Amides having two hydrogen atoms bound with the nitrogen are termed as simple amides while those having one or two aliphatic or aromatic groups are called amides of amines.

4.4 Applications of amides in medicine and agriculture

a. Anthelmintic and insecticidal activity
Premarth et al. reported the synthesis and evaluation of 4-aminoantipyrine derivatives of amino acids and peptides (110) since antipyrine is a promising structural unit in medicinal and agriculture industry. They found that these derivatives show potent anthelmintic and insecticidal activity against Eudrillus eugenia and Coptotermis formasanus at concentrations of 100mg/20mL using Chloropyrifos as standard reference. 77

b. Antioxidant activity
The compound (111), 78 amide derivative of 4-aminoantipyrine, is shown to possess antioxidant activity which is measured by diphenylpicrylhydrazyl (DPPH) radical scavenging method. 79
c. DEET as an insect repellent
DEET, an abbreviation of \( N, N\text{-diethyl-meta-toluamide} \) (112), is pale yellow oil. It is an active ingredient of insect repellent and is intended to be used on skin or on clothing, provides protection against ticks, chiggers, mosquito bites and other insects that are capable of transmitting the disease.\(^8^0\)

\[
\begin{align*}
\text{N} & \hspace{1cm} \text{O} \\
\hspace{1cm} & \hspace{1cm} \text{CH}_3
\end{align*}
\]

112

d. For treatment of schizophrenia
Pani et al., reported the synthesis of substituted benzamides such as sulpiride (113), and the clinical potential of these benzamide derivatives on the negative symptoms of schizophrenia.\(^8^1\)

\[
\begin{align*}
\text{O} & \hspace{1cm} \text{SO}_2
\end{align*}
\]

113

e. As inhibitors of HCV replication
Li et al., carried out the synthesis of novel amino-substituted N-aryl benzamide analogues (114) and evaluated their ability to inhibit hepatitis C virus (HCV) replication in acute infected Huh 7.5 cells. The results of bioevaluation indicated that these compounds
increased intracellular HA3G protein levels and thereby inhibited HCV replication in dose dependant manner.  

\[ \text{Lysergic acid amide as psychedelic agent} \]
Lysergic acid (115) is a precursor for a wide range of ergoline alkaloids that are produced by ergot fungus and some plants (also justifiably named as D-lysergic acid and (+)-lysergic acid). Lysergamides, which are amides of lysergic acid, are widely utilized by pharmaceuticals and also as psychedelic drugs (LSD).

\[ \text{Hydroxycinnamic amides prevent smooth muscle spasm} \]
Zhang et al. demonstrated that hydroxycinnamic amides (HCA amides) (116) are part of M1 receptor-inhibiting principles of S. tangutica. They found that inhibition of M1 receptor lessens smooth muscle spasm as a result of blockage of parasympathetic nerve impulse.
h. Poly ester amides (PEA): scaffolds for tissue engineering applications
Poly ester amide is a synthetic polymer which exhibits excellent thermal and mechanical properties along with biodegradability and biocompatibility which makes PEA a strong candidate for tissue engineering. 84

4.5 Synthetic methodologies
a. From chalcones
Dabhi et al. 85 reported the synthesis of amide derivatives of pyrazole-containing heterocyclic compounds (119) via condensation reaction between chalcones (117) derived from 4-aminoantipyrine and isoniazide (118) as presented in scheme 19. The synthesized amide derivatives were then screened for their antimicrobial activity.

\[
\begin{align*}
\text{RCHO/Ethanol} & \quad \text{isoniazide} \\
117 & \quad 118 \\
119 & \quad \text{Scheme 19}
\end{align*}
\]

b. Enzyme (Papin) catalyzed synthesis of Z-L-aminoacyl-antipyrine amides
The enzyme-catalyzed synthesis 86 of Z-L-aminoacyl-antipyrine amides (123) has been accomplished through the reaction between Z-protected amino acid esters (120) and 4-
amino antipyrine (121) using papin (122) catalyst in aqueous-organic biphasic media as well as in suspension as given in scheme 20.

Scheme 20

From isocyanides
Shabbani *et al.* reported a novel method for the synthesis of aryl amides (126) in high yields on account of reaction between carboxylic acid (124) and isocyanide (125) in methanol at ambient temperature (scheme 21).

Scheme 21

d. Microwave assisted synthesis
The microwave assisted synthesis of various benzamide derivatives (128) was reported by Khadse *et al.* in 2012 (scheme 22). The microwave radiations assisted in the ring opening of less reactive dimethyl amino benzylidene oxazolone (127) which was difficult to perform by making use of conventional synthesis involving heating to obtain ring-opened products. The probable reason for failure of ring opening under conventional heating procedure is explained on the basis of poor tendency of carbonyl carbon (C5) of AZ4 to undergo nucleophilic attack by mono or di-substituted anilines.
e. By aminocarbonylation of aryl/hetero halides using non-gaseous NH$_3$ and CO sources

Palladium catalyzed aminocarbonylation of aromatic halides (129) for the synthesis of primary amides (130) by using solid sources of gaseous ammonia and carbon monoxide was developed, a practically simple method by Suresh et al. as highlighted in scheme 23. 89

f. By reaction of carboxamoylsilane and $\alpha$-ketoesters

Synthesis of $\alpha$-siloxy-$\alpha$-alkoxy carbonyl amides (133) (scheme 24), in good yields was achieved via reaction between N, N-dimethylcarbamoyl(trimethyl) silane (131) with
ketoesters (132) in anhydrous toluene at 60°C. The reaction was effected by the electronic group on aryl ring.\(^9\)

![Scheme 24](image)

5. Ferrocene
In 1951, ferrocene (134) was isolated by two independent research groups. It is an orange solid, insoluble in water and melts at 173°C. Ferrocene is a prototypical metallocene, i.e., an organometallic compound in which two cyclopentadienyl rings are bound on opposite sides of a central metal atom which is iron in this case.\(^9\) On account of its fascinating chemistry, ferrocene has grasped the attention of scientific and technical community as compared to the other members of the class.\(^9\) Ferrocene is an important building block for the production of a diverse range of materials and exploration of their applications in a variety of scientific fields due to its chemical and structural stability.\(^9\)

5.1 Physical and chemical properties

Being non-polar in nature, it is soluble in most of the organic solvents and insoluble in water. It is resistant against air, moisture and heat and can withstand temperatures up to 470°C.\(^9\) It gives reactions similar to that of aromatic compounds, thus, facilitating the preparation of substituted derivatives. Due to its extra stability,\(^9\) ferrocene can stand fairly harsh reaction conditions, resulting in wide range of organic conversions including Friedal-Crafts alkylation/acylation, phosphorylation, sulphonation and lithiation.

5.2 Medicinal chemistry of ferrocene

Due to the novelty introduced by the presence of ferrocene, medicinal chemists also consider the inclusion of ferrocene into their drug-design strategies. It is a nontoxic stable compound and shows good redox properties. Ferrocene-derived compounds display
interesting cytotoxic, antitumor, anti-malarial, anti-fungal and DNA-cleaving activities. In literature many examples have been cited regarding the use of ferrocene in drug-design tactics. The activities of certain drugs are reported to be enhanced by the insertion of ferrocene moiety in their structures; e.g., the ferrocene analogue of hydroxytamoxifen and tamoxifen i.e, hydroxyferrocifen (135), and ferrocifen (136) reported by Jaouen et al. was the first molecule shown to be active in both hormone-dependent and hormone-independent breast cancer cells, besides this, it also revealed a wide therapeutic window against kidney, ovarian and prostate cancers.

![Chemical Structure]

\[(135) \ R=\text{OH- hydroxyferrocifen} \]
\[(136) \ R=\text{H- ferrocifen} \]

Biot et al. discovered that the chemotherapeutic activity of chloroquine (137) gets altered as a result of insertion of ferrocenyl group to (138), thus giving rise to the formation of ferroquine which manifested more potent antimalarial activity against \( P. falciparum \) in comparison to chloroquine.

![Chemical Structure]

\[(137) \ Q=\text{chloroquine} \]
\[(138) \ Q=\text{ferroquine} \]

Antibiotics play crucial role in fight against infectious diseases caused by bacteria, however the resistance to bacteria shown by existing antibiotics continues to develop and poses a
significant threat. Penicillin V (138)\(^\text{100}\) was the first antibacterial drug effective against many serious diseases such as syphilis and staphylococcus infections. It is still being used today but many bacteria have been resistant to it such as *Escherichia coli*.

In 1975, the synthesis of ferrocene penicillin (139a) was reported by Edward *et al.* in which they showed that the incorporation of ferrocene moiety into penicillin, resulted in significant enhancement of antibacterial activity of newly-synthesized compound, penicillin (139b).\(^\text{101}\)

5.3 Biological significance of ferrocene derived thioureas

**a. Antibacterial activity**

Yavuz *et al.*\(^\text{102}\) studied the antibacterial activity of ferrocene-derived thioureas (140) using Ciprofloxacin antibiotic as reference drug. The results indicated that the newly-synthesized compound demonstrates potent antibacterial activity against gram positive *Klebsiella pneumonia* ATCC 6633 and gram negative bacterium *Escherichia coli* ATCC 25922.

**b. In organocatalysis**

Ren *et al.* developed an efficient method for the synthesis of ferrocene-based bifunctional amine-thiourea (141) having multiple hydrogen bonding donors. The addition of acetylacetone to nitroolefins in symmetric Michael addition fashion was catalyzed by these bifunctional catalysts that results in the formation of Michael adducts in high yield with moderate to excellent enantioselectivities. The multiple hydrogen bonds help in accelerating the reaction.\(^\text{103}\)
c. Antioxidant activity

The free radical scavenging activity of ferrocene incorporated N,N’-disubstituted thioureas (142) indicate that they can be employed as useful therapeutic agents in terms of their antioxidant property.\textsuperscript{104}

\begin{center}
\textbf{(141)}
\end{center}

\begin{center}
\textbf{(142)}
\end{center}

d. As electrochemical dosimeter for Hg\textsuperscript{2+} ion recognition

Ferrocene-derived thioureas (143) have been used as a novel electrochemical dosimeter for recognition of Hg\textsuperscript{2+} ions.\textsuperscript{105}

\begin{center}
\textbf{(143)}
\end{center}

e. As dual chemosensors

Devaraj \textit{et al.} reported the synthesis and application of new ferrocene-based receptors, i.e., N-[4-ferrocenyl-2-methyl-4-oxobut-1-enyl]-N’-phenylthiourea (144) and N-[4ferrocenyl-2-
methyl-4-oxobut-1-enyl]-N’-[4-nitrophenyl]thiourea (145). They found that these newly-synthesized derivatives possess dual chemo-sensing ability observed as a result of fluorescent titrations carried out against many metal ions as well as electrochemical titrations with anions.\(^{106}\)

![Diagram of compounds](image)

(144) \(R = H\)
(145) \(R = \text{NO}_2\)

**f. Antifungal activity**
The antifungal activity of ferrocene-based bioactive bimetallic thiourea complexes (146) against pathogenic yeast species was studied by Ali.e et al. The results revealed that these compounds possess good antifungal activity against *Aspergillus niger* and poor activity against other yeasts.\(^ {107}\) From these findings it was concluded that iron is essential for microorganisms as a trace nutrient since these compounds show effective activities against selective yeasts.\(^ {108}\)

![Diagram of compounds](image)

\[M = \text{Zn(II), Cd(II), Hg(II), Pd(II), Ag(II)}\]

(146)
5.4 Synthetic approaches to ferrocene derived thioureas

a. From oxalyl chloride
Cao et al. reported the synthesis of ferrocene derived thiourea (152) as depicted in scheme 25. The first step of synthesis involved the formation of ferrocenoyl chloride (149) on account of reaction between ferrocene carboxylic acid (147) and oxalyl chloride (148). The in situ formed chloride of ferrocene was converted to its respective isothiocyanate (150), which on treatment with ethylene diamine (151), yielded ferrocenoyl thiourea in good yields. ¹⁰⁵

b. Synthesis of mononuclear ferrocenophane-based thiourea
Mononuclear ferrocenophane-based thioureas (154) are compounds in which the ferrocene moiety is simultaneously attached to two thiourea groups directly. These were synthesized directly from 1,1′-bis(isothiocyanato)ferrocene (153) by Oton et al. as produced in scheme 26. These compounds possess ion sensing properties due to the presence of redox active ferrocene unit while the bridging of thiourea acts as dual binding site for anions and cations. ¹⁰⁹
d. Iminophosphorane based synthesis of ferrocenyl thiourea
Lorenzo et al. carried out the one-flask preparation of bisferrocenyl-substituted thiourea (157) from iminophosphorane (156), formed from ferrocenemethyl azide (155) by performing aza-Wittig reaction between carbon dioxide and carbon disulfide (scheme 27).¹¹⁰

![Scheme 26](image)

**Reagents and conditions:** (a) Ph₃P, Et₂O, rt; (b) CO₂, toluene, rt

**Scheme 27**

e. Synthesis of ferrocene-based bioactive bimetallic thiourea complexes
The bioactive 1,1-(4,4-diferrocenyl)-i-phenyl thiourea (161) was synthesized by adding ethanolic solution of 3-ferrocenyl aniline (158) to the solution of carbon disulphide (159), and a few drops of triethyl amine. After stirring for overnight, the reaction mixture was
filtered to form ferrocene-based thiourea ligand, (160) which on reaction with acetonitrile solution of appropriate metal salt (Zn, Cd, Hg, Pd), afforded the target compound as given in scheme 28 (161).

\[
\text{Fe}^2 \quad \text{HCl (aq) + NaNO}_2 \quad \text{H}_2\text{O} + \text{PTC} \quad \text{Fe}^2
\]

\[
\text{CS}_2 \quad \text{EtOH} \quad \text{Et}_2\text{N}
\]

Scheme 28

M = Zn(II), Cd(II), Hg(II), Pd(II), Ag(II)
f. Synthesis of ferrocene-modified pyrimidinyl acyl-thiourea derivatives

The synthesis of ferrocene-modified pyrimidinyl acyl-thiourea derivatives, i.e., N-ferrocenoyl-N′-(2-pyrimidinyl)thiourea (162) and N-ferrocenoyl-N′-(5-pyrimidinyl)thiourea (163) has been reported by Duan et al. The synthesis was achieved by the reaction between ferrocenoyl isothiocyanate with 2-amino pyrimidine or 5-amino pyrimidine, respectively, as shown in scheme 29.  

\[ \text{2-amino-pyrimidine} \rightarrow \text{162} \]

\[ \text{5-amino-pyrimidine} \rightarrow \text{163} \]

Scheme 29

---

g. Synthesis of ferrocene-based bifunctional amine-thiourea complex-a scaffold for organocatalyst

An efficient method for the synthesis of a prototype of ferrocene-based bifunctional amine-thiourea, \((R_c, S_{Fc})\) (168) has been reported by Yao et al. The synthesis of (168) was achieved in three steps from (R)-Ugi’s amine (164) as indicated in scheme 30. Thus, lithiation of (164) with t-BuLi followed by reaction with \(p\)-toluenesulfonyl azide resulted in the formation of azide intermediate (165) which was subjected to hydrogenation, using 5% Pd-C under inert atmosphere of hydrogen gas to afford the diamine \((R_c, S_{Fc})\) (166) in 93% yield. As a final step, the reaction of (166) with 3,5bis(trifluoromethyl)phenyl isothiocyanate (167) resulted in the formation of target compound (168) which found its application as an organocatalyst in various organic transformations.  

\[(R)\text{-164} \rightarrow a,b \rightarrow \text{NaMe_2} \rightarrow c \rightarrow \text{NH_2} \]

(R, S_{Fc})-165 (R, S_{Fc})-166
6. Thiazolines
A group of isomeric heterocyclic compounds (fig. 6) containing both nitrogen and sulfur in the ring is termed as thiazolines or dihydrothiazoles. Unsubstituted thiazolines are rarely encountered, however, derivatives of thiazolines are more common and some are bioactive as well.

Fig. 6 Isomeric thiazoline structures

2-thiazoline 3-thiazoline 4-thiazoline

6.1 Biological significance of thiazoline containing natural products
A variety of natural products such as polyazoles, polyketides, linear and cyclic peptides, etc. contains thiazoline core in their structure as contained in fig. 7. The thiazoline ring-containing natural products are responsible for a wide range of bioactivities such as antiviral, antibiotic, anticancer, and anthelmintic. Anticancer activity is the most common activity reported for thiazoline ring containing natural products such as curacin A, a tubulin polymerization inhibitor and largazole, an HDAC I inhibitor. However, a few small thiazoline ring-containing natural products are found in aromas, pigments such as D-luciferin.
which is responsible for the bioluminescence of fireflies and as flavoring agents. Extensive studies were carried out on the D-luciferin/luciferase system which was utilized for \textit{in vivo} imaging.\textsuperscript{115}

![Chemical structures](image-url)
Fig. 7 Thiazoline ring containing natural products

6.2 2-Imino-1,3-thiazoline

Thiazolines with an exocyclic double bond at 2-position are termed as 2-imino-1,3-thiazolines (169).

In case of R=H, the structure exists as two tautomers, i.e., 2-imino-1,3-thiazoline and 2-aminothiazole as displayed in fig. 8

Fig. 8 Tautomeric forms of iminothiazolines
6.3 Biological significance of 2-imino-1,3-thiazolines

Wide range of biological applications has been shown by 2-imino-1,3-thiazolines and their N-aroyl derivatives.

a. Cytotoxic activity

The use of pifithrin-α (170) helps in improving therapeutic selectivity and higher doses of cytotoxic treatments are to be administered on humans. It also aids in protection against many genotoxic agents.\textsuperscript{135d}

\begin{center}
\includegraphics[width=0.2\textwidth]{170}
\end{center}

b. For treatment of Schizophrenia

Compound like (171) is useful for treatment of various mental aberrations in man such as schizophrenia by virtue of its ability to inhibit indole amine-N-methyl transferase. The function of this enzyme is to catalyze biosynthetic steps of some pysochomimetic agents in the body.\textsuperscript{116}

\begin{center}
\includegraphics[width=0.2\textwidth]{171}
\end{center}

R\textsubscript{1} = alkyl, R\textsubscript{2} = tri-fluoro methyl, R\textsubscript{3} = H

\textbf{c. For treatment of Schistosomiasis}

A variety of iminothiazoline derivatives has been found to be active against Schistosomamansoni which is responsible for Schistosomiasis, a disease in which not only...
the skin is affected but internal organs are also damaged. One example of such derivative is (172).

\[
\begin{align*}
\text{172}
\end{align*}
\]

d. Antioxidant activity
A new class of sulfone-linked thiazoline derivatives (173) was synthesized and evaluated for their antioxidant potential by Padmavathi et al. The results of their activity revealed that these compounds can act as effective antioxidant agents.

\[
\begin{align*}
\text{173}
\end{align*}
\]

e. Antimicrobial activity
*In vitro* antibacterial activity against two different strains of gram-negative (*E. coli* and *S. typhi*), gram-positive (*S. aureus* and *B. subtilis*) bacteria and the antimycobacterial activity against H_{37}Rv of *Mycobacterium tuberculosis* has been evaluated for N-[3,4-disubstituted-1,3-thiazole-2(3H)-ylidene]-2-(pyrazine-2-yl oxy)acetohydrazide (174) and N-[(2Z)-3-[4-bromophenyl]-4-oxo-1,3-thiazolidin-2-ylidene]-2-(pyrazin-2-yl oxy)acetohydrazide derivatives (175). The results of minimum inhibitory concentrations for tested compounds showed significant antibacterial and antimycobacterial activity against the microbial strains used during *in vivo* testing.

\[
\begin{align*}
\text{174} & \quad \text{and} \quad \text{175}
\end{align*}
\]
f. Herbicidal activity
Synthesis of a series of new thiazoline derivatives of bis(2-imino-4-amino-5-ethoxycarbonyl-3-phenyl-3H-thiazoline alkylene (177) via reaction between alkyl or arylamines and 4-amino-5-ethoxycarbonyl-2-methylthio-3-phenyl-3H-thiazolinium sulfate intermediate (176) was reported by Bonde et al. The results of bioevaluation of synthesized compounds revealed that these compounds manifest high to moderate herbicidal activity.

6.4 Synthetic methodologies

a. From thiosemicarbazones
3-aryl-4-formylsydnone thiosemicarbazone (178), when subjected to reaction with phenacyl bromide or ethyl chloroacetate (179) results in the formation of substituted 2-imino-1,3-thiazoles (180) shown in scheme 31.
b. Solid-phase synthesis of tetrasubstituted 2-imino-1,3-thiazolines
Utilization of functionalizing cleavage strategy for synthesis of novel tetrasubstituted 2-imino-1,3-thiazolines (183) was described by Gomez et al. The synthetic route makes use of the ambient reactivity of a dithiocarbamate functionality to achieve the key resin-bound electrophilic thiazolium intermediate (scheme 32). The desired compounds were obtained by reacting various amines (181) with thiazolium salt (182) in high purity.

\[
\begin{array}{cccccc}
& S & S & N & R_1 & \text{Br} \\
\text{182} & & & & & \\
\end{array}
\]

\[
\begin{array}{cccccc}
\text{181} & & & & \text{NH}_2 & R_4 \\
\text{183} & & & & & \\
\end{array}
\]

Scheme 32

c. Novel synthesis of 2-imino-4-thiazolines via α-bromoketimines
Kimpe et al. reported a simple and straightforward method for the synthesis of 2imino-4-thiazolines (186) via reaction between α-bromoketimines (184) and potassium thiocyanate (185) in acetonitrile solvent shown in scheme 33. Contrary to other synthetic routes of these heterocycles, this method has advantage that it occurs smoothly without any side reactions.

\[
\begin{array}{cccccc}
\text{184} & & & & \text{NH}_2 & R'' \\
\text{185} & & & & \text{S} & R' \\
\text{186} & & & & \text{R} & \text{NH} \\
\end{array}
\]

Scheme 33
d. From 1-acyl-3-aryl thioureas

The reaction between 1-acyl-3-aryl thioureas (187) and α-chloro acetonitrile (188) gives rise to 2-iminothiazolines (189) (scheme 34).  

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```

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Scheme 34

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e. From N-propargylic anilines and acyl isothiocyanates

The formation of 2-imino-1,3-thiazoline derivative from N-propargylic anilines and acyl isothiocyanates occurs in two steps (scheme 35). In the first step formation of 2(α-acylimino)-1,3-thiazolidene (192) takes place due to reaction between N-propargylic aniline (190) and acyl isocyanate (191). Then in second step, treatment of (191) with sodium methoxide results in N-acyl-5-alkyl-2-imino-1,3-thiazoline (193).  

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Scheme 35

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7. Towards the development of direct methodology to enantioenriched α-alkylated aldehydes

7.1 Background

Chiral α-alkyl-substituted aldehydes are used in wide-ranging of chemical transformations; primarily introducing asymmetry through C-C bond formation. A variety of nucleophiles has
been employed in this way which gives rise to the a number of new functionalities (alcohols, amines, imines, olefins, etc.) thus producing more complexity in molecular architecture.

These chiral α-alkyl substituted aldehydes find their direct application in perfume industry as odorant as well.\(^{123}\) Despite the long standing methods available to form racemic α-alkyl substituted aldehydes,\(^ {124}\) direct methodologies to access α-alkyl aldehydes via intermolecular nucleophilic substitution reaction remains an elusive feat.\(^ {125}\) Recently Hodgson \textit{et al.} have discovered that whilst the good levels of enantioselectivity in α-alkyl aldehydes could be delivered from homotropane auxiliary, the tropane auxiliary could not and showed reversal of selectivity.\(^ {126}\)

7.2 Previous synthesis and α-alkylation of aldenamines

An overview of the enamine synthetic approaches is given since the proposed research employs an aldenamine α-alkylation. Enamines, derived from ketones, are well known. Only those routes are outlined below which generate aldehyde-derived enamines (or aldenamines).

7.2.1 Limitations of enolates

Before the advent of enamine chemistry, for carrying out α-alkylation of aldehydes, enolate chemistry was considered a solution. Deprotonation of aldehydes (194) α- to carbonyl is affected by strong bases, and electrophiles can be attacked by enolate ion (195). Unfortunately, things are not so simple and plethora of side reactions are known to occur.\(^ {127}\) Enolates are capable of both C-alkylation (196) and O-alkylation (197) since they are ambident nucleophiles. Owing to the electrophilic nature of the original aldehyde carbonyl, aldol addition and condensation products are regularly observed (198) (scheme 36).

\begin{center}
\textbf{Scheme 36}
\end{center}

Tishchenko and Cannizzaro reactions can also occur. Although frequently seen for enolates, polyalkylation is rarely observed for enamines as a consequence of the inherent, additional
Steric bulk on nitrogen. N-alkylation (at least for ketone-derivate enamines) is less problematic since the enamines have advantage of being neutral; the ammonium ions are water-soluble and can be separated from the desired C-alkylation products.

### 7.2.2 Classical approach to aldenamines

The term “enamine” was coined by Wittig to describe the nitrogen analogue of an enol. The first enamines were reported in 1884. General synthetic methodology to prepare them was not established until the pioneering work of Mannich and Davidsen in 1936. Enamines were generated reversibly as a result of reaction of aldehydes and ketones with secondary amines. A suitable drying agent (e.g., K$_2$CO$_3$, Na$_2$SO$_4$) was used for dehydration which allowed the isolation of enamines.

For forming highly-hindered enamines, alterations to the classical approach have focused on increasing the efficiency of reaction. Lewis acid, i.e., TiCl$_4$ was employed to polarize the carbonyl which also acted as water scavenger. However, the rate of enamine formation was shown to be highly dependent on the quantities of titanium tetrachloride and amine selected. Determination of optimum amount of TiCl$_4$ and amine for each aldehyde is time consuming. An effective method of dehydration by adding benzene and ensuing azeotropic distillation has been demonstrated by Heyl. The first alkylation attempts on vinylamines gave exclusively N-alkylated products as presented in scheme 37 and led to enamine methodologies being neglected for many years. Stork et al. eventually demonstrated the first α-alkylation and α-acylation, of ketenamines (enamines derived from ketones). However, when the reaction was carried out between unhindered aldenamines (enamines derived from aldehydes) with simple alkyl halides, only the corresponding N-quarternary salts were generated as shown in scheme 37.

![Scheme 37](image-url)
7.2.3 Synthesis of hindered aldenamines

Bulky amines have been employed by Hodgson et al. to reduce the levels of alkylations seen at nitrogen. The corresponding aldenamines significantly reduce the nucleophilicity of the nitrogen lone pair due to sterically hinderance at nitrogen.

Under the standard conditions for enamine formation, pentanal (201) was condensed with N-butyl isobutyl amine to render intermediate aldenamine (202) in 71% yields. α-ethylated aldehyde (203) was generated as a result of ethylation and mild hydrolysis of the enamine (202) as given in scheme 38.

![Scheme 38](image)
Similar sort of reaction with a slightly less reactive electrophile, butyl iodide, gave only 24% of the α-alkylated aldehyde, however this was later increased up to 54% by the Hodgson group.\textsuperscript{133} This illustrates well the sensitive nature of the procedure. Encounter of moisture with the reaction mixture and small changes to substrate (e.g., increasing the bulk about nitrogen and reducing reactivity of electrophile) can lead to self-condensation of the starting aldehyde.

### 7.2.4 Solvent for aldenamines alkylation

For the alkylation of enamines the solvent of choice is predominantly acetonitrile.\textsuperscript{134} Besides minimising the likelihood of enamine protonation, the polar and aprotic nature of solvent complements the charge separation seen in the transition state (Fig. 9).

\[
\text{Fig. 9 Transition state of enamine}
\]

### 7.2.5 Hindered lithium amide strategy

Conversion of terminal epoxides to enamines by hindered lithium amides occur in excellent yields. It was shown by Hodgson \textit{et al.} that insertion of lithium amides into α-lithiated epoxide intermediates (204), and subsequent elimination of Li₂O, generates highly-hindered aldenamines (205) as described in scheme 39.\textsuperscript{135} Previous attempts had been unsuccessful to form these bulky aldenamines from aldehydes due to the inaccessibility of the nitrogen lone pair in such bulky amines; only selfcondensation of the aldehydes has been found.
7.2.6 Asymmetric α-alkylation of aldenamines

The introduction of asymmetry at the α-position of carbonyl systems by chiral enamines was first indicated by Otani et al. \(^{136}\) Enantioenriched α-substituted ketones were obtained from (S)-proline-derived ketamine (206), which were then trapped by cyclization as produced in scheme 40. Further development of this strategy led to similar asymmetric induction of aldehydes (as outlined in scheme 40) giving optically-active cyclohexanone (207) in 50% yield. \(^{137}\)

![Scheme 40](image)

7.3 Current synthetic approaches to chiral α-alkylated aldehydes

### 7.3.1 Evans/Myers

Some of the most robust examples of asymmetric enolate alkylation have been offered by Evans et al. \(^{138}\) and Myers et al. \(^{139}\) which are still in use. The Evans (oxazolidinone) (208) (scheme 41) and Myers (pseudoephedrine) (209) (scheme 42) chiral auxiliaries were engaged to direct enolate formation and subsequent alkylation in the corresponding amides. The preferential formation of the Z-enolate (>99%), and metal-chelation to the carbonyl oxygen of oxazolidinone, in the Evans’ strategy, are responsible for the high levels of stereoselective alkylation observed. The less efficiency (EtI; 36% yield; 94% dr) shown by reaction with simple alkyl halides is attributed to the enolate’s low nucleophilicity.

![Scheme 41](image)
7.3.2 Crystallization-induced dynamic resolution (CIDR)

The isolation of (R)-2-ethylhexanal (211) has been carried out via the crystallization-induced dynamic resolution (CIDR) of imine diastereomers-derived from the condensation of (210) with racemic 2-ethylhexanal. This methodology of Evans et al. to access enantioenriched α-alkylated aldehydes provides (R)-2-ethylhexanal in 94% yield and 99:1 er as presented in scheme 43. Having such high levels of enantioenrichment found for this unfunctionalized aldehyde, it might be imagined that there would be widespread adoption of this methodology. However, the need for a crystalline imine in order to resolve the diastereomers led to this vision being unrealized.

7.3.3 SAMP/RAMP

SAMP/RAMP hydrazone alkylation chemistry becomes the most prevalent of all α-alkylation methodologies,\textsuperscript{140} since its introduction by Corey and Enders in 1976.\textsuperscript{141} (S)-1-amino-2-methoxymethylpyrrolidine (SAMP) (213) and RAMP can be readily obtained from amino acids, (S)-proline and (R)-glutamic acid, respectively. The condensation of aldehydes (212) with these derivatized pyrrolidines and the ensuing deprotonation of the hydrazone species (214) by LDA, provides the key azaenolate intermediates (215). Unlike Evans’ enolate, the azaenolate anion is highly nucleophilic, and, therefore, reaction is even observed with simple alkyl halides. High enantimeric excesses have been recorded (up to 99%, 216) as shown in scheme 44. Typically, to yield the final aldehyde; the alkylated hydrazone must undergo ozonolysis; this is taken as a limitation. Moreover, it is not easy to recover the chiral auxiliaries from the toxic ozonolysis side-product (nitrosamine 217).
7.3.4 Organocatalytic methods

In recent years, despite the great surge in interest for organocatalysis, the intermolecular asymmetric α-alkylation of aldehydes appears to be an elusive transformation. \[^{142}\] Intermolecular benzylation of propionaldehyde with catalytic amounts of proline have been attempted by Vignola and List. They discovered that only N-benzylation of proline had occurred, which is typical for unhindered aldehydes. \[^{143}\] The first catalytic intramolecular asymmetric α-alkylation of its kind was the cyclization of aldehyde (218) induced by (S)-α-methylproline (219). \[^{144}\] The formation of a seven-membered ring transition state with a trans double bond during the intramolecular N-alkylation of the proline-derived enamine intermediate, is exceedingly unfavorable, and this is presumably why it is not observed in this chemistry (scheme 45).

Ibrahem and Córdova \[^{145}\] developed an organocatalytic intermolecular route to asymmetric α-substituted aldehydes. The allylation of aldehyde (230) was carried out in a 25% yield and 87:13 er by making use of both palladium and proline-type catalysts. (scheme 46). \[^{146}\] The chiral enamine generated in the reaction, carries stereoselective attack on palladium π-allyl
complexes which is the key step for forming the intermediate iminium ion. The hydrolysis of that intermediate results in aldehyde (231).

\[ \text{OH} \]

\[ \text{OAc} + \text{H} \]

\[\text{230}\]

\[\text{Pd(PPh}_3\text{)}_4 (\text{5 mol\%}) \quad \text{DMSO, rt}\]

\[\text{231}\]

\[\text{NaBH}_4 \]

\[\text{25\%, 74\% ee}\]

\[\text{Scheme 46}\]

7.3.5 SOMO activation

Iminium-enamine catalysis was pioneered by Ahrendt \(^{147}\) and List. \(^{148}\) By using this technique, they successfully fashioned a large range of enantioenriched \(\alpha\)-substituted aldehydes- the focus of enamine catalysis is based on raising the energy of the HOMO in aldehydes whilst iminium catalysis lowers the LUMO. Since these enamine catalysis reactions are well-documented, there is no relevance of iminium catalysis to this work, hence, it will not be discussed further. However, more recently a novel mode of catalysis has been reported by MacMillan \textit{et al;} SOMO catalysis, a hybrid of enamine and iminium catalysis. A 3\(\pi\)-electron radical cation is generated, with a singly-occupied molecular orbital and this proceeds with single-electron oxidation of a transient enamine (234) (scheme 47). \(^{149}\) There must be a radical stabilizing functional group associated with electrophile. The application of this catalysis has been found for a broad range of transformations. Effective \(\textit{ers}\), as high as 97.5:2.5, have been demonstrated by the intermolecular \(\alpha\)-allylation of aldehydes (232) by allylsilanes (233).

\[\text{20 mol\%}\]

\[\text{CAN, NaHCO}_3, 24 \text{ h} \quad \text{DME, -20 \textdegree C}\]

\[\text{88\% yield} \quad 97.5:2.5 \text{ er}\]

\[\text{234}\]

\[\text{232}\]

\[\text{233}\]

\[\text{Scheme 47}\]
7.4 Previous work in the Hodgson Group

The initial routes to racemic α-alkylated aldehydes were based on the reaction of terminal epoxides with lithium amides and the subsequent alkylation of enamine intermediates. After this achievement, the focus of the Hodgson Group shifted towards synthesizing enantioenriched α-alkyl substituted aldehydes. Major success was shown by Kaka when he utilized the chiral trialkyl-substituted piperidine-derived lithium amides (235), with an isopropyl group at the stereogenic C (6) center as depicted in scheme 48. Enantioenriched aldehydes (237) were generated as a result of mild acid hydrolysis of the iminium intermediate (236).

The isopropyl-substituted aldenamines possess two important features which influence both reactivity and selectivity. The alkylation is directed predominantly to one face of the enamine double bond by the i-Pr group and the enamine is forced to adopt one of two possible rotameric forms (fig. 10) by the gem dimethyl group.

Two possible chair conformations of the piperidine-denoted as 238a and 238b are shown in fig 11. The 238a, which is a reactive conformer, has the desired alignment of N lone pair and C=C orbitals for n → π* donation of electrons along with but also a 1,3-diaxial interaction between the axial methyl group and the isopropyl group; conversely, conformer 238b suffers from 1,3 allylic (A\textsuperscript{1,3}) strain but has the π orbital of the enamine double bond orthogonal to the N lone pair. When the i-Pr is axial, the 1,3-diaxial interaction is proven to be more significant than the allylic strain which is found when the i-Pr is equatorial. Computational studies predict 238b to be the ground state conformer. This explains that when the steric bulk is increased to anything larger than an i-Pr group (e.g., t-Bu), to improve facial discrimination on alkylation, it turns futile since the 1,3-diaxial interaction would become so great that the reactive conformation would not form.
Further improvement, in terms of enantioselectivity and reactivity, was observed when the reactive conformer was forced to become the ground state conformer.\textsuperscript{152b} The introduction of a conformational lock by bridging the axial methyl group to the isopropyl methylene center (illustrated in fig. 12) removed the more favorable ground state conformer (238b).

Charltron work was based on the Hansson and Wickberg approach,\textsuperscript{152b} to access enamines (241) via the formamides (240) (scheme 49) which is a deviation from the original chiral lithium piperidine synthetic strategy.

A synthesis of tropane 239 was developed;\textsuperscript{126} the corresponding formamide species (240) was generated and then converted to non-racemic enamine (241). However, alkylation of this enamine species led to an unexpectedly poor \textit{er} ($R$:S, 55:45) of 2ethyl hexanal (242) as given in scheme 50. Retrospective computational studies backed up this result suggesting a similar \textit{er} ($R$:S, 62:38).\textsuperscript{126} The presence of the 5membered ring therefore caused a surprising change in the precise ground state structural orientation in the enamine species. The enamine was indicated as leaning more over the 6-membered ring than the 5-ring. The hydrogen labelled $H^*$ is now marginally better positioned to block the $Si$ face than the exo-methyl group $Me^*$ could the $Re$ face. Importantly, in this tropane system, the \textit{gem} dimethyl group was not capable of directing $C$-alkylation to only one face.
It was revealed by the computationally-determined transition states for this tropane that the embedded pyrrolidine is influencing the preference of the enamine double bond to lean over the 6-ring, thus, negating the desired effects of the methyl group (MeA) to direct alkylation. So, to combat this issue, two rings of equal size, i.e., a homotropane (243), was considered. The gem dimethyl group on one of the two rings should now determine exclusively the facial discrimination. Ideally, an enantiomeric ratio close to 15:85 (R:S), the computational prediction for this homotropane-derived aldenamine, would be detected.

The alkylation of the homotropane-derived enamine (244) (in contrast to that seen for the [6,5] tropane system (55:45 R:S er) provided, following mild hydrolysis of the iminium intermediate, the α-ethylated aldehyde (245) (scheme 51) with the sense of asymmetric induction anticipated at the outset (28:72 R:S er). The C-alkylation has successfully been directed by the gem dimethyl group on one face of the exocyclic double bond.

2. Research Highlights

Part I:
Considering the biological significance of heterocyclic compounds based on extensive literature survey we planned to synthesize series of different classes of compounds with useful biological applications such as 3,4-dihydropyrimidine-2-thiones, thioureas, benzamides and 2-aryliminothiazolines.

The synthesized series with some important mechanisms is given below. Derivatives of 3,4-dihydropyrimidinethiones find applications in medicinal chemistry which is mainly due to the presence of pyrimidine base that constitutes the building block of nucleic acids (DNA and RNA). So, in order to explore more bioactivity of pyrimidinethione derivatives, we designed the synthesis of new 4,4,6-trimethyl-1-phenyl-3,4-dihydropyrimidine-2(1H)thiones derivatives 248a-n using one-pot strategy. The synthesis involved reaction between substituted anilines 246, 4-methylpent-3-en-2-one 247 and potassium thiocyanate 248 in acidic conditions as shown in scheme 52.

![Scheme 52 Synthesis of substituted 4,4,6-trimethyl-1-phenyl-3,4-dihydropyrimidine-2(1H)thiones (248a-n)](image)

**Mechanism:** Plausible mechanistic pathway to pyrimidine-2-thiones (248a-n)
These synthesized compounds were subjected to PAINS analysis and then screened against lymphoid tyrosine phosphatase enzyme, the malfunction of which leads to emergence of various autoimmune disorders in humans.

In the next step, we made use of a drug named as 4-aminophenazone which is available commercially and is known to be an active member of non-steroidal antiinflammatory drugs (NSAIDs). The literature survey reveals that this drug displays broad spectrum applications in medicines as well as in therapies. So, based on the medicinal importance of this drug, we decided to derivatize this drug to its benzamide derivatives and thiourea derivatives (fig. 13) both of which manifested potential bioactivities.
The synthesis was accomplished by reacting different substituted aryl isocyanates 250 a-l formed in situ with 4-aminophenazone 251 at reflux temperature for 3 hours to form the target compounds 252a-l in good yields. The synthetic steps are highlighted in scheme 53.

**Scheme 53** Synthesis of substituted 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1Hpyrazol-4-yl)-3-phenylthioureas (252a-l)
These synthesized thiourea derivatives were then evaluated for their cytotoxic and antioxidant potentials besides subjecting them to calf intestinal alkaline phosphatase inhibition assay.

The benzamides 256a-j are easily accessible by reaction of suitably substituted acid chlorides 254a-j to the solution of 4-aminophenazone 255 in dry pyridine as presented in scheme 54.

\[
\begin{align*}
\text{O} & \quad \text{O} & \quad \text{O} & \quad \text{Cl} \\
\text{C.\(\cdot\)OH} & \quad \text{H}_{3}\text{C} & \quad \text{NH}_{2} & \quad \text{H}_{3}\text{C} \\
\text{Reflux, 2h} & \quad \text{Pyridine} & \quad \text{Reflux, 3h} & \\
\text{R} & \quad \text{R} \\
253a-j & \quad 254a-j & \quad 255 & \quad 256a-j
\end{align*}
\]

**Scheme 54** Synthesis of substituted N-\((1,5\text{-}\text{dimethyl}-3\text{-}\text{oxo}-2\text{-}\text{phenyl}-2,3\text{-}\text{dihydro}-1\text{H}\text{pyrazol}-4\text{-}\text{yl})\)benzamides (256a-j)

These compounds possess potential biological applications and were screened against human recombinant alkaline phosphatase as well as human and rat ecto-5' nucleotidases. Literature survey of ferrocene derived compounds show that the presence of ferrocene moiety in numerous organic structures imparts useful biological application. So from this observation, we planned to synthesize aroyl thiourea derivatives of ferrocene and then carry out the biological activity of the synthesized compounds, besides, conducting pharmacokinetic and computational studies.

The synthesis was achieved in three steps by reacting ferrocene-derived benzoyl chloride with potassium thiocyanate to form the corresponding aroyl isothiocyanate intermediate of ferrocene which was converted to target compounds 261a-q on reaction with various suitably substituted anilines at reflux temperature as given in scheme 55.
Scheme 55 Synthesis of substituted 1-(4-ferrocenylaroyl)-3-phenylthioureas (261a-q)

The compounds 261a-q were screened for their locomotor activity. The active member of the series 261d and 261g were subjected to in silico screening for their pharmacokinetic profiling to quantify their LD₅₀, absorption, distribution metabolism and other characteristics affecting pharmacokinetics.

Synthesis of 2-imino-1,3-thiazoline derivatives was achieved in two steps.

Step I:

In this step isomeric chloro benzoyl thioureas 265a-c were synthesized via reaction between chloro-substituted aroyl isothiocyanates 264a-c with aqueous ammonia as shown in scheme 56.

Scheme 56 Synthesis of isomeric chloro benzoyl thioureas

Step II:
In the second step, these isomeric chloro-substituted aroyl thioureas 265a-c were subjected to heterocyclization reaction with different cyclizing agents such as α-halo carbonyl compounds and diethyl oxalate while the reaction was carried out under inert conditions to get the 2-imino-1,3-thiazolines (scheme 57) in good yields.

Scheme 57 Synthesis of substituted isomeric chloro-N-(thiazol-2(3H)ylidene)benzamides (266, 267, 268, 269 a-c)

Mechanism:
Part II:
The second part of present research work includes the synthesis of racemic monomethyl tropane auxiliary as a stereodirecting element to achieve $\alpha$-alkylation of aldehydes.

The previous work, in Hodgson group, focused on the intermolecular reaction of chiral non-racemic hindered aldenamines with simple alkyl halides. Charlton discovered that whilst homotropane auxiliary ([6,6] system) could deliver good levels of enantioselectivity in $\alpha$-alkylated aldehydes indicating that the presence of germinal dimethyl group has influence in directing alkylation, in case of tropane auxiliary ([6,5] system), reversal of selectivity was observed with lower $er$ as presented in fig 35.

These experimental findings were also supported by computational results. From these it was concluded that the presence of 5-membered pyrrolidine ring has influence on selectivity in [6,5]system, i.e., the five-membered ring has impacted the relative position of exocyclic double bond, that is, it sits more over the 6-membered ring, thus, exposing the face of 5-membered ring and, hence, directing alkylation to its side despite the presence of the blocking element; gem dimethyl group.

So, based on these previous findings, we planned to accomplish synthesis of new racemic 1-methyltropane auxiliary ($\pm$ 270) as explained in fig 36 considering that removal of germinal dimethyl group from [6,5] bicyclic auxiliary would drive alkylation further on the face of 5-membered ring and will offer a significantly increased $dr$. 

![Fig 36 Alkylation reaction of bicyclic auxiliaries](image)
So, for this purpose, a multistep synthetic pathway was designed for racemic 1-methyl tropane auxiliary (described below with their proposed mechanisms) and the alkylation of its corresponding aldenamine was explored in order to find the effect of 5-membered pyrrolidine ring on facial selectivity in [6,5] bicyclic monomethyl tropane auxiliary.

**Step I: Synthesis of 4-oxo pentanal ((±)-273)**

\[
\text{HOCH}_2\text{Cl} + \text{CH}_2\text{Cl}_2 \xrightarrow{\text{PCC-silica, Stirring, rt}} \text{HOCH} = \text{CH}_2
\]

**Step II: Synthesis of racemic 1-methyl-N-benzyltropinone ((±)-276)**

\[
\text{CH}_2\text{Cl}_2 + \text{NH}_3 \text{H}^+ \xrightarrow{\text{NaOAc, HCl, 70°C, pH=5, 7h}} \text{±276}
\]

**Mechanism:**

\[
\text{H}
\]
Step III: Synthesis of racemic 1-methyl-N-benzyltropane ((±)-279)
Step IV: Synthesis of hydrochloride salt of racemic 1-methyltropane ((±)-281)

\[
\text{±276} + \text{NH}_{2}\text{NH}_{2}\text{H}_{2}\text{O} \xrightarrow{\text{KOH}} \text{±279}
\]

\[
\text{±279} + \text{Pd/C} \xrightarrow{\text{H}_2 \text{gas, MeOH}} \text{±281}
\]

Step V: Synthesis of formamide ((±)-289)

\[
\text{±281} + \text{CHCl}_3 \xrightarrow{\text{BnEt}_3\text{NCl}} \text{±287}
\]

\[
\text{±287} + \text{H} \xrightarrow{\text{CH}_2\text{Cl}_2, 12.5 \text{ M aq NaOH}} \text{±288}
\]

Step VI: Synthesis of enamine ((±)-286)

\[
\text{±289} + \text{CsH}_3\text{MgCl} \xrightarrow{\text{Stirring, rt}} \text{±286}
\]
Mechanism:

The exact mechanism of enamine formation via unusual Grignard addition-elimination reaction is not known, so the proposed mechanism is given below.

Somehow there is involvement of nitrogen lone pair (to form iminium) which helps in elimination by forming this intermediate in which oxygen is coordinated to Lewis acid which weakens the C-O bond (since Grignard is used in excess), while the other magnesium alkoxide helps in deprotonation, thus, further assisting the elimination step.
3. Results and Discussion

3.1 Synthesis and characterization of substituted 4,4,6-trimethyl-1-phenyl-3,4dihydropyrimidine-2(1H)thiones (248a-n)

Literature survey reveals that pyrimidine thione heterocyclic compounds have exhibited potential in medicinal chemistry in terms of their use as therapeutic agents.\(^{155}\)

So, based on the biological importance of pyrimidine thione nucleus and the advantages associated with one-pot multicomponent strategy, a one-pot twocomponent method was developed to form variously-substituted pyrimidine thione derivatives in good yields.

Equimolar quantities of suitably substituted anilines \(248a-n\) and potassium thiocyanate were reacted with a slight excess of 4-methylpent-3-en-2-one \(247\) in the presence of a catalytic amount of concentrated HCl at room temperature, as depicted in scheme 52. The reaction mixture was heated at 50-60 °C for 3-5 h and the crude products were recrystallized from ethanol to afford the dihydropyrimidine-2-thiones \(248a-n\) in high purity and good to excellent yields.

![Scheme 52](image)

**Scheme 52** Catalyst and solvent free to 4,4,6-trimethyl-1-phenyl-3,4dihydropyrimidine-2(1H)thiones (248a-n)

A series of fourteen 3,4-dihydropyrimidine thione derivatives was synthesized and successfully characterized by FT-IR, \(^1\)H-NMR, \(^13\)C-NMR, LC-MS and CHNS elemental analyses. Analytical and spectroscopic data substantiated their depicted structures. Apart from this, the structure of two derivatives of the series (248e: 4-OMe substituent on phenyl ring, 248h: 2-Cl substituent on phenyl ring) was also confirmed by single crystal X-ray crystallography.

FT-IR spectral data of the pyrimidinethiones \(248a-n\) indicated the characteristic stretching vibration for the thioamide thiocarbonyl bond which appeared within a range of 1575-1700 cm\(^{-1}\) in addition to the stretching frequency of olefinic bond that was found between 1523 and 1617 cm\(^{-1}\).

In \(^1\)H-NMR, the signal for thioamide proton for the synthesized compounds \(248a-n\) lies within the range of 9.00-7.71 ppm while the signals for aromatic protons appear at their specific chemical shift region. In some cases \(248c\) and \(248k\), the resonances for allylic
coupling were observed, besides, other signals giving a multiplicity pattern of quartet and doublet with coupling constant of 3 Hz. The $^{13}$C-NMR further confirmed the structure by exhibiting a diagnostic signal for thiocarbonyl carbon within a range of 188.2-177.2 ppm whereas the signals for olefinic carbons of pyrimidine ring arose in the region of 100-150 ppm.

The structure of $248a$-$n$ has also been supported by LC-MS by showing the molecular ion peak in the protonated mode [M+H$^+$] as well as an adduct of potassium ion [M+K$^+$].

**Table 2**: Physical data of substituted 4,4,6-trimethyl-1-phenyl-3,4dihydropyrimidine-2(1H)thiones ($248a$-$n$)

<table>
<thead>
<tr>
<th>Cpd.No.</th>
<th>Compound</th>
<th>M.p. (°C)</th>
<th>$R_f$</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$248a$</td>
<td>2-Me</td>
<td>100-101</td>
<td>0.33</td>
<td>90</td>
</tr>
<tr>
<td>$248b$</td>
<td>4-Me</td>
<td>139-140</td>
<td>0.36</td>
<td>88</td>
</tr>
<tr>
<td>$248c$</td>
<td>2,4-diMe</td>
<td>150-151</td>
<td>0.32</td>
<td>85</td>
</tr>
<tr>
<td>$248d$</td>
<td>2-MeO</td>
<td>141-142</td>
<td>0.43</td>
<td>86</td>
</tr>
<tr>
<td>$248e$</td>
<td>4-MeO</td>
<td>163-164</td>
<td>0.47</td>
<td>87</td>
</tr>
<tr>
<td>$248f$</td>
<td>2-F</td>
<td>118-119</td>
<td>0.41</td>
<td>81</td>
</tr>
<tr>
<td>$248g$</td>
<td>4-Br-2-F</td>
<td>169-170</td>
<td>0.39</td>
<td>80</td>
</tr>
<tr>
<td>$248h$</td>
<td>2-Cl</td>
<td>123-124</td>
<td>0.31</td>
<td>92</td>
</tr>
<tr>
<td>$248i$</td>
<td>3-Cl</td>
<td>113-114</td>
<td>0.44</td>
<td>84</td>
</tr>
<tr>
<td>$248j$</td>
<td>4-Cl</td>
<td>148-149</td>
<td>0.42</td>
<td>90</td>
</tr>
<tr>
<td>$248k$</td>
<td>2,3-diCl</td>
<td>130-131</td>
<td>0.33</td>
<td>81</td>
</tr>
<tr>
<td>$248l$</td>
<td>3-Cl-4-F</td>
<td>102-103</td>
<td>0.35</td>
<td>80</td>
</tr>
<tr>
<td>$248m$</td>
<td>4-NO$_2$</td>
<td>158-159</td>
<td>0.47</td>
<td>78</td>
</tr>
<tr>
<td>$248n$</td>
<td>$\alpha$-Naphthyl</td>
<td>173-174</td>
<td>0.49</td>
<td>75</td>
</tr>
</tbody>
</table>

*Petroleum ether: ethyl acetate (4:1)

The structure of compounds $248e$ and $248h$ was also confirmed by single crystal X-ray crystallography belonging to triclinic and monoclinic crystal systems as shown in fig. 14.
The comparison of crystallographic data for 1-(2-chlorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione 248h & a reported structure 1-(3-methylphenyl)4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione 248h′ is given below in table 3.

**Table 3:** Comparison of crystallographic parameters between 248h and 248h′
### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>248h</th>
<th>248h'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>$\text{C}<em>{13} \text{H}</em>{15} \text{Cl N}_{2} \text{S}$</td>
<td>$\text{C}<em>{14} \text{H}</em>{18} \text{N}_{2} \text{S}$</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Orthorhombic</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>$a = 9.795(3)$ Å</td>
<td>$a = 10.5904(3)$ Å</td>
</tr>
<tr>
<td></td>
<td>$b = 19.423(6)$ Å</td>
<td>$b = 16.9189(5)$ Å</td>
</tr>
<tr>
<td></td>
<td>$c = 14.205(4)$ Å</td>
<td>$c = (4)$ Å</td>
</tr>
<tr>
<td>Bond Lengths</td>
<td>$\text{S}(11)-\text{C}(107)$ 1.681(4) Å</td>
<td>$\text{S}(1)-\text{C}(1)$ 1.681(2) Å</td>
</tr>
<tr>
<td></td>
<td>$\text{N}(11)-\text{C}(107)$ 1.377(4) Å</td>
<td>$\text{N}(4)-\text{C}(15)$ 1.366(3) Å</td>
</tr>
<tr>
<td></td>
<td>$\text{N}(11)-\text{C}(108)$ 1.442(4) Å</td>
<td>$\text{N}(4)-\text{C}(22)$ 1.450(3) Å</td>
</tr>
<tr>
<td></td>
<td>$\text{C}(201)-\text{C}(202)$ 1.457(5) Å</td>
<td>$\text{C}(4)-\text{C}(7)$ 1.493(3) Å</td>
</tr>
<tr>
<td></td>
<td>$\text{C}(204)-\text{C}(205)$ 1.549(5) Å</td>
<td>$\text{C}(2)-\text{C}(6)$ 1.521(3) Å</td>
</tr>
</tbody>
</table>

#### 3.1.1 Pan-Assay Interference Compounds (PAINS) analysis

Compounds which display activity across an array of assay platforms or against a range of proteins are defined by the term PAINS; a new concept that has been introduced in the area of organic research and drug discovery. Since these compounds become active against many biological assays, they lose their selectivity in terms of activity and are not considered to be useful from synthetic point-of-view as well for bio-application purposes.

The synthesized series was run through PAINS filter to eliminate compounds that were likely to be chemically-promiscuous and, therefore, not likely to be biologically useful. The synthesized pyrimidine thione series didn’t appear in the category of PAINS and was thus, considered further for bio-evaluation purpose.

#### 3.1.2 Biological activity

##### 3.1.2.1 Screening target: the lymphoid tyrosine phosphatase (LYP)

The lymphoid tyrosine phosphatase is a protein tyrosine phosphatase (PTP) with a critical negative regulatory role on signaling through the T cell receptor (TCR). The function of T cell receptor is to produce T cells which are important in human defensive mechanism. Engagement of the TCR leads to a spatially and temporally-regulated chain of PTK/PTP activation/deactivation events, which in turn, result in tyrosine phosphorylation and dephosphorylation of numerous cytosolic and trans-membrane proteins. LYP, a 105-kDa protein and its mouse ortholog (PEP), dephosphorylate and inactivate Src and Syk family kinases involved in early TCR signaling, thus, efficiently inhibiting the whole signaling pathway.
A single-nucleotide polymorphism (SNP) in the PTPN22 gene (C1858T) results in a change of gene sequence from arginine 620 (R620) to a tryptophan (W620).\textsuperscript{157} This LYP-W620 variant is associated with type 1 diabetes,\textsuperscript{158} rheumatoid arthritis,\textsuperscript{159} systemic lupus erythematosus,\textsuperscript{160} Graves’ disease,\textsuperscript{160} autoimmune Addison’s disease,\textsuperscript{160} suggesting that PTPN22 is a shared autoimmunity gene in humans.\textsuperscript{161} Considering the primary association between the C1858T polymorphism and disease, and the increased enzymatic activity of LYP-W620, it has been predicted that a specific small-molecule inhibitor of LYP will be useful to revert the effects of LYPW620 at the central and/or peripheral level, and prevent the emergence, or reappearance, of auto reactive T cells in carriers of LYP-W620 affected by autoimmunity.\textsuperscript{158} Furthermore, recent evidence indicates that inhibition of LYP could also be beneficial in anaphylaxis\textsuperscript{162} and chronic lymphocytic leukemia.\textsuperscript{161}

In addition to serving as lead compounds for potential therapeutic development, chemical inhibitors targeted toward LYP would be of invaluable help in understanding the other biological roles of LYP, by providing complementary information to genetic manipulations of the PTPN22 gene expression. For example, they will help clarify the role of this important phosphatase in T cell development, and differentiation and its effect on TCR signal transduction in isolated T cell subpopulations.

However, the protein tyrosine phosphatases or PTPs have not yet been successfully targeted, despite overwhelming evidence that they could be good therapeutic targets.\textsuperscript{163} The lymphoid tyrosine phosphatase PTPN22 is a particularly intriguing therapeutic target for the treatment of human autoimmune disorders as explained in fig 15.\textsuperscript{160, 163}
Anaphylaxis  Rheumatoid arthritis  Graves disease

Fig 15  Role of lymphoid tyrosine phosphatase PTPN22 in autoimmune disorders

So in this study, series of pyrimidine thioule compounds was screened and identified two novel inhibitors of PTPN22 activity with activity in the mid-micromolar range.

3.1.2.2 LYP Inhibitor Screening
The increase in fluorescence from the enzymatic turnover of 6,8-difluoro-4methylumbelliferyl (DiFMUP) is an established method of measuring PTP activity. Several of the synthesized compounds were initially screened at a concentration of 100 μM to test their efficacy in inhibiting the PTP LYP (figure 16).
Cpd. 248. a, c, d, e, g, h, i, j, k, l, m, n

Figure 16. Initial screening of selected compounds in their efficacy of inhibiting PTPN22. Concentration was held constant at 100 μM for all compounds.

Although compounds 248a and 248c differ only in the position of one methyl group yet show a significant difference in their ability to inhibit PTPN22. This suggests that a substituent at the 4 contributes to inhibitor activity, and is supported by the fact that 4 of the top 5 compounds identified, contain a group in the 4 position. Interestingly, the top-hit lacks a group at the 4 position, containing only a methoxy group at the two positions. Compounds 248c, 248d, 248g, 248l and 248n showed inhibition of greater than 50% at a concentration of 100 μM, and were further screened to identify their IC₅₀ values. Results are furnished in fig. 17. Compounds 248c (IC₅₀ = 45 ± 3.3 μM) was 1.5 times more potent than compound 248l (IC₅₀ = 69 ± 3.2 μM). Compound 248d, the top-hit identified in the initial screen, was found to have an IC₅₀ value of 18 ±1.4 μM, which is 2.5 times more potent than the second most potent compound. Compounds 248g and 248n had IC₅₀ values of 119 ± 6 μM and 219 ± 48 μM, respectively. These experimentally-determined IC₅₀ values are in
reasonable agreement with the initial screening data. Furthermore, the results suggest that a methoxy group in the 2 position contributes greatly to inhibition, and the addition of another group in the 4 position may further increase potency.

a. 248c

b. 248d

c. 248l

d. 248g
Figure. 17  IC₅₀ curves for compound a. 248c (IC₅₀ = 45 ± 3.3 μM), b. 248d (IC₅₀ = 18 ± 1.4 μM), c. 248l (IC₅₀ = 69 ± 3.2 μM), d. 248g (IC₅₀ = 119 ± 6 μM), and e. 248n (IC₅₀ = 219 ± 48 μM).

3.2 Synthesis and characterization of 4-aminophenazone derived aryl thiourea derivatives (252a-l)

4-Aminophenazone is a drug which is also named as 4-aminoantipyrine or ampyrone. As has already been mentioned, one of the important aspects about this drug is that it belongs to the group of pyrazolone ring-containing non-steroidal anti-inflammatory drugs (NSAIDs). The NSAIDs are over the counter drugs, i.e., they can be used worldwide without the prescription of doctors. The specific medicinal uses of 4-aminophenazone involve anti-inflammatory, antipyretic, analgesic and nonsteroidal agent, while its applications in therapies include treatment of soft tissue disorders, arthritis, lung inflammation (pneumonia) and neuralgia.

Knowing the pharmacological importance and due to our interest in 4-aminophenazone as a biologically-active pharmacophore, we decided to derivatize this 4-aminophenazone to its thiourea derivatives, its benzamide derivatives and then perform the bioevaluation of each of the synthesized series.

The synthesis of 4-aminophenazone derived aryl thiourea derivatives carried out via a multistep synthetic sequence, is demonstrated in scheme 53.

The synthesis was achieved by reacting different substituted-anilines with carbon disulfide and ammonia solution followed by reaction with aqueous solution of lead nitrate to form the intermediates 250a-l. The freshly-prepared substituted isothiocyanates were then refluxed with 4-aminophenazone to yield the target compounds 252a-l.
Scheme 53 Synthesis of substituted 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1Hpyrazol-4-yl)-3-phenylthioureas (252a-l)

The formation of target compounds 252a-l was established by FT-IR spectroscopy where characteristic symmetric stretching absorptions observed in the region of 3378-3169 cm⁻¹, were due to NH groups along with typical C=S peak appearing in the range of 1267-1229 cm⁻¹. However, the absorption band, arising in the region of 1703-1685 cm⁻¹ was indicative of the C=O of the 4-aminophenazone nucleus. The presence of unsaturation in the structure was further confirmed by the presence of absorption bands around 3155-3027 cm⁻¹.

In ¹H-NMR, these compounds exhibited dominant signals in the range of 11.72-9.05 ppm and 8.91-7.47 ppm which were assigned to N-H attached to phenyl and to the NH of 4-aminophenazone moiety, respectively. The resonances in the form of singlet characteristic of the methyl groups, one in the downfield region due to the direct attachment with aza hetero atom, and the other slightly shifted towards shielding region, confirmed the presence of 4-aminophenazone moiety in the structure. The ¹³CNMR spectral data gave resonances for the thiocarbonyl cabon in the range of 189.1181.4 ppm, in addition to those of aromatic carbons, which appeared at appropriate chemical shift values. Peaks ranging from 167.2-163.9 ppm were diagnostic for the amide carbonyl while the signals resonating around 40-10 ppm were assigned to methyl groups that further supported the presence of 4-
aminophenazone nucleus in the synthesized target compounds 252a-l. The purity of newly-prepared compounds was ascertained by elemental analysis. Physical data of all the synthesized compounds is given in table 4.

**Table 4:** Physical data of substituted 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-phenylthioureas (252 a-l)

<table>
<thead>
<tr>
<th>Cpd.No</th>
<th>Compound</th>
<th>M.p. (°C)</th>
<th>Rf*</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>252a</td>
<td>H</td>
<td>67-68</td>
<td>0.47</td>
<td>80</td>
</tr>
<tr>
<td>252b</td>
<td>2-Me</td>
<td>89-90</td>
<td>0.42</td>
<td>80</td>
</tr>
<tr>
<td>252c</td>
<td>3-Me</td>
<td>74-75</td>
<td>0.41</td>
<td>79</td>
</tr>
<tr>
<td>252d</td>
<td>3-MeO</td>
<td>95-96</td>
<td>0.50</td>
<td>80</td>
</tr>
<tr>
<td>252e</td>
<td>2,4,6-triMeO</td>
<td>103-104</td>
<td>0.49</td>
<td>80</td>
</tr>
<tr>
<td>252f</td>
<td>3-Cl</td>
<td>116-117</td>
<td>0.45</td>
<td>81</td>
</tr>
<tr>
<td>252g</td>
<td>4-Cl</td>
<td>109-110</td>
<td>0.46</td>
<td>80</td>
</tr>
<tr>
<td>252h</td>
<td>2-NO2</td>
<td>131-132</td>
<td>0.44</td>
<td>78</td>
</tr>
<tr>
<td>252i</td>
<td>3-NO2</td>
<td>121-122</td>
<td>0.40</td>
<td>79</td>
</tr>
<tr>
<td>252j</td>
<td>4-NO2</td>
<td>140-141</td>
<td>0.43</td>
<td>78</td>
</tr>
<tr>
<td>252k</td>
<td>3,5-di NO2</td>
<td>129-130</td>
<td>0.48</td>
<td>77</td>
</tr>
<tr>
<td>252l</td>
<td>2-Furanyl</td>
<td>147-148</td>
<td>0.51</td>
<td>81</td>
</tr>
</tbody>
</table>

*n- Hexane: chloroform (4:1)

3.2.1 Biological assays

3.2.1.1 Alkaline Phosphatase Assay

Activity of calf intestinal alkaline phosphatase (CIALP) was measured by spectrophotometric assay as described by Ashraf et al. The protocol used for this bio-evaluation is mentioned in section allocated for biological protocols (4.2). The entire assay experiments were repeated three times. KH₂PO₄ was used as the reference inhibitor of calf ALP. The results are summarized in table 5.

**Table 5:** Alkaline phosphatase inhibition of 4-aminophenazone-derived aryl thiourea derivatives (252a-l)
<table>
<thead>
<tr>
<th>Compound</th>
<th>Alkaline Phosphatase IC\textsubscript{50} ± SEM (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>252a</td>
<td>5.012±0.091</td>
</tr>
<tr>
<td>252b</td>
<td>0.420±0.012</td>
</tr>
<tr>
<td>252c</td>
<td>1.793±0.053</td>
</tr>
<tr>
<td>252d</td>
<td>1.233±0.036</td>
</tr>
<tr>
<td>252e</td>
<td>4.245±0.21</td>
</tr>
<tr>
<td>252f</td>
<td>8.348±0.250</td>
</tr>
<tr>
<td>252g</td>
<td>2.374±0.071</td>
</tr>
<tr>
<td>252h</td>
<td>5.455±0.163</td>
</tr>
<tr>
<td>252i</td>
<td>4.844±0.145</td>
</tr>
<tr>
<td>252j</td>
<td>4.801±0.310</td>
</tr>
<tr>
<td>252k</td>
<td>1.720±0.051</td>
</tr>
<tr>
<td>252l</td>
<td>3.765±0.112</td>
</tr>
<tr>
<td>KH\textsubscript{2}PO\textsubscript{4}</td>
<td>2.80±0.065</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM

Standard error of mean

The careful observation of the activity results, in terms of inhibition concentration (IC\textsubscript{50}), revealed that several compounds of the series possess potent alkaline phosphatase inhibition activity which is much better than the standard reference used. Compound 252b appeared to be the most active member of the series with an IC\textsubscript{50} value of 0.420 ± 0.012. This increased activity, possessed by 252b, is attributed to the presence of electron donating group, i.e., the methyl group at ortho position in phenyl ring. A slight modification in substitution pattern on phenyl ring, i.e., substitution at meta position such as in case of 252c and 252d bearing electron-donating groups also manifested good inhibition potential value in comparison to reference standard used as depicted in table 5. It was noted that substitution at 3- and 5- position, having electron-withdrawing groups also afforded higher potential than the standard reference. The least active member of the series turned out to be 252f (Cl at 3- position) with an IC\textsubscript{50} value of 8.348±0.250, which revealed that substitution at meta position with halo group results in decreased activity of the compound.
3.2.1.2 Kinetic studies

On the basis of IC\textsubscript{50} value, the most potent inhibitor \textbf{252c} was selected to determine the mechanism of enzyme inhibition. The inhibitor concentrations used were 0.0, 0.100, 0.200 and 0.400 \textmu M. Substrate p-NPP concentrations were 10, 5, 2.5, 1.25 and 0.625 mM. Pre-incubation time and other conditions were same as described in alkaline phosphatase inhibition assay section. Maximal initial velocities were determined from initial linear portion of absorbances up to 10 minutes, after addition of enzyme, at per minute interval. The inhibition type on the enzyme was assayed by Lineweaver-Burk plot of inverse of velocities (1/V) versus inverse of substrate concentration 1/ [S] mM\textsuperscript{-1} (fig. 18). The EI dissociation constant K\textsubscript{i} was determined by secondary plot of 1/V versus inhibitor concentration (fig. 19).

Fig. 18 Lineweaver-Burk plot
Fig. 19 Plot for determination of dissociation constant $K_i$
**Table 6:** Kinetic analysis of compound 252c

<table>
<thead>
<tr>
<th>Entry</th>
<th>Concentration type</th>
<th>1/V&lt;sub&gt;max&lt;/sub&gt; (µM)</th>
<th>1/V&lt;sub&gt;max&lt;/sub&gt; (ΔA/Min)</th>
<th>K&lt;sub&gt;m&lt;/sub&gt; (mM)</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; (µM)</th>
<th>Inhibition type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.00</td>
<td>200.00</td>
<td>1.0952</td>
<td>0.360</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.100</td>
<td>363.63</td>
<td>1.0952</td>
<td>0.360</td>
<td>Non-competitive</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.200</td>
<td>436.36</td>
<td>1.0952</td>
<td>0.360</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.400</td>
<td>500.00</td>
<td>1.0952</td>
<td>0.360</td>
<td></td>
</tr>
</tbody>
</table>

1/V<sub>max</sub> is the inverse of reaction velocities, K<sub>m</sub> is the Michaelis-Menten constant, K<sub>i</sub> is the inhibition constant.

The results are presented in table 6 (Kinetic parameters table). From these kinetic studies, inhibition mechanism of compound 252c was determined to be non-competitive inhibitor of calf intestinal alkaline phosphatase. The inhibition constant K<sub>i</sub> (0.360) was determined from fig 19. The value of 1/V<sub>max</sub> is increased to a new value while that of K<sub>m</sub> remains same which indicated that compound 252c simply lowers the concentration of jack bean urease by a non-competitively binding mode at enzyme (figs. 18 & 19).

### 3.2.1.3 Free radical scavenging assay

Free radical scavenging activity was carried out by bringing modification in the method reported earlier by Kamal <i>et al.</i> 165 and Ashraf <i>et al.</i> 164 using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Ascorbic acid (Vitamin C) was employed as a reference inhibitor. The percentage of free radical scavenging activity shown by the series 252a-1 is summarized in table 7. The best free radical scavenging activity was provided by compound 252b featuring a methyl substituent (electron donating group) at the ortho position on phenyl ring. Although this activity is lower than the reference standard engaged but still higher in the series. On changing substitution from phenyl group to furan ring, 252l lowered the scavenging activity. The percentage in scavenging activity was found to decrease further when there is substitution on the phenyl ring at 3-position (i.e., meta position) with alkoxy group as in case of 252d.

**Table 7:** Free radical scavenging assay of 4-aminophenazone derived aryl thiourea derivatives (252a-l)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Free radical % scavenging (100 µg/mL)</th>
</tr>
</thead>
</table>

---

148
3.2.1.4 Cytotoxicity evaluation using brine shrimp assay

A. salina nauplii (20) were counted macroscopically using Pasteur pipette against a lighted background and transferred into each sample vial. The solutions were made to 5mL with test compound using serial dilutions with brine solution. A drop of dry yeast suspension was added as food to each vial. All the vials were maintained under light. The surviving nauplii were counted with the aid of a magnifying glass after 24 hours. The mean mortality at the three dose levels for compound in question was determined and repeated thrice. Potassium dichromate was employed as reference standard. After 24 hours, the LD$_{50}$ were calculated by Probit analysis. The results are represented in table 8 in terms of lethal concentration in micro-moles.

**Table 8**: LD$_{50}$ of 4-aminophenazone-derived aryl thiourea derivatives (252a-l) engaging brine shrimp assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>252a</td>
<td>3.37±0.05</td>
</tr>
<tr>
<td>252b</td>
<td>35.50±1.06</td>
</tr>
<tr>
<td>252c</td>
<td>4.00±0.02</td>
</tr>
<tr>
<td>252d</td>
<td>2.5±0.065</td>
</tr>
<tr>
<td>252e</td>
<td>15.45±0.32</td>
</tr>
<tr>
<td>252f</td>
<td>8.32±0.08</td>
</tr>
<tr>
<td>252g</td>
<td>10.00±0.03</td>
</tr>
<tr>
<td>252h</td>
<td>12.45±0.27</td>
</tr>
<tr>
<td>252i</td>
<td>10.13±0.01</td>
</tr>
<tr>
<td>252j</td>
<td>12.35±0.6</td>
</tr>
<tr>
<td>252k</td>
<td>13.67±0.31</td>
</tr>
<tr>
<td>252l</td>
<td>15.0±0.35</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>96.91±3.0</td>
</tr>
<tr>
<td>252a</td>
<td>87.98±7.182</td>
</tr>
<tr>
<td>-------</td>
<td>-------------</td>
</tr>
<tr>
<td>252b</td>
<td>84.107±7.62</td>
</tr>
<tr>
<td>252c</td>
<td>85.259±8.11</td>
</tr>
<tr>
<td>252d</td>
<td>115.440±7.89</td>
</tr>
<tr>
<td>252e</td>
<td>106.71±9.85</td>
</tr>
<tr>
<td>252f</td>
<td>112.55±9.75</td>
</tr>
<tr>
<td>252g</td>
<td>90.402±6.42</td>
</tr>
<tr>
<td>252h</td>
<td>125.29±10.50</td>
</tr>
<tr>
<td>252i</td>
<td>70.906±5.36</td>
</tr>
<tr>
<td>252j</td>
<td>110.23±10.23</td>
</tr>
<tr>
<td>252k</td>
<td>121.42±10.23</td>
</tr>
<tr>
<td>252l</td>
<td>95.467±9.45</td>
</tr>
<tr>
<td>Potassium dichromate</td>
<td>89.1 ± 0.01</td>
</tr>
</tbody>
</table>

These results indicated that the compound 252i of the series turned out to be a lead candidate with maximum activity value of LD<sub>50</sub> equaling 70.906±5.36 µM which proved to be many folds better than the reference standard. This suggested that presence of electron-withdrawing group at 3-position is important for showing good cytotoxic activity. Moreover, the substitution at 3- and 4-positions with electron-donating groups such as methyl group (252b and 252c) is also important for exhibiting good cytotoxic activity. However, switching the position of electron-withdrawing group, from 3-position to 2-position such as 252h, totally reverted the effect in terms of activity of compound, 3-NO<sub>2</sub> derivative, the lead member of the series, lost its activity when the NO<sub>2</sub> group was positioned at 2- on phenyl ring with LD<sub>50</sub> of 125.29±10.50 µM.

3.3 Synthesis and characterization of substituted N-(1,5-dimethyl-3-oxo-2phenyl-2,3-dihydro-1H-pyrazol-4-yl)benzamides (256 a-j).

Based on the reported observations and in continuation of our research work in the drug of interest the 4-aminophenazone, we describe a simple synthetic route to form
the amide derivatives of this pharmacophore which were then screened against different types of alkaline phosphatases that are responsible for causing cancer disease. Molecular docking studies were performed that explained the active binding site interactions responsible for inhibition activity revealed by the synthesized benzamide derivatives of 4-aminophenazone against a variety of alkaline phosphatases.

The synthetic pathway adopted for syntheses of title compounds is depicted in scheme 54. The benzamides are easily accessible by reaction of suitably-substituted acid chlorides with the solution of 4-aminophenazone in dry pyridine. The solid products obtained were recrystallized by aqueous ethanol to afford the purified products 256 aj.

Scheme 54 Synthesis of substituted N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1Hpyrazol-4-yl)benzamides (256a-j)

The spectral and micro elemental data confirmed the structural assignments. The structure has also been verified by single crystal x-ray crystallography.

The FT-IR spectral data indicated the formation of target compounds 256a-j by virtue of characteristic stretching signal of strong intensity for the amide NH around 3250-3140 cm⁻¹ while the absorption band observed in the region of 1679-1634 cm⁻¹ was assigned to amide carbonyl C=O.

¹H-NMR spectra validated the formation of these synthesized compounds by displaying the characteristic singlet for the N-H proton in the range of 8.79-7.26 ppm. However, the resonances for the two methyl groups, characteristic of 4-aminophenazone structure, appeared as singlet with a shift in the downfield region for the methyl group attached directly to hetero atom, i.e., 3.42-3.05 ppm. Another shift in the up-field region for the other methyl group with the chemical shift value ranging between 2.53 and 2.10 ppm, justified the formation of target compounds. ¹³C-NMR also authenticate the formation of 4-aminophenazone-derived benzamides as the signal emerged in the region of 177.4-161.9 ppm was due to amide carbonyl carbon. The methyl carbons in the range of 39.2-10.6 ppm, in addition to, those of aromatic carbons which popped up at appropriate chemical shift values.
The structure of these compounds has also been substantiated by LC-MS as well as GC-MS, in some cases. The mass fragmentation scheme for one of the synthesized members 256c is given in fig 20, showing the formation of stable base peak (100%), as a result of homolytic cleavage (due to loss of 202 units), corresponding to the 4aminophenazone fragment from the molecular ion.

![Mass fragmentation pattern of 256c](image)

\[ \text{Fig 20: Mass fragmentation pattern of } N\{-1,5\text{-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl}\}-2\text{-methylbenzamide (256c)} \]

The purity of compounds was further ascertained from elemental analysis.

The structure of one of the representative of this series, named as 2-bromo-N-(1,5dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-2-methylbenzamide was proved by single crystal X-ray crystallography. It belonged to monoclinic crystal system as presented in fig 21.
Fig 21 Crystal structure of 2-bromo-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1Hpyrazol-4-yl)benzamide (256f)

Table 9: Physical data of substituted N-(2,3-dimethyl-5-oxo-1-phenyl-2,5-dihydro1H-pyrazol-4-yl)benzamides (256a-j)

<table>
<thead>
<tr>
<th>Cpd.No.</th>
<th>Compound</th>
<th>M.p. (°C)</th>
<th>Yield (%)</th>
<th>Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>256a</td>
<td>H</td>
<td>133-134</td>
<td>89</td>
<td>0.40</td>
</tr>
<tr>
<td>256b</td>
<td>2-Me</td>
<td>126-126</td>
<td>86</td>
<td>0.42</td>
</tr>
<tr>
<td>256c</td>
<td>4-Me</td>
<td>183-184</td>
<td>85</td>
<td>0.41</td>
</tr>
<tr>
<td>256d</td>
<td>2-Cl</td>
<td>103-104</td>
<td>82</td>
<td>0.39</td>
</tr>
<tr>
<td>256e</td>
<td>4-Cl</td>
<td>113-114</td>
<td>86</td>
<td>0.41</td>
</tr>
<tr>
<td>256f</td>
<td>2-Br</td>
<td>108-109</td>
<td>87</td>
<td>0.44</td>
</tr>
<tr>
<td>256g</td>
<td>3,5-diOH</td>
<td>163-164</td>
<td>82</td>
<td>0.45</td>
</tr>
<tr>
<td>256h</td>
<td>Phenyl acetyl</td>
<td>137-138</td>
<td>84</td>
<td>0.37</td>
</tr>
<tr>
<td>256i</td>
<td>2-Furanoyl</td>
<td>143-144</td>
<td>83</td>
<td>0.46</td>
</tr>
<tr>
<td>256j</td>
<td>Pivaloyl</td>
<td>127-128</td>
<td>86</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*Petroleum ether:ethyl acetate (4:1)
3.3.1 Alkaline phosphatase and ecto-5'-nucleotidase activity and SAR

Different benzamide derivatives of phenazone were synthesized and further evaluated for their anticancer properties. For this purpose, *in-vitro* activity against membrane-bound nucleotidases, including alkaline phosphatases and ecto-nucleotidase, were performed. These compounds were found to inhibit all APs at the concentration of 200 µM. However, at the lower concentration of 100 µM, these compounds significantly inhibited both human and rat e5-NT.

As regards AP activity, the compounds were tested against four isozymes of human recombinant APs: h-TNAP, h-IAP, h-PLAP and h-GCAP. It was found that all the derivatives exhibited significant inhibitory potential against h-TNAP within the IC$_{50} \pm$ SEM values of 0.337±0.08 µM to 5.91±0.99 µM. Among ten derivatives, compound 256i was the most potent inhibitor of h-TNAP with an IC$_{50}$ value ±SEM of 0.337±0.08 µM which is 70 folds lower than the reference standard inhibitor Levamisole (IC$_{50}$±SEM=20.2±1.9 µM). The detailed study of the structure revealed that, in this compound, the benzene ring attached to acetamide group is replaced with the furan ring. Thus, the name benzamide is replaced with carboxamide. The hydrophilic character of the compound was increased as furan ring is electron donating and it increases the electron density of the compound by donating its lone pair of electrons to it, so increasing its hydrophilicity. It is suggested that the activity of this compound might be due to the presence of furan ring. Furthermore, this statement was proved by observing inhibitory values of the other compounds. The compounds having less carbon number are more active against h-TNAP than the compounds containing higher carbon number. The presence of halogens also affects this property of the compound. The compounds with halogens are more lipophilic in character, thus, exhibiting lower IC$_{50}$ values against h-TNAP as mentioned in Table 9. On the other hand, the inhibition of h-IAP by this compound increased with the hydrophobicity or lipophilicity character of the compound. In this case, maximum inhibition was shown by 256h with an IC$_{50}$ value of ±SEM = 2.83±0.03 µM which is 35 fold lower than the known reference standard inhibitor, i.e. L-Phenylalanine (IC$_{50}$±SEM=100±3 µM). With the decrease in lipophilic character other compounds inhibited more modestly as reported in table 10.

When these compounds were tested for their inhibition potential against h-PLAP, it was observed that, with the exception of compounds 256i and 256j, all the other derivatives were strong inhibitors of h-PLAP. In comparison to h-TNAP, the inhibition of h-PLAP by these compounds was weaker but stronger than with reference inhibitor i.e., Levamisole (IC$_{50}$±SEM=120.0±2.51 µM). The maximum inhibition was obtained in presence of compound 256c and 256f with IC$_{50}$ value of 4.97±0.66 µM and 4.63±0.11 µM, respectively. Only few derivatives inhibited h-GCAP. The compounds, 256e and 256g, inhibited this enzyme at a concentration of 180 and 300 folds less than the standard inhibitor: L-phenylalanine. The structures of these compounds showed that substitution at para position is important for inhibition of h-GCAP. However, the presence of bulky groups and substitution at other sites will result in loss of h-GCAP inhibition by the compound.

When the benzamide derivatives were tested for inhibition of human and rat e5-NT, it was noticed that these compounds exhibited more inhibitory potential against e5-
NT rather than the same member of the family, i.e., APs (table 10). The compounds 256c and 256i displayed strongest inhibition of h-e5-NT with IC$_{50}$ value of 0.78±0.26 µM and 0.77±0.42 µM, respectively. The structures of these compounds exhibited electron-donating groups, i.e., methyl and furan respectively, which are responsible for their inhibitory behavior against human e5-NT. All derivatives inhibited rat e5NT. The structure-activity relationship of all derivatives revealed that the compound 256h having substitution of large carbon chain, manifested the strongest inhibition of rat e5-NT with IC$_{50}$ value of 0.174±0.02 µM which is a concentration 450 fold lower than its previously-mentioned standard inhibitor: sulfamic acid. The compound, 256g, was found to be the selective inhibitor of rat e5-NT with IC$_{50}$ value 1.38±0.29 µM which is the concentration 55 folds lower than its standard inhibitor sulfamic acid. The reported compounds herein are of considerable interest for further applications in the field of medicinal chemistry as these compounds have potential to bind nucleotide protein targets.

Table 10. Alkaline phosphatase and ecto-5'-nucleotidase inhibition in presence of the synthesized compounds (256a-j)

<table>
<thead>
<tr>
<th>Code</th>
<th>h-TNAP</th>
<th>h-IAP</th>
<th>h-PLAP</th>
<th>h-GCAP</th>
<th>h-e5-NT</th>
<th>r-e5-NT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$ (µM) ±SEM</td>
<td>IC$_{50}$ (µM) ±SEM</td>
<td>IC$_{50}$ (µM) ±SEM</td>
<td>IC$_{50}$ (µM) ±SEM</td>
<td>IC$_{50}$ (µM) ±SEM</td>
<td>IC$_{50}$ (µM) ±SEM</td>
</tr>
<tr>
<td>256a</td>
<td>4.22±0.34</td>
<td>12.3±1.08</td>
<td>8.58±0.91</td>
<td>4.94±0.23</td>
<td>1.49±0.86</td>
<td>23.5±0.87</td>
</tr>
<tr>
<td>256b</td>
<td>3.32±0.98</td>
<td>-</td>
<td>6.67±0.87</td>
<td>-</td>
<td>5.59±0.94</td>
<td>7.41±0.64</td>
</tr>
<tr>
<td>256c</td>
<td>5.91±0.99</td>
<td>3.27±0.67</td>
<td>4.97±0.66</td>
<td>7.63±0.99</td>
<td>0.78±0.26</td>
<td>0.83±0.41</td>
</tr>
<tr>
<td>256d</td>
<td>2.19±0.87</td>
<td>-</td>
<td>9.21±1.11</td>
<td>-</td>
<td>172.62±3.34</td>
<td>1.92±0.61</td>
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<tr>
<td></td>
<td>256e</td>
<td>256f</td>
<td>256g</td>
<td>256h</td>
<td>256i</td>
<td>256j</td>
</tr>
<tr>
<td>-------</td>
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</tr>
<tr>
<td></td>
<td>0.691±0.11</td>
<td>0.408±0.09</td>
<td>5.15±1.01</td>
<td>0.917±0.02</td>
<td>0.337±0.08</td>
<td>4.36±0.71</td>
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<td>6.29±0.99</td>
<td>15.1±1.06</td>
<td>2.83±0.03</td>
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<td>10.5±1.09</td>
<td>10.9±1.22</td>
<td>6.57±1.02</td>
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<td></td>
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<td>1.65±0.22</td>
<td>1.03±0.11</td>
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</tr>
</tbody>
</table>

The IC₅₀ is the concentration at which 50% of the enzyme activity is inhibited.
Alkaline phosphatase (h-TNAP, h-IAP, h-PLAP and h-GAP) activity was performed at the final concentration of 200 µM. Ecto-5'-nucleotidase (human and rat) activity was executed at final concentration of 100 µM.

### 3.3.2 Homology Modelling of h-TNAP, h-IAP and h-GCAP

Since crystal structures of human tissue non-specific alkaline phosphatase (h-TNAP), intestinal alkaline phosphatase (h-IAP) and germ-cell alkaline phosphatase (h-GCAP) are not available from the Protein Data Bank (PDB), homology models of h-TNAP, h-IAP and h-GCAP were generated using Chimera¹⁶⁶ and Modeller.¹⁶⁷ The sequence of target proteins h-TNAP (uniprot id: P05186), h-IAP (uniprot id: P09923), and h-GCAP (uniprot id: P10696) were fetched in Chimera. BLAST (Basic Local Alignment Search Tool) protein database¹⁶⁸ was employed to search for sequence similarity with target proteins. Human placental alkaline phosphatase (h-PLAP, PDB id: 1ZED) was identified among the top five matches in all cases, and was accordingly selected to be used as a template. The sequence of template protein was added to the sequence of target protein using Needleman Wunsch global alignment algorithm¹⁶⁹ embedded in Chimera. Comparative modelling was run using Modeller¹⁶⁷ via Chimera. The overall quality of the protein was evaluated, and Ramachandran plots were generated using Molprobity.¹⁷⁰ Comparative sequence alignments and Ramachandran plots are given as supporting information in figs. S6S8 and figs. S2-S4, respectively. All active-site residues among modelled proteins (hTNAP, hIALP, hGCALP), and hPALP were found to be highly-conserved (table 11). Amino acid residues: Phe107, Gln108, Ser155 and Glu429 in hPALP have been replaced by amino acid residues Glu108, Gly109, Thr156 and His434, respectively, in
hTNALP. Glu429 of hPALP, was found to be the only active site amino acid residue that was not conserved in any of the hAPs. In hIALP, it is replaced by Ser448 residue, whereas in hGCALP, it has been replaced by Gly448 residue. Figures 22-24 show comparison of active-site residues of modelled proteins hTNALP, hIALP and hGCALP against the template protein hPALP.

Table 11. Comparison of active-site residues of hTNALP, hIALP and hGCALP with hPALP (PDB id: 1ZED). Conserved residues (with respect to hPALP) are in purple font color, the residues which are not conserved are in red font color

<table>
<thead>
<tr>
<th></th>
<th>hPALP</th>
<th>hTNALP</th>
<th>hIALP</th>
<th>hGCALP</th>
</tr>
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<tbody>
<tr>
<td>Asp42</td>
<td>Asp43</td>
<td>Asp61</td>
<td>Asp61</td>
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<td>Asp91</td>
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<td>Phe107</td>
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<td>Gln108</td>
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<td>Ser155</td>
<td>Thr156</td>
<td>Ser174</td>
<td>Ser174</td>
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<td>Arg166</td>
<td>Arg167</td>
<td>Arg185</td>
<td>Arg185</td>
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<td>Glu311</td>
<td>Glu315</td>
<td>Glu330</td>
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<tr>
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<td>Glu429</td>
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<td>Ser448</td>
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<tr>
<td>His432</td>
<td>His437</td>
<td>His451</td>
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</tr>
</tbody>
</table>

**Figure 22** Comparison of active-site residues of modelled h-TNAP (purple, A) with template h-PLAP (light brown, B)

**Figure 23** Comparison of active-site residues of modelled h-IAP (green, A) with template h-PLAP (light brown, B)
3.3.2.1 Homology Modelling of Rat ecto-5′-Nucleotidase (r-e5NT)

Homology model of r-e5NT was generated using Chimera\textsuperscript{166} and Modeller.\textsuperscript{167} The sequence of target protein r-e5NT (uniprot id: Q66HL0), was opened in Chimera. BLAST (Basic Local Alignment Search Tool) protein database\textsuperscript{171} was used to search for sequence similarity with target proteins. Human ecto-5′-nucleotidase (h-e5NT, PDB id: 4H2I) was identified among the top five matches and was accordingly selected to be used as a template for modeling of r-e5NT. The sequence of template protein was added to the sequence of target protein using Needleman Wunsch global alignment algorithm\textsuperscript{169} embedded in Chimera. Comparative modelling was run using Modeller.\textsuperscript{167} The overall quality of the protein was evaluated and Ramachandran plots were generated using Molprobity.\textsuperscript{168}

Table 12. Comparison of active-site residues of h-e5NT (PDB id: 4H2I) with r-e5NT. Conserved residues (with respect to h-e5NT) are in purple font color, the residues which are not conserved are in red font color.

<table>
<thead>
<tr>
<th></th>
<th>h-e5NT</th>
<th>r-e5NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp36</td>
<td></td>
<td>Asp38</td>
</tr>
<tr>
<td>His38</td>
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<td>His40</td>
</tr>
</tbody>
</table>
Figure 25 Comparison of active-site residues of modelled r-e5NT (violet, A) with template h-e5NT (grey, B)

3.3.3 Molecular Docking

3.3.3.1 Molecular Docking Studies against Ecto-5′-Nucleotidase (CD73)

3.3.3.1.1 Human Ecto-5′-Nucleotidase (h-e5NT) Docking

Compound 256i was found to be the most active h-e5NT inhibitor and was, therefore, selected for molecular docking studies against h-e5NT (PDB id: 4H2I). No direct binding with the zinc ions was found. Detailed analysis of the docked mode of 256i indicated presence of three hydrogen bonds, the oxygen atom of furan ring was
found to act as a hydrogen bond acceptor towards amino group of Phe500 (2.34 Å). The amino group of amino acid Asn390 was making a bridging hydrogen bond with oxygen atom of carbonyl group of pyrazole ring (2.04 Å), and with oxygen atom of carboxamide group (2.14 Å). The 3D and 2D binding site interactions of 256i are given in figs. 26 and 27, respectively.

Figure 26 3D binding-site interactions of 256i
3.3.3.1.2 Rat Ecto-5'-Nucleotidase (r-e5NT) Docking

Compound 256h was found to be the most active r-e5NT inhibitor and was, therefore, selected for molecular docking studies against homology modelled r-e5NT. No direct binding with the zinc ions was found. The most hydrophobic part of the molecule, that is, ethylphenyl group was found to have a snug fit in the binding cavity of enzyme, surrounded by hydrophobic residues Phe419 and Leu186. The carboxamide group and the pyrazole ring were found to be surrounded by amino acid residues His120, Asn119 and His245. The NH of the carboxamide group was making a hydrogen bond with carbonyl oxygen atom of amino acid residue Leu186 (1.95 Å). The 3D and 2D binding-site interactions of 256h are given in fig. 28.
3.3.3.2 Molecular Docking Studies against Alkaline Phosphatases (APs)

3.3.3.2.1 Human Tissue Non-Specific Alkaline Phosphatase (h-TNAP) Docking

The most active h-TNAP inhibitor, 256i, was subjected to molecular docking studies. Detailed analysis of binding-site interactions reveals a direct binding of oxygen atom of furan ring with the catalytic zinc ion at a distance of 2.12 Å. The furan ring is surrounded by amino acid residues His321 and His324. The imidazole ring nitrogen atom of the amino acid residue His437, was found to be involved in a hydrogen bond contact with the NH of carboxamide group of 154i at a distance of 2.08 Å. The NH group of same amino acid, His437, was making another hydrogen bond (2.31 Å) with
The oxygen atom of carbonyl group of 154i. The 3D and 2D binding-site interactions of 256i are given in fig. 29.

Figure 29 3D and 2D binding-site interactions of 256i

3.3.3.2.2 Human Intestinal Alkaline Phosphatase (h-IAP) Docking

The hydrophobic part, ethylphenyl, of the most active inhibitor, 256h, was found to be surrounded by hydrophobic amino acid residues Phe126 and Val108, whereas the rest of the ligand was lying in close vicinity of the hydrophilic residues His339 and His336. The oxygen atom of the carboxamide group was making a hydrogen bond (2.12 Å) with NH group of His451 amino acid. A direct binding of carbonyl oxygen of pyrazole ring with the catalytic zinc ion was observed having a distance of 2.24 Å.
Putative binding-site interactions are given in figures below. The 3D and 2D bindingsite interactions of 256h are given in fig. 30.

![Figure 30 3D and 2D binding-site interactions of 256h](image)

### 3.3.3.2.3 Human Germ Cell Alkaline Phosphatase (h-GCAP) Docking

Detailed molecular docking studies for compound 256g, the most active h-GCAP, were accomplished engaging the homology-modelled structure of h-GCAP. The docking studies revealed putative binding site interactions, which are given in fig. 31. Compound 154g was found to bind near the active site such that the phenyl pyrazole moiety was surrounded by amino acids Phe126, Thr450, Val108, His339 and His451. NH of carboxamide group was making a hydrogen bond with His451 at a distance of 2.21 Å. One of the hydroxyl substituents on the phenyl group was making bridging hydrogen bonds with His172 (2.07 Å) and Asp335 (2.39 Å). The 3D and 2D binding site interactions of 256g are given in fig. 31.
3.3.3.2.4 Human Placental Alkaline Phosphatase (h-PLAP) Docking

Compound \textbf{256f} was found to be the most active \textit{h}-PLAP inhibitor and was selected for molecular docking studies against this enzyme, for which crystal structure is available from the Protein Data Bank (\textit{h}-PLAP, PDB id: 1ZED). No direct binding with the zinc ions was encountered. Detailed analysis of the docked mode of \textbf{154f} indicated presence of two hydrogen bonds involving carbonyl oxygen of pyrazole ring and NH of carboxamide group. The carbonyl oxygen of pyrazole ring was noticed to make a hydrogen bond with NH of amino acid residue His432 (2.08 Å), while at the same time, the nitrogen atom from the imidazole ring of same amino acid residue, His432, was making another hydrogen bond (2.31 Å) with the NH of carboxamide group. The 3D and 2D binding-site interactions of \textbf{4f} are given in the following figure. The 3D and 2D binding site interactions of \textbf{256i} are given in fig. 32.
Figure 32 3D and 2D binding-site interactions of 256f
3.4. Synthesis and characterization of ferrocene-derived aroyl thioureas (261 a-q)

It has been reported in literature that incorporation of ferrocene moiety in various organic scaffolds imparts them useful biological applications. So based on this observation, a three-step synthetic methodology was conducted to form a library of new ferrocene derived aroyl thiourea derivatives which were screened in silico for their pharmacokinetic profiling after performing locomotor activity in mice at different doses for the active compounds of the series.

For the synthesis of target compounds, 261 a-q, ferrocene-derived benzoic acid was synthesized by reported method which was converted to its respective ferrocene benzoyl chloride on refluxing with thionyl chloride. The freshly-prepared aroyl chloride 258 was allowed to react with solution of potassium thiocyanate in acetone at room temperature for two hours. The extent of reaction was followed by TLC and after completion of reaction the mixture was refluxed with variously-substituted anilines, 260 a-q, to obtain the desired compounds in satisfactory yields.

![Scheme 55 Synthesis of substituted 1-(4-ferrocenylaroyl)-3-phenylthioureas (261a-q)](image)

The structure of these compounds 261a-q has been confirmed by the spectroscopic analysis. FTIR spectra revealed the appearance of characteristic symmetric stretching signals for the NH protons in the range of 3396-3219 cm\(^{-1}\). The amide carbonyl (C=O) symmetric stretching was observed at 1697-1639 cm\(^{-1}\) while that of thiocarbonyl bond (C=S) arose at 1298-1237 cm\(^{-1}\). A strong absorption band, characteristic of Fe-cp, was found in the range of 489-482 cm\(^{-1}\). \(^1\)H-NMR spectra showed the characteristic signals for two NH protons around 11.00-8.19 ppm as singlets. The signals for the ferrocene cyclopentadienyl rings emerged in the form of two singlets.
for the substituted cp ring in the range of 4.65-4.19 ppm while that of the unsubstituted cp ring cropped up singlet at around 4.23-4.03 ppm. The $^{13}$C-NMR was also in accordance with the structure by giving the characteristic signals for the thioamide carbon at 184.7-180.1 ppm whereas that of the amide carbon appeared in the range of 177.9-168.4 ppm. On the other hand, the resonances for the Fe-cp rings popped up at 81.7-67.0 ppm.

Table 13: Physical data of substituted 1-(4-ferrocenylaroyl)-3-phenylthioureas (261 a-q)

<table>
<thead>
<tr>
<th>Cpd.No.</th>
<th>Compound</th>
<th>M.p. (ºC)</th>
<th>Rf</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
<td>261a</td>
<td>2-Me</td>
<td>131-132</td>
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<td>87</td>
</tr>
<tr>
<td>261b</td>
<td>4-Me</td>
<td>142-143</td>
<td>0.33</td>
<td>88</td>
</tr>
<tr>
<td>261c</td>
<td>2,4,6-triMe</td>
<td>156-157</td>
<td>0.35</td>
<td>85</td>
</tr>
<tr>
<td>261d</td>
<td>4-MeO</td>
<td>139-140</td>
<td>0.37</td>
<td>87</td>
</tr>
<tr>
<td>261e</td>
<td>4-OH</td>
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<tr>
<td>261f</td>
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<td>261g</td>
<td>3-Cl</td>
<td>170-171</td>
<td>0.39</td>
<td>81</td>
</tr>
<tr>
<td>261h</td>
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</tr>
<tr>
<td>261i</td>
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<tr>
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</tr>
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</table>
3.4.1 Locomotor activity, *in silico* screening and pharmacokinetic studies

*In vivo* studies were carried out in mice to find the locomotor activity of the synthesized compounds of the series. Two compounds 261d (4-methoxy substituted) and 261g (3-chloro substituted) were found to be active among the series as shown by its LD50 value which is above 2g (table 15 and 17). These active members were subjected to *in silico* screening for their pharmacokinetic profiling using Admet-SAR.
software, to quantify their LD$_{50}$, absorption, distribution, metabolism and other characteristics affecting pharmacokinetics.

3.4.2 Locomotor activity

Effect of compound 261d and 261g on locomotor activity was quantified in mice (2530 g) at dose of 25 mg and 50 mg respectively. For this purpose, mice were allowed to move freely in locomotor activity boxes measuring 45.6 X 45.6 cm, and equally divided in 28.6 X 28.6 cm, four quadrants, by lines. Separate groups of animals treated with saline, compound 261d and compound 261g, and standard were placed in locomotor box after specified treatment (saline, drug, and standard) and number of line crossings inside the locomotor box after thirty minutes of drug treatment was recorded through camera.

3.4.3 Results of in silico screening of compounds 261d and 261g

3.4.3.1 For compound 261d

Table. 14: ADMET Predicted Profile --- Classification

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<tr>
<th>Model</th>
<th>Result</th>
<th>Probability</th>
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<tr>
<td>Blood-Brain Barrier</td>
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<td>Human Intestinal Absorption</td>
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<td>Caco-2 Permeability</td>
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<td>P-glycoprotein Substrate</td>
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<td>Renal Organic Cation Transporter</td>
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</table>

<p>| | | |
|                        |             |             |
| Distribution           |             |             |
| Metabolism             |             |             |
| CYP450 2C9 Substrate   | Non-substrate | 0.5589      |
| CYP450 2D6 Substrate   | Non-substrate | 0.8244      |
| CYP450 3A4 Substrate   | Non-substrate | 0.6990      |
| CYP450 1A2 Inhibitor   | Inhibitor   | 0.8282      |
| CYP450 2C9 Inhibitor   | Inhibitor   | 0.9145      |
| CYP450 2D6 Inhibitor   | Non-inhibitor | 0.9522      |
| CYP450 2C19 Inhibitor  | Inhibitor   | 0.7985      |
| CYP450 3A4 Inhibitor   | Non-inhibitor | 0.7717      |</p>
<table>
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<tr>
<th>CYP Inhibitory Promiscuity</th>
<th>High CYP Inhibitory Promiscuity</th>
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**Excretion**

**Toxicity**

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<td>AMES Toxicity</td>
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<tr>
<td>Carcinogens</td>
<td>Non-carcinogens</td>
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<td>Carcinogenicity (Three-class)</td>
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**Table 15: ADMET Predicted Profile --- Regression**

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<tr>
<td>Rat Acute Toxicity</td>
<td>2.5368</td>
<td>LD50, mol/kg</td>
</tr>
<tr>
<td>Fish Toxicity</td>
<td>1.7142</td>
<td>pLC50, mg/L</td>
</tr>
</tbody>
</table>
Tetrahymena Pyriformis Toxicity | 0.8743 | pIGC50, ug/L

### 3.4.3.2 For compound 261g

**Table 16: ADMET Predicted Profile --- Classification**

<table>
<thead>
<tr>
<th>Model</th>
<th>Result</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood-Brain Barrier</td>
<td>BBB+</td>
<td>0.9656</td>
</tr>
<tr>
<td>Human Intestinal Absorption</td>
<td>HIA+</td>
<td>0.9656</td>
</tr>
<tr>
<td>Caco-2 Permeability</td>
<td>Caco2+</td>
<td>0.5319</td>
</tr>
<tr>
<td>P-glycoprotein Substrate</td>
<td>Non-substrate</td>
<td>0.8770</td>
</tr>
<tr>
<td>P-glycoprotein Inhibitor</td>
<td>Non-inhibitor</td>
<td>0.6952</td>
</tr>
<tr>
<td>Renal Organic Cation Transporter</td>
<td>Non-inhibitor</td>
<td>0.9466</td>
</tr>
<tr>
<td>Distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP450 2C9 Substrate</td>
<td>Non-substrate</td>
<td>0.6497</td>
</tr>
<tr>
<td>CYP450 2D6 Substrate</td>
<td>Non-substrate</td>
<td>0.8380</td>
</tr>
<tr>
<td>CYP450 3A4 Substrate</td>
<td>Non-substrate</td>
<td>0.6389</td>
</tr>
<tr>
<td>CYP450 1A2 Inhibitor</td>
<td>Inhibitor</td>
<td>0.8586</td>
</tr>
<tr>
<td>CYP450 2C9 Inhibitor</td>
<td>Inhibitor</td>
<td>0.8810</td>
</tr>
<tr>
<td>CYP450 2D6 Inhibitor</td>
<td>Non-inhibitor</td>
<td>0.8712</td>
</tr>
<tr>
<td>CYP450 2C19 Inhibitor</td>
<td>Inhibitor</td>
<td>0.9117</td>
</tr>
</tbody>
</table>
### Table 17: ADMET Predicted Profile --- Regression

<table>
<thead>
<tr>
<th>Model</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absorption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous Solubility</td>
<td>-5.5139</td>
<td>LogS</td>
</tr>
<tr>
<td>Caco-2 Permeability</td>
<td>1.6150</td>
<td>LogPapp, cm/s</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Metabolism

<table>
<thead>
<tr>
<th>Excretion</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Acute Toxicity</td>
<td>2.4452</td>
</tr>
<tr>
<td>Fish Toxicity</td>
<td>1.1678</td>
</tr>
<tr>
<td>Tetrahymena Pyriformis Toxicity</td>
<td>1.3029</td>
</tr>
</tbody>
</table>

#### 3.4.4 Effect of compound 261d and 261g on locomotor activity

Both the compound showed significant sedative effect and significantly depressed locomotor activity at both doses. Both compounds at the dose of 50 mg/kg showed results equivalent to diazepam 0.5 mg/kg dose.

#### 3.4.5 Discussion

Cytochrome P450 2C9 is one of the most abundant liver enzymes that is responsible for metabolism of both endogenous and exogenous substrates including acidic, basic and neutral drugs. The in silicio pharmacokinetic profiling of the compound 261d proved that it is an inhibitor of this compound, so concurrent use of this compound can lead to deregulation and abnormal and poor metabolism of many endogenous compounds including steroids, arachidonic acid, melatonin, and retinoids. Many synthetic drugs like diclofenac tolbutamide, glyburide, torasemide, celecoxib, phenytoin losartan, and S-warfarin are metabolized by cytochrome P450 2C9 and subsequently their metabolism may be lowered and their toxicity can occur.

Both the compounds have demonstrated significant sedative properties (fig. 33) and the computational profiling unfolds that the compounds cross blood brain barrier, with a probability of 0.9563 which is highly significant. The anxiolytic/ sedative effects of the drug corroborate computational findings of high level drug availability in CNS.

For both compounds, LD50 is above 2g as presented in the tables 15 and 17 reflecting its safety and tolerability in repeated and long-term higher doses. The tables 14 and 16 shows that the compounds 261d and 261g are neither candidate substrate for p-glycoprotein, nor its inhibitor, so apparently giving an impression that steady state shall be achieved, limiting probability of p-glycoprotein dependent drug resistance and drug tolerance. p-glycoprotein are natural cell surface carrier proteins involved in expulsion of xenobiotics, including drugs from cell.
Table 14 and 16 reveal that compound 261d and 261g have maximum human intestinal absorption value 0.9656 indicating their candidacy to be given orally, as tablet, syrup or capsule. The table 14 and 16 divulge that both the compounds are non-AMES toxic implying their non-carcinogenic/non-mutagenic character and these can be safely taken in long-term uses.

In behavioral assays, both compounds showed good sedative effect and the results at 50 mg/kg of both compounds were comparable to standard therapy (fig.33).

Fig. 33. Effect of compounds 261d and 261g on locomotor activity in mice. The results are shown as mean ± SEM. The results were statistically compared using ANOVA, followed by Tukey’s test.

3.5 Synthesis and characterization of isomeric chlorobenzoyl thioureas (265a-c)
The title compounds 265a-c were prepared by the reaction of isomeric chlorobenzoyl isothiocyanates 264a-c with aqueous ammonia at low temperature. The isothiocyanates were obtained in situ by stirring isomeric chlorobenzoyl chloride 263a-c with potassium thiocyanate in dry acetone at room temperature for one hour. Recrystallization of crude product from ethanol afforded the target compounds 265ac in good yields (scheme 56).

Scheme 56 Synthesis of isomeric chloro benzoyl thioureas
Structural characterization is based on FT-IR and FT-Raman, multinuclear \(^1\)H and \(^{13}\)C NMR, elemental analysis and single crystal x-ray crystallography. The FT-IR spectrum of isomeric chloro-benzoyl thioureas displayed a characteristic absorption band for primary NH\(_2\) along with a shoulder at at 3158-3147 cm\(^{-1}\) and for the secondary NH at 3329-3229 cm\(^{-1}\). The strong absorptions in the region of 1687-1678 cm\(^{-1}\) and 1295-1279 cm\(^{-1}\) were assigned to C=O and C=S of thiourea respectively. The \(^1\)H-NMR exhibited singlets of one and two protons between 11.64 and 9.56 ppm and 8.70-7.82 ppm for CONH and CSNH\(_2\), besides, the multiplets for aromatic protons which appeared in their specific chemical shift region. In \(^{13}\)C-NMR, the characteristic thiocarbonyl and carbonyl carbons appeared in the range of 182.0-180.5 ppm and 170.2-167.7 ppm, respectively, in addition to other characteristic signals.

**Table 18:** Physical data of isomeric chloro-substituted benzoyl thioureas (265a-c)

<table>
<thead>
<tr>
<th>Cpd.No.</th>
<th>Compound</th>
<th>M.p.(ºC)</th>
<th>R(_f)</th>
<th>Yield ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>265a</td>
<td>2-Cl</td>
<td>167-168</td>
<td>0.55</td>
<td>85</td>
</tr>
<tr>
<td>265b</td>
<td>3-Cl</td>
<td>125-126</td>
<td>0.57</td>
<td>80</td>
</tr>
<tr>
<td>265c</td>
<td>4-Cl</td>
<td>152-153</td>
<td>0.56</td>
<td>83</td>
</tr>
</tbody>
</table>

*Petroleum ether:ethyl acetate (1:4)*

The structure of one crystalline isomer, i.e., 1-(2-chlorobenzoyl)thiourea was confirmed from single crystal x-ray crystallography and it possessed triclinic crystal system as produced in fig. 34.
The vibrational analysis (FT-IR and FT-Raman) for the new 1-(2-chlorobenzoyl)thiourea species was conducted which suggested that strong intramolecular interactions affect the conformational properties. The X-ray structure determination corroborates that an intramolecular N–H...O=C hydrogen bond exists between the carbonyl (–C=O) and thioamide (–NH₂) groups. Moreover, periodic system electron density and topological analysis were applied to characterize the intermolecular interactions in the crystal. Extended N–H...S=C hydrogen-bonding networks between both the thioamide (N–H) and carbamide (NH₂) groups and the thiocarbonyl bond (C=S) determine the crystal packing. The Natural Bond Orbital (NBO) population analysis demonstrates that strong hyperconjugative remote interactions are responsible for both, intra and intermolecular interactions. The Atom in Molecule (AIM) results also show that the N–H...Cl intramolecular hydrogen bond between the 2-Cl-phenyl ring and the amide group in the free molecule changes to an N...Cl interaction as a consequence of crystal packing.

3.6 Synthesis and characterization of substituted isomeric chloro–N- (thiazol2(3H)-ylidene)benzamides (266, 267, 268, 269 a-c)

The solution of isomeric chloro-substituted benzoyl thioureas 265a-c in absolute ethanol was treated with different cyclizing agents such as alpha halo carbonyl compounds and diethyl oxalate. The reaction mixture was heated at 50°C under inert conditions of nitrogen atmosphere for 2 hours to form the corresponding substituted 2-imino thiazoline cyclized products 266, 267, 268, 269 a-c as shown in scheme 57.
The formation of 2-iminothiazolines was indicated by the disappearance of strong absorption bands of NH$_2$ and C=S groups of isomeric chloro-benzoyl thioureas and the appearance of C=N absorption in the range of 1690-1640 cm$^{-1}$ for the target compounds (266, 267, 268, 269 a-c). In case of cyclization with (d) and (e), the IR indicated stretching signals for ester carbonyl in the range of 1735-1728 cm$^{-1}$ for the compounds 266a-c and 267a-c while strong absorption bands observed in the region of 1741-1738 cm$^{-1}$, were assigned to keto carbonyl group for the cyclized products 269 a-c formed from (g) cyclizing agent.

The purity of the synthesized compounds (266, 267, 268, 269 a-c) was justified by elemental analysis.

The structure of these newly-synthesized iminothiazolines was also supported by multinuclear $^1$H and $^{13}$C-NMR and GC-MS, in some cases. The results of four different cyclizations with one of the isomeric, i.e., 1-(2-chlorobenzoyl)thiourea 265a is explained stepwise as follows:

The $^1$H-NMR spectral data for the cyclized product of 266a with ethyl-2-chloroacetoacetate (d) exhibited resonance for characteristic N-H proton as singlet at 9.52 ppm along with other aromatic protons at appropriate chemical shift values.
The disappearance of NH$_2$ signal also indicated the smooth cyclization while another singlet integrating to three protons of methyl group was found at 2.26 ppm. Two signals depicted the following multiplicity pattern triplet and quartet with coupling constant of $J=6$ Hz were assigned to ethyl group attached to ester moiety. In $^{13}$CNMR, signals were also observed at 164.1 and 162.5 ppm which were accounted for C=O of amide and C=O of ester functionalities, respectively. Another distinguishing signal found at 16.2 ppm was due to the resonance of methyl carbon attached to thiazole ring.

Further confirmation was provided by mass spectrometry where the molecular ion peak of $^{266a}$ appeared at 324 a.m.u with an isotopic cluster pattern of 1:3 characteristic for chlorine isotopes, while the origin of base peak and other daughter peaks is explained in fig. 35.

Cl
m/z = 139
Base peak

Cl
m
/z = 2
7
9
In $^1$H-NMR, the signal for N-H proton appeared at 10.62 ppm besides other characteristic multiplicity patterns observed for the ethyl group at 4.31 ppm as quartet and triplet at 1.37 ppm which indicated the desired conversion of 265a with (e) to form 267a, i.e., ethyl-2-(2-chlorobenzamido)-2,3-dihydrothiazole-4-carboxylate. In $^{13}$C-NMR, the emergence of amide C=O and ester C=O peaks at 164.5 and 161.5 ppm, and disappearance of C=S peak further confirmed the cyclization reaction.

The $^1$H-NMR for cyclization reaction of 265a with (f) depicted a characteristic singlet in the olefinic region at 7.19 ppm besides other singlet for N-H proton which resonated at 10.88 ppm, thus confirming the structure of product 268a named as N-(4(4-bromophenyl)thiazol-2(3H)-ylidene)-2-chlorobenzamide. In $^{13}$C-NMR, signals observed at 173.5 ppm and 108.7 ppm were attributed to the amide carbonyl and the olefinic carbon of thiazole ring (-S-C=). The appearance of singlet, in the $^1$H-NMR, at 10.19 ppm besides the characteristic multiplicity pattern for ortho-substituted aromatic ring around 7.84-7.40 ppm confirmed the smooth cyclization of 1-(2-chlorobenzoyl)thiourea 265a with diethyl oxalate (g) to form 2-chloro-N-(4,5-dioxothiazolidin-2-ylidene)benzamide 269a. However, the $^{13}$C-NMR showed the characteristic peak of most deshielded carbon at $\delta=186.1$ ppm (C=O), whereas C=O of amide resonated upfield at $\delta=170.7$ ppm. Peaks ranging from 163.0-128.5 were assigned to the remaining carbons of the compound 269a.

The physical data of the newly-prepared compounds (266, 267, 268, 269 a-c) is tabulated in table 19.

**Table 19**: Physical data of substituted isomeric chloro-N-(thiazol-2(3H)ylidene)benzamides (266, 267, 268, 269 a-c)

<table>
<thead>
<tr>
<th>Cpd.No</th>
<th>Compound</th>
<th>M.p. (°C)</th>
<th>Rf</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>266a</td>
<td>2-Cl</td>
<td>121-122</td>
<td>0.54</td>
<td>80</td>
</tr>
<tr>
<td>266b</td>
<td>3-Cl</td>
<td>154-155</td>
<td>0.51</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>266c</td>
<td>4-Cl</td>
<td>160-161</td>
<td>0.60</td>
<td>82</td>
</tr>
<tr>
<td>267a</td>
<td>2-Cl</td>
<td>127-129</td>
<td>0.59</td>
<td>81</td>
</tr>
<tr>
<td>267b</td>
<td>3-Cl</td>
<td>181-182</td>
<td>0.45</td>
<td>76</td>
</tr>
<tr>
<td>267c</td>
<td>4-Cl</td>
<td>170-171</td>
<td>0.53</td>
<td>80</td>
</tr>
<tr>
<td>268a</td>
<td>2-Cl</td>
<td>136-137</td>
<td>0.55</td>
<td>86</td>
</tr>
<tr>
<td>268b</td>
<td>3-Cl</td>
<td>195-196</td>
<td>0.48</td>
<td>78</td>
</tr>
<tr>
<td>268c</td>
<td>4-Cl</td>
<td>183-184</td>
<td>0.61</td>
<td>84</td>
</tr>
<tr>
<td>269a</td>
<td>2-Cl</td>
<td>121-122</td>
<td>0.52</td>
<td>83</td>
</tr>
<tr>
<td>269b</td>
<td>3-Cl</td>
<td>114-115</td>
<td>0.56</td>
<td>84</td>
</tr>
<tr>
<td>269c</td>
<td>4-Cl</td>
<td>144-145</td>
<td>0.50</td>
<td>82</td>
</tr>
</tbody>
</table>

*Petroleum ether:ethyl acetate (1:4)*
3.6.1 Anti-leishmanial activity

MTT method\textsuperscript{176} was used to measure the antileishmanial activity of new 2iminothiazoline derivatives, and % inhibition for all the synthesized compounds at different concentrations is reported in table 20.

Table 20: Anti-leishmanial activity of substituted isomeric chloro–N-(thiazol-2(3H)yldene)benzamides (266, 267, 268, 269 a-c)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Compound</th>
<th>Leishmaniasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 µM</td>
</tr>
<tr>
<td>266a</td>
<td>2-Cl</td>
<td>68.7 ± 1.5</td>
</tr>
<tr>
<td>266b</td>
<td>3-Cl</td>
<td>50.5 ± 1.8</td>
</tr>
<tr>
<td>266c</td>
<td>4-Cl</td>
<td>76.4 ± 1.2</td>
</tr>
<tr>
<td>267a</td>
<td>2-Cl</td>
<td>60.4 ± 1.4</td>
</tr>
<tr>
<td>267b</td>
<td>3-Cl</td>
<td>52.3 ± 1.5</td>
</tr>
<tr>
<td>267c</td>
<td>4-Cl</td>
<td>60.9 ± 1.4</td>
</tr>
<tr>
<td>268a</td>
<td>2-Cl</td>
<td>58.2 ± 1.7</td>
</tr>
<tr>
<td>268b</td>
<td>3-Cl</td>
<td>43.5 ± 2.1</td>
</tr>
<tr>
<td>268c</td>
<td>4-Cl</td>
<td>54.6 ± 2.1</td>
</tr>
<tr>
<td>269a</td>
<td>2-Cl</td>
<td>55.7 ± 1.9</td>
</tr>
<tr>
<td>269b</td>
<td>3-Cl</td>
<td>39.6 ± 2.3</td>
</tr>
<tr>
<td>269c</td>
<td>4-Cl</td>
<td>56.5 ± 2.1</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>—</td>
<td>80.5 ± 1.7</td>
</tr>
</tbody>
</table>

Amphotericin B was used as standard drug for anti-leishmanial activity. The careful observation of the anti-leishmanial activity results revealed that some of the tested compounds showed good inhibition of *Leishmania major* in vitro. Among the series 266c proved highest inhibition of 76.4 ± 1.2% at 100 µM indicating presence of chloro group at para position is important for producing good inhibition besides the influence of methyl and ester groups on thiazole ring towards this inhibition, while the inhibition exhibited by Amphotericin B was 80.5%. However, the compound 266c displayed highest potential at variable concentrations tested in the assay. Removal of methyl group resulted in decrease in inhibition to 60.9 ± 1.4 % at 100 µM 267c. The activity was further reduced when the methyl and ester groups were replaced by the keto functionality in thiazole ring. Thiazole derivatives, having chloro substituent at ortho position, showed moderate inhibition of *Leishmania major*. Decreased activity was by all the thiazole derivatives having chloro substitution at meta position as compared to the standard drug. Least active compound among the series was 269b having 39.6 ± 2.3% inhibition at 100 µM. The results indicate that these compounds may need further attention to be developed as therapeutic agents to treat leishmaniasis.
3.7 Synthesis of tropane auxiliary, for incorporation as a stereodirecting element to achieve $\alpha$-alkylation of aldehydes

Previous work by Charlton focused on the alkylation of bicyclic enamines to produce enantioenriched $\alpha$-alkylated aldehydes. The alkylation reaction of the two bicyclic N auxiliaries (which differ only by a single methylene group) with EtI gave opposite sense of asymmetric induction and provided modest yields of enantioenriched-2ethylhexenal upon hydrolysis of the alkylated iminium as presented in fig. 36. The results of the observed stereoselectivity was also backed up by the computational results and provided a reliable insight into the predisposition of each enamine face to alkylation.\(^\text{126}\)

![Fig. 36 Alkylation reaction of bicyclic auxiliaries](image)

The combination of experimental and theoretical results explained that tropane-derived enamine showed almost no facial selectivity. Unexpectedly, the position of the exocyclic double bond (whether it leans more over the 5-membered or the 6membered ring), in the alkylation transition state was determined mostly by the embedded pyrrolidine, hence, reducing the influence of gem dimethyl group. However, the results of selectivity in case of homotropane, i.e., [6,6] system explained the influence of gem dimethyl group in directing alkylation.

So, based on these results, it was concluded that the 5-membered ring impacted the relative position of the exocyclic double bond (i.e., the double bond sits more over the six-membered ring exposing the face of 5-membered ring) and directed alkylation towards its face despite the presence of blocking element, gem dimethyl group.

3.7.1 Aim of project

From this conclusion, it was predicted that removing the gem dimethyl group from the [6,5] bicyclic auxiliary would presumably lead to further alkylation of the enamine directed over the face of five-membered ring and will give an increased level of $dr$. So the obvious candidate to test this hypothesis was to use racemic 1-methyl tropane auxiliary 270 as shown in fig. 37.
Fig 37 Aim of project

The key points of my work in the project include,

- To design synthesis of racemic 1-methyl tropane system
- To exploit 5-membered ring’s effect on facial selectivity
- Determination of $dr$ for the transient diastereomeric iminium ions from $^1$H-NMR

3.7.2 Synthesis and alkylation of racemic 1-methyl tropane

The synthesis and alkylation of racemic 1-methyl tropane auxiliary was achieved in eight steps as explained below.

3.7.2.1 Synthesis of 4-oxopentanal ((±)-273)

As a first step of the synthesis to make the building block for tropane auxiliary oxidation of 5-hydroxy-2-pentanone 271 was carried out using the pyridinium chlorochromate 272 as an oxidizing agent. A mixture of PCC with silica was employed in DCM solvent and the oxidized product 4-oxopentanal, 273, was formed as a result of stirring of this reaction mixture under an inert atmosphere of nitrogen while using flame dried apparatus. The crude product was filtered through a pad of florisil, purified by column chromatography using a SiO$_2$, 5% Et$_2$O in DCM solvent system which gave the pure 4-oxopentanal, 273, in 56% yield as green oil (scheme 58). The product was quite volatile in nature.

\[
\begin{align*}
\text{HO} & \text{O} \\
\text{PCC-silica} & + \\
\text{CH}_2\text{Cl}_2 & \text{Stirring, rt} \\
271 & \rightarrow \text{O} \\
& \text{272} \quad \text{273}
\end{align*}
\]

Scheme 58 4-oxopentanal synthesis

The structure of the oxidized product, 273, was confirmed by $^1$H-NMR which displayed the characteristic signal in the form of singlet for the aldehyde proton at 9.81 ppm while another singlet integrating three protons at 2.21 ppm was assigned to methyl protons. However the singlet for four protons at 2.78 ppm was attributed to methylene protons. $^{13}$C-NMR exhibited the resonances for the carbonyl carbon of aldehyde and ketone at 206.3 ppm and 200.4 ppm, respectively, whereas the methyl carbon resonated at 29.7 ppm.
3.7.2.2 Synthesis of racemic 1-methyl-N-benzyltropinone ((±)-276)

A well-known Robinson Schöpf reaction was followed with few modifications in reaction conditions to form the racemic N-benzyl protected tropinone (±)-276 ring in satisfactory yields (54%). Different reaction conditions were tried and the best optimized conditions which gave improved yield are given in scheme 59.

![Scheme 59](image)

Scheme 59 Synthesis of (±)-8-benzyl-1-methyl-8-aza-bicyclo[3.2.1]actan-3-one

The synthesis was achieved by reacting the oxidized precursor, i.e., ketoaldehyde, 273, with benzyl amine 274 in the presence of acetone-1,3-dicarboxylic acid, 275, using acidic buffer of pH=5. The reaction mixture was heated at 40°C for 7 hours followed by basification with 50% aq. NaOH and extraction with dichloromethane which gave the pure racemized 1-methyl-N-benzyltropinone as reddish orange oil with an Rf of 0.15 in 4% EtOAc, 2% NEt3 in petroleum ether solvent system.

The structural elucidation of the racemic benzyl protected bicyclic tropinone was accomplished with the help of FT-IR, 1H-NMR, 13C-NMR and high resolution mass spectrometry.

The FT-IR depicted the strong symmetric stretching absorption characteristic for the carbonyl of ketone at 1712 cm⁻¹ while the absorption bands for the asymmetric and symmetric stretching of Csp³-H appeared at 2958 and 2873 cm⁻¹ respectively.

In 1H-NMR, three key proton resonances were recorded which confirmed the structure of racemic bicyclic tropinone auxiliary, (±)-276. This include the signal for the bridgehead proton which resonated at 3.45 ppm displaying the multiplicity pattern of doublet of a doublet of a doublet (dddl) while the methyl protons resonated at 1.23 ppm integrating three protons as singlet. Two distinctive doublets resonating at 3.84 ppm and 3.67 ppm with coupling constant of 13.4 Hz, were attributed to benzylic diastereotopic protons. The absence of signal in the aldehyde region was another strong indication of product formation.

The formation of title compound from 13C-NMR was indicated by the appearance of C=O of keto moiety in the title compound (±)-276 at 210.1 ppm besides other diagnostic signals which arose at 62.6 ppm and at 56.8 ppm for the quarternary (Cquart.) and methyne carbon (CH) of tropinone ring respectively. The benzylic carbon (NCH₂Ph) resonated at 48.1 ppm.

Further confirmation was provided by high resolution mass spectrometry using ESI in positive mode where molecular ion peak emerged at 230.1539 a.m.u.
3.7.2.3 Synthesis of racemic 1-methyl-N-benzyltropane ((±)-279)
The (±)-276 was subjected to reduction, using Wolff-Kishner methodology,\textsuperscript{178} to form the protected tropane species, (±)-279. The ability of the substrate to withstand high temperatures allowed the reduction to be effected at high temperature. So, the synthesis was achieved by heating (±)-276 with hydrazine hydrate, 277, using potassium hydroxide 278 pellets in the presence of diethylene glycol as a solvent at 220ºC for 24 hours as in scheme 60. The reaction yielded pure racemized product, (±)-279, as a pale yellow oil in 87 % yield.

Scheme 60 Synthesis of (±)-1-methyl-8-benzyl-8-azabicyclo[3.2.1]octane

The disappearance of stretching vibration of keto carbonyl in functional group region at 1712 cm\textsuperscript{-1} in FT-IR and the appearance of strong asymmetric and symmetric stretching bands at 2928 cm\textsuperscript{-1} and 2867 cm\textsuperscript{-1} indicated the formation of reduced product, (±)-279.

The structure of the reduced tropane auxiliary was evidenced by multinuclear \textsuperscript{1}HNMR and \textsuperscript{13}C-NMR spectroscopy. A slight shift towards the lower ppm, i.e., towards the shielding region was found for the three key proton resonances which were tracked for the structure determination of its starting precursor (±)-276. The shift was explained owing to the removal of keto group which has now been reduced to methylene, therefore, making these protons to resonate in low field region. So, the signal of bridge head proton was found at 3.03 ppm while that of methyl singlet appeared at 1.07 ppm. The signals for diastereotopic benzylic proton at 3.77 ppm and 3.50 ppm further confirmed the structure.

Similar trend was observed in case of \textsuperscript{13}C-NMR, where the quaternary (C\textsubscript{quan}) and the methyne carbon (CH) of the tropane ring resonated slightly low field at 60.5 ppm and 56.7 ppm, respectively. The same shielding effect was found for rest of the protons of the structure which resonated at their appropriate chemical shift region.

The structure of reduced racemic auxiliary was further supported by HR-MS which gave the molecular ion peak at m/z equals 216.1747 a.m.u.

3.7.2.4 Synthesis of hydrochloride salt of racemic 1-methyltropane ((±)-281)
The (±)-279 was subjected to hydrogenolytic deprotection as a result of reaction of (±)-1-methyl-8-benzyl-8-azabicyclo[3.2.1]octane, (±)-279, with 10 % Pd on charcoal, 280, as a catalyst in methanol while stirring the reaction mixture overnight under an inert atmosphere of hydrogen gas. The reaction was quite neat and gave quantitative yield (100 %) of the product, i.e., the free tropane base.
As previously reported by Charlton regarding the volatile nature of these tropane systems, and in anticipation of this, the free tropane base was converted to its stable hydrochloride salt (±)-281 by the addition of 2M HCl in Et₂O (scheme 61).

Scheme 61 Synthesis of (±)-1-methyl-8-azabicyclo[3.2.1]octanehydrochloride

The formation of target compound was proved by FT-IR spectroscopy where characteristic stretching absorption band of medium intensity for the NH₂ appeared at 3387 cm⁻¹. However, the Csp³-H bending vibrations for the CH₂ and CH₃ emerged at 1460 and 1369 cm⁻¹, respectively, which further confirmed the structure of target compound, (±)-281.

The key proton resonance, recorded in ¹H-NMR which substantiated the desired conversion turned up in the form of a broad doublet at 9.37 ppm with a coupling constant of 104 Hz. Rest of the protons appeared in the form of multiplets at their specific chemical shift values.

The disappearance of the signals of benzylic and aromatic carbons, in the ¹³C-NMR, was another strong indication of the desired conversions with the remaining bicyclic core resonating at its appropriate chemical shift values.

The presence of molecular ion at 126.1278 a.m.u further justified the desired transformation.

3.7.2.5 Addition of hexanal to tropane ((±)-286)
Curphey’s method of using hindered amines to form enamines is very sensitive in nature. Steric crowding about the nitrogen lone pair determines the successful condensation. Reaction becomes unfavorable at this center and may lead to competitive side reactions if the substituents on the nitrogen are too bulky. The addition of hexanal to disopropyl amine 282, was successful and led to attempts by Kaka at enamine formation for the slightly more hindered amine 283. Effective condensations were prevented due to the presence of one tert-butyl group which created a sterically-encumbered nitrogen lone pair. Reaction went unsuccessful when amine 284 was tried with a hope that the conformation of six-membered would lead to reduced crowding of the nitrogen lone pair (fig 38).
So, based on these experimental observations, it was considered that the presence of five-membered ring lock in tropane, \(281\), could further pin back some of the bulk, thus, leading to increased exposure of the nitrogen lone pair which would be just enough for successful addition of hexanal as shown in scheme 62.

![Scheme 62 Failure attempt towards enamine synthesis (286)](#)

Perhaps unsurprisingly, given the failure of quite similar amines to undergo condensation, \(^1\)H-NMR analysis of the crude reaction mixture exhibited only the starting amine and hexanal self-aldolization product—fig. 39—(characterized by the diagnostic triplet in the olefinic region, \(\sim 6\) ppm for \(^1\)H).

![Fig. 39 Hexanal self-aldolization product](#)

3.7.2.6 Formamide formation of racemic 1-methyl tropane auxiliary ((\(\pm\))-289)

After having failed hexanal condensation with \((\pm\))-281 to form enamine \((\pm\))-286 directly, modification was carried out in the synthetic scheme to form enamine from formamide precursor \((\pm\))-289. Blum and Nyberg conditions\(^1\) were followed to form the \((\pm\))-289. In this reaction, the stable hydrochloride salt \((\pm\))-179 was directly used so as to avoid the careful isolation of free base tropane. The formylation was achieved by reacting the \((\pm\))-281 with chloroform 287 in the presence of phase transfer catalyst, i.e., triethyl benzyl ammonium chloride (TEBA-Cl) 288 utilizing dichloromethane as a solvent while conducting out the reaction in basic conditions which ensured the formation of free tropane \textit{in situ}. The reaction was refluxed for 24 hours to form the \((\pm\))-289 in quantitative yields (scheme 63).
Scheme 63 Synthesis of (±)-1-methyl-8-azabicyclo[3.2.1]octane-8-carbaldehyde

The FT-IR indicated the formation of formamide species by the emergence of new stretching absorption peak for formamide carbonyl at 1657 cm\(^{-1}\) and by the disappearance of absorption band for NH\(_2\) at 3387 cm\(^{-1}\).

The structure of the formamide containing bicyclic auxiliary was confirmed by \(^1\)HNMR where the resonance for the formamide proton was found at 8.04 ppm as singlet besides other signals of the desired structure, while the absence of singlet in this case at 9.37 ppm was another strong indication of product formation.

In case of \(^{13}\)C-NMR, a signal at 156.3 ppm was assigned to the carbonyl of formamide moiety.

HR-MS also supported the formation of desired formylated product by giving the molecular ion peak at m/z equals 154.1226 a.m.u.

3.7.2.7 Synthesis of enamine ((±)-286)

After synthesizing pure formamide, the key enamine-forming step and alkylation could be undertaken. Hansson and Wickberg protocol\(^{152b}\) was adopted for the formation of enamine by bringing slight modification in the original procedure.\(^{39}\)

Since the enamine hydrolyzes in contact with moisture in the air, so the utmost care was exercised to ensure all components were suitably dried and purged with inert gas before starting the reaction. The reaction was carried out by reacting formamide (±)289 (100 mg) with pentyl magnesium chloride, 290, in diethylether at room temperature for 24 hours as given in scheme 64.

Scheme 64 Synthesis of (±)-8-((E)-hex-1-en-1-yl)-1-methyl-8-azabicyclo[3.2.1]octane

After this, the solvent was evaporated from the reaction mixture by blowing a stream of inert gas over the solvent surface. This method was preferred for such low quantities of solvent as it minimized the chance of hydrolysis. The crude enamine
(±)286 was purified by performing Kugelrohr distillation which gave the pure enamine in 99% (133 mg) yield. Under an inert atmosphere of argon, the pure enamine was transferred to a sealed Young’s cap NMR tube, and a 1H-NMR spectrum of the enamine in deuterated acetonitrile was recorded. Three key proton resonances were tracked through to the iminium ion the olefins and the NCH at the bridgehead. A doublet peak appearing at 5.93 ppm with \(J=14\) Hz, was assigned to NCH=CH; the magnitude of the coupling constant verified that the E-isomer of the starting enamine was present. At 4.32 ppm, doublet of triplets was ascribed to NCH=CH \(J_1 = 14.0\) Hz, \(J_2 = 7.1\) Hz while the multiplet at 3.82 ppm was attributed to NCH.

3.7.2.8 Alkylation of enamine and 1H-NMR analysis ((±)-292)

Alkylation of enamine (±)-286 was done in situ by addition of EtI, 291, in the same Schlenk NMR tube. The formation of diastereomeric iminium ions, 292, in \(d_3\)-MeCN was confirmed by 1H-NMR (fig. 43, spectrum 2) after reaction at 85°C for 16 h (scheme 65).

![Scheme 65 Alkylation of enamine (± 286)](image)

Analysis of this sealed reaction mixture by 1H-NMR showed a multitude of peaks. Most importantly, the key peaks characteristic for iminium ions were easily recognizable. At 8.08 ppm (292 A, fig. 43, spectrum 2), the principal iminium proton manifested as a doublet signalling that ethylation, rather than protonation, has occurred. Furthermore, a shift in the bridgehead (NCH) proton to ~5.0 ppm (292 B) exhibited a complex shift with an asymmetric distribution; characteristic of diastereomeric iminium ions! The determination of the quantity of each diastereomer from the integration of the bridgehead multiplet at ~5.0 ppm was challenging. However, an improvement in the resolution was observed when the analysis was conducted on high resolution NMR instrument, i.e., on 500 MHz instead of 400 MHz (fig. 40, spectrum 1), baseline separation was not evident, hence, the appearance of a complex multiplet. Unlike for homotropane (shown in fig. 36), where the gem dimethyl reduces the level of coupling, the bridgehead proton is coupled to two adjacent methylene groups. It is, therefore, inherent in the molecule that this peak has poorer resolution and more complexity.
Determination of $dr$ was executed by half-integration of the two overlapping proton shifts, and it came out to be 64:36. This experimentally determined $dr$ was in full agreement with the computationally-determined ratio performed by Kelvin Jackson of Paton group, which predicted 68:32.\textsuperscript{126} The lowest energy transition state for the addition of EtI is depicted below [technically the shortened analogue (where C$_4$H$_9$ = CH$_3$) is depicted] and demonstrates the enamine double bond in a pseudo axial position. It is clear from the transition state geometries and the corresponding free energies that the approach of ethyl iodide is on the face of the 5-membered ring (~2 kJ mol$^{-1}$ preference for $Si$ compared to $Re$ addition) (fig. 41).
In order to validate the model system’s suggestions of a good dr, it was imperative that all the side-products be identified. In the olefinic region, the presence of two well-defined peaks (293A and 293B; fig 43, spectrum 2) were the greatest cause for concern. Considerable evidence for the isomerization to Z-enamine 295 and of the protonated iminium species, 296 (fig 42), was provided by Kaka who detailed the possible alternative pathways for 2,2,6-trimethylpiperidine-derived enamines.\textsuperscript{180}

Protonation of enamine 286 was observed, giving rise to the protonated iminium 294. The N=CH proton 294\textsuperscript{A}, coupling to two protons on the adjacent carbon, was indicated by the presence of a triplet (8.19 ppm) in the $^1$H NMR spectrum of the iminium ions (fig. 43, spectrum 2), confirming that enamine protonation has indeed occurred. Attention then shifted towards the identification of the olefins displaying fine-structure splitting at ~6.0 ppm. A prominent roofing effect was found which suggested that two olefinic peaks at 6.03 ppm and 5.85 ppm are coupled to each other that were further verified by COSY 2D NMR techniques. Additionally, it was found that only the proton shift at 6.03 ppm coupled with any other proton. These two combining features of the olefinic peaks indicated the presence of an enamine. The $\Delta \delta_H$ for these two peaks is only 0.20 ppm, suggesting that the nitrogen lone pair and the $\pi^*$ of the double bond have an orthogonal relationship. However $^3J_{HH}$ was found to be 14 Hz, the ideal value for an E-alkene! Whereas the three bond coupling
constant observed in case of Kaka’s Z-enamine was $J = 7.5$ Hz which ruled out the possibility of formation of a Z-enamine via isomerization. In fact, all the traits and peaks described previously, in addition to a bridgehead (NCH) multiplet at ~4.3 ppm (293C, fig. 43, spectrum 2), define the vinyl ammonium 293 rather well, suggesting alkylation at nitrogen had occurred. As it has been discussed earlier that this is an issue typically associated with alkylation of less hindered enamines. Once again, the favored face of attack (by~13 kJ/mol) is on the side of the 5-membered ring. Provided there is no interconversion of the vinyl ammonium species to the C-alkylated product, alkylation at nitrogen will not affect the observed $dr$. In order to test this, the reaction mixture was further heated for 2 days. Interestingly, no change was encountered in the observed $dr$ implying that the proportion of vinyl ammonium present remained constant, thus, confirming that there is no significant interconversion between N-alkylated and C-alkylated products.
3.7.2.9 Solubility in $d_3$-MeCN

In order to ensure complete ethylation, an excess of EtI was used. The corresponding peak in the $^1$H NMR spectra [low ppm region is not shown in fig. 43, spectrum 2]; 2H quartet at 2.3 ppm for $CH_3CH_2$, had an integration value approximately 20 times higher than expected. With repetition of the experiment, the level of EtI reduced slightly (~15 times higher than expected). This consistent result suggests that the high levels of EtI observed were not due to practical errors. During the second attempt at ethylation, it was noted that the reaction mixture was not a homogeneous solution. This suggested that some, or all, of the alkylation products were not entirely soluble in $d_3$-MeCN. Presumably, the diastereomeric iminiums $^{292}$ have similar solubilities, and a $dr$ calculated from the relative masses present in the NMR sample is reliable. This experimentally-determined $dr$ (which is in close agreement with computational findings) for the racemic system will be further justified when the ethylation of analogous asymmetric enamine is performed by resolution of this racemic mono-methyl tropane auxiliary via chromatographic separation of derived diastereomeric $\alpha$amino amides.$^{182}$ An $er$ for the resultant aldehyde will be determined which provides an accurate description of the facial preference of this tropane system towards ethylation (the next step of current project).

3.8 Conclusions

3.8.1 A series of 14 dihydropyrimidine-2-thione derivatives were synthesized under metal-catalyst- and solvent-free conditions in good to excellent yields. Several of these compounds inhibit PTPN22 in the mid-micromolar range, with the best hit being compound $^{248}$d ($IC_{50}=$14 µM). Based on these results structure-activity relationships (SARs) were studied which indicated that these compounds could be promising leads for the future development of more potent inhibitors of PTPN22.
3.8.2 Synthesis of twelve new aryl thiourea derivatives of 4-aminophenazone which is a non-steroidal anti-inflammatory drug (NSAID) was accomplished and all the derivatives were characterized by $^1$H-NMR, $^{13}$C-NMR and FT-IR spectroscopy. The purity of the synthesized compounds was also ascertained by elemental analysis.

Different biological activities were tested, namely, alkaline phosphatase inhibition activity in the intestine of calf and radical scavenging activity, for both of which showed the 2-methyl derivative 252b of the series proved to be as the lead member. Cytotoxic activity was carried out using brine shrimp assay which presented the 3-nitro 252i derivative as the active member of the synthesized series. Besides these activities kinetic studies of the active member in terms of IC$_{50}$ value, i.e., 2-methyl derivative 252b were conducted. The kinetic studies determined the inhibition mechanism of this compound 252b to be non-competitive inhibitor of calf intestinal alkaline phosphatase, i.e., it lowers the concentration of enzyme by showing noncompetitive binding mode with enzyme.

3.8.3 The synthesis, characterization and biological evaluation of some benzamides derivatives of phenazone were performed. The obtained data showed that some of these compounds were potent inhibitors of both APs and e5-NT. The compounds tested against four APs yielded four inhibitors of h-GCAP, five inhibitors of h-IAP, eight inhibitors of h-PLAP and all as inhibitor of h-TNAP. Most of the compounds revealed more potent inhibition of e5-NT than of APs even at lower concentrations. Compounds 256c, 256g, 256h and 256i are of great interest as they inhibit e5-NT, which may be employed to inhibit total production of adenosine from AMP. The new and potent inhibitors can now be used to study the potential e5-NT as a novel drug target.

3.8.4 A new series of ferrocene-derived aryl thioureas was synthesized and its effect on locomotor activity was studied in mice at two different doses which were 25 and 50mg. Two compounds 261d (4-methoxy-substituted) and 2619g (3-chlorosubstituted) were found to be active among the series. Results obtained from the behavioral assays showed that both of these compounds displayed significant sedative effect and greatly depressed locomotor activity comparable to the standard drug, diazepam at both doses. Furthermore, the computational profiling of these compounds revealed that they crossed the blood brain barrier with a probability of 0.9563 which is highly significant. The in silico screening of these compounds for their pharmacokinetic profiling demonstrated that these compounds possess maximum human intestinal absorption value of 0.9656 supporting its candidacy to be used orally, as tablet, syrup or capsule. The computational studies further proved that these compounds can be safely employed for long-term usage. This is due to their non-AMES toxicity because of which they are considered as non-carcinogenic/mutagenic compounds.

3.8.5 Isomeric chlorobenzoyl thiourea derivatives were synthesized. Among the series, the molecular and crystal structures of the novel species 1-(2-chlorobenzoyl)thiourea was strongly determined by intramolecular and intermolecular hydrogen bond. The formation of an intramolecular C=O...H–N2 hydrogen bond is promoted by an IpO $\rightarrow$ 6*(N2–H) hyperconjugative interaction that favors the adoption of the S conformation of the 1-acyl thiourea group. Low
frequency values observed in the vibrational spectra (infrared and Raman) for the $\nu$(C=O) (1684 cm$^{-1}$) and $\nu$(NH$_2$) (3114 cm$^{-1}$) stretching modes are clear manifestations of this interaction. The topological analysis clearly identify two different intermolecular N–H...S=C hydrogen bonds forming pseudo eight membered rings between adjacent molecules. The NBO analysis allowed to characterize the hyperconjugative $lpS \rightarrow \sigma^*(N–H)$ remote interaction as important factor of covalency for these bonds, respectively. Heterocyclization of the isomeric chloro benzoyl thioureas with different cyclizing agents such as alpha halo carbonyl compounds, i.e, ethyl-2-chloroacetoacetate, ethylbromopyruvate, 2,4-dibromoacetophenone and diethyl oxalate resulted in the formation of a series of new 2-iminothiazoline derivatives that were successfully characterized by FT-IR, $^1$H-NMR, $^{13}$C-NMR, LC-MS and CHNS elemental analyses. The synthesized compounds were tested against *leishmania* major and compound 266c with 76.4 ± 1.2 % was found to be the lead member.

3.8.6 Racemic monomethyl tropane bicyclic auxiliary ($\pm$) 270 was synthesized and alkylation of its corresponding aldenamine ($\pm$) 292 was explored. $C$-alkylation of enamine ($\pm$) 286 occurred (besides the undesirable $N$-alkylation of enamine ($\pm$) 293 which was consistently observed and the full extent of which remains to be probed) with good levels of diastereofacial selectivity; analysis of the $^1$H-NMR revealed a 64:36 dr for the resultant iminiums indicating that the presence of embedded 5membered pyrrolidine ring had influence on facial selectivity as it impacted the relative position of exocyclic double bond in the precise ground state structural orientation of enamine species (i.e., it sits more over the six-membered ring exposing the face of 5-membered ring) and, hence, directed alkylation towards its face. These experimental results are in good agreement with the computationally-determined results and provide valuable insight into the origins of the observed diastereomeric induction.

3.9 Future Plan

Docking studies for the active members of 4,4,6-trimethyl-1-phenyl-3,4dihydropyrimidine-2($1H$)thiones, 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-$1H$pyrazol-4-yl)-3-phenylthioureas and 1-(4-ferrocenylaroyl)-3-phenylthioureas will be carried out based on their specific biological activities. Besides this, the new and potent inhibitors of $N$-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-$1H$-pyrazol-4-yl) benzamides will be used to investigate the potential e5-NT as a novel drug target.

Resolution of racemic monomethyl tropane auxiliary via chromatographic separation of derived diastereomeric $\alpha$-amino amides$^{182}$ will be executed to find the *er* of the resultant aldehyde.
4. Experimental

4.1 General

4.1.1 Reaction Conditions and Materials

Reactions requiring anhydrous conditions were performed using flame-dried glassware under an inert atmosphere of nitrogen and/or argon. Deuterated CD$_3$CN (0.75mL) glass ampules purchased from Sigma Aldrich were opened under an inert Ar atmosphere and distilled over CaH$_2$ prior to use. Diethylene glycol was also purged with Ar for few hours before using in reaction. Alkyl halides were passed through several plugs of basic alumina immediately prior to their use in alkylation reaction. Solids were dried to completeness by keeping them under high vacuum using a high vacuum (less than 0.1 mbar) at rt for overnight before their use. Bulb-tobulb distillation was carried out using a Kugelrohr apparatus. All other reagents were used as received.

4.1.2 Solvents

All the solvents (CH$_3$)$_2$CO, CH$_2$Cl$_2$, CH$_3$CN, C$_5$H$_5$OH, Et$_2$O, pyridine, petroleum ether (30-40ºC), EtOAc and MeOH were purchased from Sigma Aldrich and were dried and purified by following the standard methods.$^{183}$ Where necessary, the solvents were dried over 4 Å MS, then degassed and dried over activated alumina under nitrogen.

4.1.3 Chromatography

Silica gel aluminum backed plates (Kieselgel 60 F$_{254}$) of TLC (thin layer chromatography) were employed for reaction monitoring. The plates were visualized using ultraviolet light of $\lambda_{\text{max}}$ 254 and 260 nm and were developed in vanillin and basic potassium permanganate stain solutions. Column chromatography was performed using the solvent systems indicated. The stationary phase engaged was silica gel (SiO$_2$, 0.04-0.6mm particle size, Kieselgel 60), or as otherwise indicated [neutral alumina (Al$_2$O$_3$) or Florisil$^\text{®}$]. Where required, the silica gel was deactivated by stirring silica overnight in 20% Et$_3$N in petroleum ether or otherwise by direct usage of 20% Et$_3$N in solvent systems employed for following reaction profiles. The Petroleum ether refers to the low boiling petrol fraction, i.e., between 30-40 ºC.
4.1.4 Analysis

Melting points are uncorrected and were determined using digital Gallenkamp (Sanyo) model MPD BM 3.5 and Reichert melting point apparatus. Infrared spectra were recorded using a Bruker Tensor 27 FTIR spectrometer (in ATR mode) as either neat sample, thin films in CH₂Cl₂ / CHCl₃ with the absorption peaks denoted as: br (broad), s (strong), m (medium), w (weak). FT-Raman spectra of the powdered solid sample were recorded in the region of 4000-100 cm⁻¹ using a Bruker IFS 66v spectrometer equipped with Nd:YAG laser source operating at 1.064 µm line width with 200mW power of spectral width 2 cm⁻¹. ¹H and ¹³C-NMR spectra were recorded in CDCl₃ at ambient temperature (unless stated otherwise) using Bruker NMR spectrometers of different field strengths, i.e., 500 MHz, 400 MHz, 300 MHz and 200 MHz spectrometers. Chemical shifts are given in parts per million (ppm) and were referenced to residual peaks; the multiplicity of each signal is designated using the following abbreviations: s, singlet; br s, broad singlet; d, doublet; q, quartet; dd, doublet of doublets, q, m, multiplet. Where diastereoselectivity is quoted, this was determined from isolated corresponding signals of each diastereomer in the crude ¹HNMR. Various 2D NMR experiments (COSY, DEPT, HSQC, HMQC and NOE) were carried out for ¹H and ¹³C-NMR peak assignments. Elemental analyses were conducted using CHNS 932 LECO instrument. MS were recorded using an EI source of (70 eV) on Agilent technologies 6890N (GC) and an inert mass selective detector 5973 mass spectrometer. High-resolution mass spectra were obtained by electrospray ionization (ESI); values are quoted as ratio of mass to charge (m/z) in Daltons, and intensities of assignable peaks observed are quoted as a percentage value.

4.2 General procedure for the synthesis of substituted 4,4,6-trimethyl-1-phenyl3,4-dihydropyrimidine-2(1H)-thione (248 a-n)

Suitably substituted anilines 246 (1.0 g, 1.0 mmol) were added portion wise to a stirred suspension of potassium thiocyanate (0.097 g, 1.0 mmol) in 4-methylpent-3-en-2-one 247 (0.098 g, 1.2 mmol) containing 1-2 drops of HCl, at room temperature. The reaction mixture was heated at 50-60°C for 3-5 h, and the progress was followed by TLC. On completion, the reaction mixture was cooled to room temperature and poured into ice-water. The precipitated compounds were recrystallized from ethanol to afford the pure dihydropyrimidine-2-thiones 248 a-n in good to excellent yields.
4.2.1 4,4,6-Trimethyl-1-α-tolyl-3,4-dihydroprymidine-2(1H)-thione (248a)

Brown amorphous solid; Yield: 90%; Rf: 0.33 (EtOAc: Pet. Ether, 1:4); m.p.: 100-101°C; FTIR (ATR, cm⁻¹): 3251 (NH), 3050 (=C-H stretch), 1691 (CS-NH), 1523 (C=C), 1285 (C-N): ¹H-NMR ((CD₃)₂SO, 300 MHz): δ 8.93 (s, 1H, N-H), 7.16-7.42 (m, 5H, ArH), 4.95 (s, 1H, Csp²-H), 2.50 (s, 3H, Ar-CH₃), 1.44 (s, 3H, Csp²-CH₃) 1.29 (s, 3H, CH₃), 1.25 (s, 3H, CH₃); ¹³C-NMR ((CD₃)₂SO, 75 MHz): δ 176.66 (CSNH), 143.0 (C≡C-), 132.9, 131.4, 130.9, 130.3, 129.7, 128.7, 110.2 (-H-C≡C-), 52.0 ((CH₃)₂-C), 31.0 ((CH₃)₂-C), 27.5 (-C-CH₃), 20.61 (Ar-CH₃). LC-MS (m/z) %: [M+K]⁺ 284.5 (100%). Anal. Calc. for C₁₄H₁₈N₂S: C 68.27, H 7.35, N 11.34, S 13.03; found: C₁₄H₁₈N₂S: C 68.27, H 7.35, N 11.34, S 13.03.

4.2.2 4,4,6-Trimethyl-1-β-tolyl-3,4-dihydroprymidine-2(1H)-thione (248b)

Yellow crystalline solid; Yield: 88%; Rf: 0.36 (EtOAc: Pet. Ether, 1:4); m.p.: 139-140°C; FTIR (ATR, cm⁻¹): 3345 (NH), 3075 (=C-H stretch), 1695 (CS-NH), 1527 (C=C), 1287 (C-N): ¹H-NMR ((CD₃)₂CO, 300 MHz): δ 7.71 (s, 1H, N-H), 7.24-7.01 (m, 5H, Ar-H), 4.97 (s, 1H, Csp²-H), 2.35 (s, 3H, Ar-CH₃), 1.50 (s, 3H, Csp²-CH₃), 1.39 (s, 3H, CH₂), 1.35 (s, 3H, CH): ¹³C-NMR ((CD₃)₂CO,
75 MHz): δ 179.16 (CSNH), 142.9 (C=0), 138.4, 133.4, 129.1, 128.3, 111.2 (CH=CH-), 53.9 ((CH$_3$)$_2$-), 32.6 ((CH$_3$)$_2$-C), 27.9 (C=CH$_3$), 20.5 (ArCH$_3$). LC-MS (m/z) %: [M+K]$^+$ 284.5 (100%). Anal. Calc. for C$_{14}$H$_{18}$N$_2$S: C 68.25, H 7.37, N 11.33, S 13.04; found: C 68.28, H 7.35, N 11.34, S 13.02.

### 4.2.3 1-(2,4-Dimethylphenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248c)

![Structure of 1-(2,4-Dimethylphenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248c)]

White crystalline solid; Yield: 85%; R$_f$: 0.32 (EtOAc: Pet. Ether, 1:4); m.p.: 150-151°C; FTIR (ATR, cm$^{-1}$): 3320 (NH), 3082 (=C-H stretch), 1697 (CS=NH), 1535 (C=C), 1290 (C=N). $^1$H-NMR (CD$_3$)$_2$CO, 300 MHz): δ 7.72 (s, 1H, N-H), 7.08-6.89 (m, 3H, Ar-H), 4.97 (q, 1H, $J=3$ Hz, Csp$^2$-H), 2.31 (s, 3H, Ar-CH$_3$), 2.17 (s, 3H, Ar-CH$_3$), 1.46 (d, 1H, $J=3$ Hz, Csp$^2$-CH$_3$), 1.44 (s, 3H, CH$_3$), 1.39 (s, 3H, CH$_3$); $^{13}$C-NMR ((CD$_3$)$_2$CO, 75 MHz): δ 177.2 (CSNH), 138.1 (-C=0), 137.5, 136.5, 131.8, 130.8, 129.9, 128.8, 109.2 (-CH=CH-), 51.8 ((CH$_3$)$_2$-), 30.8 ((CH$_3$)$_2$-C), 20.2 (-C-CH$_3$), 19.6 (2×Ar-CH$_3$); GC-MS: 260 (M$^+$), 245 (100%), 163, 79. Anal. Calc. for C$_{15}$H$_{20}$N$_2$S: C 69.14, H 7.79, N 10.72, S 12.35; found: C 69.17, H 7.75, N 10.71, S 12.37.

### 4.2.4 1-(2-Methoxyphenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248d)
Brown amorphous solid; Yield: 86%; Rf: 0.43 (EtOAc: Pet Ether, 1:4); m.p.: 141-142°C; FTIR (ATR, cm⁻¹): 3360 (NH),

H₃CS 3070 (=C-H stretch), 1699 (CS-NH), 1575 (C=C), 1299 (C=OCH₃ N): ¹H-NMR (CDCl₃, 300 MHz): 6.803 (s, 1H, N-H), 7.07-6.35 (m, 5H, Ar-H), 4.45 (s, 1H, Csp²-H), 3.52 (s, 3H, Ar-OCH₃), 1.54 (s, 3H, Csp²-CH₃) 1.19 (s, 3H, CH₃), 1.10 (s, 3H, CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 185.7 (CSNH), 136.0 (-C=C-), 135.1, 130.0, 127.4, 125.3, 121.7, 121.2, 108.2 (-H-C=C-), 58.9 ([CH₃]₂-), 55.9 (Ar-OCH₃), 35.0 ([CH₃]₂-C), 32.0 (-C-CH₃). LC-MS (m/z) %: [M+H]⁺ 263.6 (100%). Anal. Calc. for C₁₄H₁₈N₂O₃: C 64.03, H 6.97, N 10.62, O 6.18, S 12.20; found: C 64.07, H 6.93, N 10.64, O 6.14, S 12.22.

4.2.5 1-(4-Methoxyphenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248e)

H₃CS 3C 3 1:4); m.p.: 163-164°C; FTIR (ATR, cm⁻¹): 0.47 (EtOAc: Pet Ether, ); 3355 (NH), 3045 (=C-H stretch), 1700 (CS-NH), 1585 (C=C), 1301 (C-N): ¹H-NMR (CDCl₃, 300 MHz): δ 7.81 (s, 1H, N-H), 7.65-6.17 (m, 5H, Ar-H), 4.61 (s, 1H, Csp²-H), 3.71 (s, 3H, Ar-OCH₃), 1.64 (s, 3H, Csp²OCH₃CH₃) 1.24 (s, 3H, CH₃), 1.21 (s, 3H, CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 186.5 (CSNH), 137.9 (C=C-), 134.7, 126.3, 121.9, 120.2, 111.2 (-H-C=C-), 57.9 ([CH₃]₂-C-), 54.6 (ArOCH₃), 33.8 ([CH₃]₂-), 32.4 (-
C-CH₃). LC-MS (m/z) %: [M+H]⁺ 263.6 (100%). Anal. Calc. for C₁₄H₁₈N₂O₅S: C 64.05, H 6.96, N 10.64, O 6.15, S 12.21; found: C 64.03, H 6.93, N 10.66, O 6.16, S 12.20.

4.2.6 1-(2-Fluorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248f)

```
\[ \text{CH} \]
\[ \text{\text{N}} \]
\[ \text{\text{H}_3\text{C}} \]

\(^3\) Brown crystalline solid; Yield: 81%; Rᵣ: 0.41 (EtOAc: Pet. Ether, 1:4); m.p.: 118-119°C; FTIR (ATR, \text{cm}⁻¹): 3321 (NH), 3092 (=C-H stretch), 1689 (CS-NH), 1583 (C=C), 1347 (C-N): \(^1\text{H}-\text{NMR} (\text{CDCl}_3, 300 \text{MHz}): \delta 8.02 (s, 1H, N-H), 7.15-6.27 (m, 5H, Ar-H), 4.39 (s, 1H, Csp²-H), 1.59 (s, 3H, Csp²-CH₃) 1.21 (s, 3H, CH₃), 1.19 (s, 3H, CH₃);

\(^{13}\text{C}-\text{NMR} (\text{CDCl}_3, 75 \text{MHz}): \delta 183.5 (\text{CSNH}), 136.9 (-\text{C}=-\text{C}-), 134.1, 132.4, 126.8, 126.0, 121.4, 120.9, 110.2 (-\text{H}-\text{C}=-\text{C}-), 56.0 ((\text{CH₃})₃-\text{C}-), 33.3 ((\text{CH₃})₂-\text{C}-), 32.7 (\text{C}-\text{CH₃}).
```


4.2.7 1-(4-Bromo-2-fluorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)thione (248g)

```
\[ \text{CH} \]
\[ \text{\text{N}} \]
\[ \text{\text{H}_3\text{C}} \]

\(^3\) Off white amorphous solid; Yield: 80%; Rᵣ: 0.39 (EtOAc: Pet. Ether, 1:4); m.p.: 169-170°C; FTIR (ATR, \text{cm}⁻¹): 3348 (NH), 3063 (=C-H stretch), 1679 (CS-NH), 1615 (C=C), 1349 (C-N): \(^1\text{H}-\text{NMR (CDCl}_3, 300 \text{MHz}): \delta 8.22 (s, 1H, N-H), 7.27-6.23 (m, 5H, Ar-H), 4.33 (s, 1H, Csp²-H), 1.52 (s, 3H, Csp²-CH₃) 1.27 (s, 3H, CH₃), 1.24 (s, 3H, CH₃);

\(^{13}\text{C}-\text{NMR} (\text{CDCl}_3, 75 \text{MHz}): \delta 184.6 (\text{CSNH}), 136.4 (-\text{C}=-\text{C}-),
```
136.0, 135.9, 133.8, 129.9, 128.7, 127.3, 111.7 (-H-C=CH), 57.9 ((CH₃)₂-C-), 34.8 ((CH₃)₂-C-),
33.8 (-C(CH₃)₃), LC-MS (m/z) %: [M+H]⁺ 330.1 (100%). Anal. Calc. for C₁₃H₁₃BrFN₂S: C 47.63, H 4.09, Br 24.75, F 5.29, N 8.93, S 9.32; found: C 47.66, H 4.06, Br 24.79, F 5.25, N 8.95, S 9.30.

4.2.8 1-(2-Chlorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248h)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Description</th>
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</table>
| White crystalline solid; Yield: 92%; Rf: 0.31 (EtOAc: Pet. Ether, 1:4); m.p.: 123-124°C; FTIR (ATR, cm⁻¹): 3390 (NH), 3029 (⁻C-H stretch), 1645 (CS-NH), 1576 (C=C), 1297 (C-N): ¹H-NMR (CDCl₃, 300 MHz): δ 7.88 (s, 1H, N-H), 7.22-6.45 (m, 5H, Ar-H), 4.25 (s, 1H, Csp²-H), 1.41 (s, 3H, Csp²-CH₃) 1.28 (s, 3H, CH₃), 1.24 (s, 3H, CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 187.5 (CSNH), 138.1 (-C=C-), 137.3, 135.2, 134.7, 131.7, 130.4, 129.8, 110.6 (-H-C=CH), 57.4 ((CH₃)₂-C-), 34.2 ((CH₃)₂-C-), 33.4 (-C(CH₃)₃), LC-MS (m/z) %: [M+H]⁺ 267.4 (100%). Anal. Calc. for C₁₃H₁₃ClN₂S: C 58.97, H 5.23, Cl 13.26, N 10.54, S 12.01; found: C 58.94, H 5.27, Cl 13.28, N 10.53, S 12.00.

4.2.9 1-(3-Chlorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248i)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Description</th>
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<tbody>
<tr>
<td>Yellow crystalline solid; Yield: 84%; Rf: 0.44 (EtOAc: Pet. Ether, 1:4); m.p.: 113-114°C; FTIR (ATR, cm⁻¹): 3393 (NH), 3011 (⁻C-H stretch), 1645 (CS-NH), 1576 (C=C), 1297 (C-N): ¹H-NMR (CDCl₃, 300 MHz): δ 7.88 (s, 1H, N-H), 7.22-6.45 (m, 5H, Ar-H), 4.25 (s, 1H, Csp²-H), 1.41 (s, 3H, Csp²-CH₃) 1.28 (s, 3H, CH₃), 1.24 (s, 3H, CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 187.5 (CSNH), 138.1 (-C=C-), 137.3, 135.2, 134.7, 131.7, 130.4, 129.8, 110.6 (-H-C=CH), 57.4 ((CH₃)₂-C-), 34.2 ((CH₃)₂-C-), 33.4 (-C(CH₃)₃), LC-MS (m/z) %: [M+H]⁺ 267.4 (100%). Anal. Calc. for C₁₃H₁₃ClN₂S: C 58.97, H 5.23, Cl 13.26, N 10.54, S 12.01; found: C 58.94, H 5.27, Cl 13.28, N 10.53, S 12.00.</td>
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</tr>
</tbody>
</table>
H₃C-S
stretch), 1676 (CS-NH), 1579 (C=C), 1283 (C-N): ¹H-NMR
((CD₂)₂CO, 300 MHz): δ 8.06 (s, 1H, N-H), 7.02-6.30 (m, 5H, Ar-
H), 4.36 (s, 1H, Csp²-H), 1.47 (s, 3H, Csp²-CH₃) 1.21 (s, 3H, CH₃),
1.19 (s, 3H, CH₃); ¹³C-NMR ((CD₂)₂CO, 75 MHz): δ 188.2 (CSNH), 135.1
(-C=), 134.0, 132.9, 131.3, 129.7, 129.4, 128.8, 108.7 (-H-C=C-), 56.2 ((CH₃)₂-C-), 34.7 ((CH₃)₂-),
32.5 (-C-CH₃). LC-MS (m/z) %: [M+H]⁺ 267.4 (100%). Anal. Calc. for C₁₃H₁₅ClN₂S: C 58.67, H
5.53, Cl 13.07, N 10.74, S 12.00; found: C 58.69, H 5.54, Cl 13.04, N 10.75, S 12.02.

4.2.10 1-(4-Chlorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248j)

H₃C

Brown amorphous solid; Yield: 90%; Rᵣ: 0.42 (EtOAc: Pet. Ether,
1:4); m.p.: 148-149°C; FTIR (ATR, cm⁻¹): 3397 (NH), 3093 (=C-H
H₃CS
stretch), 1685 (CS-NH), 1595 (C=C), 1300 (C-N): ¹H-NMR
(CDCl₃, 300 MHz): δ 8.11 (s, 1H, N-H), 7.02-6.41 (m, 5H, Ar-H),
4.46 (s, 1H, Csp²-H), 1.55 (s, 3H, Csp²-CH₃) 1.25 (s, 3H, CH₃),
1.23 (s, 3H, CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 185.1 (CSNH),
136.4 (-C-), 132.0, 131.7, 129.3, 127.7, 106.7 (-H-C=C-), 53.2 ((CH₃)₂-C-), 32.7
((CH₃)₂-), 31.9 (-C-CH₃). LC-MS (m/z) %: [M+H]⁺ 267.4 (100%). Anal. Calc. for C₁₃H₁₅ClN₂S: C
58.65, H 5.55, Cl 13.13, N 10.63, S 12.05; found: C 58.67, H 5.54, Cl 13.12, N 10.64, S 12.04.

4.2.11 1-(2,3-Dichlorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)-thione (248k)

H₃C

Brown crystalline solid; Yield: 81%; Rᵣ: 0.33 (EtOAc: Pet. Ether,
1:4); m.p.: 130-131°C; FTIR (ATR, cm⁻¹): 3401 (NH), 3087 (=C-H
H$_3$C$^\text{S}$ stretch), 1694 (CS-NH), 1598 (C=C), 1307 (C-N): $^1$H-NMR

Cl

((CD$_3$)$_2$CO, 300 MHz): $\delta$ 7.99 (s, 1H, N-H), 7.64-7.29 (m, 5H, Ar-H), 5.04 (q, 1H, $J=3$ Hz, Csp$^2$-H), 1.51 (d, 3H, $J=3$ Hz, Csp$^2$-CH$_3$)

1.42 (s, 6H, 2×CH$_3$); $^{13}$C-NMR ((CD$_3$)$_2$CO, 75 MHz): $\delta$ 183.1 (CSNH), 137.5 (-C=), 137.1, 135.9, 132.8, 128.5, 128.1, 127.6, 107.9 (-H-C=C-), 57.1 ((CH$_3$)$_2$C=), 33.6

(CH$_3$)$_2$-), 32.7 (-C-CH$_3$). LC-MS (m/z %): [M+HCOOH]$^+$ 347 (100%); GC-MS: 301 (M$^+$), 265 (100%), 249, 226, 185. Anal. Calc. for C$_{13}$H$_{14}$Cl$_2$N$_2$S: C 51.95, H 4.56, Cl 23.50, N 9.33, S 10.65; found: C 51.98, H 4.53, Cl 23.54, N 9.31, S 10.63.

4.2.12 1-(3-Chloro-4-fluorophenyl)-4,4,6-trimethyl-3,4-dihydropyrimidine-2(1H)thione (248l)

Yellow amorphous solid; Yield: 80%; R$_f$: 0.35 (EtOAc: Pet. Ether, 1:4); m.p.: 102-103°C; FTIR (ATR, cm$^{-1}$): 3333 (NH), 3020 (=C-H), 1683 (CS-NH), 1598 (C=C), 1307 (C-N): $^1$H-NMR

Cl

(CDCl$_3$, 300 MHz): $\delta$ 7.92 (s, 1H, N-H), 7.25-6.47 (m, 5H, Ar-H), 4.37 (s, 1H, Csp$^2$-H), 1.56 (s, 3H, Csp$^2$-CH$_3$) 1.23 (s, 3H, CH$_3$), F

1.20 (s, 3H, CH$_3$); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 186.9 (CSNH), 135.9 (-C=), 135.5, 134.4, 130.8, 128.9, 127.4, 126.9, 109.2 (-H-C=C-), 57.1 ((CH$_3$)$_2$-C-), 33.8

(CH$_3$)$_2$-), 32.0 (-C-CH$_3$). LC-MS (m/z %): M$^+$ 284.5 (100%). Anal. Calc. for C$_{13}$H$_{14}$ClFN$_2$S: C 54.96, H 4.83, Cl 12.88, F 6.24, N 10.86, S 10.24; found: C 54.99, H 4.81, Cl 12.85, F 6.27, N 10.88, S 10.21.

4.2.13 4,4,6-Trimethyl-1-(4-nitrophenyl)-3,4-dihydropyrimidine-2(1H)-thione
4.2.14 4,4,6-Trimethyl-1-(4-naphthalen-2-yl)-3,4-dihydropyrimidine-2-\(\text{H}\)-thione (248n)

White amorphous solid; Yield: 75%; Rf: 0.49 (EtOAc: Pet. Ether, 1:4); m.p.: 173-174°C; FTIR (ATR, cm\(^{-1}\)): 3258 (NH), 1653 (CS\(-\text{NH}\)), 1601 (C=C), 1327 (C\(-\text{N}\)); \(^{1}\)H-NMR ((CD\(_3\))\(_2\)SO, 300 MHz): \(\delta\) 9.00 (s, 1H, N-H), 7.99-7.70 (m, 3H, Ar-H), 7.70-7.38 (dd, 4H, J = 9 Hz, Ar-H), 5.02 (s, 1H, Csp\(^2\)-H), 1.43 (s, 3H, Csp\(^2\)-CH\(_3\)), 1.36 (s, H, CH\(_3\)), 1.33 (s, 3H, CH\(_3\)); \(^{13}\)C-NMR ((CD\(_3\))\(_2\)SO, 75 MHz): \(\delta\) 184.9 (CSNH), 138.7 (C\-=C-), 136.3, 135.2, 131.1, 128.8, 127.6, 125.7, 124.9, 123.8, 122.4, 121.5, 108.7 (C\-=C-), 109.5 ((CH\(_3\))\(_2\)-C), 54.7 ((CH\(_3\))\(_2\)-C), 33.6 (C\(-\text{CH}_3\)). LC-MS (m/z): [M+2H]\(^+\) 284.5 (100%). Anal. Calc. for C\(_{17}\)H\(_{18}\)N\(_2\)S: C 71.30, H 7.40, N 9.74, S 11.55; found: C 71.32, H 7.41, N 9.73, S 11.53.
4.3 General procedure for the synthesis of substituted 1-(1,5-dimethyl-3-oxo-2phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-phenylthioureas (252 a-l)

The substituted anilines 249 (0.1g, 1.0 mmol) were dissolved in methanol (10 mL) in a round bottom flask, fitted with a reflux condenser. The whole assembly was placed in an ice bath. Carbon disulfide (0.06 mL, 1.0 mmol) and ammonia solution (0.03 mL, 1.0 mmol) were added slowly. The temperature of the reaction mixture was maintained at 15°C (not allowed to rise above 30°C) and stirred for 12h. After stirring, the contents of the flask were transferred to a beaker. A solution of lead nitrate was prepared in water and added to the above mixture and stirred overnight. The precipitates of lead sulfide formed alongwith isothiocyanate 250 a-l. The isothiocyanate formed was isolated by steam distillation and filtrate was collected. The isothiocyanates were extracted from the filtrate by using ethyl acetate, which is evaporated under reduced pressure to get isothiocyanates. The isothiocyanates were then set to reflux with 4-aminophenazone 251 (0.2 g, 1.0 mmol) for 3h to get the crude product which was purified by recrystallization with aqueous ethanol.

4.3.1 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3phenylthiourea (252a)

Yellow amorphous solid; Yield: 80%; Rf: 0.47 (nHexane: CHCl₃, 4:1); m.p.: 67-68°C; FTIR (ATR, cm⁻¹): 3358, 3250 (N-H), 1231 (C=S), 3153 (CSP₂-H), 1685 (C=O); ¹H-NMR (CDCl₃, 300 MHz): δ 9.29 (s, 1H, NH-Ar), 8.16 (s, 1H, NH-pyrazole), 7.89-7.15 (m, 10H, Ar-H), 3.56 (s, 3H, -NCH₃), 2.43 (s, 3H, CH₃-Csp²); ¹³C-NMR (CDCl₃, 75 MHz): δ 183.2 (C=S), 165.3 (C=O pyrazole), 147.2, 138.2, 137.4, 129.4, 129.0, 128.7, 126.7, 125.8, 124.6, 33.6, 12.9. Anal. Calc. for C₁₈H₁₈N₄OS: C 63.85, H 5.39, N 16.69, S 9.40; Found: C 63.81, H 5.43, N 16.73, S 9.30.
4.3.2 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-otolylthiourea (252b)

White amorphous solid; Yield: 80%; Rf: 0.42 (n-Hexane: CHCl₃, 4:1); m.p.: 89-90°C; FTIR (ATR, cm⁻¹): 3348, 3230 (N-H), 1238 (C=S), 3128 (C-SP²-H), 1700 (C=O); ¹H-NMR (CDCl₃, 300 MHz): δ 11.12 (s, 1H, NHAr), 8.91 (s, 1H, NH-pyrazole), 7.79-6.65 (m, 9H, Ar-H), 4.06 (s, 3H, NCH₃), 3.15 (s, 3H, CH₃-Csp²), 2.13 (s, 3H, Ar-CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 184.9 (C=S), 166.1 (C=O pyrazole), 148.3, 137.3, 129.9, 127.6, 126.7, 122.0, 121.1, 120.3, 33.7, 19.3, 13.2. Anal. Calc. for C₁₉H₂₀N₄OS: C 64.57, H 5.90, N 15.84, S 9.10; Found: C 64.51, H 5.96, N 15.94, S 9.00.

4.3.3 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-mtolylthiourea (252c)

C₃H₃ Yellow crystalline solid; Yield: 79%; Rf: 0.41 (n-Hexane: CHCl₃, 4:1); m.p.: 74-75°C; FTIR (ATR, cm⁻¹): 3343, 3215 (N-H), 1229 (C=S), 3141 (C-SP²-H), 1701 (C=O); ¹H-NMR (CDCl₃, 300 MHz): δ 10.34 (s, 1H, NH-Ar), 8.59 (s, 1H, NH-pyrazole), 7.86-6.66 (m, 9H, Ar-H), 4.11 (s, 3H, NCH₃), 3.05 (s, 3H, CH₃-Csp²), 1.35 (s, 3H, Ar-CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 183.1 (C=S), 167.2 (C=O pyrazole), 146.3, 135.6, 128.2, 127.2, 125.3, 122.6, 121.9, 120.7, 33.2, 19.5, 13.0. Anal. Calc. for C₁₉H₂₀N₄OS: C 64.51, H 5.96, N 15.94, S 9.00; Found: C 64.55, H 5.92, N 15.82, S 9.40.

4.3.4 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(3methoxyphenyl) thiourea (252d)

OCH₃ Brown amorphous solid; Yield: 80%; Rf: 0.50 (n-
H$_3$C Hexane: CHCl$_3$, 4:1; m.p.: 95-96°C; FTIR (ATR, cm$^{-1}$): 3315, 3169 (N-H), 1247 (C=S), 3121 (C$_{sp^3}$-H),

H$_3$C1686 (C=O); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 11.72 (s, 1H, NH-Ar), 7.81 (s, 1H, NH-pyrazole), 8.24-7.26 (m, 9H, Ar-H), 4.04 (s, 3H, OCH$_3$), 3.37 (s, 3H, -NCH$_3$), 2.22 (s, 3H, CH$_3$-C$_{sp^3}$); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 188.7 (C=S), 165.9 (C=O pyrazole), 143.3, 135.8, 129.4, 126.4, 125.1, 123.9, 121.5, 60.5, 35.7, 12.9. Anal. Calc. for C$_{19}$H$_{20}$N$_4$O$_2$S: C 61.52, H 5.99, N 15.44, S 8.60; Found: C 61.50, H 6.01, N 15.96, S 8.40.

4.3.5 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(2,4,6-trimethoxyphenyl) thiourea (252e)

Yellow amorphous solid; Yield: 80%; Rf: 0.49 (n-Hexane: CHCl$_3$, 4:1); m.p.: 103-104°C; FTIR (ATR, cm$^{-1}$): 3364, 3254 (N-H), 3155 (C$_{sp^3}$-H), 1703 (C=O); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 10.56 (s, 1H, NH-Ar), 8.01 (s, 1H, NH-pyrazole), 7.76-6.72 (m, 7H, Ar-H), 4.05 (s, 9H, OCH$_3$), 3.72 (s, 3H, -NCH$_3$), 3.11 (s, 3H, CH$_3$-C$_{sp^3}$); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 181.4 (C=S), 165.2 (C=O pyrazole), 146.3, 137.5, 128.4, 127.4, 124.6, 122.9, 120.5, 60.7, 35.1, 12.6. Anal. Calc. for C$_{21}$H$_{24}$N$_4$O$_4$S: C 58.36, H 6.15, N 13.00, S 7.35; Found: C 58.11, H 6.40, N 13.14, S 7.15.

4.3.6 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(3chlorophenyl) thiourea (252f)

Cl White amorphous solid; Yield: 81%; Rf: 0.45 (n-Hexane: CHCl$_3$, 4:1); m.p.: 116-117°C; FTIR (ATR, cm$^{-1}$): 3263,
$^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 187.4 (C=S), 166.9 (C=O pyrazole), 144.3, 135.1, 129.2, 126.6, 124.3, 123.9, 121.5, 35.4, 13.0. Anal. Calc. for C$_{18}$H$_{17}$ClN$_4$OS: C 57.95, H 4.63, N 15.14, S 8.74; Found: C 57.63, H 4.95, N 15.22, S 8.64.

4.3.7 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1$H$-pyrazol-4-yl)-3-(4chlorophenyl) thiourea (252g)

Yellow crystalline solid; Yield: 80%; $R_f$: 0.46 (nHexane: CHCl$_3$, 4:1); m.p.: 109-110°C; FTIR (ATR, cm$^{-1}$): 3220, 3195 (N-H), 1239 (C=S), 3043 (C$_{sp^2}$-H), 1689 (C=O); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 11.00 (s, 1H, NH-Ar), 8.10 (s, 1H, NH-pyrazole), 7.18-6.66 (m, 5H, Ar-H), 7.01 (d, 2H, $J$=8.1Hz, Ar-H), 6.67 (d, 2H, $J$=8.0Hz, Ar-H), 3.91 (s, 3H, CH$_3$-C$_{sp^2}$); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 185.4 (C=S), 163.9 (C=O pyrazole), 142.3, 113.5, 128.4, 127.4, 125.9, 124.9, 121.2, 35.0, 13.3. Anal. Calc. for C$_{18}$H$_{17}$ClN$_4$OS: C 57.90, H 4.66, N 15.10, S 8.70; Found: C 57.84, H 4.74, N 15.19, S 8.60.

4.3.8 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1$H$-pyrazol-4-yl)-3-(2nitrophenyl) thiourea (252h)

Brown amorphous solid; Yield: 78%; $R_f$: 0.44 (n-Hexane: CHCl$_3$, 4:1); m.p.: 131-132°C; FTIR (ATR, cm$^{-1}$): 3329, 3239 (N-H), 1249 (C=S), 3119 (C$_{sp^2}$-H), 1692

$^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 10.63 (s, 1H,
4.3.9 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(3-nitrophenyl)thiourea (252i)

White amorphous solid; Yield: 79%; Rf: 0.40 (nHexane: CHCl₃, 4:1); m.p.: 121-122°C; FTIR (ATR, cm⁻¹): 3378, 3234 (N-H), 1251 (C=S), 3079 (C(sp²)-H), 1697 (C=O); ¹H-NMR (CDCl₃, 300 MHz): δ 10.07 (s, 1H, NH-Ar), 8.29 (s, 1H, NH-pyrazole), 8.35-7.21 (m, 9H, Ar-H), 4.10 (s, 3H, -NCH₃), 3.21 (s, 3H, CH₃-Csp²); ¹³C-NMR (CDCl₃, 75 MHz): δ 189.1 (C=S), 167.2 (C=O pyrazole), 144.1, 138.1, 126.2, 125.0, 124.9, 123.2, 122.3, 33.6. Anal. Calc. for C₁₆H₁₆N₆O₅S: C 56.37, H 4.50, N 18.16, S 8.13; Found: C 56.31, H 4.57, N 18.16, S 8.13.

4.3.10 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(4-nitrophenyl)thiourea (252j)

Yellow crystalline solid; Yield: 78%; Rf: 0.43 (n-Hexane: CHCl₃, 4:1); m.p.: 140-141°C; FTIR (ATR, cm⁻¹): 3325, 3223 (N-H), 1236 (H₃C-C=S), 3055 (C(sp²)-H), 1694 (C=O); ¹H-NMR (CDCl₃, 300 MHz): δ 11.69 (s, 1H, NH-Ar), 7.83 (s, 1H, NH-pyrazole), 8.24-7.26 (m, 9H,
4.3.11 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(3,5-dinitrophenyl) thiourea (252k)

Pale yellow amorphous solid; Yield: 77%; Rf: 0.48 (n-Hexane: CHCl₃, 4:1); m.p.: 129-130°C; FTIR (ATR, cm⁻¹): 3365, 3247 (N-H), 1258 (C=S), 3068 (Csp₂-H), 1691 (C=O); ¹H-NMR (CDCl₃, 300 MHz): δ 10.12 (s, 1H, NH-Ar), 8.23 (s, 1H, NH-pyrazole), 7.78-6.66 (m, 9H, Ar-H), 4.04 (s, 3H, -NCH₃), 3.04 (s, 3H, CH₃-Csp²); ¹³C-NMR (CDCl₃, 75 MHz): δ 188.9 (C=S), 166.2 (C=O pyrazole), 144.5, 144.4, 129.4, 126.4, 125.1, 121.5, 33.6, 12.7. Anal. Calc. for C₁₈H₁₆N₆O₅S: C 56.23, H 4.62, N 18.75, S 8.20; Found: C 56.30, H 4.57, N 18.13, S 8.36.

4.3.12 1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(furan-2-yl) thiourea (252l)

Brown amorphous solid; Yield: 81%; Rf: 0.51 (n-Hexane: CHCl₃, 4:1); m.p.: 147-148°C; FTIR (ATR, cm⁻¹): 3375, 3228 (N-H), 1267 (C=S), 3082 (Csp²-H), 1699 (C=O); ¹H-NMR (CDCl₃, 300 MHz): δ 10.17 (s, 1H, NH-Ar), 8.41 (s, 1H, NH-pyrazole), 7.73-6.45 (m, 8H, Ar-H), 3.57 (s, 3H, -NCH₃), 2.43 (s, 3H, CH₃-Csp²); ¹³C-
NMR (CDCl$_3$, 75 MHz): $\delta$ 188.7 (C=S), 166.2 (C=O pyrazole), 143.2, 136.7, 128.6, 128.1, 126.2, 111.3, 97.6, 33.5, 11.9. Anal. Calc. for C$_{16}$H$_{16}$N$_2$O$_2$: C 58.50, H 4.93, N 17.59, S 9.76; Found: C 58.83, H 4.50, N 17.79, S 9.46.

4.4 General procedure for the synthesis of substituted N-(1,5-dimethyl-3-oxo-2phenyl-2,3-dihydro-1H-pyrazol-4-yl)benzamides (256 a-j)

A calculated amount of suitably-substituted aromatic acids 151a-j (0.1 g, 0.5 mmol) was set to reflux with thionyl chloride (0.03 mL, 0.5 mmol) for the fresh preparation of respective acid halides 152a-j. The acid chlorides 152a-j were added to the solution of 4-aminophenazone 153 (0.1g, 0.5 mmol), dissolved in pyridine (0.03 mL, 0.5 mmol) and the reaction mixture was refluxed for 3 hours. The completion of the reaction was confirmed by TLC. The solids appeared were filtered and recrystallized by aqueous ethanol to afford the purified products 154a-j.

4.4.1 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl)benzamide (256a)

Brownish black amorphous solid; Yield; 89%; $R_f$: 0.40 (EtOAc: Pet Ether, 1:4); m.p.: 133-134°C; FTIR (ATR, cm$^{-1}$): 3140 (CONH), 2967, 2829, (Csp$_3$-H), 1677 (CONH), 1421, 1340 (Csp$_3$-H bending); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 8.09 (m, 5H, Ar-H, s, 1H, N-H), 7.84 (m, 4H, Ar-H), 3.07 (s, 3H), 2.10 (s, 3H); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 173.0 (CONH), 170.8 (C=C-CO-N-), 161.4, 152.2, 134.6, 133.4, 132.3, 130.1, 129.7, 129.3, 128.4, 127.4, 124.7, 35.3 (CH$_3$N-), 10.6 (CH$_3$-C$_{sp^2}$), 12.7 (CH$_3$-Ar-C). LC-MS (m/z) %: M$^+$ 308, 214, 203. Anal. Calc. for C$_{18}$H$_{17}$N$_2$O; C 68.50, H 7.97, N 11.55; Found: C 69.00, H 6.92, N 12.05.

4.4.2 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-2methylbenzamide (256b)

Brown amorphous solid; Yield; 86%; $R_f$: 0.42 (EtOAc: Pet Ether, 1:4); m.p.: 125-126°C; FTIR (ATR, cm$^{-1}$): 3150
4.4.3 N’-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-4-methylbenzamide (256c)

Yellow crystalline solid; Yield: 85%; R<sub>f</sub>: 0.41

CH<sub>3</sub>(EtOAc: Pet Ether, 1:4); m.p.: 183-184°C; FTIR (ATR, cm<sup>-1</sup>): 3145 (CONH), 2954, 2891, (C<sub>sp</sub><sup>3</sup>-H), 1637 (CONH), 1425, 1347 (C<sub>sp</sub><sup>3</sup>-H bending): ¹H-NMR (CDCl<sub>3</sub>, 300 MHz): δ 8.04 (s, 1H, N-H), 7.47-7.40 (m, 4H, Ar-H), 7.33-7.21 (m, 3H, Ar-H), 3.10 (s, 3H), 2.39 (s, 3H), 2.32 (s, 3H); ¹³C-NMR (CDCl<sub>3</sub>, 75 MHz): δ 166.0 (CONH), 161.8 (-C=CO-N-), 149.3, 142.2, 134.6, 130.8, 129.2, 129.1, 127.5, 126.9, 124.3, 109.0, 36.2 (CH<sub>3</sub>-N-), 21.4 (CH<sub>3</sub>-C<sub>sp</sub><sup>3</sup>-), 12.6 (CH<sub>3</sub>-ArC). LC-MS (m/z) %: M<sup>+</sup> 322. Anal. Calc. for C<sub>19</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>; C 73.57, H 5.02, N 12.05; Found: C 72.32, H 5.25, N 13.05.
4.4.4 2-Chloro-$N$-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-$1H$-pyrazole-4-yl) benzamide (256d)

Yellow crystalline solid; Yield; 82%; Rf: 0.39 (EtOAc: Pet Ether, 1:4); m.p.: 103-104°C; FTIR (ATR, cm$^{-1}$): 3150 (CONH), 2991, 2865, (Csp$^3$-H), 1675 (CONH), 1435, 1346 (Csp$^3$-H bending); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 7.64-7.60 (m, 3H, Ar-H), 7.45 (m, 2H, Ar-H), 7.40 (m, 3H, Ar-H), 7.37-7.26 (m, 2H, Ar-H, s, 1H, N-H), 3.13 (s, 3H), 2.41 (s, 3H); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 166.2 (CONH), 161.3 (-C=C-CO-N-), 149.4, 137.4, 134.5, 133.4, 131.4, 129.6, 129.2, 127.4, 126.9, 124.2, 119.7, 108.3, 36.1 (CH$_3$-N), 12.7 (CH$_3$-Csp$^2$-H). LC-MS (m/z) %: M$^+$ 342 (1:3), 214. Anal. Calc. for C$_{18}$H$_{16}$ClN$_3$O$_2$; C 61.97, H 6.02, N 14.40; Found: C 61.25, H 6.72, N 14.42.

4.4.5 4-Chloro-$N$-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-$1H$-pyrazole-4-yl) benzamide (256e)

Yellow crystalline solid; Yield, 86%; Rf: 0.41

(A TR, cm$^{-1}$): 3151 (CONH), 2993, 2876, (Csp$^3$-H), 1674 (CONH), 1425, 1341 (Csp$^3$-H bending); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 7.97-7.94 (m, 2H, Ar-H), 7.55-7.41 (m, 7H, Ar-H, s, 1H, N-H), 3.31 (s, 3H), 2.26 (s, 3H); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 164.6 (CONH), 161.8 (-C=C-CO-N-), 152.9, 136.4, 135.0, 132.4, 129.5, 129.0, 128.4, 126.2, 123.5, 35.9 (CH$_3$-N), 10.9 (CH$_3$-Csp$^2$-H). LC-MS (m/z) %: M$^+$ 342 (1:3), 278, 214. Anal. Calc. for C$_{18}$H$_{16}$ClN$_3$O$_2$; C 60.25, H 7.32, N 14.72; Found: C 61.00, H 6.92, N 14.47.

4.4.6 2-Bromo-$N$-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-$1H$-pyrazole-4-yl) benzamide (256f)
Brown crystals, Yield; 87%; Rf: 0.44 (EtOAc: Pet Ether, 1:4); m.p.: 108-109°C; FTIR (ATR, cm⁻¹): 3151 (CONH), 2990, 2832, (Csp³-H), 1678 (CONH), 1423, 1332 (Csp³-H bending); ¹H-NMR (CDCl₃, 300 MHz): δ 7.81 (s, 1H, N-H), 7.66-7.28 (m, 9H, Ar-H), 3.15 (s, 3H), 2.48 (s, 3H); ¹³C-NMR (CDCl₃, 75 MHz): δ 166.3 (CONH), 161.1 (-C=CO-N-), 36.0 (CH₃-N-), 12.8 (CH₃-Csp₂-), 149.3, 137.2, 134.3, 133.4, 131.5, 129.6, 129.3, 127.5, 124.4, 35.0 (CH₃-N-), 19.9 (CH₃-Csp₂-). GC-MS (m/z): 387 (M⁺), 341, 321 (100%), 202, 119, 91, 56. LC-MS (m/z) %: M⁺ 386 (1:1), 368, 214, 203. Elemental analysis found for C₁₈H₁₅BrN₃O₂; C 59.05, H 4.08, N 9.59; Found: C 59.47, H 4.06, N 9.19.

4.4.7 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-3,5dihydroxybenzamide (256g)

Brownish black amorphous solid; Yield; 82%; Rf: 0.45

(EtOAc: Pet. Ether, 1:4); m.p.: 163-164°C; FTIR (ATR, cm⁻¹): 3159 (CONH), 2927, 2815, (Csp³-H), 1679 (CONH), 1422, 1332 (Csp³-H bending); ¹H-NMR (CDCl₃, 300 MHz): δ 8.79 (s, 1H, N-H), 7.21 (m, 2H, Ar-H), 6.92 (m, 3H, Ar-H), 6.58 (m, 2H, Ar-H), 3.27 (s, 3H); ¹³C-NMR (CDCl₃, 75 MHz): δ 165.6 (CONH), 162.5 (-C=CCO-N-), 158.7, 158.2, 144.7, 142.7, 129.3, 129.0, 126.0, 124.6, 123.4, 123.2, 108.1, 106.3, 36.1 (CH₃-N-), 11.0 (CH₃-Csp₂-). LC-MS (m/z) %: M⁺ 340, 322, 214, 135. Anal. Calc. for C₁₈H₁₇N₃O₄; C 62.76, H 7.00, N 10.01; Found: C 62.00, H 6.76, N 11.01.

4.4.8 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl)-3phenylpropanamide (256h)

Yellow solid; Yield; 84%; Rf: 0.37 (EtOAc: Pet Ether,
H$_3$C$_{1352}$ (Csp$^3$-H bending): $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 8.80 (d, 2H, Ar-H), 8.37 (d, 1H, Ar-H), 8.20 (s, 1H, N-H), 3.55 (2H, t, 9Hz), 1.81 (m, 2H), 3.08 (s, 3H); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 177.3 (CONH), 173.2 (-C=C=CO-N-), 161.0, 149.2, 144.9, 141.7, 140.2, 134.0, 128.5, 128.4, 127.4, 126.8, 123.2, 54.2 (-CO-CH$_3$C-), 35.8 (CH$_3$N-), 26.5 (CH$_3$-Csp$^2$-), 12.3 (-CH$_2$=CH$_2$-Ar-), 12.2 (-CH$_2$=CH$_2$-CH$_2$-). LC-MS (m/z) %: M$^+$ 349, 348, 232, 214. Anal. Calc. for C$_{23}$H$_{21}$N$_3$O$_2$; C 70.85, H 8.66, N 9.50; Found: C 70.18, H 8.83, N 10.00.

4.4.9 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1$H$-pyrazole-4-yl)furan-2-carboxamide (256i)

Red crystalline solid, Yield: 83%; R$_f$: 0.46 (EtOAc: Pet. Ether, 1:4); m.p.: 143-144°C; FTIR (ATR, cm$^{-1}$): 3146 (CONH), 2953, 2896, (Csp$^3$-H), 1634 (CONH), 1455, 1327 (Csp$^3$-H bending): $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 8.52 (s, 1H, N-H), 7.60-7.52 (m, 4H, Ar-H), 7.41-7.36 (m, 3H, Ar-H), 6.49 (d, 1H, C-4-Furoic ring, $J$ = 1.8 Hz), 6.49 (s, 1H), 3.42 (s, 3H), 2.96 (s, 3H); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 161.9 (CONH), 156.2 (C=C=CO-N-), 155.5, 148.7, 145.8, 132.2, 130.1, 130.0, 116.9, 112.2, 107.5, 33.6 (CH$_3$-N-), 14.8 (CH$_3$-Csp$^2$-). GC-MS (m/z): 387 (M$^+$), 341, 321 (100%), 202, 119, 91, 56. LC-MS (m/z) %: M$^+$ 322, 233, 215, 214. Anal. Calc. for C$_{16}$H$_{13}$N$_3$O$_3$; Found: C 63.66, H 5.09, N 15.01; Found: C 63.56, H 5.12, N 15.08.

4.4.10 N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1$H$-pyrazole-4-yl)pivalamide (256j)
Yellow amorphous solid, Yield: 86%; Rf: 0.39 (EtOAc: Pet Ether, 1:4); m.p.: 127-128°C; FTIR (ATR, cm⁻¹): 3250 (CONH), 2978, 2932, (Csp³-H), 1644 (CONH), 1456, 1337
(Csp³-H bending); ¹H-NMR (CDCl₃, 300 MHz): δ 7.45-7.28 (m, 4H, Ar-H), 7.26 (m, 1H, Ar-H), 7.07 (s, 1H, NH), 3.05 (s, 3H), 2.26 (s, 3H), 1.31 (s, 9H, CH₃); ¹³C-NMR (CDCl₃, 75 MHz): δ 177.4 (CONH), 161.6 (-C=C-CO-N), 148.9, 134.8, 129.1, 126.6, 123.8, 39.2 (CH₃-N), 36.4 (CH₃-Csp²-H), 27.6 (-CO-C-CH₃). GC-MS (m/z): 387 (M⁺), 341, 321 (100%), 202, 119, 91, 56. LC-MS (m/z) %: M⁺ 288, 214, 135. Anal. Calc. for C₁₆H₂₁N₃O₂; C 65.20, H 7.79, N 14.93; Found: C 65.82, H 7.43, N 14.67.

4.5 General procedure for the synthesis of substituted 1-(4-ferrocenyIaroyl)-3-phenylthioureas (261a-q)

4-Ferrocenyl benzoyl chloride 258 was synthesized by reacting 4-ferrocenyl benzoic acid (0.9 g, 3.08 mmol) 257 with thionyl chloride at reflux temperature for 2 h. 257 was formed by the reported diazonium method. ¹²The freshly prepared ferrocenyl benzoyl chloride 258 (1.0 g, 3.08 mmol) was then added to a clear solution of KSCN (1 g, 10.30 mmol) in dry acetone and was stirred for 2 h to form the corresponding red color ferrocenyl isothiocyanate 259. This in situ formed ferrocenyl isothiocyanate was subjected to reaction with different substituted anilines 260a-q (0.28 g, 3.08 mmol) at reflux temperature for 4 hours to afford the target compounds 261a-q in good to excellent yield as a result of purification of crude product via recrystallization using ethanol.

4.5.1 1-(4-Ferrocenylaroyl)-3-(2-methylphenyl)thiourea (261a)

Brown amorphous solid; Yield: 87%; Rf: 0.31 (n-
Hexane: EtOAc, 4:1); m.p.: 131-132°C; FTIR
(ATR, cm⁻¹): 3240 (NH), 2962, 2819 (Csp³-H), 1680 (C=O), 1245 (C=S), 486 (Fe-cp); ¹H-NMR
((CD₃)₂SO, 300 MHz): δ 10.02 (s, 1H, CONH), 8.09 (s, 1H, CSNH), 7.49-7.06 (m, 8H, Ar-H), 4.63 (s, 2H, C₅H₄), 4.23 (s, 2H, C₅H₄), 4.02 (s, 5H, C₅H₅), 2.02 (s, 3H); ¹³C-NMR ((CD₃)₂SO, 75 MHz): δ 182.0 (CS), 176.8 (CO), 135.8, 134.9, 132.9, 131.4, 130.8, 130.3, 130.2, 129.2, 128.2, 80.2, 70.3, 69.6, 67.4, 20.5 (Ar-CH₃). LC-MS (m/z)
%: [M+H]$^+$ 455 (100%). Anal. Calc. for $C_{27}H_{28}FeN_2OS$: C 65.39, H 4.65, N 6.50 S 7.14; Found: C 65.37, H 4.68, N 6.52, S 7.11.

4.5.2 1-(4-Ferrocenylaroyl)-3-(4-methylphenyl)thiourea (261b)

Grey amorphous solid; Yield: 88%; $R_f$: 0.33

CH$_3$ (n-Hexane: EtOAc, 4:1); m.p.: 142-143°C; FTIR (ATR, cm$^{-1}$): 3220 (NH), 2922, 2823 (Csp$^3$-H), 1670 (C=O), 1241 (C=S), 482 (Fe-cp): $^1$H-NMR ((CD$_3$)$_2$SO, 300 MHz): δ 10.45 (s, 1H, CONH), 8.23 (s, 1H, CSNH), 7.62 (d, 2H, Ar-H, $J = 6.4$Hz), 7.41 (d, 2H, Ar-H, $J = 6.6$Hz) 7.10 (d, 2H, ArH, $J = 6.3$Hz), 4.61 (s, 2H, C$_5$H$_4$), 4.26 (s, 2H, C$_5$H$_4$), 4.06 (s, 5H, C$_5$H$_5$), 2.04 (s, 3H); $^{13}$C-NMR ((CD$_3$)$_2$SO, 75 MHz): δ 182.4 (CS), 167.4 (CO), 135.4, 134.9, 132.1, 132.0, 130.3, 129.4, 129.1, 127.6, 80.5, 71.2, 69.9, 67.8, 22.8 (Ar-CH$_3$). LC-MS (m/z) %: [M+H]$^+$ 455 (100%). Anal. Calc. for $C_{27}H_{28}FeN_2OS$: C 66.04, H 4.91, N 6.11, S 7.14.

4.5.3 1-(4-Ferrocenylaroyl)-3-(2,4,6-trimethyl phenyl)thiourea (261c)

Brown amorphous solid; Yield: 85%; $R_f$: 0.35

(n-Hexane: EtOAc, 4:1); m.p.: 156-157°C; CH$_3$FTIR (ATR, cm$^{-1}$): 3219 (NH), 2982, 2845 (Csp$^3$-H), 1677 (C=O), 1247 (C=S), 485 (Fe-cp): $^1$H-NMR ((CD$_3$)$_2$SO, 300 MHz): δ 10.05 (s, 1H, CONH), 8.21 (s, 1H, CSNH), 7.72-7.13 (m, 6H, Ar-H), 4.59 (s, 2H, C$_5$H$_4$), 4.26 (s, 2H, C$_5$H$_4$), 4.14 (s, 5H, C$_5$H$_5$), 2.35 (s, 3×CH$_3$); $^{13}$C-NMR ((CD$_3$)$_2$SO, 75 MHz): δ 180.1 (CS), 169.4 (CO), 138.1.
137.6, 135.6, 134.2, 132.0, 129.8, 128.3, 127.6, 80.9, 70.9, 69.1, 67.2, 24.9, 16.8. LCMS (m/z) %: [M+H]^+ 485 (100%). Anal. Calc. for C_{29}H_{32}FeN_{2}O_{2}S: C 67.25, H 5.41, N 5.83, S 6.62; Found: C 67.26, H 5.42, N 5.82, S 6.61.

4.5.4 1-(4-Ferrocenylaroyl)-3-(4-methoxyphenyl)thiourea (261d)

Brown amorphous solid; Yield: 87%; Rf: OCH₃ 0.37 (n-Hexane: EtOAc, 4:1); m.p.: 139140°C; FTIR (ATR, cm⁻¹): 3368 (NH), 2992, 2875 (Csp³-H), 1690 (C=O), 1287 (C=S), 487 (Fe-cp): ¹H-NMR ((CD$_3$)$_2$SO, 300 MHz): δ 10.12 (s, 1H, CONH), 8.71 (s, 1H, CSNH), 7.98 (d, 2H, Ar-H, J = 6.7Hz), 7.23 (d, 2H, Ar-H, J = 6.4Hz), 4.51 (s, 2H, C$_5$H$_4$), 4.20 (s, 2H, C$_5$H$_4$), 4.13 (s, 5H, C$_5$H$_5$), 3.13 (s, OCH$_3$); ¹³C-NMR ((CD$_3$)$_2$SO, 75 MHz): δ 180.5 (CS), 168.4 (CO), 147.1, 139.2, 134.2, 129.3, 128.9, 127.6, 126.7, 117.0, 110.3, 81.3, 71.2, 69.7, 67.0. LC-MS (m/z) %: [M+H]^+ 471 (100%). Anal. Calc. for C$_{27}$H$_{28}$FeN$_2$O$_2$: C 63.81, H 4.75, N 5.97, S 6.80; Found: C 63.86, H 4.74, N 5.91, S 6.82.

4.5.5 1-(4-Ferrocenylaroyl)-3-(4-hydroxy phenyl)thiourea (261e)

White amorphous solid; Yield: 85%; Rf: 0.43 OH (n-Hexane: EtOAc, 4:1); m.p.: 150-151°C; FTIR (ATR, cm⁻¹): 3315 (NH), 2992, 2875 (Csp³-H), 1696 (C=O), 1289 (C=S), 482 (Fe-cp): ¹H-NMR ((CD$_3$)$_2$SO, 300 MHz): δ 9.23 (s, 1H, CONH), 8.62 (s, 1H, CSNH), 7.88-6.95 (m, 8H, Ar-H), 4.59 (Ar-OH), 4.55 (s, 2H, C$_5$H$_4$), 4.18 (s, 2H, C$_5$H$_4$), 4.11 (s, 5H, C$_5$H$_5$); ¹³C-NMR ((CD$_3$)$_2$SO, 75 MHz): δ 182.5 (CS), 177.4 (CO), 147.1, 139.2, 134.2, 129.8, 128.6, 127.6, 126.9, 116.7, 81.7, 71.8, 69.1, 66.7. LC-MS (m/z) %: [M+H]^+ 457
(100%). Anal. Calc. for C_{26}H_{26}FeN_{2}O_{2}: C 63.09, H 4.50, N 6.07, S 7.10; Found: C 63.03, H 4.58, N 6.02, S 7.13.

4.5.6 1-(4-Ferrocenylaroyl)-3-(2-chlorophenyl)thiourea (159f)

Cl White amorphous solid; Yield: 86%; R_f: 0.36

(nHexane: EtOAc, 4:1); m.p.: 119-120°C; FTIR (ATR, cm^{-1}): 3327 (NH), 2969, 2847 (Csp^3-H), 1681 (C=O), 1252 (C=S), 486 (Fe-cp): \(^1\)H-NMR

\[(\text{CD}_3)_2\text{SO}, 300 \text{ MHz}: \delta 11.48 \text{ (s, 1H, CONH), 8.50 (s, 1H, CSNH), 7.70-7.28 (m, 8H, Ar-H), 4.61 (s, 2H, C_5H_4), 4.25 (s, 2H, C_5H_4), 4.21 (s, 5H, C_5H_5); }^{13}\text{C-NMR}\]

\[(\text{CD}_3)_2\text{SO, 75 MHz}: \delta 180.3 \text{ (CS), 172.1 (CO), 131.6, 131.3, 131.0, 129.7, 129.5, 128.9, 125.7, 122.0, 119.57, 80.3, 71.13, 69.4, 67.5. LC-MS (m/z) %: [M+H]^+ 475.5}\]

(100%). Anal. Calc. for C_{26}H_{25}ClFeN_{2}OS: C 60.74, H 4.02, N 5.80, S 6.83; Found: C 60.71, H 4.06, N 5.82, S 6.80.

4.5.7 1-(4-Ferrocenylaroyl)-3-(3-chlorophenyl)thiourea (261g)

Cl Brown amorphous solid; Yield: 81%; R_f: 0.39

(nHexane: EtOAc, 4:1); m.p.: 170-171°C; FTIR (ATR, cm^{-1}): 3357 (NH), 2988, 2817 (Csp^3-H), 1689 (C=O), 1267 (C=S), 489 (Fe-cp): \(^1\)H-NMR

\[(\text{CD}_3)_2\text{SO, 300 MHz}: \delta 10.98 \text{ (s, 1H, CONH), 8.74 (s, 1H, CSNH), 7.98-7.08 (m, 8H, Ar-H), 4.65 (s, 2H, C_5H_4), 4.29 (s, 2H, C_5H_4), 4.20 (s, 5H, C_5H_5); }^{13}\text{C-NMR}\]

\[(\text{CD}_3)_2\text{SO, 75 MHz}: \delta 184.7 \text{ (CS), 177.9 (CO), 138.6, 134.3, 131.9, 129.5, 128.1, 127.9, 126.5, 124.0, 123.5, 81.4, 71.3, 69.5, 67.2. LC-MS (m/z) %: [M+H]^+ 475.5 (100%).}\]

Anal. Calc. for C_{26}H_{25}ClFeN_{2}OS: C 60.74, H 4.00, N 5.88, S 6.81; Found: C 60.73, H 4.01, N 5.83, S 6.82.

4.5.8 1-(4-Ferrocenylaroyl)-3-(4-chloro phenyl)thiourea (261h)

Yellow amorphous solid; Yield: 88%; R_f: 0.30
4.5.9 1-(4-Ferrocenylaroyl)-3-(2,3-dichlorophenyl)thiourea (261i)

Cl Cl Yellow amorphous solid; Yield: 81%; Rf: 0.35 (n-Hexane: EtOAc, 4:1); m.p.: 179-180°C; FTIR (ATR, cm⁻¹): 3343 (NH), 2978, 2874 (Csp₃-H), 1639 (C=O), 1298 (C=S), 485 (Fe-cp): ¹H-NMR ((CD₃)₂SO, 300 MHz): δ 10.05 (s, 1H, CONH), 9.73 (s, 1H, CSNH), 7.92-7.30 (m, 7H, Ar-H), 4.58 (s, 2H, C₅H₄), 4.29 (s, 2H, C₅H₄), 4.11 (s, 5H, C₅H₅); ¹³C-NMR ((CD₃)₂SO, 75 MHz): δ 182.6 (CS), 175.9 (CO), 132.6, 132.1, 130.5, 129.2, 128.3, 79.6, 70.1, 69.2, 67.8. LC-MS (m/z) %: [M+H]⁺ 510 (100%). Anal. Calc. for C₂₆H₂₄Cl₂FeN₂O: C 56.60, H 3.67, N 5.40, S 6.30; Found: C 56.62, H 3.61, N 5.43, S 6.31.

4.5.10 1-(4-Ferrocenylaroyl)-3-(2,4-dichlorophenyl)thiourea (261j)

Cl Cl Pale yellow amorphous solid; Yield: 80%; Rf: 0.32 (n-Hexane: EtOAc, 4:1); m.p.: 160161°C; FTIR (ATR, cm⁻¹): 3396 (NH), 2978, 2874 (Csp₃-H), 1697 (C=O), 1266 (C=S), 487 (Fe-cp): ¹H-NMR ((CD₃)₂SO, 300 MHz): δ 10.05 (s, 1H, CONH), 9.73 (s, 1H, CSNH), 7.92-7.30 (m, 7H, Ar-H), 4.58 (s, 2H, C₅H₄), 4.29 (s, 2H, C₅H₄), 4.11 (s, 5H, C₅H₅); ¹³C-NMR ((CD₃)₂SO, 75 MHz): δ 182.6 (CS), 175.9 (CO), 132.6, 132.1, 130.5, 129.2, 128.3, 79.6, 70.1, 69.2, 67.8. LC-MS (m/z) %: [M+H]⁺ 510 (100%). Anal. Calc. for C₂₆H₂₄Cl₂FeN₂O: C 56.60, H 3.67, N 5.40, S 6.30; Found: C 56.62, H 3.61, N 5.43, S 6.31.
(Fe-cp): $^1$H-NMR ([(CD$_3$)$_2$SO, 300 MHz): $\delta$ 9.97 (s, 1H, CONH), 8.30 (s, 1H, CSNH), 7.760-7.33 (m, 7H, Ar-H), 4.59 (s, 2H, C$_5$H$_4$), 4.26 (s, 2H, C$_5$H$_4$), 4.20 (s, 5H, C$_5$H$_5$);

$^{13}$C-NMR ([(CD$_3$)$_2$SO, 75 MHz): $\delta$ 181.7 (CS), 174.8 (CO), 139.5, 137.4, 134.7, 135.6, 132.6, 129.9, 128.7, 127.7, 126.7, 80.8, 70.9, 69.7, 67.7. LC-MS (m/z) %: [M+H]$^+$ 510 (100%). Anal. Calc. for C$_{26}$H$_{24}$ClFeN$_2$OS: C 56.63, H 3.62, N 5.31, S 6.41; Found: C 56.61, H 3.64, N 5.32, S 6.40.

4.5.11 1-(4-Ferrocenylaroyl)-3-(3-chloro-4-fluorophenyl)thiourea (261k)

Brown amorphous solid; Yield; 86%; $R_f$: 0.40

FTIR (ATR, cm$^{-1}$): 3389 (NH), 2936, 2876 (Csp$^3$-H), 1673 (C=O), 1285 (C=S), 487 (Fe-cp): $^1$H-NMR ([(CD$_3$)$_2$SO, 300 MHz): $\delta$ 10.07 (s, 1H, CONH), 9.00 (s, 1H, CSNH), 7.80-7.25 (m, 7H, Ar-H), 4.59 (s, 2H, C$_5$H$_4$), 4.22 (s, 2H, C$_5$H$_4$), 4.16 (s, 5H, C$_5$H$_5$); $^{13}$C-NMR ([(CD$_3$)$_2$SO, 75 MHz): $\delta$ 183.3 (CS), 173.9 (CO), 143.2, 140.9, 139.6, 134.2, 131.8, 129.6, 128.1, 127.4, 120.7, 119.9, 80.5, 70.7, 69.6, 67.9. LC-MS (m/z) %: [M+H]$^+$ 492.5 (100%): Anal. Calc. for C$_{26}$H$_{24}$ClFeN$_2$OS: C 58.53, H 3.67, N 5.67, S 6.50; Found: C 58.51, H 3.64, N 5.69, S 6.53.

4.5.12 1-(4-Ferrocenylaroyl)-3-(4-fluoro phenyl)thiourea (261l)

Brown amorphous solid; Yield; 86%; $R_f$: 0.38

FTIR (ATR, cm$^{-1}$): 3387 (NH), 2956, 2876 (Csp$^3$-H), 1685 (C=O), 1285 (C=S), 486 (Fe-cp): $^1$H-NMR ([(CD$_3$)$_2$SO, 300 MHz): $\delta$ 10.03 (s, 1H, CONH), 9.00 (s, 1H, CSNH), 7.80-7.25 (m, 8H, Ar-H), 4.64 (s, 2H, C$_5$H$_4$), 4.29 (s, 2H, C$_5$H$_4$), 4.17 (s, 5H, C$_5$H$_5$); $^{13}$C-NMR ([(CD$_3$)$_2$SO, 75 MHz): $\delta$ 181.8 (CS), 173.2 (CO), 140.4, 139.6, 138.8, 134.6, 130.3, 129.7, 127.8, 120.5,
80.3, 70.6, 69.2, 67.5. LC-MS (m/z) %: [M+H]^+ 458 (100%). Anal. Calc. for C_{26}H_{25}ClFeN_{2}OS: C 62.83, H 4.19, N 6.14, S 7.02; Found: C 62.85, H 4.14, N 6.13, S 7.06.

4.5.13 1-(4-Ferrocenylaroyl)-3-(2-fluoro-4-bromophenyl)thiourea (261m)

![Chemical structure of 261m]

Grey amorphous solid; Yield: 84%; Rf: 0.41

FTIR (ATR, cm^{-1}): 3357 (NH), 2988, 2813 (Csp^3-H), 1689 (C=O), 1267 (C=S), 489 (Fe-cp):

$^1$H-NMR ([(CD$_3$)$_2$SO, 300 MHz): $\delta$ 9.68 (s, 1H, CONH), 8.24 (s, 1H, CSNH), 7.78-7.12 (m, 7H, Ar-H), 4.63 (s, 2H, C$_5$H$_4$), 4.20 (s, 2H, C$_5$H$_4$), 4.16 (s, 5H, C$_5$H$_5$);

$^{13}$C-NMR ([(CD$_3$)$_2$SO, 75 MHz): $\delta$ 180.9 (CS), 172.7 (CO), 140.4, 139.5, 134.5, 133.7, 128.9, 127.5, 126.6, 80.8, 70.3, 69.8, 67.3. LC-MS (m/z) %: [M+H]^+ 536 (100%). Anal. Calc. for C_{26}H_{24}BrFeN_{2}OS: C 53.23, H 3.79, N 5.33, S 5.87; Found: C 53.26, H 3.72, N 5.37, S 5.88.

4.5.14 1-(4-Ferrocenylaroyl)-3-(2,6-dibromo-4-fluorophenyl)thiourea (261n)

![Chemical structure of 261n]

White amorphous solid; Yield: 86%; Rf: 0.34

FTIR (ATR, cm^{-1}): 3333 (NH), 2944, 2818 (Csp^3-H), 1678 (C=O), 1233 (C=S), 485 (Fe-cp):

$^1$H-NMR ([(CD$_3$)$_2$SO, 300 MHz): $\delta$ 9.33 (s, 1H, CONH), 8.63 (s, 1H, CSNH), 7.75-7.23 (m, 7H, Ar-H), 4.65 (s, 2H, C$_5$H$_4$), 4.20 (s, 2H, C$_5$H$_4$), 4.19 (s, 5H, C$_5$H$_5$);

$^{13}$C-NMR ([(CD$_3$)$_2$SO, 75 MHz): $\delta$ 181.6 (CS), 173.1 (CO), 142.4, 140.9, 139.1, 136.5, 134.2, 128.7, 127.6, 126.3, 123.5, 80.5, 70.6, 69.5, 67.8. LC-MS (m/z) %: [M+H]^+ 915 (100%). Anal. Calc. for C_{26}H_{23}Br$_2$FeN_{2}OS: C 46.72, H 2.79, N 4.58, S 5.24; Found: C 46.76, H 2.77, N 4.57, S 5.23.

4.5.15 1-(4-Ferrocenylaroyl)-3-(4-nitrophenyl)thiourea (261o)

![Chemical structure of 261o]
Brown amorphous solid; Yield: 83%; Rf: NO2 0.42 (n-Hexane: EtOAc, 4:1); m.p.: 115116°C; FTIR (ATR, cm−1): 3357 (NH), 2988, 2817 (Csp3-H), 1689 (C=O), 1267 (C=S), 489 (Fe-cp): 1H-NMR ((CD3)2SO, 300 MHz): δ 10.98 (s, 1H, CONH), 8.74 (s, 1H, CSNH), 7.98-7.30 (m, 8H, Ar-H), 4.60 (s, 2H, C5H4), 4.23 (s, 2H, C5H4), 4.20 (s, 5H, C5H5); 13C-NMR ((CD3)2SO, 75 MHz): δ 181.7 (CS), 173.6 (CO), 141.6, 140.5, 139.8, 134.5, 130.2, 128.6, 127.5, 125.4, 80.6, 70.7, 69.9, 68.5. LC-MS (m/z) %: [M+H]+ 485 (100%). Anal. Calc. for C26H25FeN3O3S: C 59.59, H 3.79, N 8.63, S 6.60; Found: C 59.56, H 3.73, N 8.68, S 6.64.

4.5.16 1-(4-Ferrocenylaroyl)-3-(2,4-dinitrophenyl)thiourea (261p)

Yellow amorphous solid; Yield: 80%; Rf: NO2 0.39 (n-Hexane: EtOAc, 4:1); m.p.: 149150°C; FTIR (ATR, cm−1): 3383 (NH), 2912, 2852 (Csp3-H), 1676 (C=O), 1287 (C=S), 488 (Fe-cp): 1H-NMR ((CD3)2SO, 300 MHz): δ 9.89 (s, 1H, CONH), 9.56 (s, 1H, CSNH), 7.91-6.80 (m, 11H, Ar-H), 4.62 (s, 2H, C5H4), 4.22 (s, 2H, C5H4), 4.16 (s, 5H, C5H5); 13C-NMR ((CD3)2SO, 75 MHz): δ 182.5 (CS), 173.5 (CO), 145.6, 143.2, 141.7, 139.2, 137.4, 134.6, 129.5, 128.4, 126.8, 123.2, 120.1, 80.5, 70.4, 69.5, 67.9. LC-MS (m/z) %: [M+H]+ 485 (100%). Anal. Calc. for C26H24FeN4O5S: C 54.39, H 3.53, N 10.55, S 6.01; Found: C 54.33, H 3.58, N 10.57, S 6.00.

4.5.17 1-(4-Ferrocenylaroyl)-3-napthyl thiourea (261q)
173.2 (CO), 139.2, 135.5, 133.6, 127.5, 126.7, 125.6, 124.9, 121.5, 120.3, 80.5, 70.6, 70.2, 67.7. LC-MS (m/z) %: [M+H]+ 490 (100%). Anal. Calc. for C$_{30}$H$_{28}$FeN$_{2}$OS: C 68.49, H 4.62, N 5.73, S 6.51; Found: C 68.46, H 4.67, N 5.72, S 6.50.

4.6 General procedure for the synthesis of isomeric chloro substituted benzoyl thioureas (265 a-c)

Isomeric chloro substituted benzoic acids, 262a-c, (0.1 g, 0.63 mmol) were placed in a 100 mL two neck round bottom flask, fitted with a reflux condenser and a gas trap. Then (0.04 mL, 0.63 mmol) of thionyl chloride was added and the reaction mixture was heated under reflux for 2 hours to give aroyl chloride 263a-c of respective acids. These freshly prepared chloro benzoyl chlorides were then added to the solution of potassium thiocyanate (0.013 g, 0.18 mmol) in acetone to get the corresponding isothiocyanate 264a-c via stirring at room temperature for 1 h. The insitu formed isomeric chlorobenzoyl isothiocyanates were then treated with aqueous ammonia (0.02 mL, 0.63 mmol) at low temperature. On completion, the reaction mixture was poured in ice cold water and the solid product obtained was recrystallized from ethanol to afford yellow crystalline solid.

4.6.1 1-(2-Chlorobenzoyl)thiourea (265a)

\[
\begin{align*}
\text{O} \\
\text{S} \\
\text{Cl} \\
\text{N}_1 \\
\text{N}_2
\end{align*}
\]

Yellow crystals; Yield: 85%; R$_f$: 0.55 (EtOAc: Pet Ether, C NH$_2$ 4:1); m.p.: 167-168°C; FTIR (ATR, cm$^{-1}$): 3329 (N-H), 3153 (NH$_2$), 1687 (CONH), 1279 (C=S), 1H- NMR ((CD$_3$)$_2$SO, 300 MHz): $\delta$ 11.64 (s, 1H, N-H), 7.79 (s, 2H, NH$_2$), 7.55-7.47 (m, 3H, Ar-H), 7.457.38 (m, 1H, Ar-H); $^{13}$C-NMR ((CD$_3$)$_2$SO, 75 MHz): $\delta$ 182.0 (C=S), 167.7 (CONH), 129.5, 127.8. Anal. Calc. for C$_8$H$_7$ClN$_2$OS: C 44.59, H 3.46, N 13.11, S 14.94; Found: C 44.55, H 3.50, N 13.05, S 14.96.

4.6.2 1-(3-Chlorobenzoyl)thiourea (265b)
**4.6.3 1-(4-Chlorobenzoyl)thiourea (265c)**

![Chemical Structure]

Green amorphous solid; Yield: 80%; Rf: 0.57 (EtOAc: Pet Ether, 4:1); m.p.: 125-126°C; FTIR (ATR, cm\(^{-1}\)): 3229 (N-H), 3147 (NH\(_2\)), 1678 (CONH), 1281 (C=S), \(^1\)H-NMR (CDCl\(_3\), 300 MHz): δ 9.56 (s, 1H, NH), 7.82 (s, 2H, NH\(_2\)), 7.99 (s, 1H, Ar-H), 7.50-7.81 (m, 3H, Ar-H); \(^13\)C-NMR (CDCl\(_3\), 75 MHz): δ 181.0 (C=S), 169.5 (CONH), 135.2, 134.0, 132.7, 130.5, 129.6, 124.9. Anal. Calc. for C\(_8\)H\(_7\)ClN\(_2\)OS: C 44.56, H 3.49, N 13.01, S 14.90; Found: C 44.49, H 3.56, N 13.23, S 14.86.

**4.7 General procedure for the synthesis of substituted isomeric chloro–N(thiazol-2(3H)-ylidene)benzamides (266, 267, 268, 269 a-c)**

The isomeric chloro-substituted benzoyl thioureas 265a-c (0.1 g, 0.4 mmol) were dissolved in 10 mL of dry, distilled ethanol at 50°C. To this solution different cyclizing agents such as alpha halo carbonyl compounds, i.e., ethyl-2-chloroacetoacetate, ethylbromopyruvate, 2,4-dibromoacetophenone and diethyl oxalate (0.05 mL, 0.05 mL, 0.11g, 0.06 mL, 0.4 mmol) were added and the mixture was stirred at this temperature for 2h. On completion of reaction, monitored by TLC the reaction mixture was cooled to room temperature and
was neutralized by aqueous ammonia (30% \( \text{NH}_3 \text{OH} \)). Drop wise addition of water with stirring led to precipitation of the product which was separated by filtration, dried and then recrystallized by aqueous ethanol.

### 4.7.1 Ethyl-2-(2-chlorobenzamido)-4-methyl-2,3-dihydrothiazole-5-carboxylate (266a)

White amorphous solid; Yield: 80%; Rf: 0.54 (EtOAc: Pet Ether, 4:1); m.p.: 121-122°C; FTIR (ATR, cm\(^{-1}\)):

\[
3290 \text{ (N-H), 2963, 2876 (CH}_3\text{), 1733 (CO}_2\text{Et), 1669 (CONH), 1662 (C=N)}
\]

\(3290\) (N-H), 2963, 2876 (CH\(_3\)), 1733 (CO\(_2\)Et), 1669 (CONH), 1662 (C=N);

\(1\text{H-NMR (CDCl}_3, 300 MHz): \delta}

\[
9.52 \text{ (s, 1H, NH), 7.80 (d, } J=7.5\text{Hz, 1H, Ar-H), 7.51 (dt, } J=8.1 \text{ Hz, 1.5 Hz, 1H, Ar-H), 7.42 (dt, } J=8.4 \text{ Hz, 1.8 Hz, 1H, Ar-H), 7.39 (d, } J=1.8 \text{ Hz, 1H, Ar-H), 4.34 (q, 2H, } J=6 \text{ Hz), 2.26 (s, 3H), 1.38 (t, 3H, } J=6.0 \text{ Hz); } ^{13}\text{C-NMR (CDCl}_3, 75 MHz): \delta}
\]

164.1 (C=N), 162.5 (CO\(_2\)Et), 159.8, 155.9, 133.1, 132.0, 131.5, 131.6, 130.8, 127.5, 61.0, 16.2, 14.3. GC-MS (m/z): 324 (M\(^+\)), 296, 279, 261, 213, 139 (100%), 111, 75, 50. Anal. Calc. for C\(_{14}\)H\(_{13}\)ClN\(_2\)O\(_3\)S: C 51.07, H 4.73, N 8.74, S 9.79; Found: C 51.01, H 4.79, N 8.95, S 9.95.

### 4.7.2 Ethyl-2-(2-chlorobenzamido)-2,3-dihydrothiazole-4-carboxylate (267a)

White amorphous solid; Yield: 81%; Rf: 0.54 (EtOAc:

\(3292\) (N-H), 2937, 1728 (CO\(_2\)Et), 1672 (CONH), 1655 (C=N); \(1\text{H-NMR (CDCl}_3, 300 MHz): \delta}

10.62 (s, 1H, NH), 7.90 (s, 1H, C\(_{sp^2}\)H), 7.84 (dd, 1H, \( J=8.4 \text{ Hz, 1.2 Hz, Ar-H}), 7.48 (dt, 1H, \( J=4.5 \text{ Hz, 1.8 Hz, Ar-H}), 7.40 (dt, 1H, \( J=5.1 \text{ Hz, 3.3 Hz}), 7.49 (d, 1H, \( J=1.8 \text{ Hz}), 4.31 (q, 2H, \( J=7.2 \text{ Hz}), 1.39-1.35 (t, 3H, \( J=7.2 \text{ Hz}); \( ^{13}\text{C-NMR (CDCl}_3, 75 MHz): \delta}

164.5 (CONH), 161.1 (CO\(_2\)Et), 157.9, 141.1, 132.9, 131.9, 131.3, 131.1, 130.8, 127.4, 122.6, 61.4, 14.3. Anal. Calc. for C\(_{14}\)H\(_{13}\)ClN\(_2\)O\(_3\)S: C 50.47, H 3.34, N 9.40, S 10.03; Found: C 50.44, H 3.37, N 9.02, S 10.25.

### 4.7.3 N-(4-(4-bromophenyl)thiazol-2(3\(H\))-ylidene)-2-chlorobenzamide (268a)
Yellow amorphous solid; Yield: 86%; Rf: 0.55
(EtOAc: Pet Ether, 4:1); m.p.: 136-137°C; FTIR
(ATR, cm⁻¹): 3361 (N-H), 2964, 1691 (CONH), 1640 Br
(C=N); ¹H-NMR (CDCl₃, 300 MHz): δ 10.88 (s, 1H), 7.61-7.58
(d, 2H, J= 8.4 Hz, Ar-H), 7.49-7.47 (d, 2H, J= 8.4 Hz, Ar-H), 7.30-7.28 (d,
2H, J= 7.8 Hz, Ar-H), 7.19 (s, 1H); ¹³C-NMR (CDCl₃, 75 MHz): δ 173.5 (CONH), 163.2, 158.1,
141.2, 133.7, 131.9, 131.2, 129.4, 128.3, 122.5, 108.7 (-S=C=). Anal. Calc. for

4.7.4 2-Chloro-N-(4,5-dioxothiazolidin-2-ylidene)benzamide (269a)

Yellow amorphous solid; Yield: 80%; Rf: 0.51 (EtOAc: Pet
Ether, 4:1); m.p.: 154-155°C; FTIR (ATR, cm⁻¹): 3245 (N-H), 2970, 2860 (CH₃), 1732 (CO₂Et), 1677
(CONH), 1664 (C=N); ¹H-NMR (CDCl₃, 300 MHz): δ 9.4 (s, 1H, NH), 7.81 (s, 1H, Ar-H), 7.32-7.61 (m, 3H, Ar-H), 4.20 (q, 2H, J= 7.0 Hz), 2.10 (s,
3H), 1.33 (t, 3H, J= 7.1 Hz); ¹³C-NMR
(CDCl₃, 75 MHz): δ 171.5 (CONH), 164.3 (CO₂Et), 161.5, 159.7, 141.2, 133.5, 132.5, 128.6, 121.5, 61.0, 14.6. Anal. Calc. for C₁₄H₁₃ClN₂O₃S: C 51.77, H 4.03, N 8.64, S 9.95; Found: C 51.16, H 4.69, N 8.65, S 9.87.

**4.7.6 Ethyl-2-(3-chlorobenamido)-2,3-dihydrothiazole-4-carboxylate (267b)**

White amorphous solid; Yield: 76% ; Rᵣ: 0.45 (EtOAc: Pet. Ether, 4:1); m.p.: 181-182°C; FTIR (ATR, cm⁻¹) : 3255 (N-H), 1729 (CO₂Et), 1695 (CONH), 1659 (C=N); ¹H-NMR (CDCl₃, 300 MHz): δ 10.22 (s, 1H, NH), 7.91 (s, 1H, Cₛ²-H), 7.82 (s, 1H, J = 8.4 Hz, Ar-H), 7.35-7.70 (m, 3H, Ar-H), 4.19 (q, 2H, J = 7.3 Hz), 1.39 (t, 3H, J = 7.2 Hz); ¹³C-NMR (CDCl₃, 75 MHz): δ 164.0 (CONH), 161.2 (CO₂Et), 162.5, 153.4, 136.2, 135.2, 129.8, 125.4, 119.0, 60.9, 14.6. Anal. Calc. for C₁₃H₁₁ClN₂O₃S: C 50.27, H 3.54, N 9.10, S 10.23; Found: C 50.37, H 3.44, N 9.21, S 10.21.

**4.7.7 N-(4-(4-Bromophenyl)thiazol-2(3H)-ylidene)-3-chlorobenzamide (268b)**

Yellow amorphous solid; Yield: 78%; Rᵣ: 0.48 (EtOAc: Pet Ether, 4:1); m.p.: 195-195°C; FTIR (ATR, cm⁻¹): 3345 (N-H), 1687 (CONH), 1642 (C=N); ¹H-NMR (CDCl₃, 300 MHz): δ 10.91 (s, 1H, NH), 7.81 (s, 1H, Ar-H), 7.80 (s, 1H, J = 8.1 Hz, Ar-H), 7.32 (d, 2H, J = 8.1 Hz), 7.15 (d, 2H, J = 8.01 Hz), 7.05 (s, 1H, Cₛ²-H); ¹³C-NMR (CDCl₃, 75 MHz): δ 171.5 (CONH), 162.2, 154.1, 141.9, 132.7, 131.3, 131.2, 128.4, 127.3, 120.5, 118.7 (S=C=C). Anal. Calc. for C₁₆H₁₀BrClN₂OS: C 48.53, H 2.84, N 7.09, S 8.04; Found: C 48.45, H 2.91, N 7.16, S 8.04.

**4.7.8 3-Chloro-N-(4,5-dioxothiazolidin-2-ylidene)benzamide (269b)**

Pale yellow amorphous solid; Yield: 84%; Rᵣ: 0.56 (EtOAc: Pet. Ether, 4:1); m.p.: 114-115°C; FTIR (ATR, cm⁻¹): 3343 (N-H), 3051, 1738 (C=O), 1690 (CONH), 1685 (C=N); ¹H-NMR (CDCl₃, 300 MHz): δ 10.25 (s, 1H), 8.06-7.41 (m, 4H, NMR (CDCl₃)}
Ar-H; $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 184.5 (C=O), 174.7 (CONH), 162.7, 145.2, 138.1, 137.6, 133.5, 132.6, 130.8, 130.3. Anal. Calc. for C$_{10}$H$_5$ClN$_2$O$_3$: C 44.80, H 1.70, N 10.33, S 11.87; Found: C 44.30, H 2.20, N 10.03, S 11.80.

4.7.9 Ethyl-2-(4-chlorobenzamido)-4-methyl-2,3-dihydrothiazole-5-carboxylate (266c)

Yellow amorphous solid; Yield: 82%; R$_f$: 0.60

Cl(CO$_2$Et), 1678 (CONH), 1660 (C=N); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 7.85 (s, 1H), 7.75 (d, 2H, $J$ = 8.1 Hz, Ar-H), 7.46 (d, 2H, $J$ = 8.2 Hz, Ar-H), 4.19 (q, 2H, $J$ = 7.0 Hz), 1.78 (t, 3H, $J$ = 7.1 Hz); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 174.1 (CONH), 166.3 (CO$_2$Et), 163.5, 161.2, 140.5, 133.2, 131.1, 129.2, 101.3, 61.3, 14.3. Anal. Calc. for C$_{14}$H$_{13}$ClN$_2$O$_3$: C 51.57, H 4.23, N 8.60, S 9.88; Found: C 51.09, H 4.70, N 8.82, S 14.85.

4.7.10 Ethyl-2-(4-chlorobenzamido)-2,3-dihydrothiazole-4-carboxylate (267c)

White amorphous solid; Yield: 80%; R$_f$: 0.53

Cl(CO$_2$Et), 1678 (CONH), 1651 (C=N); $^1$H-NMR (CDCl$_3$, 300 MHz): $\delta$ 8.54 (s, 1H), 7.91 (s, 1H, -S-C=C-), 7.75 (d, 2H, $J$ = 8.3 Hz, Ar-H), 7.41 (d, 2H, $J$ = 8.0 Hz, Ar-H), 4.05 (q, 2H, $J$ = 7.1 Hz), 1.50 (t, 3H, $J$ = 7.0 Hz); $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 174.5 (CONH), 165.2 (CO$_2$Et), 163.5, 151.5, 140.5, 133.7, 130.6, 129.8, 101.7, 61.5, 14.1. Anal. Calc. for C$_{13}$H$_{11}$ClN$_2$O$_3$: C 50.17, H 3.64, N 9.30, S 10.53; Found: C 50.26, H 3.55, N 9.10, S 10.51.

4.7.11 N-(4-(4-Bromophenyl)thiazol-2(3H-ylidene))-4-chlorobenzamide (268c)

White amorphous solid; Yield: 84%; R$_f$: 0.61 (EtOAc: Pet. Ether, 4:1); m.p.: 183-184°C; FTIR (ATR, cm$^{-1}$): 3240 (N-H), 2959, 1690 (CONH), 1644 (C=N); $^1$H-
$^1$H NMR (CDCl$_3$, 300 MHz): δ 8.9 (s, 1H, NH), 7.75 (d, 2H, $J$= 8.0 Hz, Ar-H), 7.42 (d, 2H, $J$= 8.0 Hz, Ar-H), 7.19 (d, 2H, $J$= 7.9 Hz, Ar-H), 7.35 (d, 2H, $J$= 8.0 Hz), 7.10 (s, 1H, C$_6$H$_2^-$-H); $^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 174.5 (CONH), 163.8, 156.3, 140.1, 133.5, 133.3, 131.6, 131.3, 129.4, 128.6, 125.5, 109.3 (-S-C=C). Anal. Calc. for C$_{16}$H$_{10}$BrClN$_2$OS: C 48.83, H 2.54, N 7.13, S 8.04; Found: C 48.51, H 2.86, N 7.00, S 8.01.

4.7.12 4-Chloro-N-(4,5-dioxothiazolidin-2-ylidene)benzamide (269c)

Yellow amorphous solid; Yield: 82%; R$_f$: 0.50 (EtOAc: Pet Ether, 4:1); m.p.: 144-145°C; FTIR (ATR, cm$^{-1}$): 3373 (N-H), 2975, 1740 (C=O), 1699 (CONH)$^\text{Cl}$; $^1$H-NMR (CDCl$_3$, 300 MHz): δ 10.07 (s, 1H), 7.89 (d, 2H, $J$= 8.5 Hz, Ar-H), 7.54 (d, 2H, $J$= 8.5Hz, Ar-H); $^{13}$C-NMR (CDCl$_3$, 75 MHz): δ 182.8 (C=O), 173.9 (CONH), 165.9, 158.8, 142.7, 138.5, 133.6, 132.7. Anal. Calc. for C$_{10}$H$_5$ClN$_2$O$_3$S: C 44.50, H 2.00, N 10.63, S 11.88; Found: C 44.90, H 1.60, N 10.32, S 11.94.

4.8 Synthesis of racemic 1-methyl tropane auxiliary

4.8.1 4-Oxopentanal (273)

To a solution of 5-hydroxy-2-pentanone 271 (5.0 g, 49.0 mmol) in dichloromethane (150 mL), was added a free-flowing, properly-mixed mixture of pyridinium chlorochromate 272 (15.8 g, 73.3 mmol) and silica (15.8 g) at room temperature. The mixture was stirred for 18 h, filtered through a pad of florisil twice and the solvent was evaporated. The crude green product was purified by column chromatography (SiO$_2$, 5% Et$_2$O in DCM) and 4-oxopentanal was obtained as green oil (2.72 g, 56%) (273).

Green oil; Yield: 56%; R$_f$: 0.36 (SiO$_2$, 5% Et$_2$O in DCM); $^1$H-NMR (400 MHz): 9.81 (s, 1H), 2.78 (s, 4H), 2.21 (s, 3H) ppm; $^{13}$C-NMR
(100 MHz): 206.3 (H-C=O), 200.4 (-CH$_2$-CO-CH$_3$), 37.4 (CH$_3$), 35.4 (CH$_2$), 29.7 (CH$_3$) ppm.

4.8.2 (±)-8-Benzyl-1-methyl-8-aza-bicyclo[3.2.1]actan-3-one ((±)-276)

To a clear solution of sodium acetate (7.71 g, 94 mmol) in water in a three-neck round bottom flask, benzyl amine 274 (1.59 mL, 23 mmol) and conc. HCl (1.48 mL, 24 mmol) were added simultaneously with constant stirring below 10°C. Within 15 min acetone-1,3-dicarboxylic acid 275 (3.65 g, 25 mmol) was added to form a homogeneous reaction mixture while the pH of the system was kept at 5. This was followed by the slow addition of 4-oxopentanal 273 (2.20 g, 21 mmol) at same temperature for 10 min. The reaction mixture was then warmed to 40°C, stirred for 7 hr after which the temperature was decreased to 15°C and pH adjusted to 10 with 50% aq NaOH. This solution was extracted with dichloromethane. The organic layers were combined and dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure to give (±)-276 as reddish orange oil (2.7 g, 54%)

Reddish orange oil; Yield: 54%; $R_f$: 0.15 (4% EtOAc, 2% NEt$_3$ in petroleum ether); FTIR (ATR, cm$^{-1}$): 3085 w, 2958 O$_s$ (Csp$^3$-sym), 2873 w (Csp$^3$-asym), 1712 s (CO), 1650 m, 1494 m, 1375 s (C-N, 3$^0$ amine), 1237 w, 1217 w, 730 s, 697 vs; $^1$H-NMR (400 MHz): 7.35-7.34 (m, 2H, Ar ortho), 7.29-7.25 (m, 2H, Ar meta), 7.21-7.18 (m, 1H, Ar para), 3.84 (d, 1H, $^2$J = 13.4Hz, CH$_2$A$_3$Ph), 3.67 (d, 1H, $^2$J = 13.4Hz, CH$_2$A$_3$Ph), 3.45 (ddd, 1H, $^3$J = 6.9Hz, $^3$J = 4.6Hz, $^3$J = 2.0Hz, CHNBz), 2.60 (d, 1H, $^3$J = 15.7Hz, COCH$_3$$^4$CHN), 2.46 (d, 1H, $^2$J = 15.4Hz, COCH$_3$$^4$C$^{\text{quat.}}$), 2.13 (dd,
1H, \(^2J = 15.4\text{Hz,}\) \(^3J = 1.7\text{Hz, COCH}_2^b\text{C}_{\text{quart.}},\) 1.96 (dt, 1H, \(^2J = 15.5\text{Hz,}\) \(^3J = 1.9\text{Hz, COCH}_2^b\text{CH}_2^b\), 1.41-1.34 (m, 1H, CHCH\(_2^b\)CH\(_2^b\)), 1.23 (s, 3H, CH\(_3^b\)); \(^13\text{C-NMR (100 MHz):}\) 210.1 (CO), 139.7 (Ar\(_{\text{quart.}}\)), 128.4 (Ar\(_{\text{ortho}}\), Ar\(_{\text{meta}}\)), 127.0 (Ar\(_{\text{para}}\)), 62.6 (C\(_{\text{quart.}}\)), 56.8 (CH), 50.8 (COCH\(_2^b\text{C}_{\text{quart.}}\)), 48.1 (NCH\(_2^b\text{Ph}\)), 42.8 (COCH\(_2^b\)CH\(_2^b\)), 36.6 (CHCH\(_2^b\)CH\(_2^b\)), 27.6 (CHCH\(_2^b\)CH\(_2^b\)), 24.98 (CH\(_3^b\)); HRMS \(m/z\) (ESI\(^+\);M\(^+\)H\(^+\)) found 230.1539, calcd for C\(_{15}\)H\(_{20}\)NO

4.8.3 (±)-1-Methyl-8-benzyl-8-azabicyclo[3.2.1]octane ((±)-279)

1-Methyl-\(N\)-benzyltropanone 276 (1.90 g, 8.0 mmol), and hydrazine monohydrate 277 (3.40 g, 57.5 mmol) were added to KOH 278 (7.09 g, 126.3 mmol) in diethylene glycol (17.1 mL) with stirring at rt. The mixture was heated to 220°C for 24 h. The reaction was cooled to rt and diluted with H\(_2\)O (127 mL) and extracted against Et\(_2\)O (3 x 127 mL). The combined organic layers were dried (MgSO\(_4\)) and then evaporated under reduced pressure to give 1-methyl-\(N\)-benzyltropane (±)-279 as a pale yellow oil (1.54 g, 87%).

Pale yellow oil; Yield: 87%; \(R_f\): 0.24 (4% EtOAc, 2% NEt\(_3\) in petroleum ether); FTIR (ATR, cm\(^{-1}\)): 3025 w, 2928 vs (Csp\(_3\) sym),, 2867 m (Csp\(_3\) asym), 1603 w, 1451 w, 1372 w, 1213 m, 849 w, 736 s, 698 s; \(^1\text{H NMR (400 MHz):}\) δ 7.31 (d, 2H, J = 7 Hz, Ar\(_{\text{ortho}}\)), 7.22 (m, 2H, Ar\(_{\text{meta}}\)), 7.13 (m, 1H, Ar\(_{\text{para}}\)), 3.77 (d, 1H, J = 13.7 Hz, CH\(_3^b\)H\(_6^b\)Ph), 3.50 (d, 1H, J = 13.7 Hz, CH\(_3^b\)H\(_6^b\)Ph), 3.03 (m, 1H, NBzCH), 1.84–1.41 (m, 8H, 3 x CH\(_2^b\), 2 x CH\(_3^b\)H\(_6^b\)), 1.10 (d, 1H, J = 7.6 Hz, CH\(_3^b\)H\(_6^b\)), 1.07 (s, 3H, CH\(_3^b\)), 0.95 (m,
1H, CH₃H₈); ¹³C NMR δ (101 MHz): 141.2 (Ar₉₄), 128.6 (Ar₉₃), 128.0 (Ar₉₃), 126.3 (Ar₈), 60.5 (CH₃), 56.7 (CH), 48.6 (NCH₂Ph), 35.7 (CH₂), 33.1 (CH₂), 27.1 (CH₂), 25.5 (CH₂), 24.7 (CH₂), 18.4 (CH₂); HRMS m/z (ESI⁺; M⁺H⁺) found 216.1747, calcd for C₁₅H₂₂N 216.1746.

4.8.4 (±)-1-Methyl-8-azabicyclo[3.2.1]octane hydrochloride ((±)-281)

Palladium on charcoal 280 (10 wt%, 0.076 g, 0.715 mmol) was added to a stirred solution of 1-methyl-N'-benzyltropane (±)-279 (1.54 g, 7.15 mmol) in MeOH (196 mL) at rt. Hydrogen gas was bubbled through the solution for 1 min and the mixture was vigorously stirred under a hydrogen atmosphere (two balloons containing H₂) for 24 h. The catalyst was removed by filtration through celite and a 2.0 M solution of hydrogen chloride in diethyl ether (19 mL) was added to the filtrate. The solvent was removed under reduced pressure and the crude product was given multiple washings with DCM to yield pure 1-Methyltropane hydrochloride salt (±)-281 (1.17 g, quant) as a peach solid.

Peach solid; Yield: 1.17 g, quant; m.p.: 95°C; FTIR (ATR, cm⁻¹):
3387 bm, 2933 vs (Csp³-sym), 2881 m (Csp³-asym), 1598 m (N-H bend), 1460 m (CH₂ bend), 1416 m, 1414 s, 1381 s, 1369 m (CH₃ bend), 900 w; ¹H NMR (400 MHz); δ 9.37 (br d, 2H, J = 104 Hz, NH₂Cl), 4.03–3.93 (d, 1H, J = 3.4 Hz, NCH), 2.35–2.31 (m, 1H, CH₃H₈), 2.14–2.02 (m, 2H, CH₂), 1.96–1.61 (m, 7H, 3 x CH₂, CH₃H₈), 1.56 (s, 3H, CH₃); ¹³C NMR (101 MHz): δ 63.8 (CCH₃), 55.9 (CH), 35.6 (CH₂), 33.2 (CH₂), 27.9 (CH₂), 26.9 (CH₃), 24.2 (CH₃), 17.0 (CH₂); HRMS m/z (ESI⁺; M⁺H⁺) found 126.1278, calcd for C₈H₁₇NCl 126.1277.

4.8.5 (±)-1-Methyl-8-azabicyclo[3.2.1]octane-8-carbaldehyde ((±)-289)
To a mixture of tropane hydrochloride salt (±)-281 (0.20 g, 1.23 mmol), BnEt₃NCl 288 (0.133 g, 0.58 mmol), CHCl₃ 287 (900 µL, 11.07 mmol), CH₂Cl₂ (4.2 mL), 12.5 M aq NaOH (3.24 mL) were added with stirring. The mixture was heated at reflux for 24 h, then diluted with water (45 mL) and extracted with CH₂Cl₂ (3 x 22 mL). The organic layers were combined and washed with brine (3 x 30 mL), dried (MgSO₄) and evaporated under reduced pressure to obtain pure formamide (±)-289 as pale yellow oil (0.18 g, quant).

Pentylmagnesium chloride 290 (2.0 M in THF, 405 µL, 0.81 mmol) was added to a stirred solution of formamide (±)-289 (100 mg, 0.65 mmol) in Et₂O (500 µL) at -15 to -20°C. The reaction temperature was maintained for 15 min, then warmed to room temperature and stirred overnight. A steady stream of argon was passed over the surface of the reaction mixture to remove the volatiles. Bulb-to-bulb distillation of the crude product (40°C, 1.35 mmHg) gave enamine (±)-286 as colourless, clear oil (133 mg, 99%).

4.8.6 (±)-8-((E)-Hex-1-en-1-yl)-1-methyl-8-azabicyclo[3.2.1]octane ((±)-286)

Pentylmagnesium chloride 290 (2.0 M in THF, 405 µL, 0.81 mmol) was added to a stirred solution of formamide (±)-289 (100 mg, 0.65 mmol) in Et₂O (500 µL) at -15 to -20°C. The reaction temperature was maintained for 15 min, then warmed to room temperature and stirred overnight. A steady stream of argon was passed over the surface of the reaction mixture to remove the volatiles. Bulb-to-bulb distillation of the crude product (40°C, 1.35 mmHg) gave enamine (±)-286 as colourless, clear oil (133 mg, 99%).
NCH=CH\text{), 4.32 (dt, 1H, } J_1 = 14 \text{ Hz, } J_2 = 7.1 \text{ Hz, NCH=CH}, \text{ 3.80–3.82 (m, 1H, NCH), 2.17–1.96 (m, 3H, NCH=CH}_2 \text{ and } CH_3H_6, \text{ 1.85–1.78 (m, 6H, 3 x CH}_3, \text{ 1.67–1.51 (m, 6H, 3 x CH}_2, \text{ 1.39-1.25 (m, 1H, CH}_A=CHCH_2, \text{ 0.90), 1.16 (s, 3H, CH}_3, \text{, (t, 3H, J = 8 Hz, CH}_3CH_2)\text{; } ^{13}C \text{ NMR (101 MHz, CD}_3CN) \delta 136.3 \text{(NCH=CH), 102.8 (NCH=CH), 60.4 (NC(CH}_3), 55.3 (NCH), 36.6 (CH}_2, \text{ 36.8 (CH}_2, \text{ 34.2 (CH}_2, \text{ 31.1 (NCH=CHCH}_2), \text{ 26.9 (CH}_2, \text{ 24.6 (CH}_3, \text{ 22.4 (CH}_2, \text{ 22.3 (CH}_2, \text{ 19.1 (CH}_2, \text{ 13.8 (CH}_2CH}_3); HRMS } m/z \text{(ESI'; } M^+H^+) \text{ found 208.0390, } C_{12}H_{22}NO \text{ requires 208.2059.}

4.8.7 Alkylation of enamine to find the } dr \text{ of the transient diastereomeric } \alpha-\text{ethylatediminium ion } ((\pm)-292)

\begin{align*}
\text{Et} & \quad \text{EtI 291 (102 } \mu\text{L, 1.28 mmol), [passed through a plug of basic } \\
\text{Bu} & \quad \text{Al}_2O_3 \text{ immediately prior to the addition] was added to enamine } (\pm)-286 \text{ (133 mg, 0.64 mmol) in } d_3\text{-MeCN (630 } \mu\text{L) and heated at } 85^\circ\text{C in a sealed, argon-filled, Young's cap NMR tube with occasional shaking, until consumption of the enamine was complete by } ^1H\text{-NMR spectroscopy. After 16 h, the mixture was submitted for } ^1H\text{-NMR (500 MHz, CD}_3CN) \text{ analysis. Integration of the transient diastereomeric } \alpha-\text{ethylated-iminium resonances revealed a } dr \text{ of 64:36.}
\end{align*}
5. Biological Protocols

5.1 LYP Inhibition Assay

LYP activity and inhibition assays were performed in black 96-well microplates in a total volume of 100 μL using a Molecular Devices Spectramax M5 plate reader. Unless otherwise noted, all solutions were prepared using a Bis-Tris (50 mM, pH 6.5) buffer containing 100 mM NaCl and 0.01% Brij35. The pyrimidine thiones and TCEP stock solutions were prepared using DMSO. Each well in the 96-well plate formatted assay contained 3.58 nM LYP, 100 μM tris(2-carboxyethyl)phosphine (TCEP), 5 μM 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP),\textsuperscript{184} and either 100 μM of inhibitor (initial screen), 5-1000 μM inhibitor (IC\textsubscript{50} determination), or DMSO, resulting in a final DMSO concentration of 6.1% (v/v). Prior to testing, LYP was activated with 1 mM TCEP and allowed to incubate on ice for 30 minutes. Inhibitor compounds were added and allowed to incubate for 30 minutes at room temperature. Reactions were initiated by addition of DiFMUP and the resulting increase in fluorescence was measured every 60 s over 30 min (λ\textsubscript{ex} = 350 nm, λ\textsubscript{em} = 455 nm). Per cent inhibition was calculated by averaging the initial enzyme activities for each inhibitor and normalizing them against a set of DMSO control wells. The IC\textsubscript{50} value of each inhibitor was determined using plots of initial enzyme activity against inhibitor concentration created using Kaleidagraph. Initial screening was run in duplicate while IC\textsubscript{50} testing was run in triplicate.

5.2 Alkaline Phosphatase Assay

Activity of calf intestinal alkaline phosphatase (CIALP) was measured by spectrophotometric assay as previously described by Iqbal et al.\textsuperscript{185} The reaction mixture comprised 50 mM Tris-HCl buffer (5 mM MgCl\textsubscript{2}, 0.1 mM ZnCl\textsubscript{2}, pH 9.5), the compound (0.1 mM with final DMSO 1% (v/v)). This mixture was pre-incubated for 10 min by adding 5 μL of CIALP (0.025 U/mL). Then, 10 μL of substrate (0.5 mM p-NPP (para nitrophenylphosphate disodium salt)) was added to initiate the reaction and the assay mixture was incubated again for 30 min at 37°C. The change in absorbance of released p-nitrophenolate was monitored at 405 nm, using a 96-well microplate reader (OPTI\textsubscript{Max}, Tunable USA). All the experiments were repeated three times. KH\textsubscript{2}PO\textsubscript{4} was used as the reference inhibitor of calf ALP.

5.2.1 Free radical scavenging assay

Radical scavenging activity was determined by modifying the already reported method by Kamal et al.\textsuperscript{165} and Ashraf et al.\textsuperscript{164} using 2, 2-diphenyl-1 picrylhydrazyl (DPPH) assay. The assay solution consisted of 100 μL of DPPH (150 μM) and 20 μL of increasing concentration) of test compounds and the volume was adjusted to 200 μL in each well with DMSO. The reaction mixture was then incubated for 30 minutes at room temperature. Ascorbic acid (Vitamin C) was used as a reference inhibitor. The assay measurements were carried out by using a micro-plate reader (OPTI\textsubscript{Max}, Tunable) at 517 nm. The reaction rates were compared and the percent inhibition caused by the presence of tested inhibitors was calculated. Each concentration was analyzed in three independent experiments.
5.2.2 Cytotoxicity evaluation using brine shrimp assay (Culturing and harvesting of Artemia salina)

Artemia salina cysts were incubated for hatching in a rectangular dish with a plastic divider with several holes making two uneven compartments. The container was filled with 3.3% solution of artificial sea water and dry yeast sprinkled into the larger compartment which was darkened. The smaller compartment was illuminated with light at 28°C. After 24 hours, hatched A. salina cysts were transferred to fresh artificial seawater and incubated for a further 24 hours under artificial light and aeration. The phototropic nauplii were collected by pipette from the lighted compartment.

5.2.2.1 Brine shrimp assay
A. salina nauplii (20) were counted macroscopically using Pasteur pipette against a lighted background and the solutions were made to 5mL with test compound using serial dilutions with brine solution. A drop of dry yeast suspension was added as food to each vial. All the vials were maintained under light. The surviving nauplii were counted with the aid of a magnifying glass after 24 hours. The mean mortality at the three dose levels for compound was determined and repeated thrice. Potassium dichromate was used as reference standard. After 24 hours, the LD_{50} as calculated by Probit analysis.

5.3 Biochemical assays

5.3.1 Cell transfection with human APs and e5-NT
The COS-7 cells were transfected with plasmids expressing human APs: TNAP, IAP, PLAP & GCAP or ecto-5′-nucleotidase either human or rat in 10-cm plates, by using Lipofectamin as standard. The confluent cells were incubated for 5 h at 37°C in DMEM/F-12 in the absence of fetal bovine serum and with 6 µg of plasmid DNA and 24 µL of Lipofectamine reagent. The same volume of DMEM/F-12 containing 20% FBS was added to stop the transfection and cells were harvested 48-72 h later.

5.3.2 Preparation of membrane fractions
These transfected cells were washed three times with Tris–saline buffer at 4°C and then the cells were collected by scraping in the harvesting buffer (95 mM NaCl, 0.1 mM PMSF, and 45 mM Tris buffer, pH 7.5). The cells were washed twice by centrifugation at 300×g for 5 min at 4°C. Later, the cells were resuspended in the harvesting buffer containing 10 µg/mL aprotinin and then sonicated. Cellular and nuclear debris were discarded by 10 min centrifugation (300×g at 4°C). Glycerol (final concentration of 7.5%) was added to the resulting supernatant and all the samples were kept at -80°C until used. Bradford microplate assay was used for the estimation of protein concentration. Bovine serum albumin was employed as a reference standard.
5.3.3 Protocol of alkaline phosphatase assay (h-TNAP, h-IAP, h-PLAP and hGCAP)

The determination of phosphatase activity with h-TNAP, h-IAP, h-PLAP and hGCAP in presence or absence of the test compounds was performed with a chemiluminescent substrate, CDP-star. The conditions for the assay were optimized with the slight modifications in previously-used spectrophotometric method. The composition of assay buffer was: 2.5 mM MgCl₂, 0.05 mM ZnCl₂ and 8 M DEA (pH 9.8). Initial screening of the test compounds was accomplished at a concentration of 0.2 mM. The total volume of 50 µL contained 10 µL of test compound (0.2 mM with final DMSO 1% (v/v)), 20 µL of h-TNAP (46 ng of protein from COS cell lysate in assay buffer) or of h-IAP (57 ng protein in assay buffer) or of h-PLAP (55 ng protein in assay buffer) or of h-GCAP (51 ng protein in assay buffer), depending on assay. The mixture was pre-incubated for 5 to 7 minutes at 37°C and luminescence was measured as pre-read using microplate reader (BioTek FLx800, Instruments, Inc. USA). Then, 20 µL of CDP-star (final concentration of 110 µM) was added to initiate the reaction and the assay mixture was allowed to incubate for 15 min more at 37°C. The change in the luminescence was measured as after-read. The activity of each compound was compared with total activity control (without any inhibitor). Levamisole (2 mM per well) and L-phenylalanine (4 mM per well) were used as a positive control for the inhibition of h-TNAP and h-IAP, respectively. For the compounds which exhibited over 50% inhibition of either enzyme activity, full concentration inhibition curves were drawn to evaluate IC₅₀ values. All experiments were performed in triplicate. The IC₅₀ values were calculated by using Cheng Prusoff equation, using a non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

5.3.4 Ecto-5'-nucleotidase inhibition assay

At the beginning of each day, the capillary was conditioned with the following sequence to obtain the highly reproducible migration times and good separation peaks: (i) rinse with 0.1 N NaOH for 5 min (ii) rinse with distilled water for 5 min and (iii) rinse with running buffer (sodium tetra-borate 20 mM, pH 9.0) for 5 min. The bioactivity assay of both rat and human ecto-5-nucleotidase was performed with the slight modifications in the method as described previously. Stock solutions of 10 mM concentration of each compound were prepared in DMSO. Working solutions were made by appropriately diluting the stock solutions to 1 mM concentration in assay buffer (2 mM MgCl₂, 1 mM CaCl₂ and 10 mM Tris HCl, pH 7.4). The volume of the final assay was kept 100 µL. A 10-µL aliquot of each test compound was preincubated with 10 µL of human e5NT (6.94 µg/mL) protein extract or rat e5NT (7.17 µg/mL) for 10 min in the presence of 70 µL assay buffer (2 mM MgCl₂, 1 mM CaCl₂ and 10 mM Tris HCl, pH 7.4). Afterwards, 10 µL of AMP substrate (final concentration of 500 µM) was added to initiate the enzymatic reaction. The reaction mixture was allowed to incubate for 10 min at 37°C. To stop the enzymatic reaction, quenching by thermal denaturation at 99°C for 20 min. Aliquots of 50 µL of each reaction mixture were transferred to CE minivial and was injected into the CE instrument for data collection and analysis. Prior to each injection, the capillary was rinsed with 0.1 N NaOH for 2 min, distilled water for 2 min, and running buffer (sodium tetra-borate 20 mM, pH 9.00) for 2 min. The enzyme reaction mixture was hydrodynamically injected into the capillary by applying pressure of 0.5 psi for 5s, followed by the application of 15 kV voltages for
separation of peaks of substrate and product. The concentration of product, i.e., adenosine was determined by calculating the area under its absorbance peak at 260 nm. The compounds which exhibited over 50% inhibition of either the r-e5NT or h-e5NT activity were further evaluated for determination of IC$_{50}$ values. For this purpose serial dilutions of each compound were prepared in assay buffer and their dose response curves were obtained by assaying each inhibitor concentration against both nucleotidases using the above mentioned reaction conditions. All experiments were performed in triplicate. The IC$_{50}$ values were calculated by engaging Cheng Prusoff equation, using a non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

5.3.5 Homology modelling of human alkaline phosphatases (h-TNAP, h-IAP, hGCAP)

Since crystal structures of human tissue non-specific alkaline phosphatase (h-TNAP), intestinal alkaline phosphatase (h-IAP) and germ-cell alkaline phosphatase (h-GCAP) have not yet been determined experimentally, therefore, homology models of hTNAP, h-IAP and h-GCAP were generated using Chimera$^{166}$ and Modeller.$^{167}$ Sequences of all three human AP enzymes, h-TNAP (uniprot id: P05186), h-IAP (uniprot id: P09923), and h-GCAP (uniprot id: P10696) were fetched in Chimera. A search on BLAST database$^3$ returned h-PLAP (PDB id: 1ZED; uniprot id: P05187) among top five searched results for all APs, hence, h-PLAP was selected to be used as a template protein. The sequences of target proteins were added to that of template protein via Needleman Wunsch global alignment algorithm$^{169}$ embedded in Chimera. The structures of all target proteins h-TNAP, h-IAP and h-GCAP were modelled using Modeller.$^{167}$ Ramachandran plots were generated using Molprobity$^{168}$. Comparative sequence alignments and Ramachandran plots are given in supporting information, Figures S6-S8 and Figures S2-S4, respectively. All active-site residues among modelled proteins (h-TNAP, h-IAP, h-GCAP), and h-PLAP were found to be highly conserved (Table 1). Amino acid residues Phe107, Gln108, Ser155 and Glu429 in h-PLAP have been replaced by amino acid residues Glu108, Gly109, Thr156 and His434, respectively in h-TNAP. Glu429 of h-PLAP, was found to be the only active-site amino acid residue that was not conserved in any of the h-APs, in h-IAP it is replaced by a Ser448 residue, whereas in h-GCAP, it has been replaced by Gly448 residue. Figures 1-3 show comparison of active site residues of modelled proteins hTNAP, h-IAP and h-GCAP against the template protein h-PLAP.

5.3.6 Homology modelling of rat ecto-5′-nucleotidase (r-e5NT)

Crystal structure of rat ecto-5′-nucleotidase (r-e5NT), is not available from the Protein Data Bank (PDB), therefore, its homology model was generated using Chimera$^{166}$ and Modeller.$^{167}$ The protein sequence of r-e5NT (uniprot id: Q66HL0) was fetched in Chimera. A search on BLAST database$^{168}$ indicated human ecto-5′-nucleotidase (h-e5NT), (PDB id: 4H2I; uniprot id: P21589) among top five results (sharing 88% sequence identity), and was selected to be used as a template protein for modelling of r-e5NT. The sequences of target protein (r-e5NT) was added to that of template protein (h-e5NT) via Needleman Wunsch global alignment algorithm$^{169}$ embedded in Chimera. The structure of target protein, r-
eSNT, was modelled using Modeller. Ramachandran plots were generated using Molprobity. Five models were generated, each model was analyzed individually and the most favorable model, having 87.97% identity and 0.141 Å rmsd, was selected. Comparative sequence alignment and Ramachandran plot are given in supporting information, Figures S1 and Figures S5, respectively. Active-site residues among modelled proteins (r-eSNT), and target protein (h-ST) were found to be highly conserved. Comparison of active site residues of r-eSNT and h-eSNT are given in fig. 4.

5.3.7 Molecular Docking

In order to rationalize most plausible binding-site interactions of inhibitors, molecular docking studies were carried out against three homology built models of alkaline phosphatases (h-TNAP, h-IAP and h-GCAP), and one experimentally-determined hPLAP (PDB id: 1ZED). Crystal structure of closed (active) form human ecto-5′-nucleotidase (h-eSNT) in complex with inhibitor adenosine 5′-(α,βmethylene)diphosphate (AMPCP) was downloaded from the Protein Data Bank (PDB id 4H2I). Prior to docking, the enzymes (receptors) were prepared for docking employing DockPrep utility of Chimera, whereby all hetero and solvent molecules are removed, hydrogen atoms and Gasteiger chargers are added and incomplete side chains (if any) are repaired using Dunbrack Rotamer library. All alkaline phosphatases contain two zinc ions and one magnesium ion in their active sites, charge of +2 was assigned to each metal center. Human ecto-5′-nucleotidase (h-eSNT) contains only two zinc ions in its active site, charge of +2 was assigned to each zinc ion. Structures of all ligands were sketched in ChemSketch, the geometries of all ligands were optimized engaging semi-empirical PM3 method in ArgusLab 4.0.1. Molecular docking studies were carried out using BiosolveIT’s LeadIT software version 2.1.8. Docked conformations were analyzed using LeadIT and Discovery Studio Visualizer 4.0. For h-eSNT method, validation was executed by redocking the ligand (AMPCP) extracted from the crystal structure of h-eSNT in complex with AMPCP (PDB id 4H2I). The docking method was successfully able to reproduce the experimentally observed bound conformation of AMPCP with an rmsd of 1.7 Å.

5.4 Methodology

5.4.1 In silico screening

The compounds were screened in silico for their pharmacokinetic profiling using AdmetSAR software, to quantify their LD50, absorption, distribution, metabolism and other characteristics affecting pharmacokinetics.

5.4.2 Ethical considerations

All experiments were approved by Ethical Committee, Department of Pharmacy, CIIT Abbottabad, in accordance to the guidelines of animal scientific procedure act, 1986.

5.4.3 Animals

Balb C mice in the weight range of 25-30 were used in the experiment. All drugs were suspended in 1% CMC and 1% CMC in saline was used as saline group treatment.
5.5 Antileishmanial activity assays (MTT assay)

The antileishmanial activity of the newly-synthesized compounds was evaluated *in vitro* against the promastigote forms of *Leishmania major* using as MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide)-based microassay as a marker of cell viability. The MTT assay engaged was based on that originally described by Mosmann (1983)\(^{176a}\) modified by Niks and Otto (1990).\(^{176b}\) A stock solution of MTT (Sigma Chemical Co., St. Louis, Mo.) was prepared by dissolving in PBS at 5 mg/mL and storing in the dark at 4°C for up to 2 weeks before use. For the antileishmanial activity assays, 100 μL/well of the culture which contained 2.5 × 10⁶ cells/mL was seeded in 96-well flat-bottom plates. Then, 10 μL/well from various concentrations of synthesized compounds were added to triplicate wells and plates were incubated for 72 h at 25±1°C. The first well of 96 wells was as a blank well which only contained 100 μL culture medium without any compound, drug or parasite. Amphotericin B was used as standard drug. At the end of incubation, 10 μL of MTT was added to each well and plates were incubated for 3 h at 25±1°C. Enzyme reaction was then stopped by the addition of 100 μL of 50% isopropanol and 10% sodium dodecyl sulfate. The plates were incubated for an additional 30 min. under agitation at room temperature. Relative optical density (OD) was then measured at a wavelength of 570 nm using a 96-well microplate reader (Bio-Tek ELx 800TM, Instruments, Inc. USA). The background absorbance of plates was measured at 690 nm and subtracted from 570 nm measurement. The absorbance of the formazan produced by the action of mitochondrial dehydrogenases of metabolically-active cells is shown to correlate with the number of viable cells. All experiments were repeated at least three times. Results reported are mean of three independent experiments (± SEM) and expressed as per cent inhibitions calculated by the formula:

\[
\text{Inhibition (\%) = } [100 - (\text{abs of test comp/abs of control}) \times 100]
\]


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