CHAPTER-4

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

PAGE NO. 59 TO 82
Chapter 4

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\textit{N}-cycloamino/disubstituted/diamino thiazoles coupled
with thiophenes as adenosine receptor ligands

4.1 Introduction

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

Figure 4.1 Design of molecule \textit{N}-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes

Previous reports from our group suggested that 2-cyclicamino group at the 2\textsuperscript{nd} position of thiazole ring resulted into good adenosine receptor ligands (Inamdar 2007; Pandya 2011). So keeping in mind the previous results, here we have designed, synthesized and tested three different set of thiophenyl-thiazolyl carboxamides. In one set, we have replaced
arylamo group of 2\textsuperscript{nd} position of thiazole with \textit{N}-cyclicamino group, in second set we have coupled thiophenes with disubstituted thiazoles and in third set of molecules diamino substituted thiazoles are coupled with thiophenes (Figure 4.1).

4.2 Present work

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

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

![Scheme 4.1](image1)  
Scheme 4.1 Synthetic scheme for \textit{N}-cyclicamino thiazoles coupled with thiophenes

![Scheme 4.2](image2)  
Scheme 4.2 Synthetic scheme for disubstituted thiazoles coupled with thiophenes
As an example, the reaction of \(N\)-phenyl thiourea with DMF-DMA was done to furnish \((E)-N,N\text{-dimethyl-N’-(phenylcarbamothioyl)}\) formimidamide. This formimidamide intermediate (1 mmol) was further reacted with Ethyl 2-(2-chloroacetamido)-4, 5, 6, 7-tetrahydrobenzo[b]thiophene-3-carboxylate (1 mmol) in DMF at room temperature which resulted into PMCDP-16 with good yields. It was further confirmed by LC-MS (M+)\(^+\), \(^1\)H NMR and \(^{13}\)C NMR. The mass spectrum of PMCDP-16 (Figure 4.2) displayed the molecular ion peak at \(m/z\) 428.1(M+1) which confirm the molecular weight of the compound 427.5 calculated for \(C_{21}H_{21}N_3O_3S_2\).

**Figure 4.2** LC-MS (M+\(^+\)) of PMCDP-16
The structure of the molecule was further confirmed by $^1$H NMR (500 MHz, CDCl$_3$), which gave the characteristic peak of all the hydrogens present. The amide proton –NH singlet came at 12.02 δ and the thiazole –NH singlet proton came at 8.20 δ. The protons of ester methyl group (−CH$_3$) was observed at 1.41-1.44 δ and split into triplet due to presence of adjacent methylene group(−CH$_2$). The eight protons of four methylene group were found in the range 1.82-2.81 δ. The two protons of ester methylene group were found at 4.37-4.41 with quartet. All the five aromatic protons of phenyl ring were observed from 7.18-7.86 δ and singlet hydrogen of thiazole was observed at 7.97 δ (Figure-4.3).

Figure 4.3 $^1$H NMR of PMCDP-16
Further, the structure of the molecule was confirmed by $^{13}$C NMR (500 MHz in CDCl$_3$). The carbonyl carbon present in the ester group of thiophene is observed most downfield at 166.94 δ. The other carbonyl carbon of amide was observed at 157.48 δ. The carbon of methyl group present on ester of thiophene was observed most upfield at 14.137 δ. The four methylene group carbons of cyclohexane attached to thiophene was observed in the range of 22-26 δ. The methylene group carbon of ester was observed at 60.62. All the aromatic carbon present on phenyl, thiophene and thiazole rings were observed in the range of 98-147 δ (Figure 4.4).

![Figure 4.4 $^{13}$C NMR of PMCDP-16](image)

After establishing the synthetic methodology, we synthesized library of compounds with structural diversification (PMCDP-n, Table 4.1).
Table 4.1 Synthesis of N-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes

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4.3 Biological results and discussion

The binding affinity of the compounds was evaluated as per the procedure described in the chapter 3. The compounds PMCDP-24, PMCDP-29 and PMCDP-41 found to show affinity towards A₁, A₂A and A₃ adenosine receptors with Ki values in micromolar range. The compound PMCDP-29 was non-selective with hA₁/hA₃=1.7 and hA₂A/hA₃=2.1. The compound PMCDP-24 was hA₁/hA₃>10 and hA₂A/hA₃>10 fold selective and PMCDP-41 was hA₁/hA₃>2 and hA₂A/hA₃>2 fold selective. Presence of phenyl or 2-furyl group on the 4th position of thiazole ring was found to be responsible for good activity for this series of compounds. Whereas presence of hydrogen on the 4th position of thiazole ring was found to be deleterious for the biological activity (Table 4.2).

Table 4.2 Binding affinity of synthesized molecules

<table>
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Data are expressed as geometric means with 95% confidence limits.

\textsuperscript{a} Displacement of specific \([\textsuperscript{3}H]\)CCPA binding at human A\textsubscript{1} receptors expressed in CHO cells.

\textsuperscript{b} Displacement of specific \([\textsuperscript{3}H]\)NECA binding at human A\textsubscript{2A} receptors expressed in CHO cells.

\textsuperscript{c} Displacement of specific \([\textsuperscript{3}H]\)HEMADO binding at human A\textsubscript{3} receptors expressed in CHO cells.

\textsuperscript{d} Inhibition of NECA-stimulated adenylyl cyclase activity at human A\textsubscript{2B} receptors expressed in CHO cells.
4.4 Conclusion

In conclusion, here we have designed new series of new N-cycloamino/disubstituted/diamino thiazoles coupled with thiophenes. Further, the molecules were synthesized and evaluated for their radio ligand binding assay against adenosine receptors. The compounds were active towards adenosine receptors and particularly they were found selectivity against A₃ adenosine receptor with high selectivity. But overall the affinity of the molecules to adenosine receptor was found to be less as compared to affinity of the molecules from chapter-3.

4.5 Experimental

Melting points were recorded on scientific melting point apparatus (Veego; Model: VMP-DS) and are uncorrected. The ¹H NMR spectra were recorded on Bruker NMR spectrometer (400 MHz) using TMS as an internal standard and ¹³C NMR spectra were recorded on Bruker NMR spectrometer at 75 MHz. Proton chemical shifts are expressed in ppm relative to internal tetramethylsilane. Mass spectra were recorded on Perkin Elmer Sciex API 165. TLC was carried out on Merck Kieselgel 60 PF₂₅₄. IUPAC name of the compounds were generated using Cambridge soft ChemBioDraw ultra 12.0. The preparations of substituted 2-chloroacetamido thiophenes are described in the chapter-3.

4.5.1. General procedure for the synthesis of Aroyl isothiocyanates

\[
\begin{array}{c}
\text{R}_1\text{Cl} \\
\text{KSCN, TBAB} \\
\text{0-10°C} \\
\rightarrow \\
\text{R}_1\text{NCS}
\end{array}
\]

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

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

4.5.1.2. *Synthesis of Furoyl Isothiocyanate*

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

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

To a stirred solution of aroyl isothiocynate (1 mmol) in THF at room temperature, morpholine (1eq) was added dropwise via syringe. The reaction was stirred for 3h and reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into the cold water to give precipitates of the desired product. The resultant solid was filtered through high vacuum suction pump and then dissolved in chloroform and dried over anhydrous Na₂SO₄. The solvent was removed on rotary evaporator to give desired compounds which was sufficiently pure and directly used for the next step.

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

The title compound was synthesized using the procedure as described in 4.5.2 by the reaction of benzoyl isothiocyanate and morpholine to afford the product. *%Yield*: 58, *LCMS*: (M+1) at 251, *Molecular Formula*: C₁₂H₁₄N₂O₂S, *m.p.* = 141-3°C, *Rf*: 0.71 (Tol :ACN :: 7:3).

4.5.2.2. *Synthesis of N-(4-Methylpiperazine-1-carboxothioyl)benzamide (C2)*
The title compound was synthesized using the procedure as described in 4.5.2 by the reaction of benzoyl isothiocyanate and 4-methyl piperazine to afford the product.

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

![Chemical structure of the title compound](image.png)

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

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

![Chemical structure of the title compound](image.png)

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

4.5.3. General procedure for the synthesis of Amidinothiourea

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

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

![Chemical structure of the title compound](image.png)

The title compound was synthesized using the procedure as described in 4.5.3 by the reaction of N-phenyl thiourea and N,N-dimethylformamide dimethylacetal(DMF-DMA) to afford the title product.
4.5.3.2. Synthesis of (E)-N,N-Dimethyl-N’-(p-tolylcarbamothioyl) formimidamide (C6)

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

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

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

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

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

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

To a stirred suspension of guanidine hydrochloride (3 mmole) and phenyl isothiocyanate (1 mmole) in THF, was added sodium hydroxide (3 mmole) dissolved in water at 0°C in
10 min. Stirring was continued at ambient temperature for 4 hours. The solid generated during the reaction was filtered with 10 ml water and washed with water and dried to give the title product. TLC : Mobile Phase : Ethylacetaee, Rf : 0.63. Mass (LC-MS Found, M+1) : 195

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

![Chemical structure of 1-Amidino-3(4-methylphenyl)thiourea (C10)](image)

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

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

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

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

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

Yield: 63%, Off white solid, mp: 226-229 °C, **Molecular formula:** C$_{21}$H$_{21}$N$_{3}$O$_{3}$S$_{2}$, LC-MS calculated: 427.1, found: 428.3(M+1), **$^1$H NMR (400 MHz, CDCl$_3$)** δ: 1.41(t, 3H), 1.82(d, 4H), 2.69(s, 2H), 2.81(s, 2H), 4.37(q, 2H), 7.18(d, 1H), 7.40-7.46(m, 4H), 7.97(s, 1H), 8.20(s, 1H, -NH), 12.02(s, 1H, -NH). **$^{13}$C NMR (400 MHz, DMSO-d$_6$)** δ: 14.37, 22.84, 23, 24.43, 26.41, 30.96, 60.62, 111.51, 119.23, 124.54, 126.96, 129.80, 130.99, 139.15, 142.61, 147.70, 157.48, 166.94.
PMCDP-17: Ethyl 2-(2-(p-tolylamino)thiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate

Yield: 67%, Light yellow solid, mp: 235-239 °C, Molecular formula: C_{22}H_{23}N_{3}O_{3}S_{2}, LC-MS calculated: 441.3, found: 442.3(M+1). \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ: 1.41(t, 3H), 1.82(s, 4H), 2.38(s, 3H), 2.68(s, 2H), 2.81(s, 2H), 4.36(q, 2H), 7.23(d, 2H), 7.28(d, 2H), 7.92(s, 1H), 7.95(s, 1H, -NH), 11.98(s, 1H, -NH).

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

Yield: 67%, Light yellow solid, mp: 230-233 °C, Molecular formula: C_{21}H_{20}ClN_{3}O_{3}S_{2}, LC-MS calculated: 461.1, found: 462.3(M+1). \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ: 1.31(t, 3H), 1.72(s, 4H), 2.61(s, 2H), 2.71(s, 2H), 4.28(q, 2H), 7.39(d, 2H), 7.65(d, 2H), 7.92(s, 1H), 10.95(s, 1H, -NH), 11.58(s, 1H, -NH).

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

Yield: 68%, White solid, mp: >250 °C, Molecular formula: C_{20}H_{18}ClN_{3}O_{5}S_{2}, LC-MS calculated: 479.1, found: 480.4(M+1). \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ: 1.35(t, 3H),
2.67(s, 3H), 3.79(s, 3H), 4.34(q, 2H), 7.39(d, 2H), 7.65(d, 2H), 7.98(s, 1H), 11.03(s, 1H, -NH), 11.73(s, 1H, -NH).

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

Yield: 74%, Off white solid, mp: 235-237 °C, Molecular formula: C_{25}H_{27}N_{3}O_{4}S_{2}, LC-MS calculated: 497.2, found: 498.4(M+1).\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\): 1.25(t, 3H), 1.78(s, 4H), 2.65(s, 2H), 2.72(s, 2H), 3.62(s, 4H), 3.85(s, 4H), 4.06(q, 2H), 7.42(d, 3H), 7.67(d, 2H), 11.20(s, 1H).

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

Yield: 72%, Off white solid, mp: 219-222 °C, Molecular formula: C_{24}H_{25}N_{5}O_{1}S_{2}, LC-MS calculated: 463.4, found: 464.4(M+1).\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\): 1.74(s, 4H), 2.46(s, 2H), 2.59(s, 2H), 2.85(s, 3H), 3.36(s, 8H), 7.43(s, 3H), 7.62(s, 2H), 10.49(s, 1H), 10.84(s, 1H).
PMCDP-27: \( N-(3\text{-Cyano-4,5,6,7-tetrahydrobenzo}\{b\}\text{thiophen-2-yl})-4\text{-}(furan-2-yl)-2\text{-}(4\text{-methylpiperazin-1-yl})\text{thiazole-5-carboxamide} \)

Yield: 69\%, Off white solid, mp: >250 \( ^\circ \)C, Molecular formula: \( C_{22}H_{23}N_5O_2S_2 \), LC-MS calculated: 453.4, found: 454.4(M+1). \( ^1H \) NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \): 1.77(s, 4H), 2.67(s, 2H), 2.74(s, 2H), 3.52(t, 4H), 3.71(t, 4H), 6.70(s, 1H), 7.08(d, 1H), 7.85(s, 1H), 11.49(s, 1H, -NH).

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

Yield: 59\%, Off white solid, mp: 182-185 \( ^\circ \)C, Molecular formula: \( C_{22}H_{23}N_3O_7S_2 \), LC-MS calculated: 505.1, found: 506.7(M+1). \( ^1H \) NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \): 1.41(t, 3H), 2.80(s, 3H), 3.64(s, 4H), 3.87(s, 7H), 6.58(s, 1H), 7.18(s, 1H), 7.73(s, 1H), 12.52(s, 1H). \( ^{13}C \) NMR (400 MHz, DMSO-\textit{d}_6) \( \delta \): 14.27, 15.60, 47.93, 51.59, 60.89, 66.07, 111.81, 113.92, 114.63, 115.57, 117.42, 143.24, 144.31, 144.82, 148.86, 152.93, 158.98, 163.60, 165.85, 170.86.
PMCDP-29: Ethyl 2-(4-(furan-2-yl)-2-morpholinothiazole-5-carboxamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate

Yield: 59%, White solid, mp: 199-202 °C, Molecular formula: C_{23}H_{25}N_{5}O_{5}S_{2}, LC-MS calculated: 487.1, found: 487.5(M+1). $^1$H NMR (400 MHz, CDCl$_3$) δ: 1.24(t, 3H), 1.73(s, 4H), 2.61(s, 2H), 2.72(s, 2H), 3.52(t, 4H), 3.72(t, 4H), 4.21-4.28(s, 2H), 6.68(q, 1H), 7.10 (d, 1H), 7.86(s, 1H), 11.99(s, 1H, -NH).

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

Yield: 59%, White solid, mp: 197-199 °C, Molecular formula: C_{24}H_{25}N_{5}O_{6}S_{2}, LC-MS calculated: 515.1, found: 515.6(M+1). $^1$H NMR (400 MHz, CDCl$_3$) δ: 1.41(t, 3H), 2.80(s, 3H), 3.64(s, 4H), 3.87(s, 7H), 6.58(s, 1H), 7.18(s, 1H), 7.73(s, 1H), 12.52(s, 1H) $^{13}$C NMR (400 MHz, DMSO-d$_6$) δ: 14.27, 15.60, 47.93, 51.59, 60.89, 66.07, 111.81, 113.92, 114.63, 115.57, 117.42, 143.24, 144.31, 144.82, 148.86, 152.93, 158.98, 163.60, 165.85, 170.86.

PMCDP-31: 2-((4-Chlorophenyl)amino)-N-(3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)thiazole-5-carboxamide
Yield: 60%, White solid, mp: 203-206 °C, Molecular formula: C₁₉H₁₅ClN₄O₂S₂, LC-MS calculated: 414.1, found: 415.6(M+1). ¹H NMR (400 MHz, CDCl₃) δ: 1.73(s, 4H), 2.60(s, 2H), 2.72(s, 2H), 7.39(d, 2H), 7.65(d, 2H), 7.92(s, 1H), 10.98(s, 1H, -NH), 11.72(s, 1H, -NH).

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

Yield: 57%, White solid, Molecular formula: C₂₀H₁₉N₃O₅S₂, LC-MS calculated: 445.1, found: 446.4(M+1). ¹H NMR (400 MHz, DMSO-d₆) δ: 1.32(t, 3H), 2.26(s, 3H), 2.90(s, 6H), 3.78(s, 3H), 4.33(q, 2H), 7.19(d, 1H), 7.40-7.46(m, 4H), 7.97(s, 1H), 10.20(s, 1H, -NH), 12.02(s, 1H,-NH).

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

Yield: 54%, White solid, Molecular formula: C₁₉H₁₇ClN₄O₂S₂, LC-MS calculated: 432.1, found: 433.7(M+1). ¹H NMR (400 MHz, CDCl₃) δ: 1.71(s, 4H), 2.61(s, 2H), 2.74(s, 2H), 6.39(s, 2H,-NH₂), 7.40(d, 2H), 7.63(d, 2H), 7.92(s, 1H), 10.98(s, 1H, -NH), 11.72(s, 1H, -NH).
PMCDP-34: Methyl 4-carbamoyl-5-(4-(furan-2-yl)-2-morpholinothiazole-5-carboxamido)-3-methylthiophene-2-carboxylate

Yield: 50%, White solid, **Molecular formula:** $\text{C}_{22}\text{H}_{22}\text{N}_4\text{O}_5\text{S}_2$, LC-MS calculated: 486.1, found: 487.7(M+1).

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

Yield: 53%, off white solid, **Molecular formula:** $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_4\text{S}_2$, LC-MS calculated: 468.1, found: 469.4(M+1). $^1\text{H NMR (400 MHz, CDCl}_3\text{)}$ $\delta$: 2.82(s, 3H), 3.65(s, 4H), 3.78(s, 3H), 3.87(s, 4H), 6.58(s, 1H), 7.18(s, 1H), 7.73(s, 1H), 12.52(s, 1H).

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

Yield: 58%, Yellow solid, mp: 193-196 $^0$C, **Molecular formula:** $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_3\text{S}_2$, LC-MS calculated: 427.1, found: 428.7(M+1). $^1\text{H NMR (400 MHz, CDCl}_3\text{)}$ $\delta$: 2.38(s, 3H), 2.80(s, 3H), 3.77(s, 3H), 6.05(s, NH$_2$), 6.58(s, 1H), 7.37-7.40(d, 2H), 7.65-7.68(d, 2H), 10.98(s, 1H, -NH), 11.70(s, 1H, -NH).
Chapter 4

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

Yield: 55%, Yellow solid, **Molecular formula:** C$_{21}$H$_{21}$N$_5$O$_4$S$_2$, LC-MS calculated: 471.1, found: 472.6(M+1).

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

Yield: 49%, Yellow solid, **Molecular formula:** C$_{16}$H$_{11}$FNN$_4$OS$_2$, LC-MS calculated: 358.1, found: 359.6(M+1).

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

Yield: 49%, Yellow solid, **Molecular formula:** C$_{21}$H$_{21}$N$_5$O$_3$S$_2$, LC-MS calculated: 455.1, found: 455.6(M+1).

4.5.6. Method used for pharmacological evaluation

The methods described in the chapter-3 were used for the pharmacological evaluation of the compounds.
Supporting Information
(LC-MS, $^1$H-NMR, $^{13}$C NMR, of representative compounds)
Chapter 4

LC-MS spectrum of PMCDP-28

Exact Mass: 505.10

$^1$H spectrum of PMCDP-28
\(^{13}\text{C}\) spectrum of PMCDP-28