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Synthesis, Characterization and Biological Evaluation of Novel Thienopyrimidine and Triazolothienopyrimidine Derivatives as Anti-Tubercular and Antibacterial Agents

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ABSTRACT

A series of novel triazole fused thienopyrimidine derivatives are synthesized by Gewald reaction, which are well characterized by IR, ^{1}HNMR, ^{13}CNMR and Mass spectral analysis. These compounds are screened for their in vitro anti-tubercular and antibacterial activities. Most of these compounds exhibited MIC values in the range of 20 – 100 µM against *Mycobacterium tuberculosis H37Rv*. In the series, compound **5c** was most active with MIC 20 µM. Furthermore, the title compounds were screened for antibacterial activity against *Staphylococcus aureus ATCC 29213* (gram positive) and *Escherichia coli ATCC 25922* (gram negative) bacteria. Some of these compounds exhibited MIC values in the range 8 - 64µM. Compound 5c was found to be the most active with an MIC of 5 and 8µM respectively.

Keywords: Gewald reaction, Thienopyrimidine, Triazolothienopyrimidine, Anti-tubercular activiy, Anti-bacterial activity.

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INTRODUCTION

Tuberculosis (TB) is one of the most airborne contagious diseases caused by Mycobacterium tuberculosis (MTB). TB is the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). TB is known to be one of the major causes of death in HIV patients[1]. Despite the fact that it is treatable and preventable, the disease has been spreading at a steady rate over the pastdecade[2]. According to the World Health Organization (WHO), 8.7 million people were infected with TB in 2013 resulting in the death of 1.4 million people. Furthermore, about 0.43 million deaths due to TB were reported amongst people affected with HIV. A large number of Multidrug Resistant-TB (MDRTB) cases were reported from India, China and Russian federation. The MTB commonly attacks the lungs, kidney, spine, and brain. Hence, if TB is not treated properly, it can be serious and fatal[3].

During last three decades, numerous reviews on pyrimidine derivatives having significant biological/pharmacological activities have been reported in the literature. These are the structural analogs of the biogenic purines and can be considered as potential nucleic acid anti-metabolites. The importance of pyrimidine derivatives and heterocyclic annulated pyrimidine based on their use as an antimicrobial[4,5], anticancer[6], antiviral[7], antiinflamatory[8] and antitumor[9]. Hence with a view to further asses pharmacological profile of these compounds and in continuation of our work for biologically active heterocyclic compounds[10-13], we herein report the synthesis of novel thienopyrimidine and triazolothienopyrimidine derivatives for the evaluation of their anti-tubercularand anti-bacterial activities.

MATERIALS AND METHOD

The reagents and solvents used in this study were of analytical grade and used without further purification. All the reactions were monitored on Merck aluminium thin layer chromatography (TLC, UV_{254nm}) plates. Column chromatography was carried out on silica gel (60-120 mesh). Melting points were determined by open capillary method and are uncorrected. Commercial reagents were used without purification. ^{1}H NMR, and ^{13}C NMR spectra were recorded on a Bruker WM-300 or 400 using $CDCl_3$ and DMSO-d_6 as the solvent. Chemical shift are reported in parts per million (δ-value).IR spectra (KBr disc) were recorded on a Nicolet -5700 FT-IR spectrophotometer and reported in wave number (cm^{-1}). ESI mass spectra were recorded on Shimadzu LC-MS after dissolving the compounds in acetonitrile and methanol.

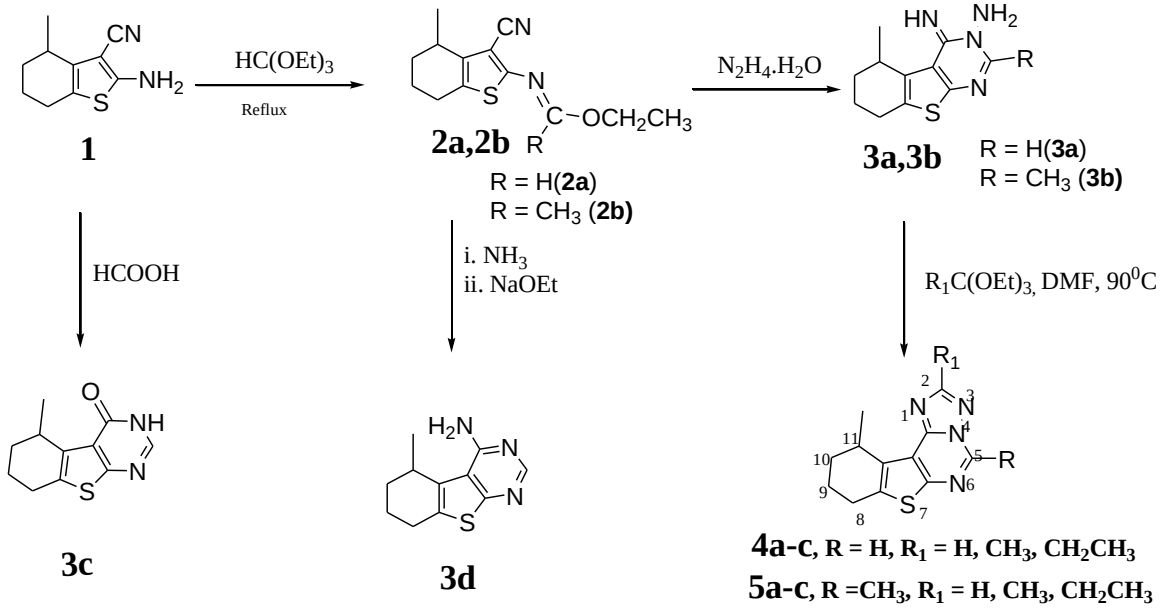

Figure 1. Synthetic scheme for the target compounds.

Experimental procedure

Preparation of 2-amino-4-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile(1)

A mixture of 2-methylcyclohexanone (71 mmole) and malononitrile (71 mmole) in ethanol (45 mL) was stirred at 0 ^{o}C and then elemental sulfur (72 mmol) and diethylamine (5 mL) was added with vigorous stirring during 1 min. Reaction mixture was stirred at 0 ^{o}C for about 1 hr. The solid separated was filtered, washed with hot ethanol and was purified by column chromatography to get analytically pure compound.

General procedure for the preparation of 2a and 2b

A solution of **1** (57 mmole) and triethylorthoformate/triethylorthoacetate (12 mL) was refluxed for 18 h, excess triethylorthoformate/triethylorthoacetate was removed under vacuum. The residue was treated with petroleum ether. Separated solid was filtered and re-crystallized from ethanol to get the analytically pure product.

General procedure for synthesis of 3a and 3b

A mixture of **2a** (55 mmol) and hydrazine hydrate (7 mL) was stirred for 2 h at room temperature and then diluted with ethanol (20 mL). The resulting fine solid was filtered, washed with ethanol.

Preparation of 4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]pyrimidin-4(3H)-one (3c)

A mixture of **1 (**52 mmole**)** and formic acid (15 mL) was refluxed for 5 h. The excess of formic acid was removed under reduced pressure. The resulting residue was crystallized from ethanol.

Preparation of 4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]pyrimidin-4-amine (3d)

Compound **2a** (55 mmole) was treated with anhydrous ethanolic ammonia (35 mL) at 0^{o}C. The solid that separated was further stirred overnight. Solvent was removed under vacuum and the residue was dissolved in DMF to which sodium ethoxide (0.8 g) was added and the stirred solution was heated to 100-112^{o}C for 1 h. The solution was concentrated under reduced pressure. The residue was treated with warm water and the product was filtered and re-crystallized with aq. ethanol.

General procedure for synthesis of compounds 4a–c and 5a-c

A mixture of (3a/3b) (53 mmole), triethylorthoformate/triethylorthoacetate/ triethylorthopropionate (1 mL) and dimethylformamide (5 mL) was heated to 90^{o}C for 4h. The reaction mixture was cooled to room temperature and diluted with petroleum ether (2 mL). The solid that separated was washed with benzene and petroleum ether and dried. The crude product was purified by means of column chromatography.

Anti-tubercular activity

MIC was determined by broth dilution method against *M. tuberculosis H37Rv* using micro-broth dilution method[14] The two fold serial dilutions of compounds were prepared in Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.5% (v/v) glycerol, 0.25% (v/v) Tween 80, and 10% ADC (albumin dextrose catalase, Becton Dickinson, Sparks, MD) in 96-well U bottom microtitre plates. The bacterial suspension was prepared from an actively growing bacterial culture and adjusted to 1 McFarland standard equivalent to 1.0x10^{7} CFU/mL. The suspension was further diluted in the ratio 1:50 in the Middlebrook 7H9 media. Hundred microlitres of this diluted inoculum was added to each well of the plate resulting in the final inoculum of 1.0x10^{6} CFU/mL in the well and the final concentrations of compounds ranged between 0.03 and 64 mg/mL. The plates were incubated at 37 ^{0}C for 3-weeks in 5% CO_2. The plates were read visually and the minimum concentration of the compound showing no turbidity was recorded as MIC.

Anti-bacterial activity

The antibacterial activity of the compounds was performed using micro dilution method[15] against S. aureus ATCC 29213 (gram positive) and E. coli ATCC 25922 (gram negative). Bacterial suspensions were prepared in sterile normal saline from 24-h grown culture. The MIC was performed in Muller Hinton Broth. Two-fold serial dilutions of the compounds were prepared in MHB in 100 ml volume in a 96 well U bottom microtitre plates. The final concentrations of the

compounds ranged between 0.06 and 128 mg/mL. The turbidity of bacterial suspensions was adjusted to 0.5 McFarland (w1.5 x10^8 CFU/mL), which was further diluted in MHB and, a 100 mL volume of this diluted inoculum was added to each well of the plate, resulting in a final inoculum of 5 x10^6 CFU/mL. The plates were incubated at 37 ^{0}C for 24 h and were read visually. The minimum concentration of the sample showing no turbidity was recorded as MIC. The MBC was also determined from the same microtitre plates after 24 h incubation. 20 ml of the suspension from the well showing MIC value and wells containing 2x, 4x, 8x and 16x concentration of MIC value was spotted onto the Muller Hinton Agar plate. The spotted plate was incubated for 24 h and the CFU count was taken simultaneously. The minimum concentration of the compound showing 3 log reductions in the inoculum size as compared to the original inoculum size was considered as the MBC.

RESULTS AND DISCUSSION

The required precursor 2-amino-4-methyl-4,5,6,7-tetrahydro-1-benzothiophene-3-carbonitrile (**1)**for the synthesis of thienopyrimidines and triazolothienopyrimidines was prepared by the reaction of 2-methylcyclohexanone under conditions reported in literature[16-17] Formation of **1** was characterized by the presence of band at 2210 cm^{-1} due to cyano group and N-H stretching bands at 3338 and 3192 cm^{-1}. ^{1}H NMR displayed D_2O exchangeable broad singlet at δ 6.9 due to NH_2 group. Imidoformates (**2a, 2b)** were prepared by treating **(1)** with triethylorthoformate and triethylorthoacetate in refluxing temperature. The structure of **(2a)** was assigned by the absence of ν_{N-H} in IR and the presence of a triplet at δ 1.31 and a quartet at 3.99 corresponding to protons of the ethoxy group and peak around δ 7.93 due to N=CH in the ^{1}HNMR spectrum, along with the other expected signals. The structure of **2b** was assigned by the absence of ν_{N-H} in IR and ^{1}HNMR spectrum showed triplet at δ 1.31 and a quartet at 3.9 corresponding to protons of the ethoxy group and peak around δ 1.2 due to N=C-CH_3.Reactions of imidoformates **(2a, 2b)** with hydrazine hydrate afforded the thienopyrimidines**(3a, 3b)**. Formation of the products is confirmed by the ^{1}HNMR spectrum which showed D_2O exchangeable singlet at δ 7.04, 5.4 for 8a and δ 6.6, 5.06(**3a**) due to amino and imino groups respectively and the C2-H of pyrimidine resonated at δ 7.8 (**3b**) as singlet along with other expected signals.

Similarly the reaction of imidoformates **(2a)** with ethanolic ammonia followed by the cyclisation of intermediate with sodium ethoxide in dimethyl formamide resulted in the formation of aminothienopyrimidine **(3d)**. Formation of the product was established by the presence of characteristic band due to amino group in IR. The ^{1}HNMR spectrum showed the presence of D_2O

exchangeable broad singlet at δ 6.74 due to NH_2 group and the C2-H of pyrimidine at δ 8.2 as a singlet. The compound **(3c)** was prepared by refluxing 2-amino-3-cyanothiophenes **(1)** with formic acid.

The compounds **(3a, 3b)** were further converted into triazolothienopyrimidine derivatives (**4a-c, 5a-c)** by treatment with triethylorthoesters in dimethylformamide. The formation of triazole ring involving both amino and imino groups was evident by the absence of absorption bands due to either of these groups. Further ^{1}HNMR spectrum also exhibited the presence of two characteristic protons each as singlet at δ 8.4 and δ 9 due to pyrimidine and triazole protons respectively.

Analytical data:

2-amino-4-methyl-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile(1)

IR (KBr) *v* cm^{-1}:2210,3338,3192 ^{1}H NMR (300MHz,$CDCl_3$) *δ*: 1.16 (*d*, 3H, CH_3), 2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2);),6.9(*s*,NH_2) ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7, 18.9, 19.5, 37.2, 39.3, 41.0, 59.5, 112.8, 115.9, 133.4, 136.0, 144.2, 163.9. m/z: 192.07

Ethyl N-(3-cyano-4-methyl-4, 5, 6, 7-tetrahydrobenzo[b]thiophen-2-yl) formimidate (2a)

Yield (83%); IR (KBr) *v* cm^{-1}:3090, 2921, 2860, 2212, 1573, 1541.^{1}H NMR (300MHz,$CDCl_3$): 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), 7.93 (*s*, 1H, N=CH); 3.99 (*q*, *J* = 7.08Hz, 2H, CH_2CH_3, 1.31(*t*, *J* = 7.1Hz, 3H, CH_2CH_3), ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7, 18.9, 19.5, 37.2, 39.3, 41.0, 59.5, 112.8, 115.9, 133.4, 136.0, 144.2, 163.9. Mass m/z: 248.10

Ethyl N-(3-cyano-4-methyl-4, 5, 6, 7-tetrahydrobenzo[b]thiophen-2-yl) acetimidate (2b)

Yield (80%); IR (KBr) *v* cm^{-1}:3090, 2921, 2866, 2215, 15 ^{1}H NMR (300MHz,$CDCl_3$): 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2),); 3.9 (*q*, *J* = 7.08Hz, 2H, CH_2CH_3, 1.31(*t*, *J* = 7.1Hz, 3H, CH_2CH_3),1.2(s,3H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7,16.2, 18.9, 19.5, 37.2, 39.3, 41.0, 59.5, 112.8, 115.9, 133.4, 136.0, 144.2, 163.9Massm/z: 262.11

2-amino-4-methyl-N-vinyl-4,5,6,7-tetrahydrobenzo[b]thiophcarboximidhydrazide (3a)

Yield 1g (77%); IR (KBr)ν cm^{-1} 3382, 3261, 3175, 3036, 2920, 1614, 1548; ^{1}H NMR (300MHz, d_6) NMR (300MHz,$CDCl_3$): 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), , 5.4 (*br s*, 2H, NH_2, D_2O exchangeable), 7.04 (*br s*, 1H, NH, D_2O exchangeable), 7.8 (*s*, 1H, C_2-H, pyrimidine); ^{13}C NMR (75 MHz, DMSO-*d6*) δ 18.2, 20.1, 32.5, 38.0, 43.9, 119.8, 128.2, 135.0, 142.4, 162.5, 163.9 m/z: 250.13

2-amino-4-methyl-N-(prop-1-en-2-yl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboximidhydrazide (3b)

Yield (77%); IR (KBr) ν cm^{-1} 3382, 3271, 3180, 3037, 2929, 1614, 1548; ^{1}H NMR (300MHz, DMSO-d_6) NMR (300MHz,$CDCl_3$): 1.12 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), , 5.06 (*br s*, 2H, NH_2, D_2O exchangeable), 6.6 (*br s*, 1H, NH, D_2O exchangeable), 3.02 (*s*,3H); ^{13}C NMR (75 MHz, DMSO-*d6*)17.8, δ 18.2, 20.1, 32.5, 38.0, 43.9, 119.8, 128.2, 135.0, 142.4, 162.5, 163.9m/z: 264.14

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]pyrimidin-4(3H)-one (3c)

Yield (80%); IR (KBr) ν cm^{-1} 3158, 3070, 1664, 1580, 1364, 974; , NMR (300MHz,$CDCl_3$): 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2) 7.7 (*s*, 1H, C_2-H, pyrimidine), 11.9 (*br s*, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.4, 18.1, 31.9, 38.2, 42.1, 130.9, 1 (75 MHz, $CDCl_3$) δ 16.4, 18.1, 31.9, 38.2, 42.1, 130.9, 136.0, 142.5, 143.1, 161.9, 169.9; m/z: 236.10

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]pyrimidin-4-amine (3d)

Yield (80%); IR (KBr) ν cm^{-1} 3360, 3313, 3113, 1645, 1571; ^{1}H NMR (300MHz, $CDCl_3$) 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2) , 6.74 (*br s*, 2H, NH_2, D_2O exchangeable), 8.2 (*s*, 1H, C2-H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 20.9, 30.5, 38.1, 44.1, 121.2, 129.2, 133.3, 142.0, 157.4, 167.7; m/z: 235.11

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d][1,2,4]triazolo[1,5-f]pyrimidine (4a)

Yield (70%);IR (KBr) ν cm^{-1} 3076, 2949, 2921, 1620, 1485; ^{1}H NMR (300MHz, $CDCl_3$) 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), 8.4 (*s*, 1H, C_2-H, pyrimidine), 9.1 (*s*, 1H, triazole); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.1, 19.8, 22.2, 35.6, 41.9, 122.4, 129.3, 133.6, 142.7, 145.9, 149.1, 157.2; m/z: 244.08

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]-2-methyl[1,2,4]triazolo[1,5-f]pyrimidine (4b)

Yield (66%); IR (KBr) ν cm^{-1} 3044, 2953, 2927, 1616, 1555; ^{1}H NMR (300MHz, $CDCl_3$) δ1.16 (*d*, 3H, CH_3), 2.6(*m*,1H,CH), 2.38 (*t*, 2HCH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), 3.10 (*s*, 3H, CH_3), 9.2 (*s*, 1H, C2-H, pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 19.9, 22.4, 34.5, 38.0, 43.1, 121.1, 127.6, 133.0, 139.9, 148.0, 156.7, 159.0m/z: 258.09

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]-2-ethyl[1,2,4]triazolo[1,5-f]pyrimidine (4c)

Yield (76%) IR (KBr) ν cm^{-1} 3051, 2960, 2911, 1610, 1573; ^{1}H NMR (300MHz, $CDCl_3$) NMR (300MHz,$CDCl_3$): 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), 1.44 (*t*, *J*=6.6Hz, 3H, CH_2CH_3), 4.48 (*q*, *J*=7.02Hz, 2H, CH_2CH_3), 8.90 (*s*, 1H, C_2-H,

pyrimidine); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.9, 19.3, 22.6, 24.3, 33.1, 36.5, 41.9, 122.0, 127.3, 135.1, 140.6, 145.9, 157.2, 160.0 m/z: 272.11.

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]-5-methyl[1,2,4]triazolo[1,5-f]pyrimidine (5a)

Yield (66%); IR (KBr) ν cm^{-1} 3076, 2949, 2921, 1620, 1485; ^{1}H NMR (300MHz, $CDCl_3$), 3.02(*s*, 3H, C_2-H,), 8.3 (*s*, 1H, triazole); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.1, 19.8, 22.2, 35.6, 41.9, 12 NMR (300MHz,$CDCl_3$): 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), 2.4, 129.3, 133.6, 142.7, 145.9, 149.1, 157.2; ^{13}C NMR (75 MHz, $CDCl_3$) δ17.8, 18.1, 19.8, 22.2, 35.6, 41.9, 122.4, 129.3, 133.6, 142.7, 145.9, 149.1, 157.2 m/z: 258.09

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]-2,5-dimethyl[1,2,4]triazolo[1,5-f]pyrimidine (5b)

Yield (70%); IR (KBr) ν cm^{-1} 3044, 2953, 2927, 1616, 1555; ^{1}H NMR (300MHz, $CDCl_3$) δ1.16 (*d*, 3H, CH_3), 2.6(*m*,1H,CH), 2.38 (*t*, 2HCH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2**)**, 2.92 (*s*, 3H, CH_3), 3.10 (*s*, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4,17.8, 19.9, 22.4, 34.5, 38.0, 43.1, 121.1, 127.6, 133.0, 139.9, 148.0, 156.7, 159m/z: 272.11

4,5,6,7-tetrahydro-5-methylbenzothieno[2,3-d]-2-ethyl-5-methyl[1,2,4]triazolo[1,5-f]pyrimidine(5c)

Yield (74%); IR (KBr) ν cm^{-1} 3051, 2967, 2911, 1616, 1573; ^{1}H NMR (300MHz, $CDCl_3$) NMR (300MHz,$CDCl_3$): 1.16 (*d*, 3H**,** CH_3),2.6(*m*,1H,CH), 2.38 (*t*, 2H,CH_2), 1.74 (*m*, 2H, CH_2),1.5 (*q*, 2H,CH_2), 1.44 (*t*, *J*=6.6Hz, 3H, CH_2CH_3), 4.48 (*q*, *J*=7.02Hz, 2H, CH_2CH_3), 2.9 (*s*, 3H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.9,17.8, 19.3, 22.6, 24.3, 33.1, 36.5, 41.9, 122.0, 127.3, 135.1, 140.6, 145.9, 157.2, 160.0; m/z: 286.13

Anti-tubercular activity

Compounds, **3a-d, 4a-c and 5a-c** were screened for anti-tubercular activityagainst MTB H_{37}Rv strain. These novel compounds exhibited MIC values in the range of 20 - 100µM. Compounds **4c** and **5c** were the most active compounds with MIC 30.1 and 20.0µM respectively(Table 1). The SAR study revealed that when 'R' is ethyl (**5c,** MIC 20.0µM) the activity was enhanced by two fold as compared to **4a**. The enhanced activity of **5c** might be attributed to the liphophillicity of the alkyl chain. Also, immediate compounds **3a-d**showed moderate activity. These encouraging results further pave the way to explore different substituents on the triazolopyrimidine moiety. All these compounds are less active compare to standard Rifampicin.

Table 1: Anti-tubercular activity

Compounds	MIC(µg/mL)
3a	65.17
3b	60.38
3c	100.06
3d	80.33
4a	40.54
4b	34.19
4c	31.03
5a	28.32
5b	25.76
5c	**20.03**
Rifampicin	0.14

The MIC values were evaluated at concentration range, 0.1-100µg/ml.

Anti-bacterial activity

The title compounds exhibited good antibacterial activityagainst S. aureus ATCC 29213 (grampositive) and E. coli ATCC 25922(gram negative) bacteria. The MIC for these compounds wasobserved in the range of 5 - 50µM. When 'R' is proton (**4a**, MIC 30µM) the compound showed moderate activityagainst gram positive and gram negative bacteria. Whereas when R is methyl group, the activity was enhanced by two fold (**5a**, MIC 15µM). Inclusion of ethyl group further enhanced the activity by three fold (**5c**, MIC 5µM).The enhanced activity of **5c** might be attributed to the liphophillicity of the alkyl chain. Compound **5c** (R = methyland R_1 = ethyl) was found to be the mostactive with MIC 5 and 8µM respectively towards S. aureusATCC 29213 and E. coli ATCC 25922 bacteria. (Table 2). However, in general the titlecompounds displayed good activity against S. aureus ATCC 29213 ascompared to E. coli ATCC 25922 strain. All these derivatives wereless active compared to the standard ciprofloxacin.

Table 2: Antibacterial activity as MIC values (µ/mL)

Compounds	***S. aureus ATCC 29213***	***E. coli ATCC 25922***
3a	42.39	45.24
3b	40.41	44.75
3c	48.92	50.13
3d	45.33	46.19
4a	30.56	35.76
4b	28.88	31.23
4c	25.82	29.34
5a	15.37	19.11
5b	12.51	16.10
5c	**5.10**	**8.56**
Ciprofloxacin	0.37	0.021

CONCLUSION

In conclusion, we have carried out the synthesis and *in vitro* antimycobacterial as well as antibacterial activity of the novel thienopyrimidine and triazolothienopyrimidine derivatives. Amongst, the synthesized compounds, **5c** exhibited excellent activity towards MTB H37Rv strain with MIC 20µM. The intermediate compounds **3a-d**showed moderate activity. In general, antibacterial activity was found to be more prominent than anti-tubercular activity. Particularly, compound **5c** inhibit 99% of S. aureus ATCC 29213 with MIC5µM and E. coli ATCC 25922 was inhibited at MIC 8µM. The SAR profile suggests that tailoring alkyl chains into the thienopyrimidine and triazolopyrimidines provide valuable inputs for further lead molecule development towards inhibiting existing drug resistant forms of bacterial pathogens.

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