Synthesis, Characterisation and Biological Evaluation of Some Novel Thieno [2,3-*d*] Pyrimidine Derivatives

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Abstract

*A series of novel **4-Substituted/Heterocyclic-N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)benzamide (8a-j)** derivatives were synthesized by a 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 Staphylococcus aureus (S. aureus) and Bacillus subtilis (B. subtilis) from Gram positive group of bacteria and Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E. coli) from Gram negative group of bacteria and antifungal activity against Aspergillus niger (A. niger) and Candida albicans (C. albicans). Anti-bacterial and anti-fungal activities were Evaluated and compared with the standard drugs Such as Amoxicillin & Flucanazole From anti-bacterial and anti-fungal activity screening results, it has been observed that compounds 8j, 8i, 8e and 8f possess good activity.*

Key words: *Thieno [2, 3-d] Pyrimidine, 4-(2-chloro thieno[2,3-d]pyrimidin-4-yl)morpholine, synthesis, Anti-bacterial; Anti-fungal activity, HATU.*

Introduction

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).

Thieno pyrimidines are a class of fused heterocycles, which are common sources for the development of new potential therapeutic agents. There are three isomeric thienopyrimidines corresponding to the three possible types of annulations of thiophene to the pyrimidine ring: thieno[2,3-*d*]pyrimidine, thieno[3,4-*d*]pyrimidine, and thieno[3,2*d*]pyrimidine.

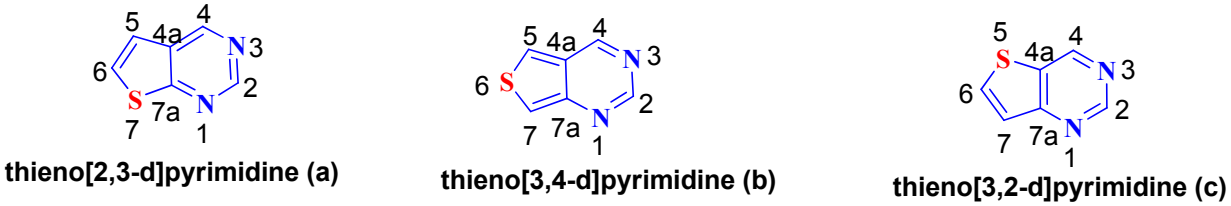


Fig.1.The three isomeric thienopyrimidines

The formation of novel fused heterocycles is an important for heterocyclic chemists from various points of view for the development of living things. Furthermore, many condensed heterocyclic systems especially, when linked to a Pyrimidine ring have attracted attention in the past few years as they are found in variety of natural products (eg. purines, pyrrolopyrimidines, pyridopyrimidines, and pteridines).

Among these heterocycles, the thienopyrimidine class is also of interest because some derivatives such as Tiprinast have been shown to clinically effective antiallergic [**1-7**]. In addition, antianaphilactic, antineoplastic[**8**], antiatherosclerotic[**9**], antibacterial [**10-17**], anti-depressive[**18-19**], antidiabetic[**20**], antihypertensive[**21-24**], antihistaminic[**25-26**], analgesic, anti-inflammatory[**27-44**], antiviral[**45-46**], spasmolytic[**47**], antipyretic[**48-50**], anticonvulsant[**51**], fungicidal[**52**], antiplatelet[**53-55**] and other Central Nervous System(CNS) affecting[**56**] activities have been reported for certain thieno-pyrimidine derivatives.

This work aimed to synthesize some new thieno [2, 3-*d*] Pyrimidine derivatives starting with methyl 3-aminothiophene-2-carboxylate and to evaluate their biological activities. Encouraged by the diverse biological activities of Thieno [2, 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 4-morpholinothieno[2,3-*d*]pyrimidin-2-amine (6) was reacted with different substituted acids (7a-j) in DMF by using HATU at Room Temperature to form novel Thieno [2,3-*d*]Pyrimidine derivatives 8(a-j). The synthesis of the compounds as per the following Scheme **I** given below. The synthetic route was depicted in scheme **I.** The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^{1}H & ^{13}C NMR spectral data analysis. Further these compounds were subjected for antifungal and antibacterial activity.

Materials and Methods

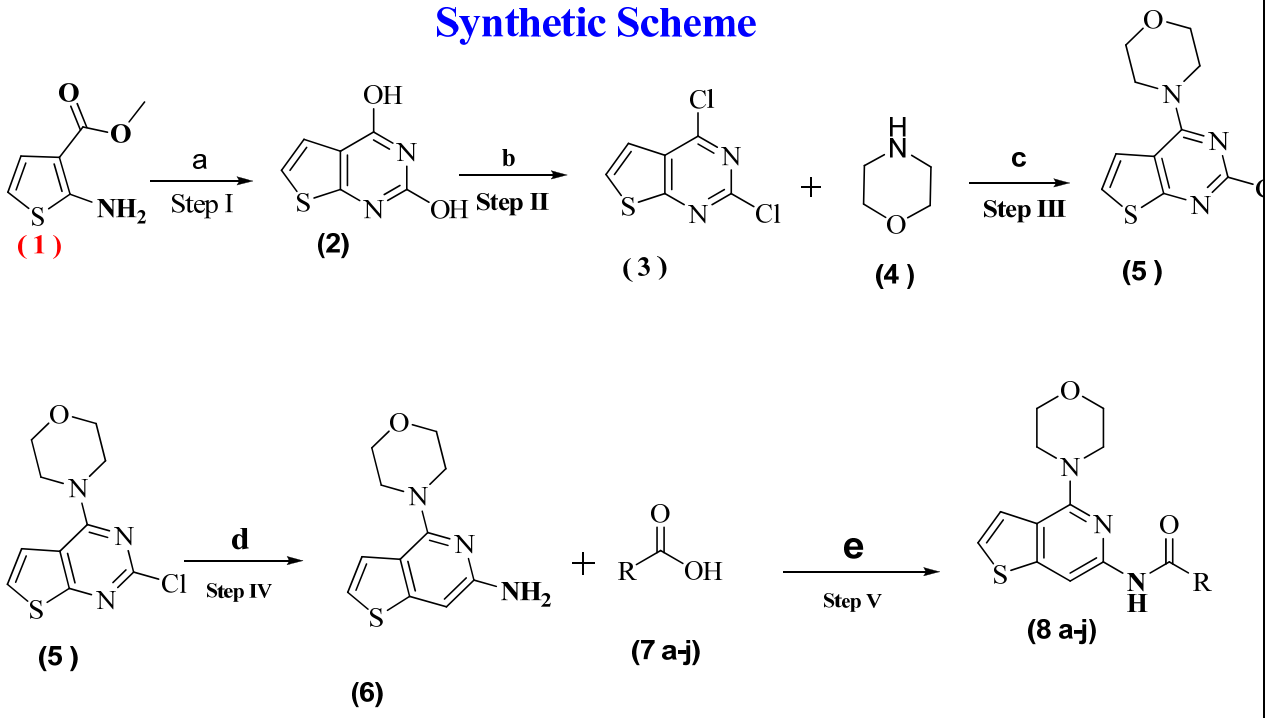
In this Investigation chemicals were purchased from local dealer with S.D fine make was used. Chemicals were 99 % pure; purity has been checked by thin layer chromatography and melting point. Conventional method has been used for synthesis of Thieno [2, 3-*d*] Pyrimidine derivatives. Stirring and reflux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

The title compounds8 (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 were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass) and analytical data.

Experimental Work

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 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 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 (^{1}H NMR and ^{13}C NMR) were recorded using tetra methyl silane (TMS) in the solvent of $CDCl_3$-d_1 or 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).

Synthetic Scheme



R = -Phenyl, -4 Methyl phenyl, -4 Methoxy phenyl, -4 tri fluoro methoxy phenyl, -4 Tri fluoro phenyl, -4 Nitro phenyl, - 1-methyl-1H-indole-2-, Furan-2, Benzo Thiophene -2, Thiophene- 2, acids .

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^{O}C, 3 hrs (b) $POCl_3$, DMF(cat), Reflux, 6 hrs (c) MeOH, 0^{O}C-RT, 2-3 hrs. (d) Aqueous Ammonia, THF, 90^{O}C, 6hrs (e) HATU, Hunigs base(N,N- di isopropyl ethyl amine), DMF, RT, 10 hrs;

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

A mixture of methyl 2-aminothiophene-3-carboxylate (100g, 0.64 mol) and urea (191g, 3.2 mol) was heated at 190^{o}C for 3 h. upon cooling to about 120^{o}C; the reaction mixture was poured into sodium hydroxide (2000ml, 1N) solution and any insoluble material removed by filtration. The mixture was then acidified with 2N HCl, to yield thieno [2, 3-*d*] pyrimidine-2, 4-diol (2) as a white precipitate, which was collected by filtration and dried (105g, 98%).m.p. >300^{o}C

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 11.14 (S, 1H, -OH), 11.61 (S, 1H, -OH), 6.94 (d, 1H, J_{HH} = 5.4 Hz, Ar-H), 8.05 (d, J_{HH} = 5.4 Hz, 1H, Ar-H).

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

ESI-MS m/z = 167.1 $(M-H)^{+}$.

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

A mixture of thieno [2, 3-*d*] pyrimidine-2, 4-diol (2) (105g, 0.63mol), phosphorous oxychloride (1000 ml) and catalytic amount of DMF (2ml), was heated at reflux for 8 h and the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and the residue was poured on to ice/water with vigorous stirring yielding a precipitate. The mixture was then filtered to yield 2,4-dichlorothieno[2,3-*d*]Pyrimidine (3) as a white solid (93.6g,73%).

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

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.98 (d, 1H, J_{HH} = 7.0 Hz, Ar-H),), 7.39 (d, J_{HH} = 7.0 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: at RT 10.96 shows m/z = 204(M^+), 206(M+2), 208(M+4), 9:6:1 it indicates molecule contain two chlorine atoms.

General procedure for synthesis of 4-(2-chlorothieno[2,3-d]pyrimidin-4-yl)morpholine [compound (5)]:

To the mixture of 2, 4-di chloro-thieno [2, 3-*d*] Pyrimidine (3) (10g, 0.048 mol) and MeOH (100ml), morpholine (4) (2.1 eq.), was added drop wise at 0^OC. The reaction mixture then was stirred at room temperature for 2 hrs. After completion of reaction indicated by TLC, the reaction mixture was then filtered, washed with water and MeOH, to yield the title compound as a yellow solid (98%, 11.4g).

IR (KBr, cm^{-1}): 756.40(-C-Cl), 3112(Ar C-H), 1640 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.95 (d, 1H, J_{HH} = 7.3 Hz, Ar-H),), 7.29 (d, J_{HH} = 7.3 Hz, 1H, Ar-H), 3.53(4H, t, J_{HH}=7.2Hz, N-CH_2×2), 3.94(4H, t, J_{HH}=7.2Hz, O-CH_2×2).

^{13}C NMR (100 MHz; $CDCl_3$): δC 31, 53, 105.23, 126, 130, 149, 162.34.

ESI-MS m/z: 256.78 $[M+H]^+$.

General procedure for synthesis of 4-morpholinothieno[2,3-d]pyrimidin-2-amine [compound (6)]:

A solution of 25% aqueous ammonia solution (5 mol) and compound (5) (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 compound (6).

IR (KBr, cm^{-1}): 3240 & 3353(N-H Stretching), 3112(Ar C-H), 1640 (Ar C=C Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.95 (d, 1H, J_{HH} = 6.9 Hz, Ar-H), 7.24(d, J_{HH} = 6.9 Hz, 1H, Ar-H), 3.55(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.71(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 7.06(2H, bs).

^{13}C NMR (100 MHz; $CDCl_3$): δC 28, 54, 105.73, 126.5, 130.55, 149, 158.8, 162.34.

ESI-MS m/z: 236.18 $[M+H]^+$.

General procedure for synthesis of

N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)benzamide (8a),

4-methyl-N-(4-morpholino thieno [2, 3-*d*] pyrimidin-2-yl) benzamide *(8b),*

4-methoxy-N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)benzamide (8c),

N-(4-morpholino thieno[2,3-*d*]pyrimidin-2-yl)-4-(trifluoro methoxy)benzamide *(8d),*

N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)-4-(trifluoromethyl)benzamide (8e),

N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)-4-nitrobenzamide (8f),

1-methyl-N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)-1H-indole-2-carboxamide (8g),

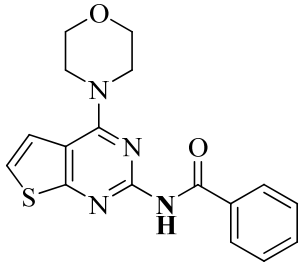
N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)furan-2-carboxamide (8h),

N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)isonicotinamide (8i),

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

To a solution of benzoic acid (7a) (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 Compound (6) (10.00 m.mol] at RT for 16 hrs, Then Reaction mixture was diluted with ice cold water, filtered the obtained Solid and Dried, Finally Purified by Flash Column Chromatography. The similar procedure was adopted to synthesize 8(b-j).

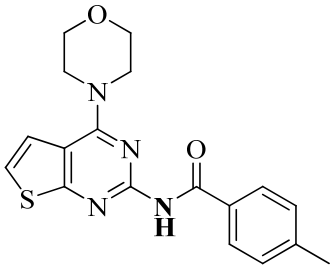
***N-(4-morpholinothieno[2,3-d]pyrimidin-2-yl)benzamide* (8a):**



***N*-(4-morpholinothieno[3,2-*c*]pyridin-6-yl)benzamide (8a)**

This compound was obtained as off-white solid in 75% yield.
IR (KBr, cm^{-1}): 3243(N-H Stretching), 3110(Ar C-H), 1685.20 (C=O Stretching).
^{1}H NMR (400 MHz; $CDCl_3$): δH 6.95 (d, 1H, J_{HH} = 6.9 Hz, Ar-H), 7.24(d, J_{HH} = 6.9 Hz, 1H, Ar-H), 3.43(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.77(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.06(1H, bs), 8.08(2H,t, J_{HH}=7.2 Hz), 7.65(2H,q,J_{HH}=7.2Hz), 7.73(1H,t,J_{HH}=7.2Hz).
^{13}C NMR (100 MHz; $CDCl_3$): δC 49, 65, 105.73, 125.5, 128.89, 130.55, 133.45,149, 158.8, 165.34.
m/z = 340.465.

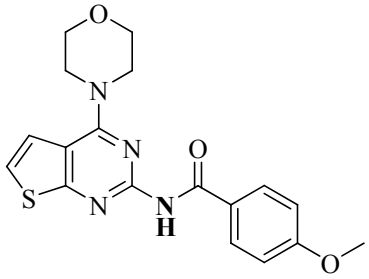
4-methyl-N-(4-morpholino thieno [2, 3-*d*] pyrimidin-2-yl) benzamide (8b):



4-methyl-*N*-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)benzamide

This compound was obtained as off-white solid in 80% yield.
m.p. 247-249^{O}C.
IR (KBr, cm^{-1}): 2920(SP^3C-H), 3243(N-H Stretching), 3110(Ar C-H), 1687.20 (C=O Stretching).
^{1}H NMR (400 MHz; $CDCl_3$): δH 2.33(3H,S),6.96(d, 1H, J_{HH} = 6.9 Hz, Ar-H), 7.25(d, J_{HH} = 6.9 Hz, 1H, Ar-H), 3.43(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.87(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.16(1H, bs), 7.98(2H,d, J_{HH}=7.2 Hz), 7.45(2H,d,J_{HH}=7.2Hz).
^{13}C NMR (100 MHz; $CDCl_3$): δC 22, 32.51, 52.88, 105.73, 125.5, 128.89, 130.55, 133.45,141,149, 158.8, 168.34.
m/z = 355.465 $[M+H]^+$.

4-methoxy-N-(4-morpholino thieno[2,3-*d*]pyrimidin-2-yl)benzamide (8c):



4-methoxy-*N*-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)benzamide

This compound was obtained as off-white solid in 77.8% yield.

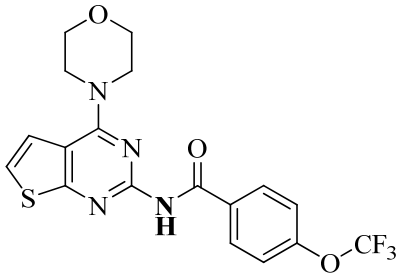
IR (KBr, cm^{-1}): 1160(C-O-C stretching), 2910.54(SP^3C-H), 3245(N-H Stretching), 3115(Ar C-H), 1686.20 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 3.83(3H,S,-OCH_3),6.96(d, 1H, J_{HH} = 6.9 Hz, Ar-H), 7.25(d, J_{HH} = 6.9 Hz, 1H, Ar-H), 3.43(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.65(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.12(1H, bs), 7.94(2H,d, J_{HH}=7.2 Hz), 7.15(2H,d,J_{HH}=7.2Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 32.51, 52.88, 55.83, 105.73, 125.5, 128.89, 130.55, 133.45,141,149, 158.8, 168.34.

m/z = 371.085 $[M+H]^+$.

N-(4-morpholino thieno[2,3-*d*]pyrimidin-2-yl)-4-(trifluoro methoxy)benzamide (8d):



N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)-4-(trifluoromethoxy)benzamide

This compound was obtained as light yellow solid in 80% yield.

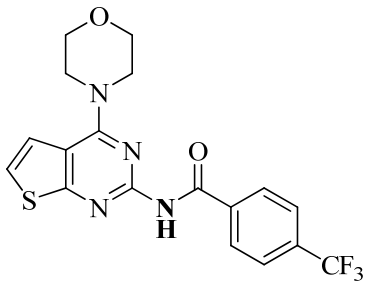
IR (KBr, cm^{-1}): 1180(C-O-C stretching), 1260(C-F Stretching), 2910.54(SP^3C-H), 3245(N-H Stretching), 3115(Ar C-H), 1690.20 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.96(d, 1H, J_{HH} = 6.9 Hz, Ar-H), 7.25(d, J_{HH} = 6.9 Hz, 1H, Ar-H), 3.45(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.95(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.15(1H, bs), 7.95(2H,d, J_{HH}=7.2 Hz), 7.25(2H,d,J_{HH}=7.2Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 32.51, 52.88, 105.73, 125.5, 128.89, 130.55, 133.45,141,149, 155.65, 158.8, 166.34.

m/z = 425.015 $[M+H]^+$.

N-(4-morpholino thieno[2,3-*d*]pyrimidin-2-yl)-4-(trifluoro methyl)benzamide (8e):



N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)-4-(trifluoromethyl)benzamide

This compound was obtained as off white solid in 80% yield.

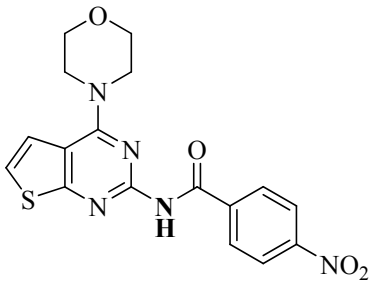
IR (KBr, cm^{-1}): 1250(C-F Stretching), 3245(N-H Stretching), 3115(Ar C-H), 1688.20 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.94(d, 1H, J_{HH} = 7.2 Hz, Ar-H), 7.22(d, J_{HH} = 7.2 Hz, 1H, Ar-H), 3.43(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.68(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.15(1H, bs), 7.95(2H,d, J_{HH}=8.2 Hz), 7.85(2H,d,J_{HH}=8.2Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 32.54, 52.85, 105.73, 124.5, 128.89, 130.55, 133.45,141,149, 155.65, 158.8, 168.34.

m/z = 409.46 $[M+H]^+$.

N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)-4-nitrobenzamide (8f):



N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)-4-nitrobenzamide

This compound was obtained as pale yellow solid in 82% yield.

m.p. 134-135^{O}C.

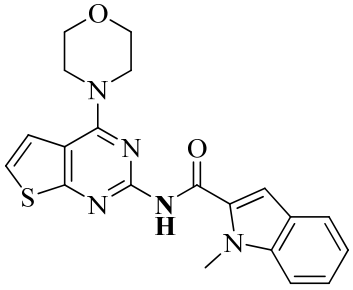
IR (KBr, cm^{-1}): 1340 & 1520(N-O Stretching), 3236(N-H Stretching), 3110(Ar C-H), 1692.20 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.94(d, 1H, J_{HH} = 7.2 Hz, Ar-H), 7.22(d, J_{HH} = 7.2 Hz, 1H, Ar-H), 3.43(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 2.68(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.05(1H, bs), 8.45(2H,d, J_{HH}=8.2 Hz), 8.15(2H,d,J_{HH}=8.2Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 28.54, 52.85, 105.73, 124.5, 128.89, 130.55, 133.45,141,149, 155.65, 158.8, 169.34.

m/z = 386.065 $[M+H]^+$.

1-methyl-N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)-1H-indole-2-carboxamide (8g):



1-methyl-*N*-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)-1*H*-indole-2-carboxamide

This compound was obtained as light yellow solid in 78% yield.

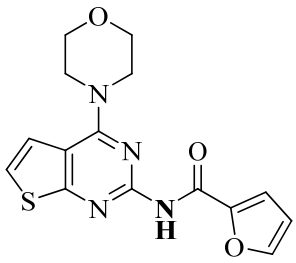
IR (KBr, cm^{-1}): 1140(N-C Stretching), 3256(N-H Stretching), 3110(Ar C-H), 1684.20 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.94(d, 1H, J_{HH} = 7.2 Hz, Ar-H), 7.22(d, J_{HH} = 7.2 Hz, 1H, Ar-H), 4.14(3H,S),3.43(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.68(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.46(1H, bs), 7.45(1H,S), 7.6(1H,d, J_{HH}=7.3 Hz), 7.12(1H,t, J_{HH}=7.3 Hz), 7.6(1H,t, J_{HH}=7.2 Hz),7.45(1H,d, J_{HH}=7.2 Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 28.54, 32.7, 52.85, 105.73, 124.5, 128.89, 130.55, 133.45,141,149, 155.65, 158.8, 174.34.

m/z = 394.103 $[M+H]^+$.

N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)furan-2-carboxamide (8h):



N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)furan-2-carboxamide

This compound was obtained as light yellow solid in 80% yield.

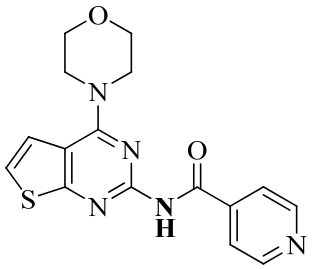
IR (KBr, cm^{-1}): 1190(C-O-C Stretching), 3246(N-H Stretching), 3110(Ar C-H), 1689.36 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.94(d, 1H, J_{HH} = 7.2 Hz, Ar-H), 7.22(d, J_{HH} = 7.2 Hz, 1H, Ar-H), 3.45(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 3.74(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.16(1H, bs), 7.45(1H,S), 6.96(1H,t, J_{HH}=7.3 Hz), 8.12(1H,d, J_{HH}=7.3 Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 28.44, 52.55, 105.73, 124.5, 128.89, 130.55, 133.45,141,149, 155.65, 157.8, 174.34.

m/z = 331.403 $[M+H]^+$.

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



N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)isonicotinamide

This compound was obtained as pale yellow solid in 81% yield.

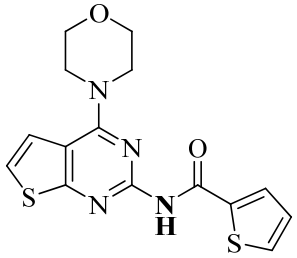
IR (KBr, cm^{-1}): 675(C-S-C Stretching), 3240(N-H Stretching), 3115(Ar C-H), 1690.55 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.94(d, 1H, J_{HH} = 7.2 Hz, Ar-H), 7.22(d, J_{HH} = 7.2 Hz, 1H, Ar-H), 3.45(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 2.72(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.26(1H, bs), 8.95(2H,d, J_{HH}=7.4Hz), 7.92(2H,d, J_{HH}=7.4 Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 28.44, 52.55, 105.73, 124.5, 128.89, 130.55, 133.45,141,149, 155.65, 157.8, 176.34.

m/z = 342.043 $[M-H]^+$.

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



N-(4-morpholinothieno[2,3-*d*]pyrimidin-2-yl)thiophene-2-carboxamide

This compound was obtained as pale yellow solid in 80% yield.

IR (KBr, cm^{-1}): 680(C-S-C Stretching), 3240(N-H Stretching), 3115(Ar C-H), 1690.36 (C=O Stretching).

^{1}H NMR (400 MHz; $CDCl_3$): δH 6.94(d, 1H, J_{HH} = 7.2 Hz, Ar-H), 7.22(d, J_{HH} = 7.2 Hz, 1H, Ar-H), 3.55(4H, t, J_{HH}=7.3Hz, N-CH_2×2), 2.77(4H, t, J_{HH}=7.3Hz, O-CH_2×2), 9.26(1H, bs), 8.45(1H,S), 7.36(1H,t, J_{HH}=7.3 Hz), 8.12(1H,d, J_{HH}=7.3 Hz).

^{13}C NMR (100 MHz; $CDCl_3$): δC 28.44, 52.55, 105.73, 124.5, 128.89, 130.55, 133.45,141,149, 155.65, 157.8, 170.34.

m/z = 347.033 $[M+H]^+$.

Biological Activity

Antibacterial studies

The newly prepared compounds were screened for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa (P. aeruginosa)* and *Escherichia coli* (clinical isolate) bacterial strains by disc diffusion method [**57, 58**]. A standard inoculums (1-2×107 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 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>8e>8f>8g >8h >8d>>8b>8c>8a.**

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

Zone of inhibition measure in mm								
Synthesised Compounds	**Gram positive**				**Gram negative**			
	Bacillus subtilis		**Staphylococcus aureus**		**Pseudomonas aeruginosa (P. aeruginosa)**		**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
8a	6	3	7.5	5	8	6	9.5	6
8b	7.5	3.5	8	7	9.5	7	10.5	7.5
8c	7	4.5	7	4.5	8.5	6.5	9	7
8d	8.5	6.5	9.0	6.5	10.15	8	11	8
8e	11.5	9	12.5	11	14.5	11.5	15.5	12
8f	11	9.5	11.5	8.5	12.5	12	13	11.5
8g	10	8	11.1	9.5	12	11	13.5	11
8h	9.5	7	9.5	7.5	12	10	12.5	10.5
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**)	---	---	----	-----	----	-----	-----	-----

Antifungal studies

The newly prepared compounds were screened for their antifungal activity against Candida albicans and Aspergillus flavus in DMSO by agar diffusion method [**59**]. Sabourauds agar media was prepared by dissolving peptone (1 g), D-glucose (4 gms) and agar (2 gms) in distilled water (100 ml) and adjusting p^H 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 are given in (**Table 2**).

Table 2: Anti-fungal activity of compounds 8a-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	5	7.5	5.5
8c	8	5.5	7	3.5
8d	9.5	7.5	8	6.5
8e	13	11.5	10.5	8
8f	11.5	6.5	9	6
8g	9.5	7.5	8	6.5
8h	11	9	10	9
8i	14.5	12	12.5	9.5
8j	17.5	12.5	16	12
Flucanazole	21	16	18.5	14
Control (**DMSO**)	---	---	---	---

Result and Discussions

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-amino thiophene-3-carboxylate (1) and Urea According to the reported procedure **[60]**.Next Step is 2,4-dichlorothieno[2,3-*d*]Pyrimidine (3) was prepared by using $POCl_3$ at reflux for 6 hrs According to the reported procedure **[60]**.The 2,4-dichlorothieno[2,3-*d*]Pyrimidine (3) was Coupling with morpholine (4) in methanol at RT to get compound (5)According to the reported procedure **[60].** Which are further treatment with Aqueous Ammonia at 90^{O}CAccording to the reported procedure **[60]**, which on further treatment with different various carboxylic acids (7a-j) to get target novel thieno [2, 3-*d*] Pyrimidine derivatives (8a-j) According to the reported procedure **[61].** 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 1340 & 1520 cm^{-1}(N-O Stretching in Nitro group),3340 cm^{-1} (-NH), 760 cm^{-1} (C-Cl) and 3320 &3250cm^{-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.

Anti-microbial studies

The newly synthesized compounds (8a-j) were screened for their in-vitro anti-bacterial activity against Bacillus subtilis, Staphylococcus aurous, Pseudomonas aeruginosa and Escherichia coli using Amoxicillin as standard by disc diffusion method (zone of inhibition. The test compounds were dissolved in dimethylsulfoxide (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, 8e, 8f** were found to be more active against tested bacterial strains as compared to the standard.

Conclusion

The research study reports the successful synthesis and anti-microbial activity of novel thieno [3,2-*d*] Pyrimidine as a core unit . The anti-microbial activity study revealed that all the tested compounds showed good antibacterial and antifungal activities against pathogenic strains and hence compounds **8j, 8i, 8e and 8f** 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 [3, 2-*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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