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DEVELOPMENT OF THIENO [2, 3-*d*] PYRIMIDINE DERIVATIVES AS LEADS FOR INHIBITION OF *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Tuberculosis (TB) is a leading infectious killer despite the reduction of new infections. India recorded a rise in the number of cases recently. Though there is a reduced rate of multi-drug resistant TB cases, it is a serious threat. Fluoroquinolones are the class of second-line drugs used in the treatment of MDR-TB. Development of resistance towards fluoroquinolones has been observed. All these facts indicate the necessity of the research for the discovery of new leads. Because thienopyrimidines are the bioisosteres of fluoroquinolones, in the current research work, various derivatives of Thieno [2,3-*d*]pyrimidines are synthesized and screened against *Mycobacterium tuberculosis.* The syntheses of 4-(4-oxothieno [2,3-d]pyrimidin-3(4H)-yl)methyl)amino)-N-substituted-phenylbenzenesulfonamide **6(a-j)** was done and spectra confirmed the formation of the final compounds. Six compounds **(6a, 6b, 6e, 6f, 6i, 6j)** were screened against *M. tuberculosis* $H_{37}Rv$ at 100 µg/ml. All six compounds exhibited moderate inhibitory activity. Newly synthesized compounds can serve as lead for better antitubercular compounds.

INTRODUCTION

Tuberculosis (TB) is one of the leading causes of death and also a leading infectious killer falls after covid according to the latest WHO report.[1] Though it is curable and preventable its burden is more till today and India, leading the count. India recorded a rise in the number of TB cases in 2021.[2]Despite new infections reducing yearly at the global rate of 2%,multi-drug resistant TB (MDR-TB) remains a public health crisis and posess a serious threat to the health of society. As MDR-TB does not respond to first-line drugs, it has to be treated with second-line anti-tubercular drugs which are expensive and also exhibit more severe side effects. The duration of treatment with second-line drugs may even extend upto two years. Sometimes, this can also lead to the development of resistance to These drugs where patients will not get any treatment options. All these facts demand further research and the discovery of new anti-tubercular drugs. Fluoroquinolones are commonly used in the treatment of TB as second-line drugs more and more newer fluoroquinolones are being included in the regimen. Though they are effective, they reported the development of intra-class cross-resistance including resistance between older and later generation fluoroquinolones.[3] Thieno pyrimidines are generally known as isosteres of fluroquinolones. Hence current work focuses on the synthesis of newer thienopyrimidine derivatives for screening against *Mycobacterium tuberculosis*. In the current research work, various derivatives of

Thieno[2,3-*d*]pyrimidines are synthesized and screened against *Mycobacterium tuberculosis.*

MATERIALS & METHODS

Synthesis of Ethyl 2-Aminothiophene-3-carboxylate (3)

Triethylamine (0.05 mol) was added dropwise over 10 min to a mixture of 2,5-Dihydroxy-1,4-dithiane (0.05 mol), ethylcyanoacetate (0.1 mol) and dimethylformamide (40 mL). The mixture was stirred at 15 °C for 30 min, diluted with 0.4 M acetic acid, and extracted with ether. The ethereal layer was washed with water 2 to 3 times and dried over sodium sulphate. The solvent was removed and the residue cooled to get the product.[4]

Melting Point: 44°C, Yield: 66.5%. **IR (KBr) cm^{-1}**: 3412.19, 3304.17(NH_2), 3173.01 (Ar. C-H str), 3090.07(Ali. C-H str), 1654.98(C=O), 1604.83 (C=N str), 1521.98, 1490 (C=C str), 1276.92 (C-N str), 650 (C-S-C str**).** **^{1}H-NMR (DMSO) δ in ppm**: 6.246-6.26 (d, 1H, C-CH Thiophene), 6.79-6.80 (d, 1H, S-CH Thiophene), 7.20 (s,2H, NH_2), 4.13-4.18 (m, 2H, CH_2), 4.13-4.18 (m, 2H, CH_2), 1.21-1.28 (t, 2H, CH_3).

Synthesis of 3H-Thieno [2,3-*d*]pyrimidin-4-one (4)

A mixture of ethyl-2-aminothiophene-3-carboxylate **(3)** (0.05 mol) and formamide (20 mL) was refluxed for 8 h and allowed to cool overnight and added to water. The crystals were filtered, dried, and recrystallized with hot alcohol. [4]

Melting Point: 260 °C, Yield:76.6%.

IR(KBr) cm^{-1}: 3285(N-H), 3150 (Ar.C-H str), 3050 (Ali.C-H str), 1660(C=O), 1590 (C=N str), 1570, 1520 (C=C), 1260 (C-N str), 700 (C-S-C str) .**^{1}H-NMR (DMSO) δ in ppm**: 7.00-7.02 (d, 1H, C-CH Thiophene), 7.24-7.28 (d, 1H, S-CH Thiophene), 7.58 (s, **1**H, 1H, N-CH-N Pyrimidine), 9.87 (s, 1H, NH).

General procedure for the synthesis of 4-(((4-oxothieno [2,3-d]pyrimidin-3(4H)-yl)methyl)amino)-N-substituted phenylbenzenesulfonamide 6(a-j)

To a mixture of 3H-thieno[2,3-*d*]pyrimidin-4-one (2) (1.0 g, 0.006 mol) and para-formaldehyde (0.009 mol) and different 4-amino-N-substituted phenyl benzenesulfonamides were added (0.006 mol) and refluxed in ethyl alcohol (25 mL) for 4 h. The solvent was removed by distillation and the residue obtained was cooled to room temperature, poured into ice-cold water, filtered, washed with water, dried, and recrystallized from ethanol.[5]

4-(((4-oxothieno[2,3-d]pyrimidin-3(4H)-yl)methyl)amino)-N-phenylbenzenesulfonamide (6a)

Melting Point: 216 °C, Yield: 90.90%. **IR(KBr) cm^{-1}**: 3341.18(NH str), 3430.48 (NH str), 3132.86 (Ar.C-H str), 2543.84 (Ali.C-H str), 1681.34 (C=O str), 1600.61 (C=N str), 1506.60 (Ar. CH=CH str), 1272.2 (C-N str), 1320.43 (C-O str), 694.33 (C-S-C str). **^{1}H-NMR (DMSO) δ in ppm**:7.41 (d, 1H, C-CH Thiophene), 7.59 (d, 1H, S-CH Thiophene), 8.10 (s, 1H, N-CH-N Pyrimidine), 8.52 (s, 1H, NH), 6.80-7.38 (m, 9H, CH of Phenyl rings), 5.52 (d, 2H, CH_2).

N-(4-fluorophenyl)-4-(((4-oxothieno[2,3-d]pyrimidin-3(4H)-yl)methyl)amino)benzenesulfonamide (6b)

Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3331.18(NH str), 3390.41 (NH str), 3122.86 (Ar.C-H str), 2553.84 (Ali.C-H str), 1672.34 (C=O str), 1610.61 (C=N str), 1502.60 (Ar. CH=CH str), 1269.2 (C-N str), 1317.43 (C-O str), 696.33 (C-S-C str).

4-(((4-oxothieno [2,3-d]pyrimidin-3(4H)-yl)methyl)amino)-N-(o-tolyl)benzenesulfonamide (6c)

Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3348.18(NH str), 3392.41 (NH str), 3112.86 (Ar.C-H str), 2463.14 (Ali.C-H str), 1685.12 (C=O str), 1618.56 (C=N str), 1513.16 (Ar. CH=CH str), 1274.2 (C-N str), 1325.23 (C-O str), 706.83 (C-S-C str). **^{1}H-NMR (DMSO) δ in ppm**: 7.50 (d, 1H, C-CH Thiophene), 7.63 (d, 1H, S-CH Thiophene), 8.30 (s, 1H, N-CH-N Pyrimidine), 8.73 (s, 1H, NH), 6.92- 7.72 (m, 8H, CH of Phenyl rings), 5.50 (d, 2H, CH_2), 2.31 (s, 3H, H of methyl group).

4-(((4-oxothieno [2,3-d]pyrimidin-3(4H)-yl)methyl)amino)-N-(p-tolyl)benzenesulfonamide (6d)

Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3348.28(NH str), 3392.44 (NH str), 3112.47 (Ar.C-H str), 2463.19 (Ali.C-H str), 1686.46 (C=O str), 1620.56 (C=N str), 1513.98 (Ar. CH=CH str), 1274.31 (C-N str), 1325.76 (C-O str), 706.18 (C-S-C str).

4-(((4-oxothieno [2,3-d] pyrimidin- 3 (4H)-yl) methyl) amino) - N -(m tolyl) benzene sulfonamide (6e) Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3348.24(NH str), 3392.34 (NH str), 3112.28 (Ar.C-H str), 2463.19 (Ali.C-H str), 1686.35 (C=O str), 1620.46 (C=N str), 1513.27 (Ar. CH=CH str), 1274.56 (C-N str), 1325.21 (C-O str), 706.20 (C-S-C str).

N-(4-chlorophenyl)-4 -(((4-oxothieno [2,3-d]pyrimidin-3 (4H)- yl) methyl) amino) benzenesulfon amide (6f) Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3340.24(NH str), 3411.34 (NH str), 3127.28 (Ar.C-H str), 2457.11 (Ali.C-H str), 1691.35 (C=O str), 1600.26 (C=N str), 1520.12 (Ar. CH=CH str), 1279.36 (C-N str), 1330.21 (C-O str), 701.02 (C-S-C str). **^{1}H-NMR (DMSO) δ in ppm**: 7.58 (d, 1H, C-CH Thiophene), 7.71 (d, 1H, S-CH Thiophene), 8.18 (s, 1H, N-CH-N Pyrimidine), 8.83 (s, 1H, NH), 7.10- 7.83 (m, 8H, CH of Phenyl rings).

N-(3-chlorophenyl)-4-(((4-oxothieno[2,3-d]pyrimidin-3(4H)-yl)methyl)amino)benzenesulfonamide (6g) Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3340.21(NH str), 3411.24 (NH str), 3127.30 (Ar.C-H str), 2457.23 (Ali.C-H str), 1691.38 (C=O str), 1604.26 (C=N str), 1522.12 (Ar. CH=CH str), 1283.36 (C-N str), 1335.21 (C-O str), 704.02 (C-S-C str

N-(2-chlorophenyl)-4-(((4-oxothieno[2,3-d]pyrimidin-3(4H)-yl) methyl) amino) benzenesulfonamide(6h) Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3340.23(NH str), 3411.17 (NH str), 3127.24 (Ar.C-H str), 2457.75 (Ali.C-H str), 1691.25 (C=O str), 1604.26 (C=N str), 1520.12 (Ar. CH=CH str), 1281.36 (C-N str), 1334.21 (C-O str), 702.02 (C-S-C str).

N- (4-fluorophenyl) -4 - (((4-oxothieno [2,3-d]pyrimidin -3 (4H)- yl) methyl) amino) benzene sulfonamide (6i) Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3362.23(NH str), 3401.12 (NH str), 3134.16 (Ar.C-H str), 2449.25 (Ali.C-H str), 1691.12 (C=O str), 1612.36 (C=N str), 1524.13 (Ar. CH=CH str), 1278.61 (C-N str), 1328.21 (C-O str), 707.02 (C-S-C str). **^{1}H-NMR (DMSO) δ in ppm**: 7.50 (d, 1H, C-CH Thiophene), 7.68 (d, 1H, S-CH Thiophene), 8.40 (s, 1H, N-CH-N Pyrimidine), 8.94 (s, 1H, NH), 6.98- 7.76 (m, 8H, CH of Phenyl rings).

N-(4-methoxyphenyl)-4-(((4-oxothieno [2,3-d]pyrimidin-3(4H)-yl)methyl)amino)benzenesulfonamide (6j) Melting Point: °C, Yield: %. **IR(KBr) cm^{-1}**: 3404.23(NH str), 3443.30 (NH str), 3130.06 (Ar.C-H str), 2438.16 (Ali.C-H str), 1698.05 (C=O str), 1616.04 (C=N str), 1536.13 (Ar. CH=CH str), 1285.33 (C-N str), 1320.96 (C-O str), 703.02 (C-S-C str).

Synthesis of 4-Acetamidobenzenesulfonyl chloride (iii) Chlorosulphonic acid was added drop wise to acetanilide in dichloromethane at 0 °C and stirred continuously for one hour at room temperature. The reaction mixture was heated to 90 °C and continued stirring for one hour. Dichloromethane layer concentrated poured to crushed ice to get the precipitate. Precipitated 4-Acetamidobenzenesulfonyl chloride filtered, dried, and crystallized with chloroform. [6]

Melting Point: 146 °C, Yield: 68.4 %. **^{1}H-NMR (DMSO) δ in ppm**: 8.10-8.11 (d, 2H, S-C-CH Phenyl), 7.19-7.21 (d, 2H, N-C-CH Phenyl), 12.49 (s, **1**H, NH Pyrimidine), 9.87 (s, 1H, NH), 1.74 (s, 3H, CH_3).

General procedure for the synthesis of 4-amino-N-substituted phenyl benzene Sulfonamides [5(a-j)]

To the mixture of an equimolar quantity of 4-Acetamidobenzenesulfonyl chloride (6) and various substituted amines in ethanol, pyridine was added and refluxed at 95 °C for 3-5 hours. 10 ml of HCl was added to the reaction mixture after confirming the completion of the reaction by TLC using DCM: Methanol in the ratio of 1:1. Refluxing continued for 2 -3 hours and neutralized with Sodium carbonate to get the precipitate of the product which is filtered, dried, and recrystallised with ethanol. [7]

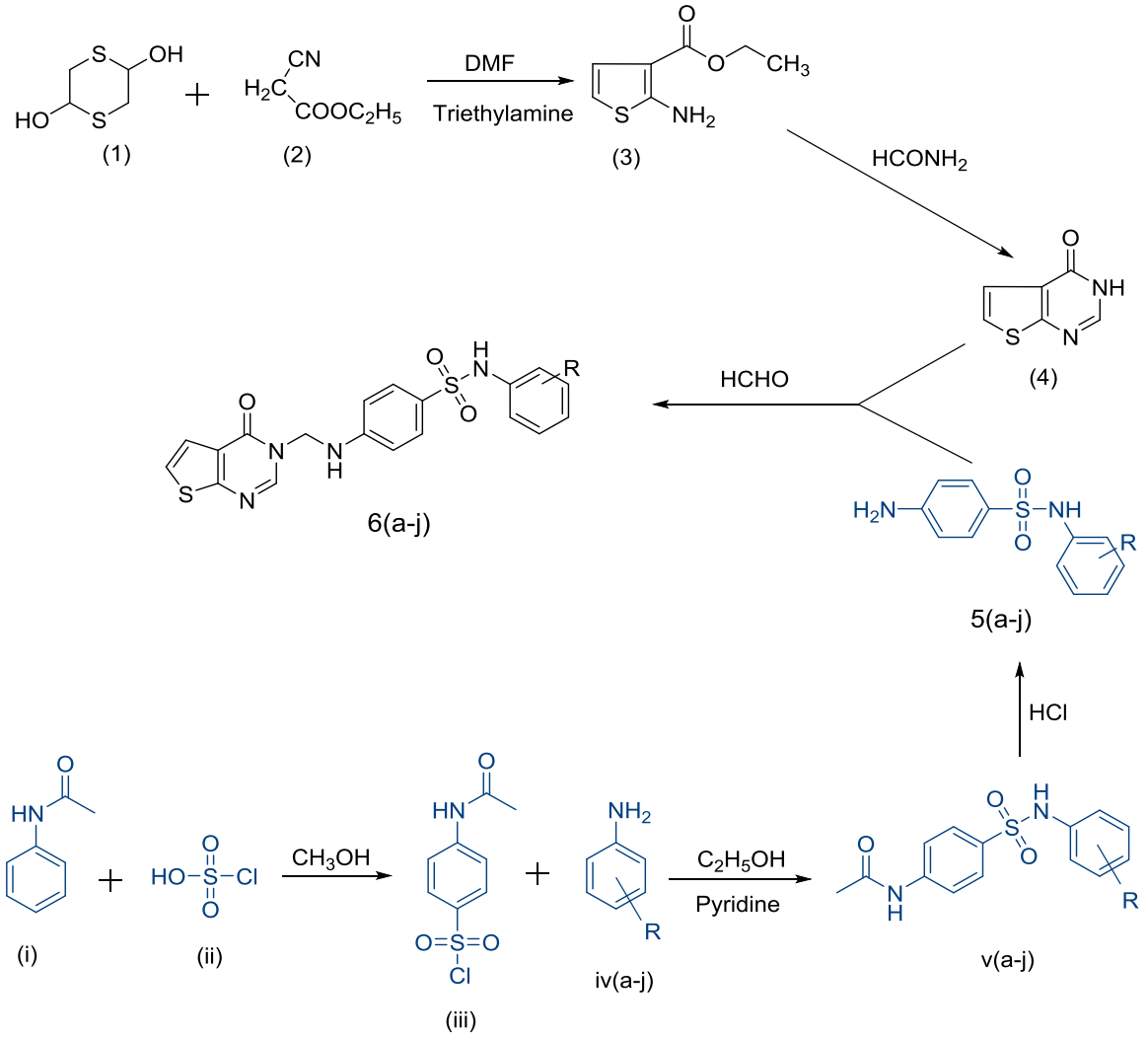
4-amino-N-phenylbenzenesulfonamide

Melting Point: 216 °C, Yield: 90.90%. **IR(KBr) cm^{-1}**: 3392.36 (NH_2 str), 3421.37 (NH_2 str), 3121.22 (Ar.C-H str), 1524.36 (Ar. CH=CH str), 1279.36 (C-N str).

Antimycobacterial activity[8]

Total 6 compounds **(6a, 6b, 6e, 6f, 6i, 6j)** were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* H_{37}Rv. The ability of compounds to inhibit

the growth of *Mycobacterium* species was determined by the Ziehl-Neelsen stain method. Organisms were grown in Middlebrook 7H9 broth and standard strain of *M. Tuberculosis* $H_{37}Rv$ (ATCC 27294). The medium was prepared according to the instructions of the manufacturer. To this, a solution of newly synthesized compounds was added at the concentration of 100µg/ml. Finally, a 10 µL suspension of *M. Tuberculosis* strain (100000 organisms/mL, adjusted by McFarland's turbidity standard) is transferred to each of the tubes and incubated at 37 °C. Along with this, one growth control without compound and standard drug control having Isoniazid were also set up. The bottles were observed for three weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth is confirmed by making a smear from each bottle and performing a Ziehl-Neelsen stain.



Scheme 1. Synthesis of 4-(((4-oxothieno[2,3-*d*]pyrimidin-3(4*H*)-yl)methyl)amino)-*N*-substituted phenylbenzenesulfonamide

Table 1. *In vitro* antimycobacterial activities of tested compounds.

Sl. No.	Compound code	Inhibition of *Mycobacterium Tuberculosis (%)*
1	6a	42 .67 %
2	6b	39.98 %
3	6e	49.27 %
4	6f	51.7 %
5	6i	32 .1 5 %
6	6j	20.47 %

RESULTS & DISCUSSION

The syntheses of 4-(((4-oxothieno [2,3-d]pyrimidin-3(4H)-yl)methyl)amino) - N substituted phenylbenzenesulfonamide **6(a-j)** was achieved through synthetic route outlined in **Scheme 1**. Progress of the reactions was monitored by TLC using precoated silica gel plates using different solvent systems as eluents. The disappearance of doublet peaks due to NH_2 in IR spectra and the appearance of two NH peaks confirmed the formation of the final compounds. Among the synthesised compounds, six compounds **(6a, 6b, 6e, 6f, 6i, 6j)** were selected for the screening of antimycobacterial activity *M. tuberculosis* $H_{37}Rv$ at a concentration of 100 µg/ml. All six compounds exhibited moderate inhibitory activity (**Table 1**). The results indicate the fact that anti-mycobacterial activity may be due to the sulphonamide group and they can be considered lead compounds.

CONCLUSION

Current research work was intended to develop a new pharmacophore having Theino[2,3-*d*] pyrimidines containing sulphonamide group. New compounds were synthesised having various substitutions on the phenyl ring and all of them showed moderated inhibitory activity against *Mycobacterium Tuberculosis*. Newly synthesised compounds can serve as lead for further development to arrive at better antitubercular compounds.

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Authors Contribution: Dr. Rashmi P is the principal investigator and Dr.Gurubasavaraja Swamy is the co-investigator.

Conflicts Of Interest: Authors hereby declare that there are no conflicts of interest.

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