Design and synthesis of substituted clubbed triazolyl thiazole as XDR & MDR antituberculosis agents Part-II

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ABSTRACT

Medicinal chemist are striving to arrive at a promising anti-tubercular agents which can be effective against XDR & MDR tuberculosis. This has made them to work non-conventionally to arrive at promising "Hit". This article describes synthesis and antimycobacterial activity of clubbed thiazolyl-triazole molecule which are synthesised overriding the Lipinski rule of five. The compounds have shown potency comparable with isoniazid even with 25 XDR & MDR clinical isolates. The candidate is under further development status and is showing promising activity with minimal toxicity.

Key words: XDR, MDR, tuberculosis, triazole, thiazole.

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INTRODUCTION

Almost 2 millions of lives annually are claimed by *Mycobacterium tuberculosis* (MTB), the etiological agent of tuberculosis (TB), and is still on the rise. The use of two antimicrobials, pyrazinamide & rifampicin in the 1960s have revolutionised the antimycobacterial therapy. And now in combination with isoniazid, ethambutol and/or streptomycin, this therapy represents a landmark in the treatment of human tuberculosis and resulted in the implementation of short-course chemotherapy (SCC), reducing the time of treatment from 18 to 6 months [1-3]. SCC contributed towards controlling tuberculosis burden for the next 20 years. Shockingly, tuberculosis cases started to rise again in the 1990s under the pressure of the HIV pandemic and the emergence of multidrug-resistant (MDR) and extremely drug-resistant (XDR) tuberculosis strains. MDR strains are resistant to at least isoniazid (INH) and rifampicin (RIF), whereas XDR strains are MDR isolates that are additionally resistant to fluoroquinolones and to one of the three injectable drugs capreomycin, amikacin and kanamycin. The emergence and dissemination of MDR and XDR isolates, estimated to account for more than 400,000 new cases per year, impart new challenges in tuberculosis control [4]. Indeed, current treatment of drug-resistant tuberculosis requires 18–36 months and is associated with an unacceptable rate of treatment failure and relapse. Consequently, developing new compounds active against MDR and XDR tuberculosis constitutes a main objective in anti-tuberculosis drug discovery. In addition, new antimycobacterial agents should ideally contribute to shorten tuberculosis treatment to 2 months

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or less [5, 6]. Few promising drug candidates fulfilling these criteria have been discovered in recent years [7-9]. Nonetheless, given the number of tuberculosis cases and the rate of emergence of drug resistance, more compounds are clearly needed to combat and have a significant impact on the control and spread of tuberculosis. Thus as a part of our in-house antimycobacterial program, we herein report the synthesis and activity of new "Hit" molecule of our lab.

MATERIALS AND METHODS

Chemistry

^{1}H NMR spectra were recorded on a Bruker Avance 300 MHz instrument using TMS as internal standard; the chemical shifts (δ) are reported in ppm and coupling constants (*J*) are given in Hertz. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet), and bs (broad singlet). Mass spectra were recorded on a Finnigan LCQ mass spectrometer. Microwave irradiation was carried out in Raga Scientific Microwave Systems, Model RG31L at 2450 MHz. Elemental analysis was performed on a Heracus CHN-Rapid Analyser. Analysis indicated by the symbols of the elements of functions was within ± 0.4% of the theoretical values. The purity of the compounds was checked on silica gel coated Al plates (Merck).

*4.1.1.Preparation   of   N-(5-(4-((4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **9**.*
Above titled compound was prepared as per the reported method of chemo-transformation. [10-12].

*4.1.2. General preparation of N-(5-(4-((4-Substituted-amido-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **10**.*

The triazole **9** (1 mmol) was dissolved in 20 ml of 10% sodium hydroxide and then drop wise equimolar amount of the 4-chlorobenzoyl chloride was added to the reaction mixture at 0 °C. The reaction mass was then stirred for 30-45 min. At the end of stirring, precipitate was observed. It was then filtered, washed thoroughly with water and crystallized to furnish **10**.

*4.1.2.1. N-(5-(4-((4-Acetamido-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide (**10a**).*
Yield 78 %; mp 280 –285 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.04 (s, 6H, C*H*$_3$), 2.30 (s, 3H, C*H*$_3$), 3.16 (s, 1H, S*H*), 3.81 (s, 2H, C*H*$_2$), 9.06 (s, 2H, pyrimidine-Ar*H*), 9.17 (bs, 2H, N*H*); MS m/z (%) 405 (M+, 100); Anal. Calcd. for C$_{15}$H$_{16}$N$_8$O$_2$S$_2$ (404.47): C, 44.54; H, 3.99; N, 27.70; O, 7.91; S, 15.86. Found: C, 44.54; H, 3.99; N, 27.70; O, 7.91; S, 15.86.

*4.1.2.2. N-(5-(4-((4-Benzamido-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **10b**.*
Yield 77 %; mp 261 –266 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.03 (s, 3H, C*H*$_3$), 2.31 (s, 3H, C*H*$_3$), 3.18 (s, 1H, S*H*), 3.80 (s, 2H, C*H*$_2$), 7.63-8.04 (m, 5H, Ar*H*), 9.08 (s, 2H, pyrimidine-Ar*H*), 9.10 (bs, 2H, N*H*); MS m/z (%) 468 (M+, 100); Anal. Calcd. for C$_{20}$H$_{18}$N$_8$O$_2$S$_2$ (466.54): C, 51.49; H, 3.89; N, 24.02; O, 6.86; S, 13.75. Found: C, 51.49; H, 3.89; N, 24.02; O, 6.86; S, 13.75.

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*4.1.2.3. N-(5-(4-((4-(2-Chloroacetamido)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)
pyrimidin-2-yl)acetamide **10c.***

Yield 71 %; mp 251 –256 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.02 (s, 3H, C*H*$_3$), 2.35 (s, 3H,
C*H*$_3$), 3.13 (s, 1H, S*H*), 3.82 (s, 2H, C*H*$_2$), 4.27 (s, 2H, -C*H*$_2$Cl), 9.02 (s, 2H, pyrimidine-Ar*H*),
9.11 (bs, 2H, N*H*); MS m/z (%) 440 (M+, 100); Anal. Calcd. for C$_{15}$H$_{15}$ClN$_8$O$_2$S$_2$ (438.92): C,
41.05; H, 3.44; Cl, 8.08; N, 25.53; O, 7.29; S, 14.61. Found: C, 41.05; H, 3.44; Cl, 8.08; N,
25.53; O, 7.29; S, 14.61.

4.1.3. General preparation of 11.

The triazole **10** (1 mmol), diiodomethane (1.5 mmol) and potassium hydroxide (1 mmol) were
dissolved in 20 ml of dichloromethane. To the said mixture acidic alumina (20 g) was added.
Dichloromethane was evaporated *invacuos*, and mixture was kept inside the alumina bath and
irradiated for 5-6 min at the power level of 300W. The mixture was cooled. The solid thus
separated was recrystallised in hot ethanol.

*4.1.3.1.   N,N'-(5,5'-(4,4'-(5,5'-Methylenebis(sulfanediyl)bis(4-acetamido-4H-1,2,4-triazole-5,3-diyl))   bis
(methylene)bis(5-methylthiazole-4,2-diyl))bis(pyrimidine-5,2-diyl))diacetamide **11a.***

Yield 80%; mp 242 –246 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (s, 12H, C*H*$_3$), 2.31 (s, 6H,
C*H*$_3$), 3.87 (s, 4H, C*H*$_2$), 4.52 (s, 2H, S-C*H*$_2$-S), 9.02 (s, 4H, pyrimidine-Ar*H*), 9.13 (bs, 4H,
N*H*); MS m/z (%) 822 (M+, 100); Anal. Calcd. for C$_{31}$H$_{32}$N$_{16}$O$_4$S$_4$ (820.95): C, 45.35; H, 3.93;
N, 27.30; O, 7.80; S, 15.62. Found: C, 45.35; H, 3.93; N, 27.30; O, 7.80; S, 15.62.

*4.1.3.2.   N,N'-(5,5'-Methylenebis(sulfanediyl)bis(3-((2-(2-benzamidopyrimidin-5-yl)-5-methylthiazol-4-yl)
methyl)-4H-1,2,4-triazole-5,4-diyl))diacetamide **11b.***

Yield 70 %; mp 232 –236 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.06 (s, 6H, C*H*$_3$), 2.37 (s, 6H,
C*H*$_3$), 3.84 (s, 4H, C*H*$_2$), 4.50 (s, 2H, S-C*H*$_2$-S), 7.61-8.07 (m, 10H, Ar*H*), 9.09 (s, 4H,
pyrimidine-Ar*H*), 9.19 (bs, 4H, N*H*); MS m/z (%) 946 (M+, 100); Anal. Calcd. for
C$_{41}$H$_{36}$N$_{16}$O$_4$S$_4$ (945.09): C, 52.10; H, 3.84; N, 23.71; O, 6.77; S, 13.57. Found: C, 52.10; H,
3.84; N, 23.71; O, 6.77; S, 13.57.

*4.1.3.3.   N,N'-(5,5'-(4,4'-(5,5'-Methylenebis(sulfanediyl)bis(4-(2-chloroacetamido)-4H-1,2,4-triazole-5,3-
diyl))bis(methylene)bis(5-methylthiazole-4,2-diyl))bis(pyrimidine-5,2-diyl))diacetamide **11c.***

Yield 65 %; mp 221 –226°C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (s, 6H, C*H*$_3$), 2.33 (s, 6H,
C*H*$_3$), 3.83 (s, 4H, C*H*$_2$), 4.25 (s, 4H, -C*H*$_2$Cl), 4.54 (s, 2H, S-C*H*$_2$-S), 9.03 (s, 4H, pyrimidine-
Ar*H*), 9.18 (bs, 4H, N*H*); MS m/z (%) 891 (M+, 100); Anal. Calcd. for C$_{31}$H$_{30}$Cl$_2$N$_{16}$O$_4$S$_4$
(889.84): C, 41.84; H, 3.40; Cl, 7.97; N, 25.19; O, 7.19; S, 14.41. Found: C, 41.84; H, 3.40; Cl,
7.97; N, 25.19; O, 7.19; S, 14.41.

*4.1.4.   General   preparation   of   N-(5-(4-((4-Substituted-amido-5-(cyanomethylthio)-4H-1,2,4-triazol-3-
yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **12.***

The triazole **10** (1 mmol) was mixed with 1.2 ml (2 mmol) of chloroacetonitrile and dissolved in
25 ml of water:ethanol (95:05). Neutralization with sodium carbonate gave a precipitate, which
was filtered, washed with cold water (2 x 20 ml), and column purified.

*4.1.4.1.   N-(5-(4-((4-Acetamido-5-(cyanomethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)
pyrimidin-2-yl)acetamide **12a.***

Yield 66 %; mp 236 –241 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.09 (s, 6H, C*H*$_3$), 2.35 (s, 3H,
C*H*$_3$), 3.86 (s, 2H, C*H*$_2$), 4.41 (s, 2H, S-C*H*$_2$), 9.08 (s, 2H, pyrimidine-Ar*H*), 9.13 (bs, 2H, N*H*);
MS m/z (%) 445 (M+, 100); Anal. Calcd. for C$_{17}$H$_{17}$N$_9$O$_2$S$_2$ (443.51): C, 46.04; H, 3.86; N,
28.42; O, 7.21; S, 14.46. Found: C, 46.04; H, 3.86; N, 28.42; O, 7.21; S, 14.46.

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4.1.4.2.   N-(5-(4-((4-Benzamido-5-(cyanomethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)
pyrimidin-2-yl)acetamide **12b.**

Yield 68 %; mp 225 –230 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.05 (s, 3H, C*H*$_3$), 2.34 (s, 3H,
C*H*$_3$), 3.88 (s, 2H, C*H*$_2$), 4.43 (s, 2H, S-C*H*$_2$), 7.66-8.07 (m, 5H, Ar*H*), 9.02 (s, 2H, pyrimidine-
Ar*H*), 9.10 (bs, 2H, N*H*); MS m/z (%) 507 (M+, 100); Anal. Calcd. for C$_{22}$H$_{19}$N$_9$O$_2$S$_2$ (505.58):
C, 52.26; H, 3.79; N, 24.93; O, 6.33; S, 12.68. Found: C, 52.26; H, 3.79; N, 24.93; O, 6.33; S,
12.68.

4.1.4.3.   N-(5-(4-((4-(2-Chloro   acetamido)-5-(cyanomethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methyl
thiazol-2-yl)pyrimidin-2-yl)acetamide **12c.**

Yield 65 %; mp 228 –232 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.00 (s, 3H, C*H*$_3$), 2.38 (s, 3H,
C*H*$_3$), 3.87 (s, 2H, C*H*$_2$), 4.24 (s, 2H, -C*H*$_2$Cl), 4.48 (s, 2H, S-C*H*$_2$), 9.08 (s, 2H, pyrimidine-
Ar*H*), 9.13 (bs, 2H, N*H*); MS m/z (%) 479 (M+, 100); Anal. Calcd. for C$_{17}$H$_{16}$ClN$_9$O$_2$S$_2$
(477.95): C, 42.72; H, 3.37; Cl, 7.42; N, 26.38; O, 6.69; S, 13.42. Found: C, 42.72; H, 3.37; Cl,
7.42; N, 26.38; O, 6.69; S, 13.42.

4.1.5. General preparation of N-(5-(4-((4-Substituted-amido-5-(2-hydrazinyl-2-oxoethylthio)-4H-1,2,4-
triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **14.**

A solution of triazole **10** (1 mmol), sodium hydroxide (1 mmol) and methyl bromoacetate (1
mmol) was prepared in dichloromethane. To this, acidic alumina was added in 1:5 equivalent of
triazole. The reaction mixture was mixed, DCM was evaporated and mixture was kept inside the
alumina bath and irradiated for 4-5 min at the power level of 300W. The mixture was cooled and
poured on ice. The solid thus separated was extracted with hot ethanol, filtered. After cooling,
filtrate gave almost pure product **13**.

A solution of compound **13** (1 mmol) with 5 ml (1 mmol) hydrazine hydrate (98%) was prepared
in 10 ml ethanol. To this acidic alumina (10 g) was added. Ethanol then was evaporated *invacuo*,
and mixture was kept inside the alumina bath and irradiated for 5-6 min at the power level of
300W. The mixture was cooled and the product was extracted with ether. Ether was distilled off
and product thus obtained was crystallized to give pure **14**.

4.1.5.1.   N-(5-(4-((4-Acetamido-5-(2-hydrazinyl-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthi
azol-2-yl)pyrimidin-2-yl)acetamide **14a.**

Yield 73 %; mp 238 –243 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.06 (s, 6H, C*H*$_3$), 2.37 (s, 3H,
C*H*$_3$), 3.85 (s, 2H, C*H*$_2$), 4.06 (s, 2H, C*H*$_2$), 4.97 (d, 2H, N*H*$_2$), 9.04 (s, 2H, pyrimidine-Ar*H*),
9.13 (b, 3H, N*H*); MS m/z (%) 478 (M+, 100); Anal. Calcd. for C$_{17}$H$_{20}$N$_{10}$O$_3$S$_2$ (476.54): C,
42.85; H, 4.23; N, 29.39; O, 10.07; S, 13.46. Found: C, 42.85; H, 4.23; N, 29.39; O, 10.07; S,
13.46.

4.1.5.2. N-(5-(4-((4-Benzamido-5-(2-hydrazinyl-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthi
azol-2-yl)pyrimidin-2-yl)acetamide **14b.**

Yield 70 %; mp 231 –236 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.09 (s, 3H, C*H*$_3$), 2.37 (s, 3H,
C*H*$_3$), 3.85 (s, 2H, C*H*$_2$), 4.03 (s, 2H, C*H*$_2$), 4.92 (d, 2H, N*H*$_2$), 7.61-8.04 (m, 5H, Ar*H*), 9.08 (s,
2H, pyrimidine-Ar*H*), 9.16 (b, 3H, N*H*); MS m/z (%) 540 (M+, 100); Anal. Calcd. for
C$_{22}$H$_{22}$N$_{10}$O$_3$S$_2$ (538.61): C, 49.06; H, 4.12; N, 26.01; O, 8.91; S, 11.91. Found: C, 49.06; H,
4.12; N, 26.01; O, 8.91; S, 11.91.

4.1.5.3. N-(5-(4-((4-(2-Chloroacetamido)-5-(2-hydrazinyl-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5
-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **14c.**

Yield 72 %; mp 240–245 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (s, 3H, C*H*$_3$), 2.33 (s, 3H,
C*H*$_3$), 3.85 (s, 2H, C*H*$_2$), 4.07 (s, 2H, C*H*$_2$), 4.29 (s, 2H, -C*H*$_2$Cl), 4.90 (d, 2H, N*H*$_2$), 9.02 (s, 2H,

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pyrimidine-Ar*H*), 9.14 (b, 3H, N*H*); MS m/z (%) 512 (M+, 100); Anal. Calcd. for C$_{17}$H$_{19}$ClN$_{10}$O$_3$S$_2$ (510.98): C, 39.96; H, 3.75; Cl, 6.94; N, 27.41; O, 9.39; S, 12.55. Found: C, 39.96; H, 3.75; Cl, 6.94; N, 27.41; O, 9.39; S, 12.55.

4.1.6. General preparation of N-(5-(4-((4-Substituted-amido-5-(2-(2-acylhydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15.

To a solution of compound **14** (1 mmol) in dry dichloromethane (excess amount), appropriate acid chloride (1 mmol) was added drop-wise with constant vigorous stirring. After 25 min of stirring, acidic alumina (10g) was added. Dichloromethane then was evaporated invacuo, and mixture was kept inside the alumina bath and irradiated for 5-6 min at the power level of 300W. The mixture was cooled and the product was extracted with ether. Ether was distilled off and product thus obtained was crystallized from n-hexane-carbon tetrachloride mixture. Note: After the reaction is over, keep the microwave door open for at least 1h before initiating another reaction in order to avoid side reactions with the fumes of other acid chloride.

4.1.6.1. N-(5-(4-((4-Acetamido-5-(2-(2-acetylhydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15a.

Yield 80 %; mp 251 –256 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.05 (s, 9H, C*H*$_3$), 2.37 (s, 3H, C*H*$_3$), 3.89 (s, 2H, C*H*$_2$), 4.00 (s, 2H, S-C*H*$_2$), 6.21-6.23 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, N*H*), 8.46-8.58 (b, 2H, N*H*), 9.02 (s, 2H, pyrimidine-Ar*H*); MS m/z (%) 520 (M+, 100); Anal. Calcd. for C$_{19}$H$_{22}$N$_{10}$O$_4$S$_2$ (518.57): C, 44.01; H, 4.28; N, 27.01; O, 12.34; S, 12.37. Found: C, 44.01; H, 4.28; N, 27.01; O, 12.34; S, 12.37.

4.1.6.2. N-(5-(4-((4-Acetamido-5-(2-(2-benzoylhydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15b.

Yield 68 %; mp 241–246 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.07 (s, 6H, C*H*$_3$), 2.39 (s, 3H, C*H*$_3$), 3.81 (s, 2H, C*H*$_2$), 4.03 (s, 2H, S-C*H*$_2$), 6.25-6.28 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, N*H*), 7.60-8.02 (m, 5H, Ar*H*), 8.44-8.56 (b, 2H, N*H*), 9.08 (s, 2H, pyrimidine-Ar*H*); MS m/z (%) 582 (M+, 100); Anal. Calcd. for C$_{24}$H$_{24}$N$_{10}$O$_4$S$_2$ (580.64): C, 49.64; H, 4.17; N, 24.12; O, 11.02; S, 11.04. Found: C, 49.64; H, 4.17; N, 24.12; O, 11.02; S, 11.04.

4.1.6.3. N-(5-(4-((4-Acetamido-5-(2-(2-(2-chloroacetyl)hydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15c.

Yield 80 %; mp 231–236 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.02 (s, 6H, C*H*$_3$), 2.34 (s, 3H, C*H*$_3$), 3.86 (s, 2H, C*H*$_2$), 4.08 (s, 2H, S-C*H*$_2$), 4.20 (s, 2H, -C*H*$_2$Cl), 6.21-6.23 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, N*H*), 8.45-8.57 (b, 2H, N*H*), 9.09 (s, 2H, pyrimidine-Ar*H*); MS m/z (%) 554 (M+, 100); Anal. Calcd. for C$_{19}$H$_{21}$ClN$_{10}$O$_4$S$_2$ (553.02): C, 41.27; H, 3.83; Cl, 6.41; N, 25.33; O, 11.57; S, 11.60. Found: C, 41.27; H, 3.83; Cl, 6.41; N, 25.33; O, 11.57; S, 11.60.

4.1.6.4. N-(5-(4-((5-(2-(2-Acetylhydrazinyl)-2-oxoethylthio)-4-benzamido-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15d.

Yield 75 %; mp 238–243 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.04 (s, 6H, C*H*$_3$), 2.38 (s, 3H, C*H*$_3$), 3.82 (s, 2H, C*H*$_2$), 4.06 (s, 2H, S-C*H*$_2$), 6.23-6.26 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, N*H*), 7.69-8.02 (m, 5H, Ar*H*), 8.45-8.58 (b, 2H, N*H*), 9.01 (s, 2H, pyrimidine-Ar*H*); MS m/z (%) 582 (M+, 100); Anal. Calcd. for C$_{24}$H$_{24}$N$_{10}$O$_4$S$_2$ (580.64): C, 49.64; H, 4.17; N, 24.12; O, 11.02; S, 11.04. Found: C, 49.64; H, 4.17; N, 24.12; O, 11.02; S, 11.04.

4.1.6.5. N-(5-(4-((4-Benzamido-5-(2-(2-benzoylhydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15e.

Yield 65 %; mp 216–221 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.06 (s, 3H, C*H*$_3$), 2.39 (s, 3H, C*H*$_3$), 3.82 (s, 2H, C*H*$_2$), 4.05 (s, 2H, S-C*H*$_2$), 6.28-6.21 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz,

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NH), 7.64-8.07 (m, 10H, ArH), 8.40-8.53 (b, 2H, NH), 9.06 (s, 2H, pyrimidine-ArH); MS m/z (%) 644 (M+, 100); Anal. Calcd. for $C_{29}H_{26}N_{10}O_4S_2$ (642.71): C, 54.19; H, 4.08; N, 21.79; O, 9.96; S, 9.98. Found: C, 54.19; H, 4.08; N, 21.79; O, 9.96; S, 9.98.

4.1.6.6. N-(5-(4-((4-Benz amido-5-(2-(2-(2-chloroacetyl) hydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15f.
Yield 77 %; mp 198–203 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.06 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 3.88 (s, 2H, CH_2), 4.04 (s, 2H, S-CH_2), 4.20 (s, 2H, -CH_2Cl), 6.26-6.22 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, NH), 7.68-8.04 (m, 5H, ArH), 8.40-8.57 (b, 2H, NH), 9.04 (s, 2H, pyrimidine-ArH); MS m/z (%) 616 (M+, 100); Anal. Calcd. for $C_{24}H_{23}ClN_{10}O_4S_2$ (615.09): C, 46.86; H, 3.77; Cl, 5.76; N, 22.77; O, 10.40; S, 10.43. Found: C, 46.86; H, 3.77; Cl, 5.76; N, 22.77; O, 10.40; S, 10.43.

4.1.6.7. N-(5-(4-((5-(2-(2-Acetylhydrazinyl)-2-oxoethylthio)-4-(2-chloroacetamido)-4H-1,2,4-triazol-3-yl) methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15g.
Yield 90 %; mp 146–150 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (s, 6H, CH_3), 2.38 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 4.02 (s, 2H, S-CH_2), 4.29 (s, 2H, -CH_2Cl), 7.66-8.03 (m, 5H, ArH), 6.20-6.28 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, NH), 8.46-8.54 (b, 2H, NH), 9.02 (s, 2H, pyrimidine-ArH); MS m/z (%) 554 (M+, 100); Anal. Calcd. for $C_{19}H_{21}ClN_{10}O_4S_2$ (553.02): C, 41.27; H, 3.83; Cl, 6.41; N, 25.33; O, 11.57; S, 11.60. Found: C, 41.27; H, 3.83; Cl, 6.41; N, 25.33; O, 11.57; S, 11.60.

4.1.6.8. N-(5-(4-((5-(2-(2-Benzoylhydrazinyl)-2-oxoethylthio)-4-(2-chloroacetamido)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15h.
Yield 60 %; mp 212–217 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.09 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 4.06 (s, 2H, S-CH_2), 4.25 (s, 2H, -CH_2Cl), 6.24-6.23 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, NH), 7.62-8.01 (m, 5H, ArH), 8.40-8.54 (b, 2H, NH), 9.02 (s, 2H, pyrimidine-ArH); MS m/z (%) 616 (M+,100); Anal. Calcd. for $C_{24}H_{23}ClN_{10}O_4S_2$ (615.09): C, 46.86; H, 3.77; Cl, 5.76; N, 22.77; O, 10.40; S, 10.43. Found: C, 46.86; H, 3.77; Cl, 5.76; N, 22.77; O, 10.40; S, 10.43.

4.1.6.9. N-(5-(4-((4-(2-Chloro acetamido)-5-(2-(2-(2-chloro acetyl)hydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 15i.
Yield 73 %; mp 225–229 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.06 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 4.03 (s, 2H, S-CH_2), 4.26 (s, 4H, -CH_2Cl), 6.25-6.28 (dd, 2H, J_{NH-NH}=4.28Hz, J_{NH-NH}=4.59Hz, NH), 8.47-8.59 (b, 2H, NH), 9.01 (s, 2H, pyrimidine-ArH); MS m/z (%) 588 (M+,100); Anal. Calcd. for $C_{19}H_{20}Cl_2N_{10}O_4S_2$ (587.46): C, 38.85; H, 3.43; Cl, 12.07; N, 23.84; O, 10.89; S, 10.92. Found: C, 38.85; H, 3.43; Cl, 12.07; N, 23.84; O, 10.89; S, 10.92.

4.1.7. General preparation of N-(5-(4-((4-Substituted-amido-5-(2-(2-benzylidenehydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide 16.
A solution of compound **14** (1mmol) with benzaldehyde (1 mmol) was prepared in 10 ml ethanol. To this acidic alumina (10 g) was added. Ethanol then was evaporated *in-vacuo*, and mixture was kept inside the alumina bath and irradiated for 1 min at the power level of 300W. The mixture was cooled and poured on ice. The solid thus separated was filtered and extracted with DCM. DCM was then evaporated and product thus obtained was crystallized from hot ethanol.

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4.1.7.1. N-(5-(4-((4-Acetamido-5-(2-(2-benzylidene hydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl) methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **(16a).**
Yield 80 %; mp 226–228 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (s, 6H, CH_3), 2.39 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 4.08 (s, 2H, S-CH_2), 7.50-8.07 (m, 5H, ArH), 8.33 (s, 1H, N=CH), 9.06 (s, 2H, pyrimidine-ArH), 9.14 (bs, 3H, NH); MS m/z (%) 566 (M+, 100); Anal. Calcd. for C$_{24}$H$_{24}$N$_{10}$O$_3$S$_2$ (564.64): C, 51.05; H, 4.28; N, 24.81; O, 8.50; S, 11.36. Found: C, 51.05; H, 4.28; N, 24.81; O, 8.50; S, 11.36.

4.1.7.2. N-(5-(4-((4-Benzamido-5-(2-(2-benzylidene hydrazinyl)-2-oxoethylthio)-4H-1,2,4-triazol-3-yl) methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **16b.**
Yield 70 %; mp 188 –193 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.09 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.88 (s, 2H, CH_2), 4.03 (s, 2H, S-CH_2), 7.57-8.02 (m, 10H, ArH), 8.36 (s, 1H, N=CH), 9.01 (s, 2H, pyrimidine-ArH), 9.15 (bs, 3H, NH); MS m/z (%) 628 (M+, 100); Anal. Calcd. for C$_{29}$H$_{26}$N$_{10}$O$_3$S$_2$ (626.71): C, 55.58; H, 4.18; N, 22.35; O, 7.66; S, 10.23. Found: C, 55.58; H, 4.18; N, 22.35; O, 7.66; S, 10.23.

4.1.7.3. N-(5-(4-((5-(2-(2-Benzylidenehydrazinyl)-2-oxoethylthio)-4-(2-chloroacetamido)-4H-1,2,4-triazol -3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **16c.**
Yield 88 %; mp 243–248 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.03 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 4.07 (s, 2H, S-CH_2), 4.24 (s, 2H, -CH_2Cl), 7.58-8.01 (m, 5H, ArH), 8.38 (s, 1H, N=CH), 9.00 (s, 2H, pyrimidine-ArH), 9.19 (bs, 3H, NH); MS m/z (%) 600 (M+, 100); Anal. Calcd. for C$_{24}$H$_{23}$ClN$_{10}$O$_3$S$_2$ (599.09): C, 48.12; H, 3.87; Cl, 5.92; N, 23.38; O, 8.01; S, 10.70. Found: C, 48.12; H, 3.87; Cl, 5.92; N, 23.38; O, 8.01; S, 10.70.

4.1.8. General preparation of N-(5-(4-((4-Substituted-amido-5-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **17**.
The compound **14** (1 mmol) was dissolved in alcoholic potassium hydroxide (1 mmol) and kept for stirring. Carbon disulphide (1.5 mmol) was added drop wise to this solution with stirring. Thick solid mass was obtained, to which 50 ml of absolute alcohol was added. Stirring was continued for 16 h. At the end of 16th h, dry ether was added to the mixture. The precipitate (thiocarbazate) obtained was taken immediately for the next step.

A solution of thiocarbazate (1 mmol) with hydrazine hydrate (1 mmol) was prepared in 10 ml ethanol. To this acidic alumina (10 g) was added. Ethanol then was evaporated invacuo, and mixture was kept inside the alumina bath and irradiated for 5-6 min at the power level of 300W. The mixture was cooled and poured on ice and pH was adjusted to 2-3 with (1:1) HCl:water. The solid thus separated was filtered, extracted with ether, ether was distilled off and product thus obtained was purified to offer **17**.

4.1.8.1. N-(5-(4-((4-Acetamido-5-((4-amino-5-mercapto-4H-1,2,4- triazol-3-yl) methylthio)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **17a.**
Yield 77 %; mp 205–210 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.04 (s, 6H, CH_3), 2.30 (s, 3H, CH_3), 3.13 (s, 1H, SH), 3.81 (s, 2H, CH_2), 4.01 (s, 2H, S-CH_2), 5.77 (s, 2H, NH_2), 9.06 (s, 2H, pyrimidine-ArH), 9.17 (bs, 3H, NH); MS m/z (%) 534 (M+, 100); Anal. Calcd. for C$_{18}$H$_{20}$N$_{12}$O$_2$S$_3$ (532.63): C, 40.59; H, 3.78; N, 31.56; O, 6.01; S, 18.06. Found: C, 40.59; H, 3.78; N, 31.56; O, 6.01; S, 18.06.

4.1.8.2. N-(5-(4-((5-((4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl thio)-4-benzamido-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **17b.**
Yield 81 %; mp 211–216 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.01 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.14 (s, 1H, SH), 3.85 (s, 2H, CH_2), 4.06 (s, 2H, S-CH_2), 5.71 (s, 2H, NH_2), 7.68-8.09 (m,

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5H, ArH), 9.00 (s, 2H, pyrimidine-ArH), 9.11 (bs, 3H, NH); MS m/z (%) 596 (M+, 100); Anal. Calcd. for $C_{23}H_{22}N_{12}O_2S_3$ (594.69): C, 46.45; H, 3.73; N, 28.26; O, 5.38; S, 16.18. Found: C, 46.45; H, 3.73; N, 28.26; O, 5.38; S, 16.18.

*4.1.8.3. N-(5-(4-((5-((4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)methylthio)-4-(2-chloroacetamido)-4H-1,2,4-triazol-3-yl)methyl)-5-methylthiazol-2-yl)pyrimidin-2-yl)acetamide **17c.***
Yield 70 %; mp 214–219 °C; ^{1}H NMR (300 MHz, CDCl$_3$): δ 2.08 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.18 (s, 1H, SH), 3.80 (s, 2H, CH_2), 4.02 (s, 2H, S-CH_2), 4.22 (s, 2H, -CH_2Cl), 5.76 (s, 2H, NH_2), 9.08 (s, 2H, pyrimidine-ArH), 9.10 (bs, 3H, NH); MS m/z (%) 568 (M+, 100); Anal. Calcd. for $C_{18}H_{19}ClN_{12}O_2S_3$ (567.07): C, 38.12; H, 3.38; Cl, 6.25; N, 29.64; O, 5.64; S, 16.96. Found: C, 38.12; H, 3.38; Cl, 6.25; N, 29.64; O, 5.64; S, 16.96.

4.2. Antimycobacterial activity
4.2.1. Strains and growth conditions
M. tuberculosis H37Rv (ATCC, cat. no. 27294), derivative strains and clinical isolates were maintained in Middlebrook 7H9 broth medium supplemented with 0.2% glycerol, 0.05% Tween 80 and 10% ADS supplement. Culture media were supplemented with hygromycin (50 μg ml^{-1}) or kanamycin (20 μg ml^{-1}) when required.

4.2.2. High-throughput cell-based screen
M. bovis BCG was cultured to an OD$_{600}$ of 0.5–0.6 in complete 7H9 broth medium. In preparation for 1536-well dispensing, the culture was diluted to an OD$_{600}$ of 0.01 using complete 7H9 media. A volume of 4 μl of complete 7H9 media was dispensed into a white, solid bottom 1536-well plate using a custom Bottle Valve liquid dispenser (GNF). A volume of 100 nl of test compound in DMSO (1 mM) was then transferred into each assay plates using a custom 1536 Pintool (GNF). Diluted culture (4 μl) was subsequently added to the assay plates using a Bottle Valve liquid dispenser (final OD$_{600}$ in 8 μl is 0.005). The plates were incubated at 37 °C for 48 h. Growth was assessed by measuring ATP levels using the BacTiter-Glo Microbial Cell Viability Assay (Promega). Luminescence was measured using a ViewLux plate reader.

4.2.3. MIC$_{50}$ determination
MIC$_{50}$ were determined as previously described, with slight modifications [13]. Briefly, compounds dissolved in 90% DMSO were twofold serial-diluted in duplicates and spotted by mosquito HTS (TTP LabTech) to 384-well clear plates, resulting in 10 dilutions of each compound. A volume of 50 μl of M. tuberculosis culture (final OD$_{600}$ of 0.02) was added to each well, and the assay plates were incubated at 37 °C for 5 days. OD$_{600}$ values were recorded using a SpectraMax M2 spectrophotometer, and MIC$_{50}$ curves were plotted using GraphPad Prism 5 software. Under the assay setting, MIC$_{50}$ values, which fall in the linear part of the inhibition curve, are more robust and reproducible than MIC$_{90}$. Therefore, only MIC$_{50}$ values are reported. Clinical isolates used in drug susceptibility testing were strain typed by IS6110 analysis as described [14].

4.2.4. Cytotoxicity
Cytotoxicity was tested against cell lines HepG2 (ATCC, cat. no. HB-8065) and BHK21 (ATCC, cat. no. CCL-10) in 96-well microplates. The cells were seeded at a density of 105 cells per well, incubated at 37 °C for 24 h and exposed to twofold serial-diluted compounds for 3 days. Cell viability was monitored using the Cell Proliferation Kit II (Invitrogen).

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4.2.5. Determination of intracellular ATP levels

The intracellular ATP level was quantified as previously described [15]. Briefly, 25 μl of *M. tuberculosis* culture was mixed with an equal volume of freshly prepared BacTiter-Glo reagent in white 384 flat-bottom plates and incubated in the dark for 5 min. Luminescence was measured using a Tecan Safire^2 plate reader.

4.2.6. Drug preparation

Unless specified, all the compounds were obtained from Sigma and were prepared in sterile de-ionized water. The experimental compounds were prepared in dimethyl sulphoxide (Sigma) for in vitro drug susceptibility testing.

RESULTS AND DISCUSSION

2.1. Chemistry

The chemo-transformation which led to the synthesis of **9** was performed as per the reports [10-12]. Compound **9** was treated with different acid chlorides at 0 °C to yield **10a-c** (Figure 1).

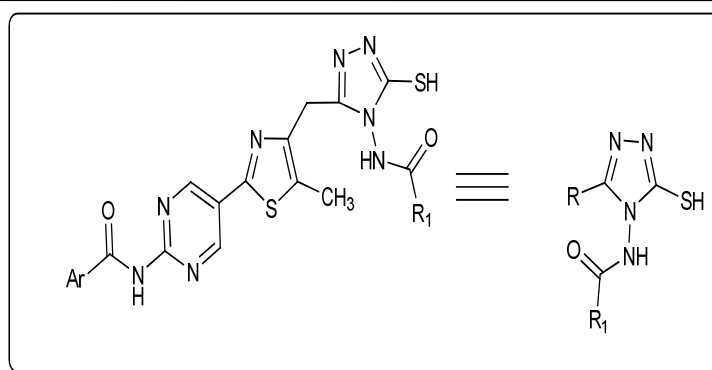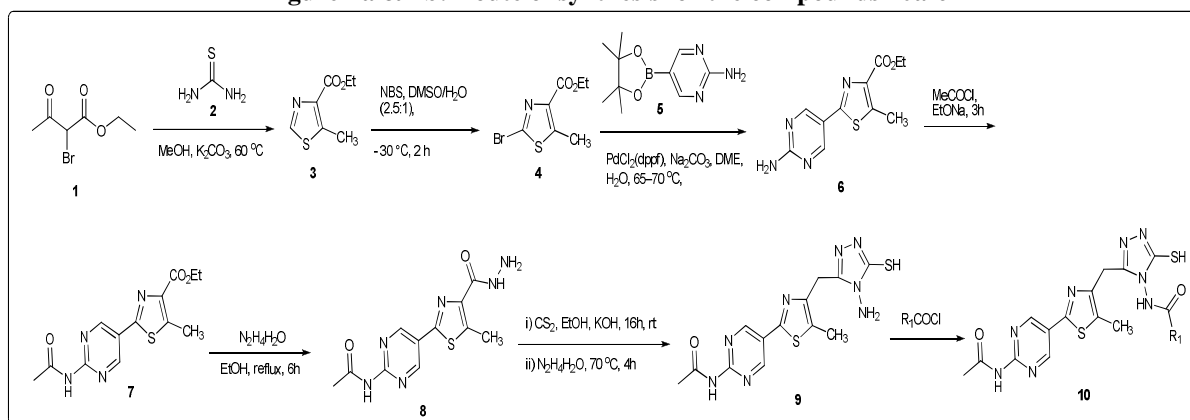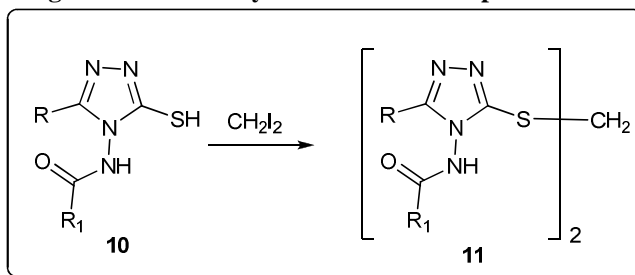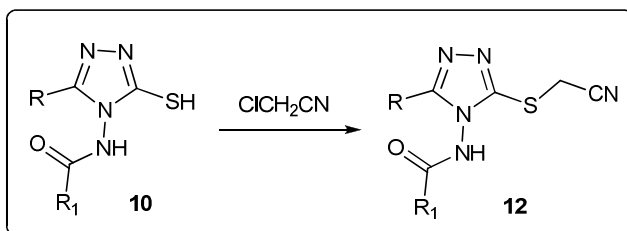**Figure-1a & 1b: Route of synthesis for the compounds 10a-c**

Figure-2: Route of synthesis for the compounds 11a-c

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The transformed compounds **10a-c** was used as a lead compounds for analog synthesis. The **10a-c** on treatment with diiodomethane in the presence of strong alkali i.e. sodium hydroxide gave **11a-c** which were found as stable dimer of **10a-c** (Figure 2).

Later on compounds **10a-c** were treated with chloroacetonitrile, which on neutralization with sodium carbonate gave a precipitates of compounds **12a-c** (Figure 3).

**Figure-3: Route of synthesis for the compounds 12a-c.**

Also, compounds **10a-c**, when treated with methyl bromoacetate in basic condition produced **13a-c**, which in-turn, under influence of hydrazine hydrate transformed to **14a-c** (Figure 4).

Figure-4: Route of synthesis for the compounds 14a-c.
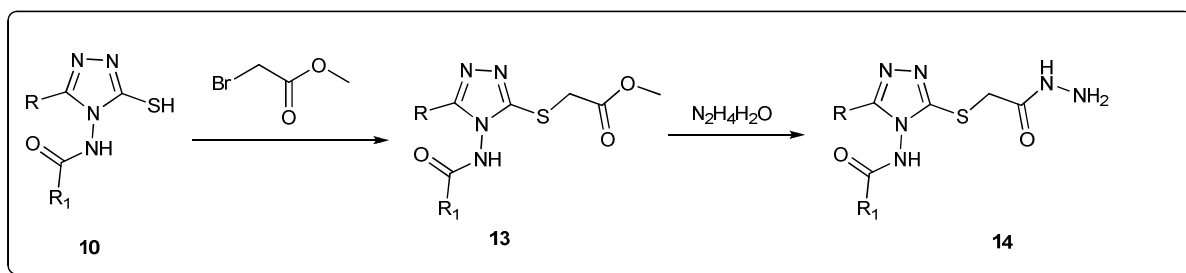
The synthesised compounds **14a-c** were then treated with appropriate acid chlorides to furnish **15a-i** (Figure 5).

Figure-5: Route of synthesis for the compounds 15a-i.
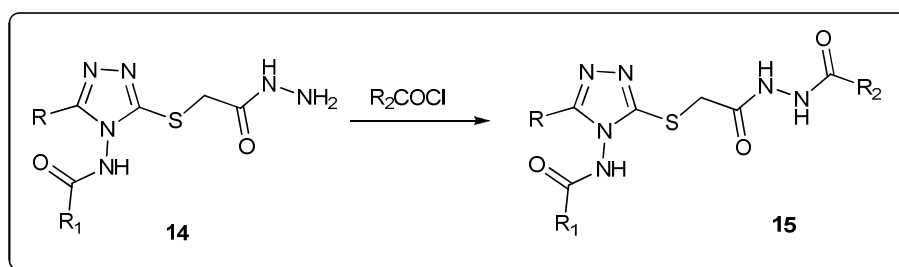
It was observed that these reactions when carried out in microwave, fumes of one acid chloride participate in next reaction if sufficient vapour clearance time is not allotted to clear off the vapours. Schiff bases, the condensation products of **14a-c**, were synthesized by treating them with benzaldehyde and confirmed by absence of triplet of NH of hydrate (Figure 6).

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Figure-6: Route of synthesis for the compounds 16a-c.
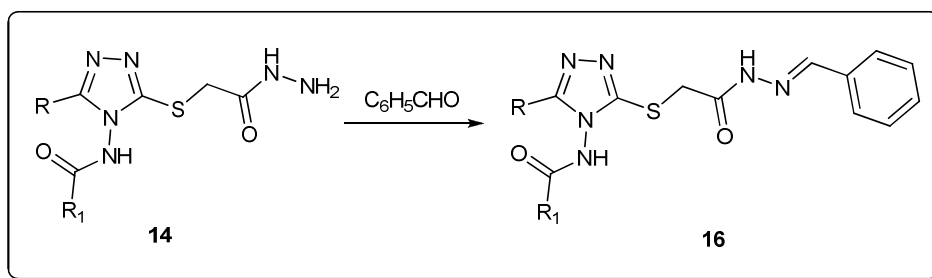
Compounds **14a-c** were then converted to thiocarbazate salts by treatment with carbon disulphide and potassium hydroxide, and then treated with hydrazine hydrate to give **17a-c** (Figure 7). The NMR spectra confirmed formation of triazole derivative from hydrazide, which shows presence of sulfhydryl proton at near δ 3.1.
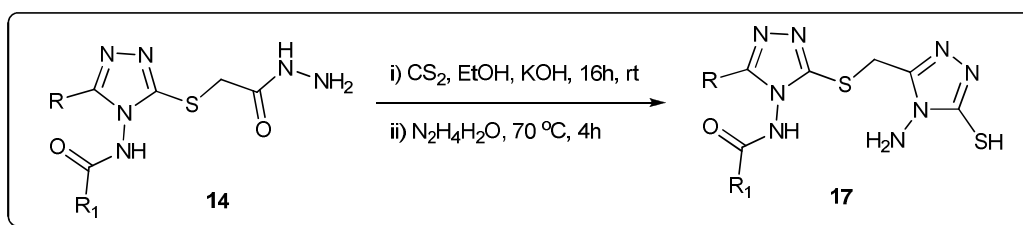
Figure-7: Route of synthesis for the compounds 17a-c.

2.2. Antitubercular activity
A cellular screen was developed to identify mycobacterial growth inhibitors. The screen was carried out against *M. bovis* BCG using intracellular ATP content as a surrogate marker of bacillary growth. Compound hits with confirmed activity against M. tuberculosis were chemically clustered to identify any emerging SAR. Our attention was drawn to a cluster of clubbed compounds comprising three major compounds, **A**, **B** & **C** (detailed synthesis and activity data is communicated to Indian Journal of Chemistry, section B; scheme 8) with an MIC_{50} ranging from 0.14 to > 0.19 μM (Table 1).

Figure-8: Structures of compounds A, B & C.
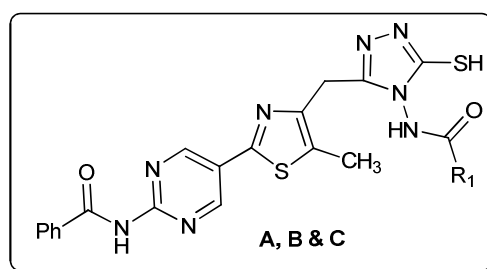
The compounds were bactericidal and the cytotoxic profile was within an acceptable range. Therefore, a lead optimization programme was initiated with the goal of achieving potent antitubercular activity.

To begin with we synthesised **10a**, **10b** &**10c** which have shown better activity overall than **A**, **B** & **C**. This promising results have prompted us to initiate chemo-transformation of compounds **10a**, **10b** &**10c**. Initially a raw thought stroke us which involved dimerisation of the "Hit"

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compounds. Although the Lipinski rule of five does not allow these modifications, but not all the inventions follow the rule. Thus taking odd step, we have synthesised the dimer compounds **11a**, **11b** &**11c**. To our disappointment none of them were better than the "Hits". We postulate the labile S-CH$_2$-S bond breakage which may lead to ionic compound which may be representative of the parent compound. Thus it was thought to increase the alkyl chain with a cyano at end to check if they prove to be cytotoxic for bacterial cells. This led us to the synthesis of **12a-c**. As expected these compounds were active but were cytotoxic too (Table-1).

Table 1: Preliminary Structure Activity Relationship of compounds A, B, C, 10a-c, 11a-c, 12a-c, 14a-c, 15a-c, 16a-i &17a-c.

ID	R$_1$	R$_2$	MIC$_{50}$ (µm)	MBC$_{90}$ (µm)	CC$_{50}$-BHK21 (µm)	CC$_{50}$-HepG2 (µm)
A	-CH$_3$	-----	0.14±0.15	1.25-2.5	5.71±0.27	8.55±0.21
B	-C$_6$H$_5$	-----	0.17±0.19	1.25-2.5	4.12±0.44	4.17±0.21
C	-CH$_2$Cl	-----	0.19±0.14	1.25-2.5	4.25±0.41	4.57±0.56
10a	-CH$_3$	-----	0.14±0.11	1.25-2.5	5.10±0.20	8.71±0.20
10b	-C$_6$H$_5$	-----	0.12±0.18	1.25-2.5	4.38±0.40	4.27±0.20
10c	-CH$_2$Cl	-----	0.13±0.11	1.25-2.5	4.57±0.40	4.30±0.51
11a	-CH$_3$	-----	0.14±0.11	1.25-2.5	6.21±0.20	9.28±0.82
11b	-C$_6$H$_5$	-----	0.40±0.17	2.5-5	7.29±0.40	8.19±0.51
11c	-CH$_2$Cl	-----	0.51±0.23	2.5-5	10.65±0.40	9.10±0.90
12a	-CH$_3$	-----	0.12±0.18	1.25-2.5	0.16±0.03	0.52±0.04
12b	-C$_6$H$_5$	-----	0.18±0.21	1.25-2.5	1.10±0.03	1.42±0.05
12c	-CH$_2$Cl	-----	0.17±0.17	1.25-2.5	0.96±0.05	1.38±0.08
14a	-CH$_3$	-----	0.15±0.03	1.25-2.5	>50	>50
14b	-C$_6$H$_5$	-----	0.60±0.18	2.5-5	>50	>50
14c	-CH$_2$Cl	-----	0.69±0.25	2.5-5	>50	>50
15a	-CH$_3$	-CH$_3$	>20	n.d.	n.d.	n.d.
15b	-CH$_3$	-C$_6$H$_5$	>20	n.d.	n.d.	n.d.
15c	-CH$_3$	-CH$_2$Cl	>20	n.d.	n.d.	n.d.
15d	-C$_6$H$_5$	-CH$_3$	>20	n.d.	n.d.	n.d.
15e	-C$_6$H$_5$	-C$_6$H$_5$	>20	n.d.	n.d.	n.d.
15f	-C$_6$H$_5$	-CH$_2$Cl	>20	n.d.	n.d.	n.d.
15g	-CH$_2$Cl	-CH$_3$	>20	n.d.	n.d.	n.d.
15h	-CH$_2$Cl	-C$_6$H$_5$	>20	n.d.	n.d.	n.d.
15i	-CH$_2$Cl	-CH$_2$Cl	>20	n.d.	n.d.	n.d.
16a	-CH$_3$	-----	0.04±0.01	1.25-2.5	>50	>50
16b	-C$_6$H$_5$	-----	0.05±0.01	1.25-2.5	4.19±0.40	4.27±0.20
16c	-CH$_2$Cl	-----	0.05±0.01	1.25-2.5	4.20±0.45	4.35±0.40
17a	-CH$_3$	-----	>20	n.d.	n.d.	n.d.
17b	-C$_6$H$_5$	-----	>20	n.d.	n.d.	n.d.
17c	-CH$_2$Cl	-----	>20	n.d.	n.d.	n.d.
Isoniazid			0.03±0.01	n.d.	n.d.	n.d.

The inhibitory activity (MIC$_{50}$) was determined against M. tuberculosis H37Rv. The cidal activity (MBC$_{90}$) and cytotoxicity (CC$_{50}$) were determined after 5 days of exposure to a single dose of compound. Assays were carried out at least two times. MIC$_{50}$: Minimum Inhibitory Concentration 50%; MBC$_{90}$: Minimum Bactericidal Concentration 90%, CC$_{50}$: Cyotoxic concentration 50%. n.d.: not determined.

Thus it was thought to increase the length, have a nitrogen but not in the form of cyano, but amino/hydrazide. To our imagination, these compounds **14a-c** have shown comparable results to their parent with less to no cytotoxicity. Thus to check if the activity may increase we have reacted them with acid chlorides and synthesised **15a-i**. None of them, surprisingly turned out to be active. It may be topic of research as why only a small change led to such a vast different activity. As the Schiff bases reported to have their antibacterial activity and are also reported to help in penetration, we then synthesised **16a-c**. To our expectations, **16a** emerged as a hit which is almost equipotent to isoniazid and without any notable cytotoxicity. Other compounds **16b** & **16c** have also shown better activity but have also shown some cytotoxicity. Then we further went

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for hetercyclisation which gave **17a-c**, which on contrary to our expectations, none of the compound was as promising as **16a**.

After having **16a** as "lead", we decided to further evaluate **16a** to compare with standard drug isoniazid (INH). First we compared **16a** with INH for their susceptibilities on 18 clinical isolates (Table 2) of MTB (M. tuberculosis), out of which 16 were pan-susceptible and 2 were mono-rifampin resistant isolates.

Table-2: 16a drug susceptibilities for MTB (pan-susceptible & mono-rifampin resistant) clinical isolates

SN	Strain	MIC (μgml^{-1})	
		Isoniazid	**16a**
1	H37Rv	0.03	0.15
2	TN675*	0.03	0.011
3	TN913	0.03	0.03
4	TN994*	0.03	0.03
5	TN1008	0.06	0.03
6	TN1037	0.03	0.06
7	TN1040	0.03	0.03
8	TN1051	0.03	0.03
9	TN1082	0.03	0.06
10	TN2351	0.06	0.25
11	TN2524	0.06	0.25
12	TN3183	0.03	0.3
13	TN3979	0.06	0.6
14	TN4259	0.03	0.06
15	AH9584	0.19	0.25
16	BE11677	0.20	0.25
17	E8133	0.08	0.13
18	W4	0.03	0.06

Compound 16a and Isoniazid drug susceptibilities were determined on 16 pan-susceptible and 2 mono-rifampin resistant (asterisk) clinical isolates.

Table-3: 16a drug susceptibilities for MTB (MDR & poly-resistant) clinical isolates

SN	Strain	Drug Resistance	**16a** MIC (μgml^{-1})
1	TN565	R,S,EM,ET,K.Cl	0.03
2	TN576	I,R,S,EM,ET,K	0.06
3	TN702	I,S,EM,P	0.25
4	TN715	I,R,EM,P	0.06
5	TN768	I,R,S,EM,ET	0.03
6	TN772	I,R,EM	0.03
7	TN1195	I,S,EM	0.25
8	TN1314	I,R	0.06
9	TN1618	I,R,S,EM,ET,CI	0.06
10	TN1811	I,R,S,EM	0.03
11	TN2557	I,R,S,EM,CA	0.06

The 16a susceptibilities were also tested on 9 multi-drug resistant (MDR) and 2 poly-resistant MTB strains. (b) Twenty of the twenty-five sensitive and resistant clinical isolates tested were previously determined to be genetically distinct by IS6110 genotyping. I, isoniazid; R, rifampin; S, streptomycin; EM, ethambutol; ET, ethionamide; K, kanamycin; P, pyrazinamide; Cl, ciprofloxacin; CA, capreomycin.

We are glad to report that our compound **16a** have been shown almost equipotent to that of INH. Having seen its potential, we decided to evaluate **16a** against 9 multi drug resistant (MDR) and 2 poly-drug resistant MTB strains (Table-3).

We are happy to report that, compound have shown promising activity against almost all the resistant strains. The compound **16a** is now under further evaluation stage, which shall be shortly communicated.

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CONCLUSION

Keeping XDR & MDR tuberculosis at the background, current report reveals a series of molecules that have shown promising activity against the H_{37} strain. These easy to synthesise molecules will certainly be cheaper and can be synthesised in short duration. The compounds have also shown comparable activity against different 25 clinical isolates. These tractable molecules have been identified as "Hit" and our future matrix revolves around the modification and observing the SAR of the synthesised compounds. Finally, it may be concluded considering the activity of reported molecule that, Lepinski's rule of five can be bend and molecules which do not obey it, can be treated as drug candidates.

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