

Synthesis, Structural Elucidation and Anti-Bacterial Evaluation of Some Novel Heterocyclic Molecules Derived from Thieno [2,3-*d*] Pyrimidine as a Core Unit

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Abstract

A series of novel 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-substituted phenyl/Heterocyclic Thieno[2,3-*d*] Pyrimidine (8 a-j) derivatives were synthesized by a facile Five-step procedure that afforded advantages of mild reaction conditions, simple protocol and good yields. The structures of the final compounds were confirmed by IR, NMR, EI-MS. The final compounds were screened for their anti-bacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* from Gram positive group of bacteria and *Escherichia coli* and *Klebsiella pneumonia* from Gram negative group of bacteria and antifungal activity against *Candida albicans* and *Aspergillus flavus*. Anti-bacterial and anti-fungal activities were Evaluated and compared with the standard drugs Such as Amoxicillin and Ketoconazole. From anti-bacterial and anti-fungal activity screening results, it has been observed that compounds 8i, 8h, 8e and 8j possess good activity.

Keywords: Thieno[2,3-*d*] pyrimidine; Pyrazoles; Facile synthesis; Anti-bacterial; Antifungal activity; Suzuki cross coupling

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 hetero aromatic 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 a 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 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, bronchodilatory activity, inhibition of Phosphodiesterases, tyrosine kinase and VEGFR kinase. It is evident that purine as an endogeneous scaffold plays an important biochemical role in variety of regular physiological functions such as respiration, inflammation, cell proliferation and so forth. As a bio isoster 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 bi cyclic heterocyclic compound consists of a five membered thiophene ring is fused to a six-membered hetero cyclic 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).

Heterocycles containing the Thieno Pyrimidine moiety (Figure 1) are of interest because of their interesting pharmacological and biological activities [1-6]. Thus, over the last two decades many thieno Pyrimidines have been found to exhibit a variety of pronounced activities, for example, as anti-inflammatory [3,7], anti-microbial [3,8], antiviral [9] and analgesic [7,10] agents. Some Thieno Pyrimidine derivatives showed good antitumor activity [11].

As a logical consequence of thiophene – phenyl isosterism, similarly thieno pyrimidines can be considered as bio isosteres 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 (Figure 2) structure has therefore, become a routine strategy in modern drug design and development. Thieno pyrimidines as isosteres of quinazolines are shown here.

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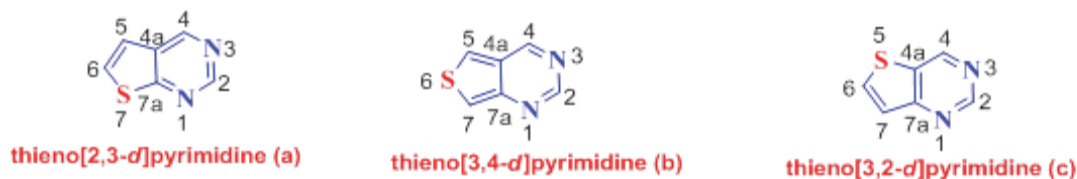


Figure 1: Structures of different isomers of Thieno Pyrimidine.

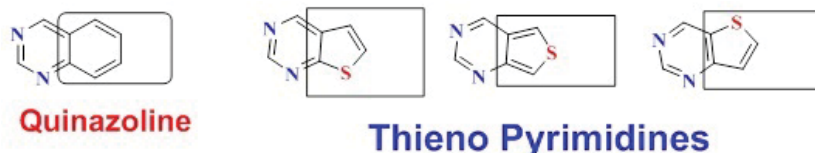


Figure 2: Structures of thiophene-phenyl isosterism in Quinazolines and thieno Pyrimidine.

Thienopyrimidines can also be considered as structural analogues of five-membered heterocycles such as purines and thiazolopyrimidines. 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 the following Figure 3.

Synthesis of thienopyrimidines

The building of thieno[2,3-*d*]pyrimidine moiety has been achieved either by annulations of pyrimidine nucleus on the parent thiophene ring or annulations of thiophene nucleus on the parent pyrimidine ring. Also, they obtained from acyclic compounds.

Annulations of pyrimidine on thiophene ring

The simple approach to the formation of a new pyrimidine ring involves introducing a one-carbon fragment between two suitable and vicinal functional groups in thiophene ring.

Using thiophene having vicinal amino ester groups

Thiophene derivatives having vicinal amino ester groups are considered a suitable synthon for the synthesis of thieno pyrimidines via its interaction with various suitable reagents.

With isocyanate and isothiocyanate derivatives: Reaction of ethyl 2-amino-5-benzoyl-4-methylthiophene-3-carboxylate (1) [12] (Figure 4) with phenyl isothiocyanate and/or phenyl isocyanate in presence of a catalytic amount of triethylamine afforded the corresponding thioureidothiophene and/or ureidothiophene 2a, b, which underwent cyclization in Ethanolic sodium ethoxide to yield thieno[2,3-*d*]pyrimidinone derivatives 3a, 3b respectively.

Moreover, treatment of compound 1 with benzoyl isothiocyanate in Ethanolic sodium hydroxide gave **6benzoyl-2-thioxothieno[2,3-*d*]pyrimidine derivative (4)** [13].

With formamide: 5-Methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-carboxamide (2) was prepared via reaction of compound (1) with formamide (Figure 5) [14].

With urea and their derivatives: Methyl 3-aminothiophene-2-carboxylate (1) was condensed with urea (2) at 190°C to yield thieno[3,2-*d*]pyrimidin-2,4(1H,3H)-dione (3) in high yield (Figure 6) [15].

Moreover, thieno[2,3-*d*]pyrimidines 4 and 5 (Figure 7) showed

potent anticancer activity at low concentrations against most of the used human tumor cell lines when compared to **doxorubicin** as potent anticancer drug [16].

Reactions of Thienopyrimidines.

Reactions attributed to thiophene ring.

Reactions at thiophene carbons.

Electrophilic substitutions like halogenation, Vilsmeier formylation, nitration and alkylation, were demonstrated in **thieno[2,3-*d*]pyrimidines (I)** and **thieno[3,4-*d*]pyrimidines (III)** (Figure 8) involved position 6 and equivalent position 7, respectively, which is typical of thiophene itself and suggested a weak influence of annelation with the pyrimidine ring. A different situation is observed for electrophilic substitution in **thieno[3,2-*d*]pyrimidines (II)**, where the influence of annelation of the Pyrimidine ring is stronger than the effect of orientation of the sulphur atom in the thiophene ring and, consequently, the attack occurred at position 7.

Halogenation

Bromination of compound (1) with mild brominating agent, N-bromosuccinimide (NBS), in dimethyl formamide afforded 4-amino-6-bromo-2-substitutedthieno[2,3-*d*]pyrimidines (2) [17] (Figure 9).

Vilsmeier-Haack reaction

The Vilsmeier-Haack reaction of compound (1) using phosphorus oxychloride and DMF resulted in the formation of 6-formyl-1,3-dimethylthieno[2,3-*d*]pyrimidine-2,4(1H, 3H)-dione (2) [18] (Figure 10).

Nitration

Thieno[2,3-*d*]pyrimidine (1) was nitrated using a solution of fuming nitric acid in concentrated sulfuric acid to afford 6-nitro-1,3-dimethylthieno[2,3-*d*]pyrimidine-2,4 (1H,3H)-dione (2) [18] (Figure 11).

Also, **6-bromo-1,3-dimethylthieno[2,3-*d*]pyrimidine 2,4(1H, 3H)-dione (4)** (Figure 12) was formed by addition of a solution of bromine dissolved in acetic acid to thienopyrimidine (3).

This work aimed to synthesize some new thieno[2,3-*d*]pyrimidine 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-

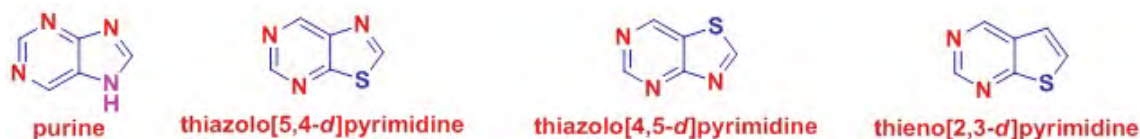


Figure 3: Structures of purines and thiazolo pyrimidines.

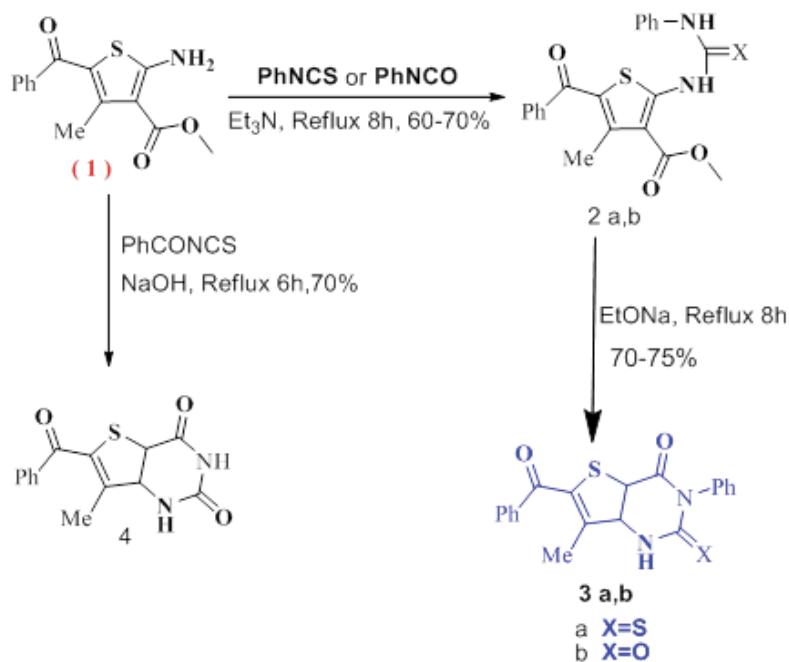


Figure 4: Synthesis of 6-benzoyl-2-thioxo-thieno[2,3-*d*]pyrimidine derivative (4).

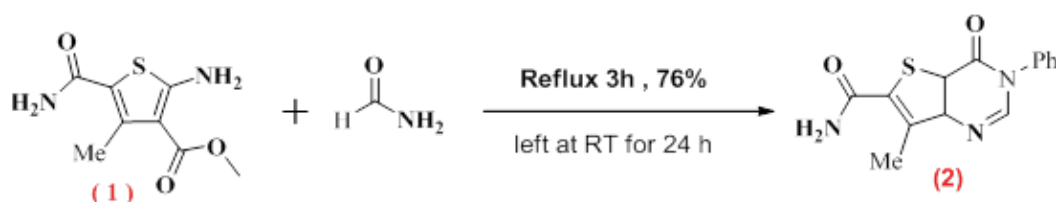


Figure 5: Synthesis of 5-Methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-carboxamide (2).

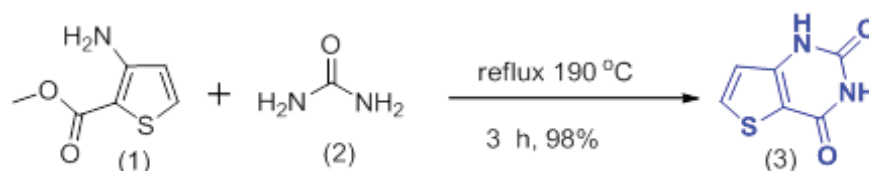


Figure 6: Synthesis of thieno[3,2-*d*]pyrimidin-2,4(1H,3H)-dione (3).

d]pyrimidine Heterocyclic compounds, 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-chloro-4-hydrazinyl thieno[2,3-*d*] pyrimidine (4) was reacted with Acetyl acetone (5) in Ethanol at Reflux Temperature to form Pyrazole Thieno Pyrimidine[2,3-*d*] derivative (6), which was further reacted with different types of boronic acids (7 a-j) under Suzuki reaction conditions

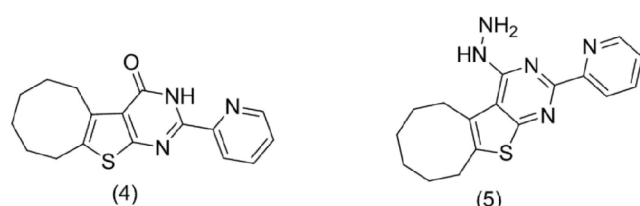


Figure 7: Structure of thieno[2,3-*d*]pyrimidines 4 and 5.

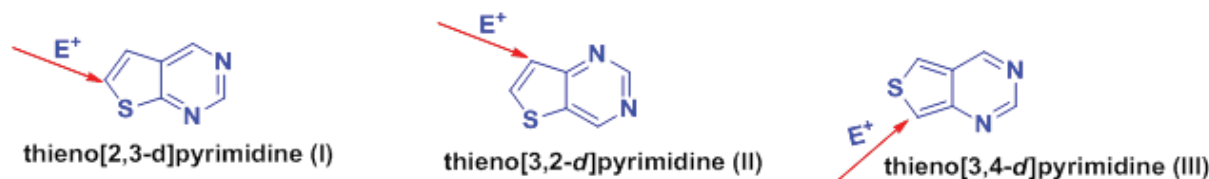


Figure 8: Electrophilic substitution reaction of various Isomers of thieno[2,3-*d*]pyrimidine.

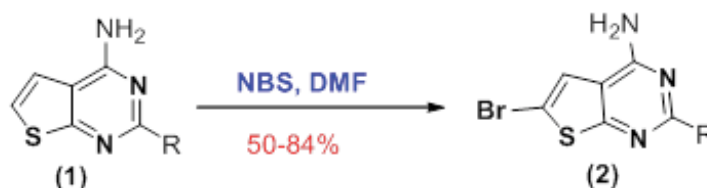


Figure 9: Synthesis of 4-amino-6-bromo-2-substituted thieno[2,3-*d*]pyrimidines.

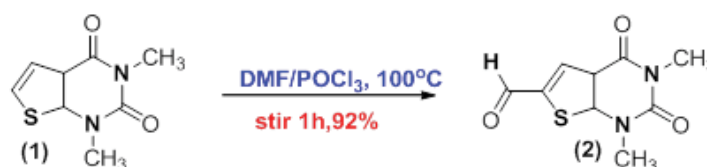


Figure 10: Synthesis of formyl-1,3-dimethylthieno[2,3-*d*]pyrimidine-2,4(1H, 3H)-dione (2).

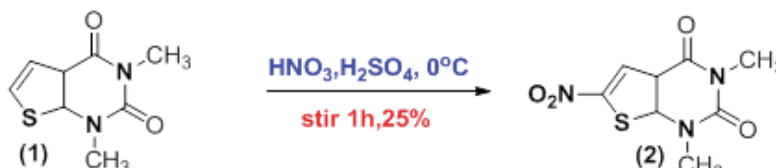


Figure 11: Synthesis of 6-nitro-1,3-dimethylthieno[2,3-*d*]pyrimidine-2,4(1H, 3H)-dione (2).

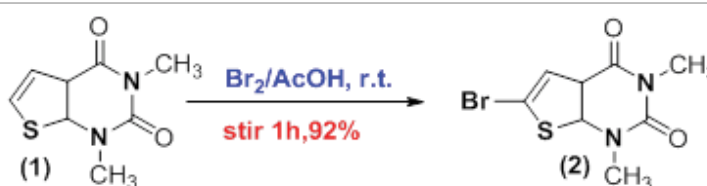


Figure 12: Synthesis of 6-bromo-1,3-dimethylthieno[2,3-*d*]pyrimidine-2,4(1H, 3H)-dione (2).

to get target compounds (8a-8j) (Figure 13). The synthesis of the compounds as per the following given below. The synthetic route was depicted in (Figure 14).

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

Materials and Methods

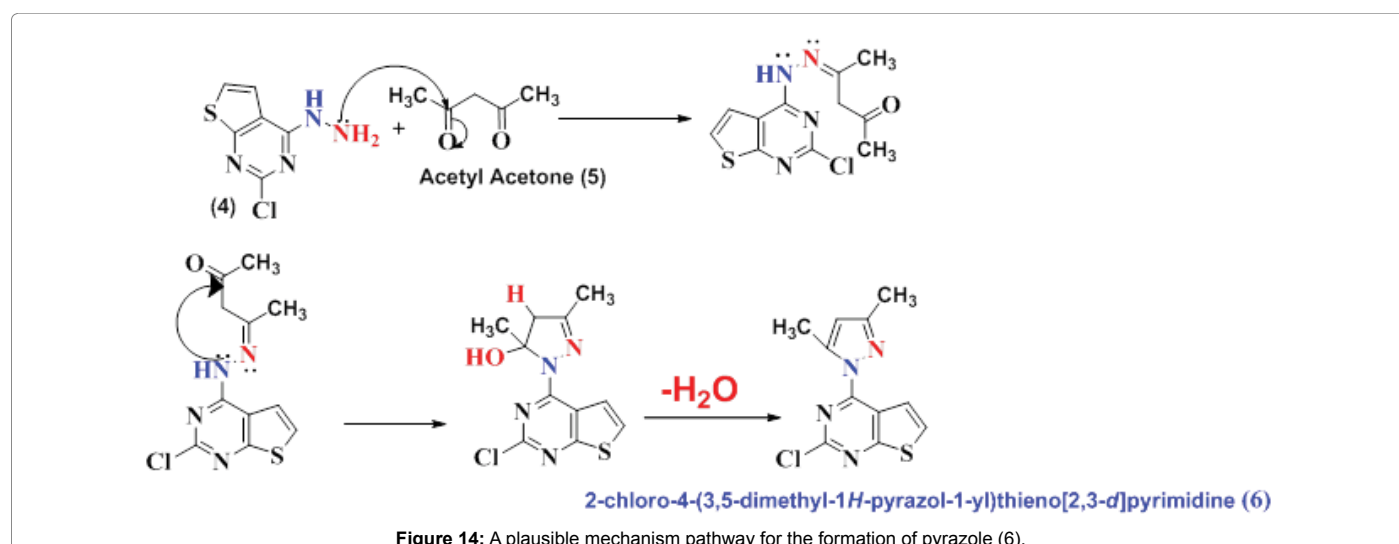
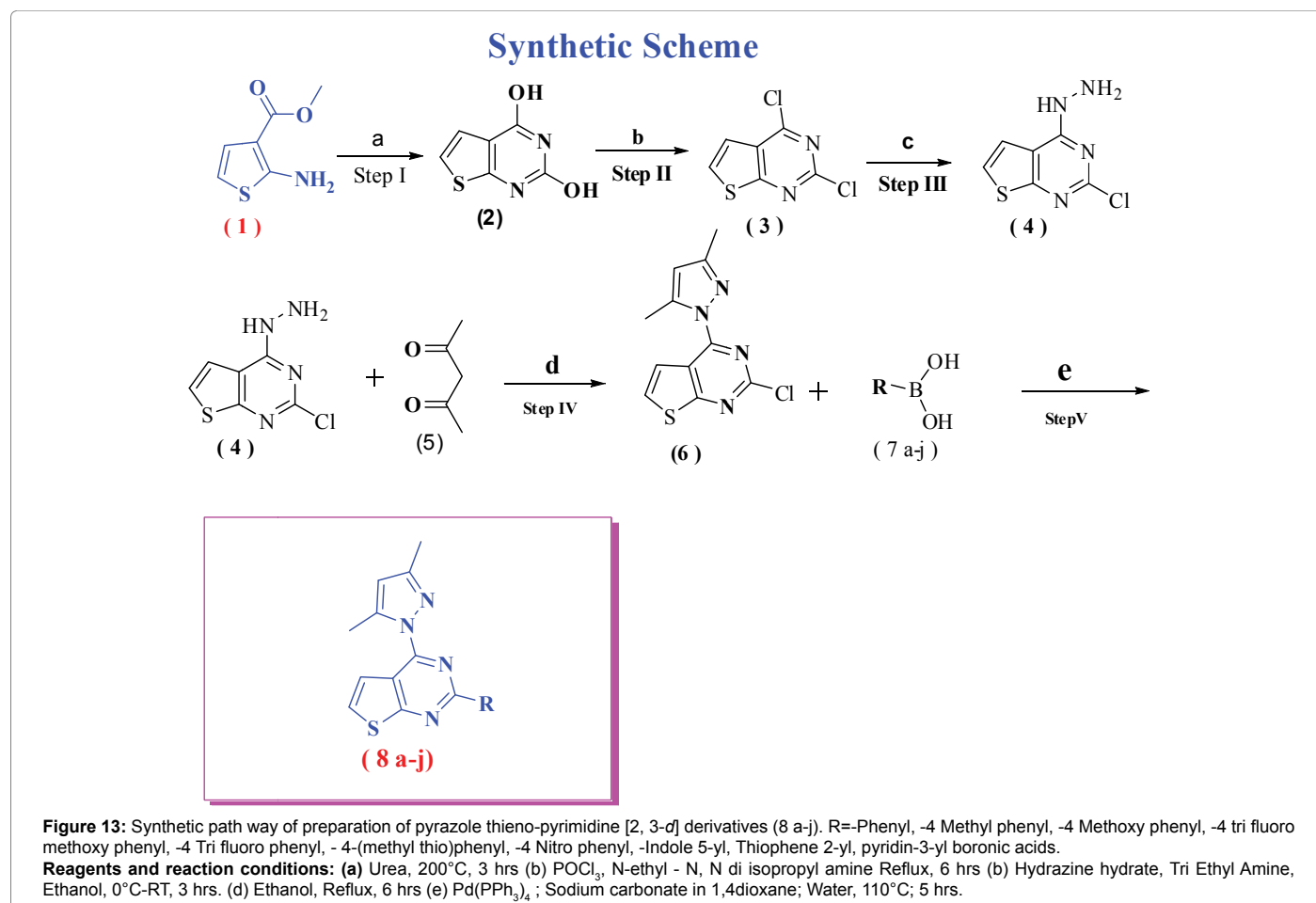
In this Investigation chemicals were purchased from local dealer with S.D fine make was used. Chemicals were 99% pure; purity has been checked by thin layer chromatography and melting point. Conventional method has been used for synthesis of thieno [2,3-*d*] Pyrimidine derivatives. Stirring and reflux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

Thieno [2,3-*d*] Pyrimidine derivatives 8 (a-j) respectively. The synthetic route was depicted in Figure 13.

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, ^1H -NMR, ^{13}C -NMR and mass) data.

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. THF was distilled from sodium benzo phenone ketyl and degassed thoroughly with dry argon directly before



use. Unless otherwise noted, organic extracts were dried with anhydrous Na₂SO₄, 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 ¹H for ¹³C, respectively, in CDCl₃ solution with tetra methyl silane 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 (¹H NMR and ¹³C NMR) were recorded using tetra methyl silane (TMS) in the solvent of CDCl₃-d₁ or DMSO-d₆ as the internal standard (¹H NMR: TMS at 0.00 ppm, CDCl₃ at 7.26 ppm, DMSO at 2.50 ppm; ¹³C NMR: CDCl₃ at 77.16 ppm, DMSO at 40.00 ppm).

Synthesis

General procedure for synthesis of thieno[2,3-*d*]pyrimidine-2,4-diol[compound (2)]: Methyl 2-aminothiophene-3-carboxylate (**0.1 mol, 15.7 g**) and urea (**0.5 mol, 30 g**) were mixed with each other, and the mixture was heated for two hours 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 72% (**16.8 gms**) of thieno[2,3-*d*]pyrimidine-2,4-diol, M.P. 300°C above.

Yield: 90% (white color solid).

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

¹H NMR (400 MHz; CDCl₃): 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).

¹³C NMR (100 MHz; CDCl₃): δC 128.92, 124.03, 128.11, 159.62, 151.67, 154.75.

LC-MS (70 eV): m/z = 169 (M+H)⁺.

General procedure for synthesis of 2,4-dichlorothieno[2,3-*d*]pyrimidine [compound (3)]: A mixture consisting of (8.4 gm, **0.05 mol**) 2,4-di hydroxy-thieno[2,3-*d*] Pyrimidine (2) (**8.4 gm, 0.05 mol**) and 100 ml. of phosphorus oxy chloride was refluxed for 10 hours, whereby a clear solution was formed. Thereafter, the excess un reacted phosphorus oxy chloride 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 gm. (**75% 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).

¹H NMR (400 MHz; CDCl₃): δH 6.98 (d, 1H, J_{HH}=7.0 Hz, Ar-H), 7.39 (d, J_{HH}=7.0 Hz, 1H, Ar-H).

¹³C NMR (100 MHz; CDCl₃): δC 126.92, 123.03, 126.11, 153.62, 161.67, 154.75.

LC-MS (70 eV): m/z=205(M+H)⁺, 207(M+2), 209(M+4), 9:6:1. It indicates molecule contain two chlorine atoms.

General procedure for synthesis of 2-chloro-4-hydrazinylthieno[2,3-*d*]pyrimidine[compound (4)]: A mixture of 2,4-dichlorothieno[2,3-*d*]pyrimidine [compound (3)] (**0.1 mol, 20.4 gms**) in methanol was taken and cooled to 0°C-5°C in an ice bath. Tri Ethyl amine (**0.3 mol, 30.3 gms**) was added to the cold reaction mixture and then hydrazine hydrate (95% Purity) (**0.15 mol, 8 gms**) was added slowly at 5°C-10°C. The reaction mass was allowed to stir at room temperature for 3 hrs, after completion of starting compound, the excess amount of methanol and Tri Ethyl amine was removed under vacuum. The residue was washed with water, finally petroleum ether then they obtain solid was filtered and Dried under vacuum (Figure 15).

Yield: 85% (17 gms, pale brown colour solid); m.p. 202-204°C.

IR (KBr, cm⁻¹): 760(-C-Cl), 3102(Ar C-H), 1650 (Ar C=C Stretching), 3342 (-N-H Stretching, two bands indicates 1° Amine) (Figure 16).

¹H NMR (400 MHz; CDCl₃): δH 4.68 (s, 2H), 7.60 (s, 2H, Ar-H), 9.6 (1H, S) (Figure 17).

General procedure for synthesis of 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*]pyrimidine compound(6): To a mixture of compound(4) (**0.1 mol, 20 g**) in methanol (50 ml), acetyl acetone compound (5) (**0.12 mol, 12 g**) was added the reaction mixture was refluxed for 2 hrs and then The obtained solid was filtered off, dried and re-crystallized from Ethanol to give compound (6).

Yield: 84% (16.8 gms, pale brown colour solid); m.p. 202-204°C.

IR (KBr, cm⁻¹): 744(-C-Cl), 3112(Ar C-H), 2942 (SP³ -C-H Stretching).

¹H NMR (400 MHz; DMSO-*d*₆): δH 6 (1H,S, Ar-H, Pyrazole ring), 7 (1H,d, J_{HH}=7.2 HZ, Thiophene ring Ar-H), 7.2 (1H,d, J_{HH} = 7.2

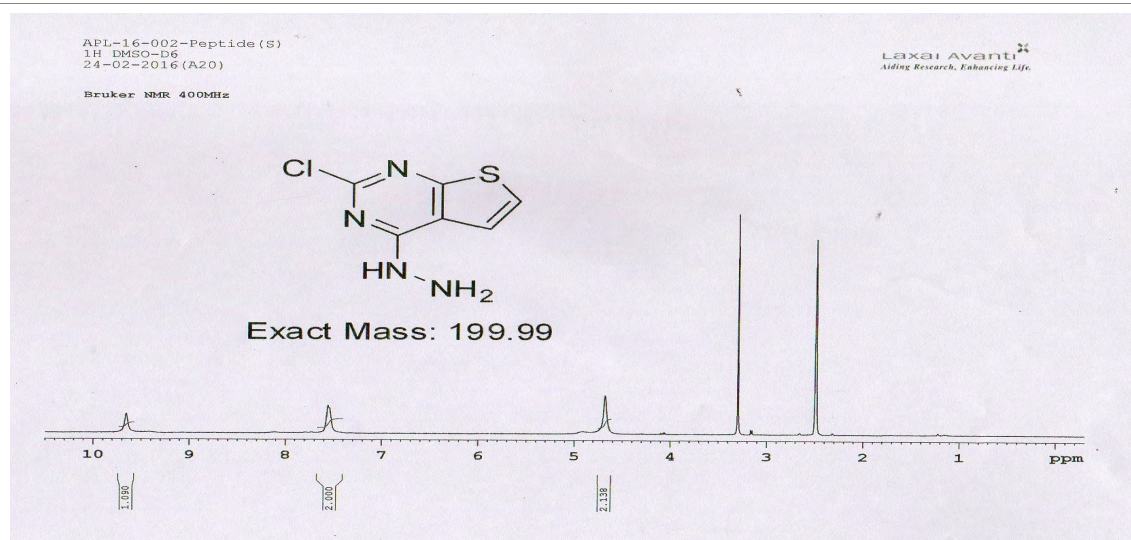


Figure 15: ¹H NMR Spectra of 2-chloro-4-hydrazinylthieno[2,3-*d*]pyrimidine (Compound 4).

HZ, Thiophene ring Ar-H), 2.43 (s, 6H, CH₃ × 2).

¹³C NMR (100 MHz; CDCl₃): δC 126.92, 123.03, 116.11, 170, 163.62, 141.67, 110.25, 153, 13 (Methyl carbon).

MS (70 eV): m/z = 265(M+H)⁺, 267(M+2), 3:1 It indicates molecule contain one chlorine atom.

General procedure for synthesis of 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-phenylthieno[2,3-*d*]Pyrimidine (8a), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-p-tolylthieno[2,3-*d*]Pyrimidine (8b), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-methoxyphenyl)thieno[2,3-*d*]Pyrimidine (8c), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(trifluoromethoxy)phenyl)thieno[2,3-*d*]pyrimidine (8d), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(trifluoromethyl)phenyl)thieno[2,3-*d*]Pyrimidine (8e), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(methylthio)phenyl)thieno[2,3-*d*]Pyrimidine (8f), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-nitrophenyl)thieno[2,3-*d*]Pyrimidine (8g), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(1H-indol-5-yl)thieno[2,3-*d*]Pyrimidine (8h), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(thiophen-2-yl)thieno[2,3-*d*]Pyrimidine (8i), 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(pyridin-3-yl)thieno[2,3-*d*]Pyrimidine(8j): A Solution of 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*]Pyrimidine compound(6) in dioxane (5v) (3.8 m.mol, 1 g), was added to a appropriate different boronic acids (7a-j) (5.7 m.mol), aqueous Na₂CO₃ (0.5M, 11.4 m.mol, 1.2 gms) Pd(PPh₃)₄ (0.38 m.mol, 440 mg, 10 mol%) was then added as a solid, Then degassed for 10 mints, the vial was capped and the solution was heated to 110°C for 5 hrs. Reaction progress was monitored by TLC. Then reaction mixture was diluted with water and Extracted with Dichloro methane, dried over Na₂SO₄ filtered and evaporated to dryness. The crude product was purified by

Column Chromatography affording product 8(a)-8(j).

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-phenylthieno[2,3-*d*]Pyrimidine (8a): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*]Pyrimidine compound(6) (3.8 m.mol, 1 g) and phenyl boronic acid (7a) (4.56 m.mol, 695 mg). The compound was obtained as pale light crystals, 63% (730 mg) (Figure 18).

yield, m.p. 217-219°C.

IR (KBr) cm⁻¹: 3060 (CH aryl), 2960 (CH alkyl), 1600 (C=N), 1550 (C=C).

¹H-NMR (400 MHz, DMSO-*d*₆) ppm: δH 2.34(s, 6H, CH₃ × 2), 6(1H,s, Ar-H, Pyrazole ring), 7.1 (1H,d, J_{HH} = 7.2 Hz, Thiophene ring Ar-H), 7.3 (1H,d, J_{HH} = 7.2 HZ, Thiophene ring Ar-H), 7.5-8.4 (5H,m).

¹³C NMR (100 MHz; DMSO-*d*₆): δC 13(Methyl carbons), 110.25, 117.11, 123.03, 1267, 130, 135, 141.67, 155, 164.62, 167.

MS (70 eV): m/z = 307(M+H, 100%)⁺, 308(M+1, 18.4%). It indicates molecule contain 17 carbon atoms.

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-p-tolyl thieno[2,3-*d*]Pyrimidine (8b): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*]Pyrimidine compound(6) (3.8 m.mol, 1 g) and 4-methyl phenyl boronic acid (7b) (4.56 m.mol, 620 mg). The compound was obtained as light white crystals, 61% (Figure 19).

Yield (0.738 mg), m.p. 259-261°C.

IR (KBr) cm⁻¹: 3070 (CH aryl), 2960 (CH alkyl), 1610 (C=N), 1590 (C=C).

¹H-NMR (400 MHz, DMSO-*d*₆) ppm: δH 2.33 (s, 9H, CH₃ × 3),

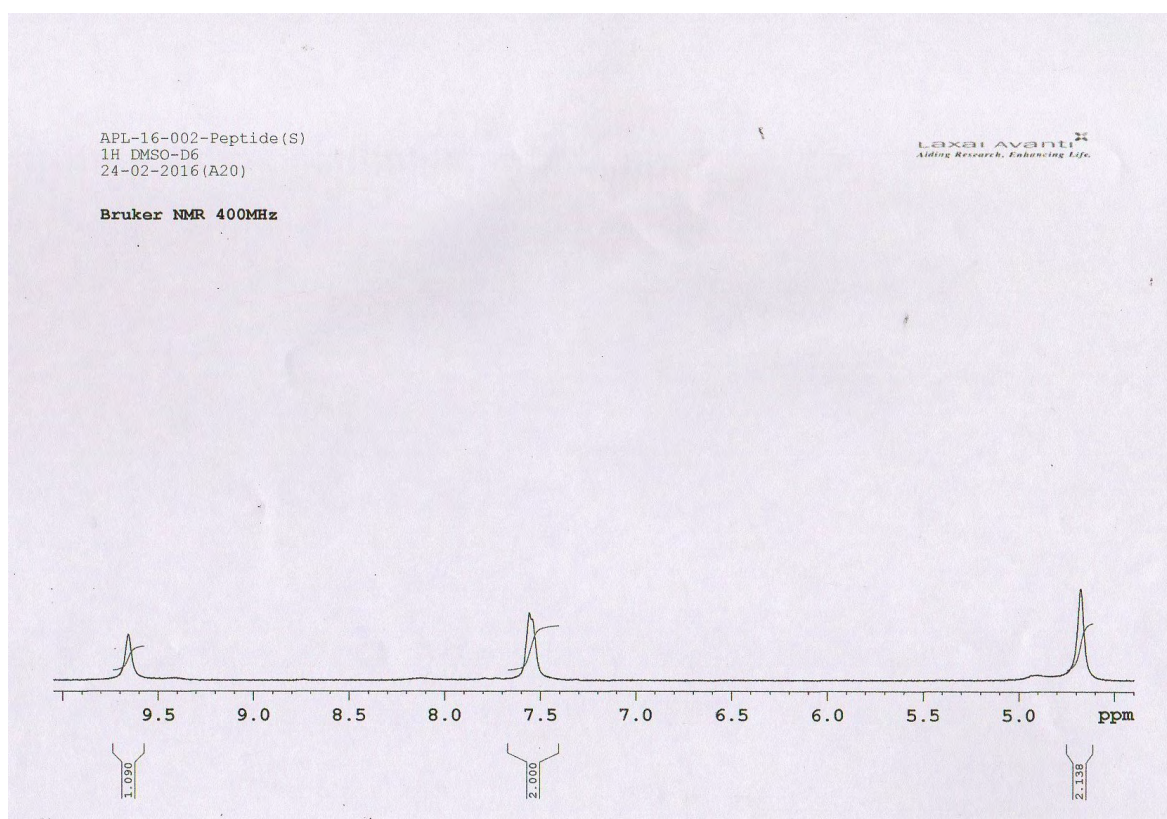


Figure 16: ¹H NMR Expansion Spectra of 2-chloro-4-hydrazinylthieno[2,3-*d*]pyrimidine (Compound 4).

6.2 (1H,S, Ar-H, Pyrazole ring), 7.1(1H,d, $J_{\text{HH}}=7.0$ HZ, Thiophene ring Ar-H), 7.23(2H,d, $J_{\text{HH}}=8$ Hz), 7.3 (1H,d, $J_{\text{HH}}=7.0$ HZ, Thiophene ring Ar-H), 8.5(2H,d, $J_{\text{HH}}=8$ Hz).

^{13}C NMR (100 MHz; DMSO- d_6): δC 13(Methyl carbons in pyrazole ring), 23(methyl carbon, Aromatic methyl carbon), 110.25, 117.11, 126.92, 128,130, 132,155, 141.67, 151, 135, 165, 169.

MS (70 eV): $m/z = 321(\text{M}+\text{H}, 100\%)^+$, $322(\text{M}+1, 19.4\%)$, It indicates molecule contain 18 carbon atoms.

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-methoxyphenyl)thieno[2,3-*d*]Pyrimidine (8c): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] Pyrimidine compound(6) (3.8 m.mol, 1 g) and 4-methoxy phenyl boronic acid (7c) (4.56 m.mol, 693 mg). The compound was obtained as pale light colourless crystals (Figure 20).

60% yield(763 mg), m.p. 231-233°C.

IR (KBr) cm^{-1} : 3090 (CH aryl), 2960 (CH alkyl), 1160(C-O-C),1600 (C=N), 1590 (C=C).

^1H -NMR (400, MHz, DMSO- d_6) ppm: δH 2.32 (S, 6H, $\text{CH}_3 \times 2$), 3.9 (3H,S, -O- CH_3),6.1 (1H,S, Ar-H, Pyrazole ring),7.2(1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.05 (1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.1(2H,d, $J_{\text{HH}}=8$ Hz), 7.8(2H,d, $J_{\text{HH}}=8$ Hz).

^{13}C NMR (100 MHz; DMSO- d_6): δC 513 (Methyl carbons in pyrazole ring), 6 (methyl carbon, Aromatic methoxy carbon), 126.92, 123.03, 117.11, 169,165, 155,141.67, 110.25, 151, 125, 130,114, 161.

MS (70 eV): $m/z = 337(\text{M}+\text{H}, 100\%)^+$, $338(\text{M}+1, 19.5\%)$. It indicates molecule contain 18 carbon atoms.

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(trifluoromethoxy)phenyl)thieno[2,3-*d*]pyrimidine (8d): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] (Figure 21) Pyrimidine compound(6) (3.8 m.mol, 1 g) and 4-Trifluoro methoxy phenyl boronic acid (7d) (4.56 m.mol, 939 mg). The compound was obtained as pale yellow crystals, 65% yield (960 mg), m.p. 248-250°C;

IR (KBr) cm^{-1} : 3080 (CH aryl), 2960 (CH alkyl), 1350(C-F), 1190(C-O-C),1610 (C=N), 1595 (C=C);

^1H -NMR (400 MHz, DMSO- d_6) ppm: δH 2.36 (S, 6H, $\text{CH}_3 \times 2$), 6.15(1H,S, Ar-H, Pyrazole ring),7.2(1H,d, $J_{\text{HH}}=7.0$ HZ, Thiophene ring Ar-H), 7.25 (1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.35(2H,d, $J_{\text{HH}}=8$ Hz), 7.9(2H,d, $J_{\text{HH}}=8$ Hz).

^{13}C NMR (100 MHz; DMSO- d_6): δC 13(Methyl carbons in pyrazole ring), 126.92, 123.03, 117.11, 169,165, 155,141.67, 110.25, 151, 128(- CF_3 carbon, Aromatic tri fluoro methoxy carbon), 125, 130,114, 161.

MS (70 eV): $m/z = 389(\text{M}-\text{H}, 100\%)^+$, $390(\text{M}+1, 19.5\%)$, It indicates molecule contain 18 carbon atoms.

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(trifluoromethyl)phenyl)thieno[2,3-*d*]Pyrimidine (8e): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] Pyrimidine compound(6) (Figure 22) (3.8 m.mol,1 g) and 4-Trifluoro methyl phenyl boronic acid (7e) (4.56 m.mol, 866 mg). The compound was obtained as yellow crystals, 65%

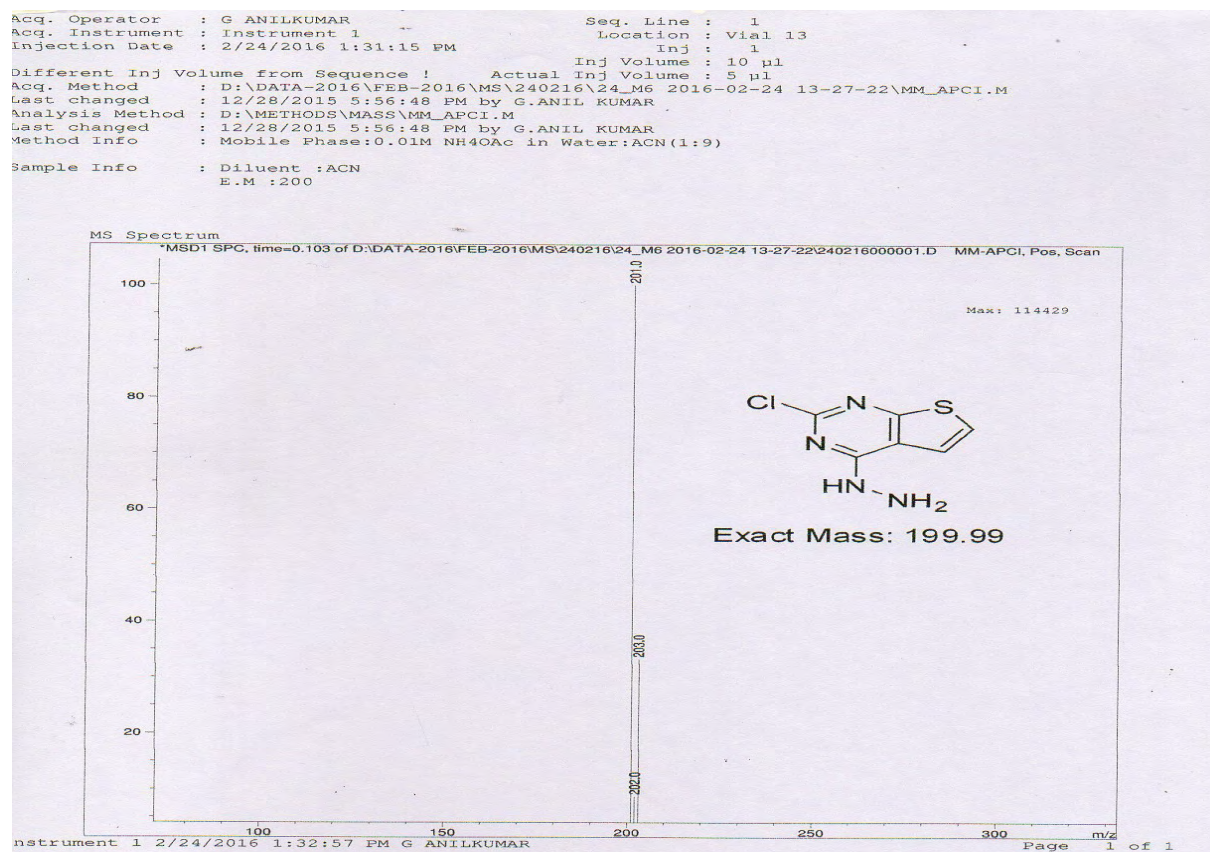


Figure 17: Mass Spectra of 2-chloro-4-hydrazinylthieno[2,3-*d*]pyrimidine (Compound 4).

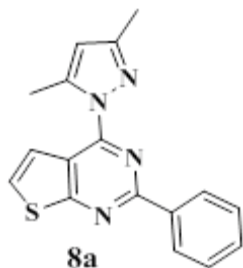


Figure 18: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-phenylthieno[2,3-*d*]pyrimidine.

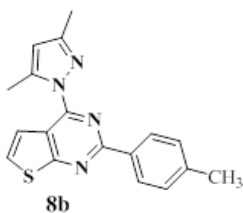


Figure 19: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-p-tolylthieno[2,3-*d*]pyrimidine.

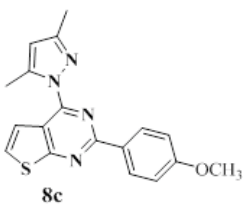


Figure 20: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-methoxyphenyl)thieno[2,3-*d*]pyrimidine.

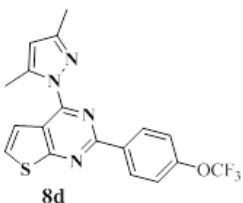


Figure 21: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(trifluoromethoxy)phenyl)thieno[2,3-*d*]pyrimidine.

yield (918 mg), m.p. 278-280°C.

IR (KBr) cm^{-1} : 3010 (CH aryl), 2960 (CH alkyl), 1340(C-F), 1610 (C=N), 1615 (C=C).

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) ppm: δ H 2.31 (S, 6H, $\text{CH}_3 \times 2$), 6.05(1H,S, Ar-H, Pyrazole ring), 7.05 (1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.2(1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.8(2H,d, $J_{\text{HH}}=8$ Hz), 8.9 (2H, d, $J_{\text{HH}}=8\text{Hz}$).

$^{13}\text{C NMR}$ (100 MHz; DMSO-d_6): δ C 16(Methyl carbons in pyrazole ring), 126.92, 123.03, 117.11, 169,165, 155,141.67, 110.25, 124(- CF_3 carbon, Aromatic tri fluoro methyl carbon), 125, 130,114, 131,151.

MS (70 eV): $m/z = 373(\text{M-H}, 100\%)^+$, 374(M+1, 19.5%), It indicates molecule contain 18 carbon atoms.

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(methylthio)phenyl)

thieno[2,3-*d*]Pyrimidine (8f): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] Pyrimidine compound(6) (Figure 23) (3.8 m.mol,1 g) and 4-(methylthio) phenyl boronic acid (7f) (4.56 m.mol, 766 mg). The compound was obtained as pale white crystals, 66% yield (877 mg), m.p. 293-295°C.

IR (KBr) cm^{-1} : 3090 (CH aryl), 2960 (CH alkyl), 1600 (C=N), 1560 (C=C).

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) ppm: δ H 2.30 (S, 6H, $\text{CH}_3 \times 2$), 2.59(3H,S, -S- CH_3), 6.1(1H,S, Ar-H, Pyrazole ring), 6.98 (1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.2(1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.5(2H,d, $J_{\text{HH}}=8\text{Hz}$), 7.7(2H,d, $J_{\text{HH}}=8\text{Hz}$).

$^{13}\text{C NMR}$ (100 MHz; DMSO-d_6): δ C 14(Methyl carbons in pyrazole ring), 18(methyl carbon, Aromatic methyl Thio carbon), 126.92, 123.03, 117.11, 169,165, 155,141.67, 110.25, 151, 125, 130,114, 161.

MS (70 eV): $m/z = 353(\text{M+H}, 100\%)^+$, 354(M+1, 19.5%), It indicates molecule contain 18 carbon atoms.

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-nitrophenyl)thieno[2,3-*d*]Pyrimidine (8g): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] Pyrimidine compound(6) (3.8 m.mol, 1 g) (Figure 24) and 4-nitro phenyl boronic acid (7 g) (4.56 m.mol, 761 mg). The compound was obtained as pale white crystals, 66% yield(875 mg), m.p. 293-295°C.

IR (KBr) cm^{-1} : 3090 (CH aryl), 2980 (CH alkyl), 1600 (C=N), 1560 (C=C); 1340 and 1560 (N-O Symmetric and Asymmetric stretching in Nitro Group).

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) ppm: δ H 2.33 (S, 6H, $\text{CH}_3 \times 2$), 6.1(1H,S, Ar-H, Pyrazole ring), 6.98 (1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.2(1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 8.1(2H,d, $J_{\text{HH}}=8\text{Hz}$), 8.45(2H,d, $J_{\text{HH}}=8\text{Hz}$).

$^{13}\text{C NMR}$ (100 MHz; DMSO-d_6): δ C 15(Methyl carbons in pyrazole ring), 126.92, 123.03, 117.11, 169,165, 155,141.67, 110.25, 151, 141, 126,125, 149.

MS (70 eV): $m/z = 352(\text{M+H}, 100\%)^+$, 353(M+1, 18.5%), It indicates molecule contain 18 carbon atoms.

4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(1H-indol-5-yl)thieno[2,3-*d*]Pyrimidine (8h): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] Pyrimidine compound(6) (3.8 m.mol, 1 g) and 1H-indol-5-ylboronic acid (7h) (4.56 m.mol, 735 mg) (Figure 25). The compound was obtained as pale white crystals, 56% yield(730 mg), m.p. 257-259°C;

IR (KBr) cm^{-1} : 3110 (CH aryl), 3360 (br, NH),2990 (CH alkyl), 1600 (C=N), 1560 (C=C).

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6) ppm: δ H 2.35 (S, 6H, $\text{CH}_3 \times 2$), 6.1(1H,S, Ar-H, Pyrazole ring), 6.45(1H,d, $J_{\text{HH}}=8\text{Hz}$), 6.98 (1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.2(1H,d, $J_{\text{HH}} = 7.0$ HZ, Thiophene ring Ar-H), 7.48(1H,d, $J_{\text{HH}}=8\text{Hz}$), 7.7(1H,d), 7.9(2H,d), 7.65(1H,S), 10(1H,bs).

$^{13}\text{C NMR}$ (100 MHz; DMSO-d_6): δ C 16(Methyl carbons in pyrazole ring), 126.92, 123.03, 117.11, 169,165, 155,141.67, 110.25, 151, 130, 117, 111, 120, 116, 130, 135, 104, 125.

MS (70 eV): $m/z = 346(\text{M+H}, 100\%)^+$, 347(M+1, 20.5%), It indicates molecule contain 19 carbon atoms.

4-(3, 5-dimethyl-1H-pyrazol-1-yl)-2-(thiophen-2-yl)thieno[2,3-*d*]Pyrimidine (8i): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] Pyrimidine compound(6) (Figure 26) (**3.8 m.mol, 1 g**) and Thiophene-2-yl boronic acid (7i) (**4.56 m.mol, 585 mg**). The compound was obtained as pale white crystals, 66% yield (**778 mg**), m.p. 293-295°C.

IR (KBr) cm^{-1} : 3090 (CH aryl), 2980 (CH alkyl), 1600 (C=N), 1560 (C=C);

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) ppm: δ H 2.43 (S, 6H, $\text{CH}_3 \times 2$), 6.1(1H,S, Ar-H, Pyrazole ring), 6.98 (1H,d, J_{HH} = 7.0 HZ, Thiophene ring Ar-H), 7.15(1H,t), 7.7(1H,d),7.2(1H,d, J_{HH} = 7.0 HZ, Thiophene ring Ar-H), 7.9(1H,d, J_{HH} =8Hz),.

$^{13}\text{C NMR}$ (100 MHz; DMSO- d_6): δ C 18(Methyl carbons in pyrazole ring), 126.92, 123.03, 117.11, 155,141.67, 110.25, 151, 163, 143, 130, 127, 129, 169,165.

MS (70 eV): m/z = 313(M+H, 100%)⁺, 314(M+1, 16.2%), It indicates molecule contain 15 carbon atoms.

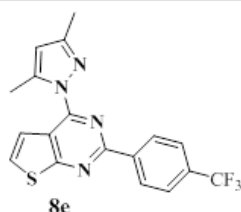


Figure 22: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(trifluoromethyl)phenyl)thieno[2,3-*d*]pyrimidine.

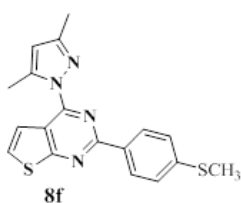


Figure 23: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-(methylthio)phenyl)thieno[2,3-*d*]pyrimidine.

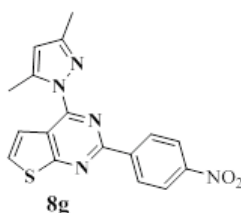


Figure 24: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(4-nitrophenyl)thieno[2,3-*d*]pyrimidine.

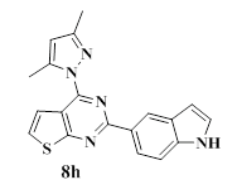


Figure 25: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(1H-indol-5-yl)thieno[2,3-*d*]pyrimidine.

4-(3, 5-dimethyl-1H-pyrazol-1-yl)-2-(pyridin-3-yl) thieno [2, 3-*d*] Pyrimidine (8j): From 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*] Pyrimidine compound(6) (**3.8 m.mol, 1 g**) and pyridine-3-yl boronic acid (7a) (**4.56 m.mol, 560 mg**) (Figure 27). The compound was obtained as pale light crystals, 63% yield (**730 mg**), m.p. 217-219°C;

IR (KBr) cm^{-1} : 3060 (CH aryl), 2960 (CH alkyl), 1600 (C=N), 1550 (C=C);

$^1\text{H-NMR}$ (400 MHz, DMSO- d_6) ppm: δ H 2.43 (S, 6H, $\text{CH}_3 \times 2$), 6(1H,S, Ar-H, Pyrazole ring),7.1(1H,d, J_{HH} = 7.2 HZ, Thiophene ring Ar-H), 7.3 (1H,d, J_{HH} = 7.2 HZ, Thiophene ring Ar-H), 7.6(1H,t), 8.5(1H,d), 8.8(1H,d), 9.3(1H,S),.

$^{13}\text{C NMR}$ (100 MHz; DMSO- d_6): δ C 20(Methyl carbons), 126.92, 123.03, 117.11,155, 167,164.62, 141.67, 110.25, 155, 162, 133, 137, 126, 148,158.

MS (70 eV): m/z = 308(M+H, 100%)⁺, 309(M+1, 17.4%), It indicates molecule contain 16 carbon atoms.

Biological activity

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. A standard inoculums ($1-2 \times 10^7$ c.f.u./ml 0.5 McFarland standards) were introduced on to 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

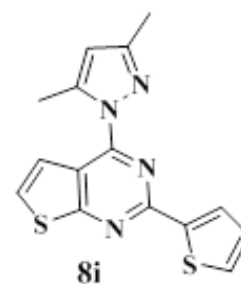


Figure 26: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(thiophen-2-yl)thieno[2,3-*d*]pyrimidine.

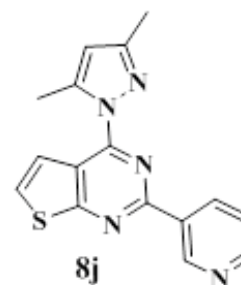


Figure 27: 4-(3,5-dimethyl-1H-pyrazol-1-yl)-2-(pyridine-3-yl)thieno[2,3-*d*]pyrimidine.

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 is given in Table 1. The order of activity was 8i>8h>8e>8j>8d >8f >8g>>8a>8b>8c.

Antifungal studies: The newly prepared compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus flavus* in DMSO by agar diffusion method. 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. Twenty millilitres 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 Ketoconazole as a standard drug. Inhibition zone were measured and compared with the controls. The fungal zone of inhibition values is given in Table 2.

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

Table 1: Anti-bacterial activity of compounds 8(a-j).

Synthesised Compounds	Zone of inhibition measure in mm			
	<i>Candida albicans</i>		<i>Aspergillus flavus</i>	
	100 µg/mL	50 µg/mL	100 µg/mL	50 µg/mL
8a	8.5	5	7.5	5.5
8b	8	5.5	7	3.5
8c	6.5	4.5	7	4
8d	11.5	6.5	9	6
8e	13	11.5	10.5	8
8f	11	9	10	9
8g	9.5	7.5	8	6.5
8h	14.5	12	12.5	9.5
8i	17.5	12.5	16	12
8j	12.5	8	10.5	10
Ketoconazole	21	16	18.5	14
Control (DMSO)	---	---	---	---

Table 2: Anti-fungal activity of compounds 8a-j.

Results and Discussion

Chemistry

The reaction sequences Employed for synthesis of title compounds are shown in. In the present work, the starting thieno[2,3-*d*]pyrimidine-2,4-diol(2) was prepared from methyl 2-amino thiophene-3-carboxylate (1) and Urea according to synthetic procedure was prepared according to synthetic procedure [19]. 2,4-dichlorothieno[2,3-*d*]pyrimidine (3) was prepared according to synthetic procedure [20]. The 2-chloro-4-hydrazinylthieno[2,3-*d*]pyrimidine (4) was prepared according to synthetic procedure [21], which on further treatment with Acetyl acetone (5) in Ethanol to get 2-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)thieno[2,3-*d*]pyrimidine (6) according to synthetic procedure [21], which were treated with different substituted phenyl boronic acids and Heterocyclic boronic acids under Suzuki reaction conditions to get Target Novel Thieno[2,3-*d*]Pyrimidine derivatives (8a-j) according to synthetic procedure [22]. All compounds displayed IR, ¹H and ¹³C NMR and mass spectra consistent with the assigned structures. ¹H 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 1140 cm⁻¹ (C-O-C), 760 cm⁻¹ (C-Cl) and 1324 and 1552 cm⁻¹ (N-O Stretching in Nitro group). The mass spectra of all the final derivatives showed comparable molecular ion peak with respect to molecular formula.

Anti-microbial studies

The newly synthesized compounds (8a-j) were screened for their *in-vitro* anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* using Amoxicillin as standard by disc diffusion method (zone of inhibition) [23,24]. The test compounds were dissolved in di methyl sulfoxide (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 8i, 8h, 8e and 8j were found to be more active against tested bacterial strains as compared to the standard. Compound 8f exhibited moderate antibacterial activity against all tested bacterial stains. The activities exhibited by the synthesized compounds were due to both pyrazole and different substituted phenyl and Heterocyclic rings linked with Thieno[2,3-*d*] Pyrimidine as a Core ring. The *in-vitro* antifungal activities for compounds 8a-8j were determined by agar diffusion method [25]. The results indicate that, among the tested compounds 8i, 8h, 8e and 8j were active against all tested fungal strains. The enhanced activities are due to electron withdrawing groups viz., -CF₃ and nitro attached to thieno [2,3-*d*] pyrimidine ring. All other compounds such as, pyrazole and phenyl ring with methyl and methoxy groups in thieno [2,3-*d*] pyrimidine showed lesser antifungal activity as compared with standard Ketoconazole. Tables 1 and 2 depict the antimicrobial screening results of the final compounds.

Conclusion

The research study reports the successful synthesis and anti-microbial activity of novel thieno[2,3-*d*] Pyrimidine as a core unit containing Pyrazole and different Substituted Phenyl / Heterocyclic derivatives. The anti-microbial 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 -CF₃ and -NO₂ groups attached to the phenyl

ring and thiophene, Indole, pyridine rings were responsible for good antimicrobial activity and hence compounds 8i, 8h, 8e and 8j exhibited more potent anti-microbial activity of all tested pathogenic strains.

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