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Design, Synthesis, Characterization and Biological Activity of Novel Thieno[2,3-*d*]pyrimidine Derivatives

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ABSTRACT

A new series of N-(4-(substituted amino) thieno[2,3-d]pyrimidin-2-yl) thiophene/Furan-2-carboxamide (7 a-j) derivatives were synthesized by a five-step procedure that afforded advantages of mild reaction conditions, simple protocol and good yields. Several thieno[2,3-d]pyrimidines have been prepared from methyl 2-aminothiophene-3-carboxylate (1). The structures of the final compounds were confirmed by infrared, nuclear magnetic resonance, and electron impact mass spectrum. The final compounds were screened for their antibacterial activity against Staphylococcus aureus and Bacillus subtilis from Gram-positive group of bacteria. Pseudomonas aeruginosa and Escherichia coli from Gram-negative group of bacteria. Antifungal activity against Aspergillus niger and Candida albicans. Antibacterial and antifungal activities were evaluated and compared with the standard drugs such as amoxicillin and fluconazole. From antibacterial and antifungal activity screening results, it has been observed that compounds 8j, 8i, 8h and 8g possess good activity.

Key words: *Thieno[2,3-d]pyrimidine, Acid-amine coupling reaction, 2, 4-di chloro thieno[2,3-d]pyrimidine, Anti-bacterial, Anti-fungal activity, Amoxicillin, Fluconazole.*

1. INTRODUCTION

Pyrimidine has always been a unique interesting heterocyclic moiety for the medicinal chemists; an exhaustive research has been done on the pyrimidines that led to the discovery and introduction of several drugs into the market. From the standpoint of biological activity, fused heteroaromatic systems are often of much greater interest than the constituent monocyclic compounds. The appearance of qualitatively new properties of an annulated molecule, enlargement of the possibility of varying pharmacophore groups in different positions of the molecule and the ability of the latter to interact with a wider spectrum of receptors adopting various conformations are apparently of crucial importance. In addition, the structure of the molecule can be varied by annealing at different positions of individual heterocyclic fragments.

Fused pyrimidines have also been attracted considerable interest in medicinal chemistry research due to their versatility and a broad bioactive potential. Thieno pyrimidine is among those fused pyrimidines found to have a wide variety of pharmacological and biological applications. Since the last four decades research has been focused on the design and synthesis of novel thieno pyrimidines as medicinal agents, a large number of reports have been documented on thieno pyrimidines as they found to exhibit a variety of biological activities such as antimicrobial, anti-inflammatory, bronchodilator activity, inhibition of phosphodiesterases, tyrosine kinase, and vascular endothelial growth factor receptor kinase. It is evident that purine as an endogenous scaffold plays an important biochemical role in variety of regular physiological functions such as respiration, inflammation, cell proliferation, and so forth. As a bioisoster to purines, thieno[2,3-*d*]pyrimidines were also found to exhibit numerous biological activities probably due to the interaction with various physiological elements.

Thieno pyrimidine is a bicyclic heterocyclic compound consists of a five-membered thiophene

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ring is fused to a six-membered heterocyclic ring with two nitrogen atoms. The fusion may occur in three different orientations that results in three important types of thieno pyrimidines, namely, thieno[2,3-*d*] pyrimidine (a), thieno[3,2-*d*]pyrimidine (b), and thieno[3,4-*d*]pyrimidine (c). Most of the isomeric thienopyrimidines occur as colored amorphous form, some exists as crystalline form.

Synthetic approaches for the construction of a number of thieno pyrimidines are well established. There exists three possible types of fusion of thiophene to pyrimidines ring results in corresponding isomeric thienopyrimidines, namely; thieno[2,3-*d*] pyrimidines (a), thieno[3,4-*d*]pyrimidines (b), and thieno[3,2-*d*]pyrimidines (c) (Figure 1).

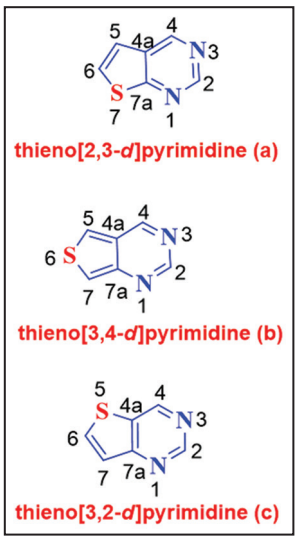


Figure 1: Structures of different isomers of thieno pyrimidine.

Thiophene containing compounds are well known to exhibit various biological effects. Heterocycles containing the thieno pyrimidine moiety are of interest because of their interesting pharmacological and biological activities [1-3]. They bear structural analogy and isoelectronic relation to purine and several substituted thieno[2,3-*d*]pyrimidine derivatives shown to exhibit prominent and versatile biological activities [4,5]. Over the last two decades, many thieno pyrimidines have been found to exhibit a variety of pronounced activities. Many of their derivatives have been synthesized as potential anticancer [6], analgesic [7], antimicrobial [8], antifungal [9], and antiviral agents [10].

Some reviews on pyrimidine thiones [11] and condensed pyrimidines, namely pyrazolopyrimidines [12] and furo-pyrimidines [13]. Thieno pyrimidines are interesting heterocyclic compounds and a number of derivatives of these compounds display therapeutic activity as antimicrobial [14-17], antiviral [18,19], anti-inflammatory [20,21], antidiabetic [22], antioxidant [23], antitumor [24-28] and anticancer agents [29,30], antidepressant [31], antiplatelet [32], antihypertensive [33], herbicidal [34], and plant growth regulatory properties [35].

As a logical consequence of thiophene – phenyl isosterism, similarly thieno pyrimidines can be considered as bioisosteres of quinazolines, which are extensively described in scientific and patent literature as displaying a plethora of biological activities. The synthesis of thieno pyrimidine derivatives as potential surrogates for the quinazoline core structure has, therefore, become a routine strategy in modern drug design and development. Thieno pyrimidines as isosteres of quinazolines are shown in Figure 2.

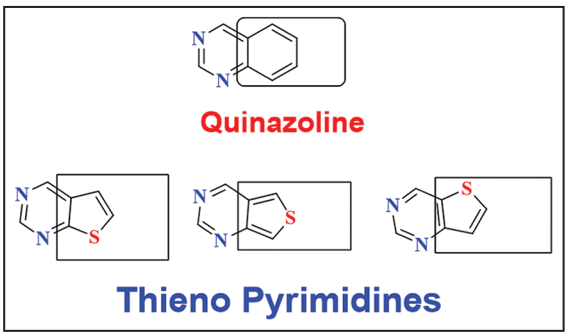


Figure 2: Structures of thiophene – phenyl isosterism in quinazolines and thieno pyrimidine.

Thienopyrimidines can also be considered as structural analogues of five-membered heterocycles such as purines and thiazolo-pyrimidines. As interesting anti-HIV activity was discovered within the thiazolo[5,4-*d*] pyrimidine series, whereas the thiazolo[4,5-*d*] pyrimidines lack antiretroviral activity. The structures of purines and thiazolo pyrimidines are shown in Figure 3.

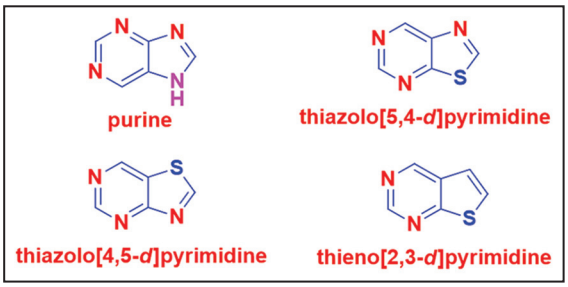


Figure 3: Structure of purines and thiazolo pyrimidines.

This work aimed to synthesize some new thieno[2,3-*d*] pyrimdine derivatives starting with methyl 2-aminothiophene-3-carboxylate and urea, to evaluate their biological activities.

Encouraged by the diverse biological activities of thieno[2,3-*d*]pyrimidine heterocyclic compounds and it was decided to prepare a new series of thieno[2,3-*d*] pyrimidine heterocyclic compounds. Literature survey revealed that incorporation of different groups in thieno[2,3-*d*]pyrimidine heterocyclic ring enhanced antibacterial and antifungal activity. In the present

communication 2,4-di chloro thieno[2,3-*d*]pyrimidine (3) was reacted with various amines in T-butanol to form novel thieno pyrimidine[2,3-*d*] derivatives (4 a-e), which was further converted into amine by using aqueous ammonia to form compounds (5 a-e), which was reacted with different acids (6 a-b) under acid-amine coupling reaction conditions by using HATU to get target compounds (8 a-8j). The synthesis of the compounds as per the following Scheme 1 given below.

The synthetic route was depicted in Scheme 1.

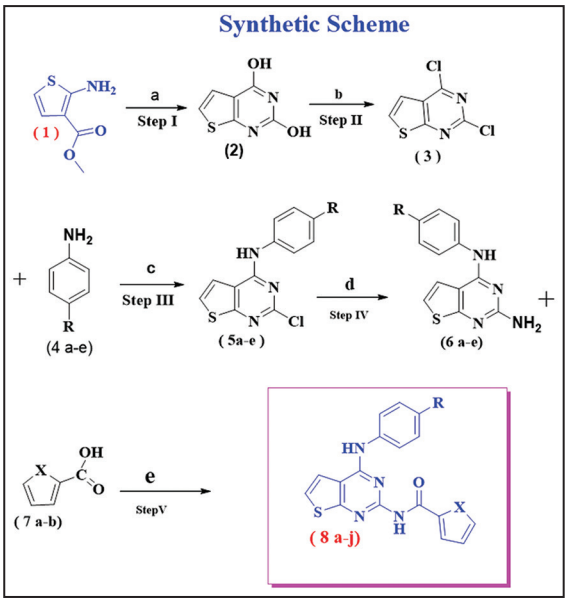
The structures of all synthesized compounds were assigned on the basis of infrared (IR), mass, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data analysis. Further, these compounds were subjected for antifungal and antibacterial activity.

2. MATERIALS AND METHODS

In this Investigation, chemicals were purchased from local dealer with S.D fine make was used. Chemicals were 99% pure; purity has been checked by thin layer chromatography and melting point. Conventional method has been used for synthesis of thieno[2,3-*d*] pyrimidine derivatives. Stirring and reflux method were used for synthesis of thieno[2,3-*d*]pyrimidine derivatives 8 (a-j), respectively.

The synthetic route was depicted in Scheme 1.

The title compounds 8 (a-j) were synthesized in five sequential steps using different reagents and reaction conditions, the 8 (a-j) were obtained in moderate yields. The structure was established by spectral (IR,^{1}H-NMR,^{13}C-NMR and mass) data (Figure 4).



R_1= -H, -4 CH_3, -4 OCH_3, -4 OCF_3, -4 CF_3, X= -O, -S.

Scheme 1: Synthetic path way of preparation of Novel Thieno-Pyrimidine [2,3-*d*] derivatives (8 a-8j).

Reagents and reaction conditions: (a) 5 eq Urea, 190°C, 3 h, (b) $POCl_3$, Reflux, 6 h, (c) tert-butyl alcohol, Hunig's base (NEthyl, N,N-di isopropyl amine), 50-60°C, 6 h. (d) aqueous ammonia, 90°C, 6 h (e) HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexa fluoro phosphate), Hunig's base (*N,N*-di isopropyl ethylamine), DMF, RT,16 h.

HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexa fluoro phosphate) is a reagent used in peptide coupling chemistry to generate an active ester from a carboxylic acid. HATU is used along with Hunig's base (*N,N*-di isopropyl ethylamine) to form amide bonds. generally DMF is used as solvent, although other polar organic solvents can also be used.

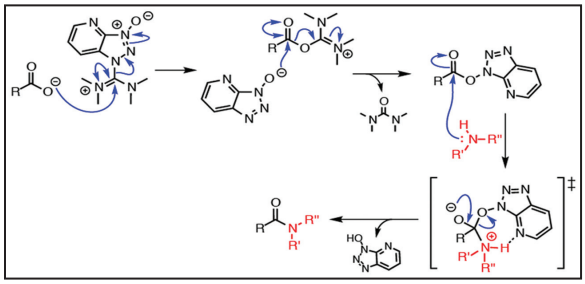


Figure 4: A plausible mechanism pathway for the formation of amide bond formation 8 (a-j) From acid 7 (a-b) and amine 6 (a-e) using HATU:

Steps:

1. The base deprotonates the carboxylic acid. The resulting carboxylate anion attacks the electron deficient carbon atom of HATU.
2. The resulting HOBt anion reacts with the newly formed activated carboxylic acid derived intermediate to form an OBt activated ester.
3. The amine reacts with the OBt activated ester to form the amide bond.

3. EXPERIMENTAL SECTION

All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. All solvents were reagent grade. Tetrahydrofuran was distilled from sodium benzophenone ketyl and degassed thoroughly with dry argon directly before use. Unless otherwise noted, organic extracts were dried with anhydrous Na_2SO_4, filtered through a fitted glass funnel, and concentrated with a rotary evaporator (20-30 Torr). Flash chromatography was performed with silica gel (200-300 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz, for^{1}H for^{13}C, respectively, in $CDCl_3$ solution with tetramethyl silane (TMS) as internal standard. Chemical shifts are given in ppm (δ) and are referenced to the residual proton resonances of

the solvents. Proton and carbon magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded using TMS in the solvent of $CDCl_3$-d_1 or dimethylsulfoxide (DMSO)-d_6 as the internal standard (^{1}H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm).

3.1. Synthesis

General procedure for synthesis of thieno[2,3-d] pyrimidine-2,4-diol [compound (2)]:

A mixture of methyl 2-aminothiophene-3-carboxylate (0.13 mol, 20 g) and urea (1 mol, 60 g) were mixed with each other, and the mixture was heated for 2 h at 200°C. A clear, brown molten mass was formed which solidified upon standing; the solid product was dissolved in warm 1 N sodium hydroxide, and then acidified with 2 N hydrochloric acid. The crystalline precipitate formed thereby was collected by vacuum filtration and re-crystallized from water, yielding 65% (13.8 g) of thieno[2,3-*d*]pyrimidine-2, 4-diol.

Yield: 65% (white color solid);

IR (KBr, cm^{-1}): 3440(-OH), 1160 (C-O-C Stretching), 3090 (Ar C-H), 1630 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 11.44 (S, 1H, -OH), 9.18 (S, 1H, -OH), 6.94 (d, 1H, J_{HH} = 8.0 Hz, Ar-H), 7.29 (d, J_{HH} = 8.0 Hz, 1H, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 128.92, 124.03, 128.11, 159.62, 151.67, 154.75.

MS (ESI): m/z = 167 $(M-H)^+$.

General procedure for synthesis of 2,4-dichlorothieno[2,3-d]pyrimidine [compound (3)]:

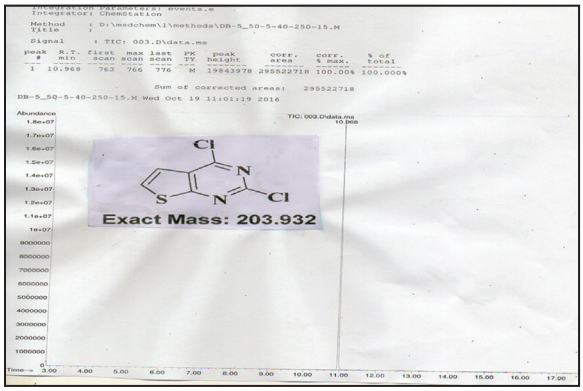
A mixture consisting of (8.4 g, 0.05 mol) 2, 4-di hydroxy-thieno[2,3-*d*]pyrimidine (2) (8.4 g, 0.05 mol) and 100 ml. of phosphorus oxychloride was refluxed for 10 h, whereby a clear solution was formed. After completion of reaction as monitored by thin layer chromatography (TLC), the excess UN reacted phosphorus oxychloride was evaporated in vacuo, the residual oil was poured into ice water, and the aqueous mixture was extracted with chloroform. The chloroform phase was isolated, washed with water until neutral, then dried over Sodium sulfate, the chloroform was evaporated in vacuo, and the solid residue was re-crystallized from ethanol. 7.65 g. (55% of yield) of 2, 4-dichloro thieno[2,3-*d*]pyrimidine, M.P. 161-162°C., were obtained.

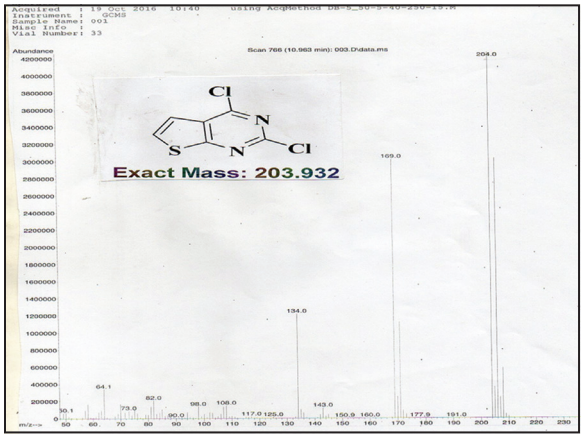
IR (KBr, cm^{-1}): 740 (-C-Cl), 3110 (Ar C-H), 1660 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 7.65 (d, 1H, J_{HH} = 6.5 Hz, Ar-H), 7.45 (d, J_{HH} = 6.5 Hz, 1H, Ar-H).

^{13}C NMR (100 MHz; $CDCl_3$): δC 126.92, 123.03, 126.11, 153.62, 161.67, 154.75.

GC-MS: RT at 10.968 (100%), m/z = 204$(M+H)^+$, 206(M+2), 208(M+4), 9:6:1 it indicates molecule contain two chlorine atoms.





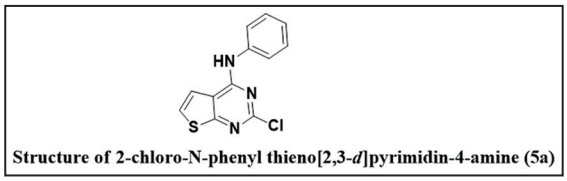
3.2. GC-MS Analysis of 2, 4-dichloro Thieno[2,3-d] pyrimidine

General procedure for synthesis of 2-chloro-N-phenyl thieno[2,3-d]pyrimidin-4-amine(5a), 2-chloro-N-p-tolylthieno[2,3-d]pyrimidin-4-amine (5b), 2-chloro-N-(4-methoxyphenyl)thieno[2,3-d]pyrimidin-4-amine (5c), 2-chloro-N-(4-(trifluoromethoxy)phenyl) thieno[2,3-d]pyrimidin-4-amine (5d), 2-chloro-N-(4-(trifluoromethyl)phenyl)thieno[2,3-d]pyrimidin-4-amine (5e):

To a well stirred solution of 2,4-dichlorothieno[2,3-*d*] pyrimidine (3) (1 m.mol) and N, N Di isopropyl ethyl amine (1.3 m.mol) in *tert*-BuOH (15 ml) at room temperature, then various substituted amine compounds-(4 a-e) (1 m.mol) was added. The reaction mixture was allowed to stir at 60-70°C for 9-10 h. After completion of reaction as monitored by TLC, *tert*-BuOH was evaporated under reduced pressure and the residue was treated with water. The mixture was extracted with EtoAc (3 × 30 ml), and the combined organic phase was dried over Na_2SO_4 and concentrated in vacuo. The

crude product was purified by column chromatography to give the corresponding compounds 5 (a-e).

2-chloro-N-phenyl thieno[2,3-*d*]pyrimidin-4-amine(5a):



Structure of 2-chloro-N-phenyl thieno[2,3-*d*]pyrimidin-4-amine (5a)

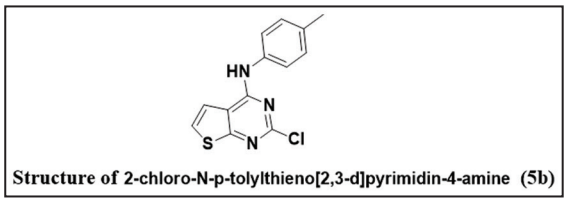
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm 5.5 (bs, 1H), 7.3 (d, 1H, J = 7.2 HZ), 7.05 (1H, d, J = 7.2 HZ), 6.8 (1H.t), 7.3 (2H, m), 7.7 (2H, m).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 118, 123, 126, 129, 140, 155, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C=N (1646.15), C=C (1575), C-Cl (739), N-H (3292).

ESI-MS m/z 262[M+H]$^+$**.**

2-chloro-N-p-tolyl thieno[2,3-*d*]pyrimidin-4-amine (5b):



Structure of 2-chloro-N-p-tolylthieno[2,3-d]pyrimidin-4-amine (5b)

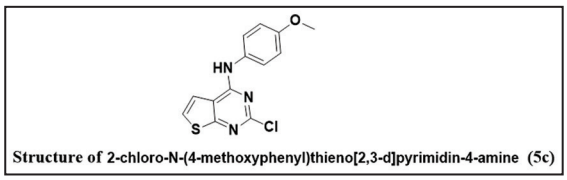
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 2.35 (3H, s), 4.8 (bs, 1H), 7.3 (d, 1H, J = 7.1 HZ), 7.1 (1H, d, J = 7.1 HZ), 7.4 (2H, d, J = 6.7 HZ), 7.2 (2H, d, J = 6.7 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 22, 118, 123, 126, 132, 140, 156, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-H (2940), C-Cl (739), C=C (1575), N-H (3310).

ESI-MS m/z 276[M+H]$^+$**.**

2-chloro-N-(4-methoxyphenyl) thieno[2,3-*d*] pyrimidin-4-amine (5c):



Structure of 2-chloro-N-(4-methoxyphenyl)thieno[2,3-d]pyrimidin-4-amine (5c)

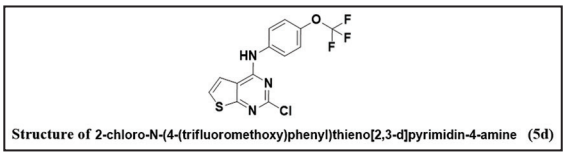
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 3.8 (3H, s), 4.6 (bs, 1H), 7.3 (d, 1H, J = 7.4 HZ), 6.9 (1H, d, J = 7.4 HZ), 7.1 (2H, d, J = 6.9 HZ), 7.6 (2H, d, J = 6.9 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 58, 118, 123, 126, 132, 140, 156, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-H (2955), C-Cl (760), C-O-C (1160), C=C (1575), N-H (3310).

ESI-MS m/z 290[M-H]$^+$**.**

2-chloro-N-(4-(tri fluoro methoxy) phenyl) thieno[2,3-*d*]pyrimidin-4-amine (5d):



Structure of 2-chloro-N-(4-(trifluoromethoxy)phenyl)thieno[2,3-d]pyrimidin-4-amine (5d)

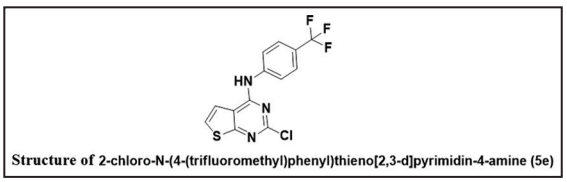
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 5.3 (bs, IH), 7.2 (d, 1H, J = 7.4 HZ), 6.95 (1H, d, J = 7.4HZ), 6.8 (2H, d, J = 6.8 HZ), 7.6 (2H, d, J = 6.8 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 118, 123, 126, 133, 142, 156, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-F (1280), C-Cl (750), C-O-C (1160), C=C (1575), N-H (3310).

ESI-MS m/z 346[M+H]$^+$**.**

2-chloro-N-(4-(trifluoro methyl) phenyl) thieno[2,3-*d*] pyrimidin-4-amine (5e):



Structure of 2-chloro-N-(4-(trifluoromethyl)phenyl)thieno[2,3-d]pyrimidin-4-amine (5e)

^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.8 (bs, 1H), 7.2 (d, 1H, J = 7.6 HZ), 6.95 (1H, d, J = 7.6 HZ), 7.4 (2H, d, J = 6.7 HZ), 7.5 (2H, d, J = 6.7 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 118, 123, 126, 133, 142, 156, 158, 160.

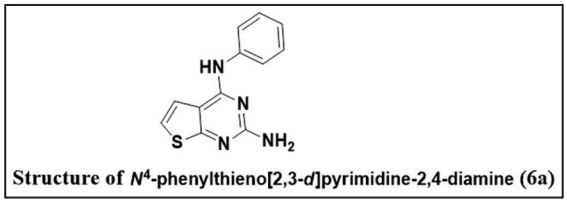
IR (KBr, cm–^{1}): Ar stretch C-H (3110), C-F (1260), C-O-C (1160), C-Cl (745), C=C (1575), N-H (3310).

ESI-MS m/z 330[M+H]$^+$**.**

General procedure for synthesis of N 4-phenylthieno[2,3-d]pyrimidine-2,4-diamine (6a), N 4-p-tolylthieno[2,3-d]pyrimidine-2,4-diamine (6b), N 4-(4-methoxyphenyl)thieno[2,3-d]pyrimidine-2,4-diamine(6c), N 4-(4-(trifluoromethoxy) phenyl)thieno[2,3-d]pyrimidine-2,4-diamine (6d), N 4-(4-(trifluoromethyl)phenyl)thieno[2,3-d] pyrimidine-2,4-diamine(6e):

A solution of 25% aqueous ammonia solution (5 mol) and compounds (5a-5e) (0.08 mol) was stirred at 90°C for 5 h. The precipitate was collected by filtration and washed with water and dried to give compounds (6a-6e).

N 4-phenyl thieno[2,3-*d*]pyrimidine-2, 4-diamine (6a):



Structure of N^4-phenylthieno[2,3-*d*]pyrimidine-2,4-diamine (6a)

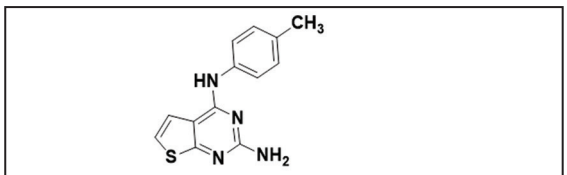
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.15 (bs, 1H), 6.86 (2H, bs), 7.35 (d, 1H, J = 7.3 HZ), 7.05 (1H, d, J = 7.3 HZ), 6.83 (1H.t), 7.32 (2H, m), 7.76 (2H, m).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 118, 123, 126, 129, 140, 155, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C=N (1646.15), C=C (1575), N-H (3292 and 3370).

ESI-MS m/z 243[M+H]$^+$**.**

N 4-p-tolyl thieno[2,3-*d*]pyrimidine-2,4-diamine (6b):



Structure of N^4-*p*-tolylthieno[2,3-*d*]pyrimidine-2,4-diamine (6b)

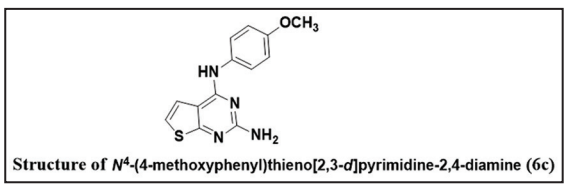
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 2.35 (3H, s), 4.65 (bs, 1H), 6.56 (2H, bs), 7.35 (d, 1H, J = 7.2 HZ), 7.15 (1H, d, J = 7.2 HZ), 7.45 (2H, d, J = 7.4 HZ), 7.27 (2H, d, J = 7.4 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 22, 118, 123, 126, 132, 140, 156, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3120), C-H (2960), C=C (1570), N-H (3295 and 3350).

ESI-MS m/z 257[M+H]$^+$**.**

N 4-(4-methoxyphenyl) thieno[2,3-*d*]pyrimidine-2,4-diamine(6c):



Structure of N^4-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidine-2,4-diamine (6c)

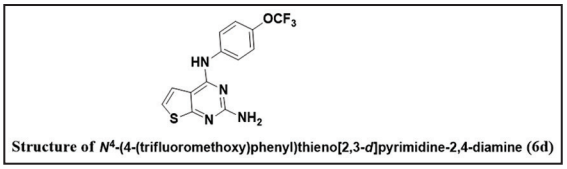
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 3.85 (3H, s), 4.25 (bs, 1H), 6.46 (2H, bs), 7.35 (d, 1H, J = 7.3 HZ), 6.96 (1H, d, J = 7.3 HZ), 7.15 (2H, d, J = 7.2 HZ), 7.64 (2H, d, J = 7.2 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 58, 118, 123, 126, 132, 140, 156, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-H (2955), C-O-C (1150), C=C (1575), N-H (3290 and 3385).

ESI-MS m/z 273[M+H]$^+$**.**

N 4-(4-(tri fluoro methoxy) phenyl) thieno[2,3-*d*] pyrimidine-2,4-diamine (6d):



Structure of N^4-(4-(trifluoromethoxy)phenyl)thieno[2,3-*d*]pyrimidine-2,4-diamine (6d)

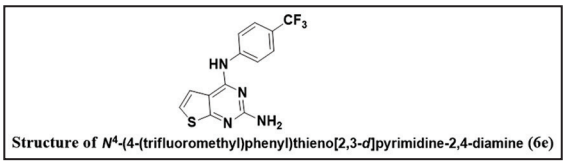
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.25 (1H, bs), 6.66 (2H, bs), 7.23 (d, 1H, J = 7.2 HZ), 6.95 (1H, d, J = 7.2 HZ), 6.83 (2H, d, J = 7.1 HZ), 7.65 (2H, d, J = 7.1 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 118, 123, 126, 133, 142, 156, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-F (1285), C-O-C (1150), C=C (1575), N-H (3285 and 3320).

ESI-MS m/z 325[M-H]$^+$**.**

N 4-(4-(trifluoro methyl) phenyl) thieno[2,3-*d*] pyrimidine-2, 4-diamine(6e):



Structure of N^4-(4-(trifluoromethyl)phenyl)thieno[2,3-*d*]pyrimidine-2,4-diamine (6e)

^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.54 (bs, 1H), 6.64 (2H, bs), 7.23 (d, 1H, J = 7.2 HZ), 6.95 (1H, d, J = 7.2 HZ), 7.43 (2H, d, J = 7.1 HZ), 7.55 (2H, d, J = 7.1 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 118, 123, 126, 133, 142, 156, 158, 160.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-F (1260), C-O-C (1160), C=C (1575), N-H (3265 and 3340).

ESI-MS m/z 309[M-H]$^+$**.**

General procedure for synthesis of:

N-(4-(phenylamino)thieno[2,3-d]pyrimidin-2-yl) furan-2-carboxamide (8a),

N-(4-(phenylamino)thieno[2,3-d]pyrimidin-2-yl) thiophene-2-carboxamide (8b),

N-(4-(p-tolylamino)thieno[2,3-d]pyrimidin-2-yl) furan-2-carboxamide (8c),

N-(4-(p-tolylamino)thieno[2,3-d]pyrimidin-2-yl) thiophene-2-carboxamide (8d),

N-(4-(4-methoxyphenylamino) thieno[2,3-d] pyrimidin-2-yl)furan-2-carboxamide (8e),

N-(4-(4-methoxyphenylamino) thieno[2,3-d] pyrimidin-2-yl)thiophene-2-carboxamide (8f),

N-(4-(4-(trifluoromethoxy) phenylamino) thieno[2,3-d]pyrimidin-2-yl)furan-2-carboxamide (8g),

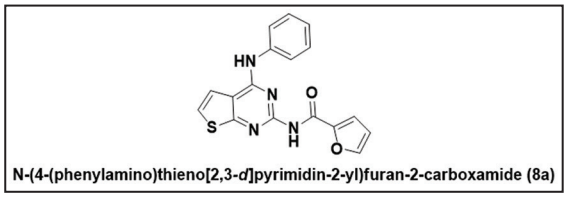
N-(4-(4-(trifluoromethoxy)phenylamino) thieno[2,3-d]pyrimidin-2-yl)thiophene-2-carboxamide (8h),

N-(4-(4-(trifluoromethyl)phenylamino)thieno[2,3-d] pyrimidin-2-yl)furan-2-carboxamide (8i),

N-(4-(4-(trifluoromethyl) phenylamino)thieno[2,3-d] pyrimidin-2-yl)thiophene-2-carboxamide (8j):

To a solution of furan/Thiophene-2-carboxylic Acids (5a-b) (10.2 m.mol) in DMF (5v), HATU (10 m.mol), Hunig's base (*N,N*-di isopropyl ethyl amine, DIPEA) (20 m.mol), Stir at RT for 10 min under Nitrogen atmosphere, Then add Compounds (4 a-e) (10.00 m. mol] at RT for 16 h, Then Reaction mixture was diluted with Ice Cold Water, Filtered the obtained Solid and Dried, Finally Purified by Flash Column Chromatography.

N-(4-(phenylamino) thieno[2,3-*d*] pyrimidin-2-yl) furan-2-carboxamide (8a):



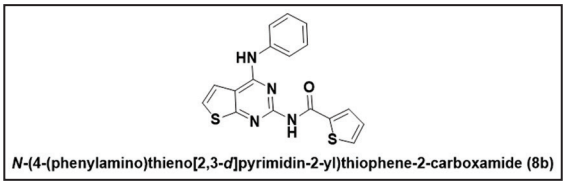
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.15 (bs, 1H), 9.86 (1H, bs), 7.35 (d, 1H, J = 7.3 HZ), 7.05 (1H, d, J = 7.3 HZ), 6.83 (1H.t), 7.32 (2H, m), 7.73 (2H, m), 8.13 (1H, d, J = 7.4 HZ), 6.92 (1H, t, J = 7.4 HZ), 7.35 (1H, d, J = 7.4 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 110, 118, 123, 126, 129, 140, 145, 155, 158, 160, 175.

IR (KBr, cm^{-1}): Ar stretch C-H (3109), C=O (1686.15), C=C (1575), N-H (3290), 1130 (C-O-C).

ESI-MS m/z 359[M+Na]$^+$**.**

N-(4-(phenylamino) thieno[2,3-*d*] pyrimidin-2-yl) thiophene-2-carboxamide (8b):



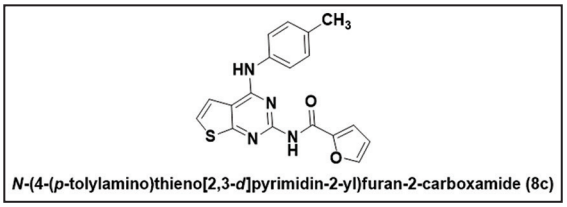
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.35 (bs, 1H), 9.26 (1H, bs), 7.25 (d, 1H, J = 7.3 HZ), 7.08 (1H, d, J = 7.3 HZ), 6.83 (1H.t), 7.32 (2H, m), 7.73 (2H, m), 8.15 (1H, d, J = 7.4 HZ), 7.34 (1H, t, J = 7.4 HZ), 8.35 (1H, d, J=7.4 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 110, 114, 116, 123, 126, 129, 140, 145, 155, 158, 160, 170.

IR (KBr, cm^{-1}): Ar stretch C-H (3119), C=O (1689.15), C=C (1575), N-H (3270), 680 (C-S-C).

ESI-MS m/z 375[M+Na]$^+$**.**

N-(4-(p-tolylamino)thieno[2,3-*d*]pyrimidin-2-yl) furan-2-carboxamidee (8c):



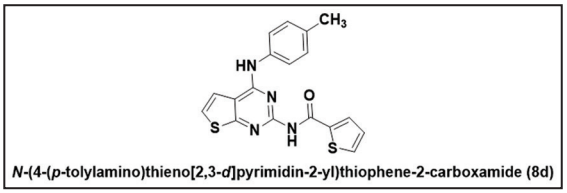
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 2.35 (3H, s), 4.15 (bs, 1H), 9.36 (1H, bs), 7.25 (d, 1H, J = 7.2 HZ), 7.05 (1H, d, J = 7.2 HZ), 7.35 (2H, d, J = 6.9 HZ), 7.27 (2H, d, J = 6.9 HZ), 8.18 (1H, d, J = 7.3 HZ), 7.34 (1H, t, J = 7.3 HZ), 8.32 (1H, d, J =7.3 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 23, 118, 123, 126, 132, 140, 156, 158, 160, 168.

IR (KBr, cm^{-1}): Ar stretch C-H (3120), C-H (2960), C=O (1683.56), N-H (3295).

ESI-MS m/z 351[M+H]$^+$.

N-(4-(p-tolylamino) thieno[2,3-*d*] pyrimidin-2-yl) thiophene-2-carboxamide (8d):



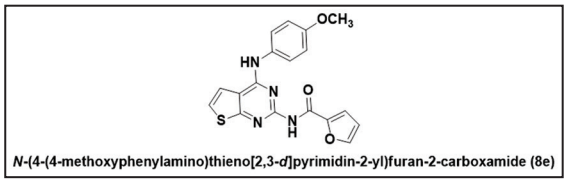
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 2.33 (3H, s), 4.13 (bs, 1H), 9.16 (1H, bs), 7.26 (d, 1H, J = 7.3 HZ), 7.08 (1H, d, J = 7.3 HZ), 7.35 (2H, d, J = 7.4 HZ), 7.27 (2H, d, J = 7.4 HZ), 8.18 (1H, d, J = 7.3 HZ), 7.24 (1H, t, J = 7.3 HZ), 8.32 (1H, d, J = 7.3 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 23, 118, 123, 126, 132, 140, 156, 158, 160, 168 (C=O).

IR (KBr, cm^{-1}): Ar stretch C-H (3120), C-H (2960), C=O (1693.56), N-H (3295), 676 (C-S-C).

ESI-MS m/z 367[M+H]$^+$.

N-(4-(4-methoxyphenylamino) thieno[2,3-*d*] pyrimidin-2-yl) furan-2-carboxamide (8e):



N-(4-(4-methoxyphenylamino)thieno[2,3-*d*]pyrimidin-2-yl)furan-2-carboxamide (8e)

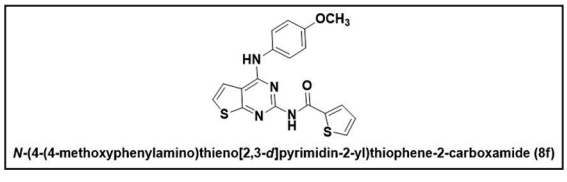
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 3.85 (3H, s), 4.25 (bs, 1H), 9.46 (1H, bs), 7.35 (d, 1H), 6.96 (1H, d), 7.15 (2H, d), 7.64 (2H, d), 8.88 (1H, d, J = 7.2 HZ), 7.34 (1H, t, J = 7.2 HZ), 8.12 (1H, d, J = 7.2 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 58, 118, 123, 126, 132, 140, 156, 158, 160, 168 (C=O).

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-H (2955), C-O-C (1150), C=O (1675), N-H (3285).

ESI-MS m/z 365[M-H]$^+$.

N-(4-(4-methoxyphenylamino) thieno[2,3-*d*] pyrimidin-2-yl) thiophene-2-carboxamide (8f):



N-(4-(4-methoxyphenylamino)thieno[2,3-*d*]pyrimidin-2-yl)thiophene-2-carboxamide (8f)

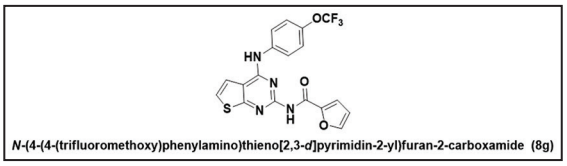
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 3.88 (3H, s), 4.38 (bs, 1H), 9.16 (1H, bs), 7.35 (d, 1H, J = 7.3 HZ), 6.86 (1H, d, J = 7.1 HZ), 7.15 (2H, d, J = 7.3 HZ), 7.64 (2H, d, J = 7.3 HZ), 8.88 (1H, t, J = 7.1 HZ), 7.34 (1H, d, J = 7.1 HZ), 8.12 (1H, d, J = 7.1 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 58, 118, 123, 126, 132, 140, 156, 158, 160, 168 (C=O).

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-H (2955), C-O-C (1150), C=O (1675), N-H (3285).

ESI-MS m/z 383[M+H]$^+$**.**

N-(4-(4-(trifluoromethoxy)phenyl amino) thieno[2,3-*d*]pyrimidin-2-yl)furan-2-carboxamide (8g):



N-(4-(4-(trifluoromethoxy)phenylamino)thieno[2,3-*d*]pyrimidin-2-yl)furan-2-carboxamide (8g)

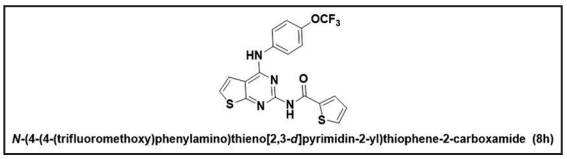
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.38 (bs, 1H), 9.16 (1H, bs), 7.65 (d, 1H, J = 7.2 HZ), 6.76 (1H, d, J = 7.1 HZ), 7.55 (2H, d, J = 7.3 HZ), 6.64 (2H, d, J = 7.3 HZ), 8.85 (1H, t, J = 7.1 HZ), 7.29 (1H, d, J = 7.1 HZ), 8.15 (1H, d, J = 7.1 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 115, 118, 123, 126, 133, 142, 156, 158, 160, 170.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-F (1268), C-O-C (1150), C=O (1685), N-H (3265).

ESI-MS m/z 421[M+H]$^+$.

N-(4-(4-(trifluoro methoxy) phenyl amino) thieno[2,3-*d*] pyrimidin-2-yl) thiophene-2-carboxamide (8h):



N-(4-(4-(trifluoromethoxy)phenylamino)thieno[2,3-*d*]pyrimidin-2-yl)thiophene-2-carboxamide (8h)

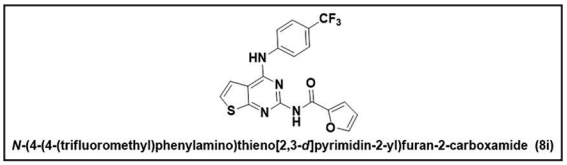
^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.38 (bs, 1H), 9.16 (1H, bs), 7.25 (d, 1H, J = 7.3 HZ), 6.90 (1H, d, J = 7.1 HZ), 6.82 (2H, d, J = 7.3 HZ), 7.54 (2H, d, J = 7.3 HZ), 8.85 (1H, d, J = 7.1 HZ), 7.24 (1H, t, J = 7.1 HZ), 8.10 (1H, d, J = 7.1 HZ).

^{13}C NMR (DMSO-d$_6$) (δ/ppm): 118, 123, 126, 133, 142, 156, 158, 160, 168.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-F (1275), C-S-C (685), C=O (1680), N-H (3280).

ESI-MS m/z 435[M-H]$^+$.

N-**(4-(4-(trifluoro methyl) phenyl amino) thieno[2,3-*d*] pyrimidin-2-yl) furan-2-carboxamide (8i):**



N-(4-(4-(trifluoromethyl)phenylamino)thieno[2,3-*d*]pyrimidin-2-yl)furan-2-carboxamide (8i)

^{1}H NMR (DMSO-d$_6$) (δ/ppm) δ ppm: 4.18 (bs, 1H), 9.26(1H, bs), 7.25 (d, 1H, J = 7.3 HZ), 6.95 (1H, d, J = 7.1 HZ), 7.41 (2H, d, J = 7.3 HZ), 7.35 (2H, d, J = 7.3

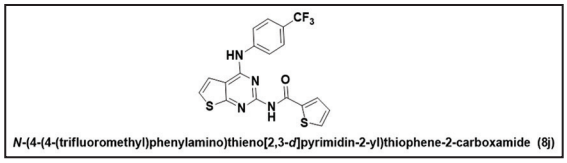
HZ), 7.35 (1H, d, J = 7.1 HZ), 6.94 (1H, t, J = 7.1 HZ), 8.15 (1H, d, J = 7.1 HZ).

^{13}C NMR (DMSO-d_6) (δ/ppm): 118, 123, 126, 133, 142, 156, 158, 160, 167.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-F (1260), C-O-C (1160), C=O (1675), N-H (3265).

ESI-MS m/z 405[M+H]$^+$.

N-(4-(4-(trifluoro methyl) phenyl amino) thieno[2,3-*d*] pyrimidin-2-yl) thiophene-2-carboxamide (8j):



N-(4-(4-(trifluoromethyl)phenylamino)thieno[2,3-*d*]pyrimidin-2-yl)thiophene-2-carboxamide (8j)

^{1}H NMR (DMSO-d_6) (δ/ppm): δ 4.28 (bs, 1H), 9.66 (1H, bs), 7.20 (d, 1H, J = 7.3 HZ), 6.90 (1H, d, J = 7.1 HZ), 7.40 (2H, d, J = 7.3 HZ), 7.35 (2H, d, J = 7.3 HZ), 7.35 (1H, t, J = 7.1 HZ), 8.45 (1H, d, J = 7.1 HZ), 8.15 (1H, d, J = 7.1 HZ).

^{13}C NMR (DMSO-d_6) (δ/ppm): 118, 123, 126, 133, 142, 156, 158, 160, 168.

IR (KBr, cm^{-1}): Ar stretch C-H (3110), C-F (1240), C-S-C (680), C=O (1668), N-H (3245).

ESI-MS m/z 421[M+H]$^+$.

3.3. Biological Activity

3.3.1. Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia,* and *Escherichia coli* (clinical isolate) bacterial strains by disc diffusion method [36,37]. Standard inoculums (1-2×107 c.f.u./ml 0.5 McFarland standards) were introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculums. The disks measuring 6 mm in diameters were prepared from what man no. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile disks previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. Amoxicillin (30 µg) was used as positive control and the disk poured in DMSO was used as negative control and the test compounds were dissolved in DMSO at concentration of 100 and 50 µg/ml. The plates were inverted and incubated for 24 h at 37°C. The susceptibility was assessed on the basis of diameter of zone of inhibition against Gram-positive and Gram-negative strains of bacteria. Inhibition of zone of measured and compared with controls. The bacterial zone of inhibition values are given in Table 1. The order of activity was 8j>8i>8h>8g>8d>8f>8b>8c>8e>8a.

3.3.2. Antifungal studies

The newly prepared compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus flavus* in DMSO by agar diffusion method [38]. Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH 5.7. Normal saline was used to make suspension of corresponding species. 20 ml of agar media was poured into each Petri dish. Excess of suspension was decanted, and the plates were dried by placing in an incubator at 37°C for 1 h using an agar punch, wells were made and each well was labelled. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The fungal activity of each compound was compared with fluconazole as a standard drug. Inhibition zone were measured and compared with the controls (Figure 5). The fungal zone of inhibition values are given in Table 2.

4. RESULT AND DISCUSSIONS

4.1. Chemistry

The reaction sequences Employed for synthesis of title compounds are shown in Scheme 1. In the present work, the starting thieno[2,3-*d*] pyrimidine-2,4-diol(2) was prepared from methyl 2-aminothiophene-3-carboxylate (1) and urea according to the reported procedure [39]. Next Step 2 is 2,4-dichlorothieno[2,3-*d*]pyrimidine (3) was prepared using $POCl_3$ at reflux for 6 h according to the reported procedure [40]. The 2,4-dichlorothieno[2,3-*d*]pyrimidine (3) was coupling with different amines (4 a-e) in t-Butanol by using Hung's base at 60-70°C to get compounds 5 (a-e) according to the reported procedure [41]. Which are further treatment with aqueous ammonia at 90°C according to the reported procedure [42], which on further treatment with different various carboxylic acids (7 a-b) to get target novel thieno[2,3-*d*]pyrimidine derivatives (8 a-j) according to the reported procedure [43]. All compounds displayed IR, ^{1}H and ^{13}C NMR and mass spectra consistent with the assigned structures. ^{1}H NMR and IR spectrum of compounds (8 a-j) showed singlet at 2.3 ppm, 3.8 ppm are due to the aromatic methyl group protons and Aromatic methoxy group protons. The most characteristic IR absorption bands are at 3340 cm^{-1} (-NH), 760 cm^{-1} (C-Cl) and 3320 and 3250 cm^{-1} (N-H stretching in amine group). The mass spectra of all the final derivatives showed comparable molecular ion peak with respect to molecular formula.

4.1.1. Anti-microbial studies

The newly synthesized compounds (8 a-j) were screened for their *in vitro* antibacterial activity

Table 1: Antibacterial activity of novel thieno[2,3-*d*] pyrimidine derivatives 8 (a-j)

Zone of inhibition measure in mm								
Synthesized compounds	**Gram-positive**				**Gram-negative**			
	Bacillus subtilis		***Staphylococcus aureus***		***Klebsiella pneumonia***		***Escherichia coli***	
	100 µg/mL	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**
8	6	3	7.5	5	8	6	9.5	6
8b	8.5	6.5	9.0	6.5	10.15	8	11	8
8c	7.5	3.5	8	7	9.5	7	10.5	7.5
8d	10	8	11.1	9.5	12	11	13.5	11
8e	7	4.5	7	4.5	8.5	6.5	9	7
8f	9.5	7	9.5	7.5	12	10	12.5	10.5
8g	11	9.5	11.5	8.5	12.5	12	13	11.5
8h	11.5	9	12.5	11	14.5	11.5	15.5	12
8i	12.5	10	14.5	10.5	15	13.5	16.5	12.5
8j	13	10.5	15	11.5	16.5	14	17	13
Amoxicillin	15.7	12.6	17.4	13	18	14.6	19.6	15.5
Control (DMSO)	-	-	-	-	-	-	-	-

DMSO: Dimethylsulfoxide

Table 2: Anti-fungal activity of novel thieno[2,3-*d*] pyrimidine derivatives 7(a-j)

Zone of inhibition measure in mm				
Synthesised compounds	***Candida albicans***		***Aspergillus flavus***	
	100 µg/mL	**50 µg/mL**	**100 µg/mL**	**50 µg/mL**
8a	6.5	4.5	7	4
8b	8.5	6.5	9.0	6.5
8c	7.5	3.5	8	7
8d	10	8	11.1	9.5
8e	8	5.5	7	3.5
8f	9.5	7	9.5	7.5
8g	11	9.5	11.5	8.5
8h	13	11.5	10.5	8
8i	14.5	12	12.5	9.5
8j	17.5	12.5	16	12
Fluconazole	21	16	18.5	14
Control (DMSO)	-	-	-	-

DMSo: Dimethylsulfoxide

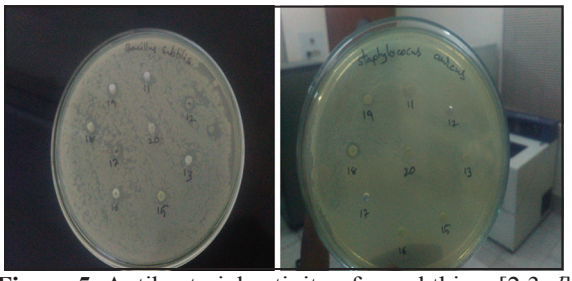


Figure 5: Antibacterial activity of novel thieno[2,3-*d*] pyrimidine derivatives (8a-8j).

against *B. subtilis*, *S. aureus*, *K. pneumonia,* and *E. coli* using amoxicillin as standard by disc diffusion method (zone of inhibition). The test compounds were dissolved in DMSO at concentrations of 50 and 100 µg/mL. The antibacterial screening revealed that all the tested compounds showed good inhibition against various tested microbial strains compared to the standard drug. Along with the synthesized compounds 8j, 8i, 8h, 8g were found to be more active against tested bacterial strains as compared to the standard.

5. CONCLUSION

The research study reports the successful synthesis and antimicrobial activity of novel thieno[2,3-*d*] pyrimidine as a core unit. The antimicrobial activity study revealed that all the tested compounds showed good antibacterial and antifungal activities against pathogenic strains. The structure and biological activity relationship of title compounds indicate that the presence of electron withdrawing groups like $-CF_3$ and $-OCF_3$ attached to the phenyl ring and thiophene, Furan rings were responsible for good antimicrobial activity and hence compounds 8j, 8i, 8h and 8g exhibited more potent anti-microbial activity of all tested pathogenic strains. Few of synthesized compounds might be useful as antimicrobial agents in future. These novel thieno[2,3-*d*]pyrimidine derivatives have proved to be promising candidates for further efficacy evaluation. On the basis of their activity, these derivatives were identified as viable leads for further studies.

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