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Design, Synthesis and Evaluation of Linked Pyridinyl-Oxadiazoles as Treatment of XDR and MDR Tuberculosis-Part II

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Abstract

Recently, *Mycobacterium tuberculosis* (MTB) strains have transformed from about to be eradicated to most resistant microbe, thank to Multi-drug resistant (MDR) and extremely drug resistant (XDR) MTB. Various novel approaches are being explored including antimutagen, efflux inhibitors etc which can act as adjuvant therapy. But there is still need to develop real anti-mycobacterial drug. This research reports small molecule anti-infectives which are specifically potent against several strains and isolates of TB. The hit compound **7f** has also proved to be active against almost 25 clinical isolates comparable to marketed anti-TB agents.

Keywords: MDR-TB; *M. Tuberculosis*; Pyridine; Oxadiazole; Resistance

Introduction

Mycobacterium tuberculosis (MTB, *M.tuberculosis*), the etiological agent of tuberculosis (TB), is the leading cause of mortality due to bacterial pathogens, claiming about 2 million lives annually. The field of anti-tuberculosis drug discovery culminated in the 1960s with the incorporation of rifampicin and pyrazinamide in the tuberculosis drug regimen. The use of these two antimicrobials, in combination with isoniazid, ethambutol and/or streptomycin, 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]. Short-course chemotherapy contributed towards controlling tuberculosis burden for the next 20 years. Nevertheless, 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 or less [5,6]. Few promising drug candidates fulfilling these criteria have been discovered in recent years [7-9]. Mainly, TMC207, which has been shown to be highly active in proof-of-concept trials, and shows the potential to shorten the duration of therapy [10,11].

Recently, there are several reports citing pyridine and oxadiazoles as potential antibacterial and anti-tubercular agent [12-15]. Inspired by the citations, we decided to design around the heterocycles and evaluate the antimycobacterial activity of the same. 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 in-continuation with the search of new drug candidate we herein discuss this report about development of a lead to hit molecule.

Experimental

Chemistry

^{1}H NMR spectra were recorded on a Bruker Avance 500 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. 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) (Table 1).

Synthesis of 2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(substituted)phenylacetamide ***5a-h,*** *2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(substituted)phenylpropanamide* ***6a-h,*** *3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(substituted)phenylpropanamide****7a-h*** *and 3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-2-methyl-N-(substituted) phenylpropanamide* ***8a-h.***

To a cool solution of metallic sodium (1 mol) in an absolute ethanol; 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thiol (1 mol) (4) was added with stirring, at about 15°C. The solution was filtered. The excess of solvent was removed under suction and cold water was added to get a clear solution. The solution was again filtered to remove suspended particles. Then N-substituted-chloroalkylamide (1 mol) was added in small portion at room temperature with stirring, then stirring was continue between 60 to 65°C for 8 h cooled and extracted with EtOAc. The EtOAc layer was dried with sodium sulphate and the column purified.

ID	R	MIC_{50} (μM)	MBC_{90} (μM)	CC_{50}-BHK21 (μM)	CC_{50}-HepG2 (μM)
4	--	0.20 ± 0.09	1.25-2.5	19.70 ± 3.77	14.7 ± 5.89
5a	$-C_6H_5$	0.18 ± 0.08	1.25-2.5	15.21 ± 2.65	16.2 ± 8.26
5b	$-CH_2-C_6H_5$	0.19 ± 0.07	1.25-2.5	17.65 ± 0.68	11.3 ± 5.67
5c	$-CH_2$-2-ClC_6H_4	9.67 ± 1.45	n.d.	n.d.	n.d.
5d	$-CH_2$-4-ClC_6H_4	11.72 ± 3.57	n.d.	>50	>50
5e	- 2-ClC_6H_5	8.52 ± 1.33	n.d.	>50	>50
5f	-4-ClC_6H_4	6.81 ± 1.08	n.d.	>50	>50
5g	-2,5-$Cl_2C_6H_3$	>20	n.d.	n.d.	n.d.
5h	-2,4-$Cl_2C_6H_3$	>20	n.d.	n.d.	n.d.
6a	$-C_6H_5$	1.48 ± 1.28	5-10.	>50	>50
6b	$-CH_2-C_6H_5$	1.15 ± 1.03	5-10	>50	>50
6c	$-CH_2$-2-ClC_6H_4	1.26 ± 1.19	5-10	>50	>50
6d	$-CH_2$-4-ClC_6H_4	>20	n.d.	n.d.	n.d.
6e	- 2-ClC_6H_5	1.04 ± 0.01	5-10	>50	>50
6f	-4-ClC_6H_4	0.12 ± 0.46	1.25-2.5	>50	>50
6g	-2,5-$Cl_2C_6H_3$	1.19 ± 0.39	5-10	>50	>50
6h	-2,4-$Cl_2C_6H_3$	2.45 ± 1.22	5-10	>50	>50
7a	$-C_6H_5$	0.61 ± 0.13	2.5-5	>50	>50
7b	$-CH_2-C_6H_5$	0.67 ± 0.24	2.5-5	13.97 ± 0.71	18.7 ± 7.4
7c	$-CH_2$-2-ClC_6H_4	0.65 ± 0.15	2.5-5	>50	>50
7d	$-CH_2$-4-ClC_6H_4	0.63 ± 0.24	2.5-5	15.62 ± 0.81	21.5 ± 6.9
7e	- 2-ClC_6H_5	0.62 ± 0.17	2.5-5	>50	>50
7f	-4-ClC_6H_4	0.07 ± 0.04	1.25-2.5	7.26 ± 0.51	8.21 ± 0.22
7g	-2,5-$Cl_2C_6H_3$	0.59 ± 0.11	2.5-5	12.6 ± 0.84	10.26 ± 8.4
7h	-2,4-$Cl_2C_6H_3$	0.55 ± 0.18	2.5-5	>50	>50
8a	$-C_6H_5$	2.26 ± 0.84	5-10.	>50	>50
8b	$-CH_2-C_6H_5$	2.51 ± 1.36	5-10	>50	>50
8c	$-CH_2$-2-ClC_6H_4	1.89 ± 0.86	5-10	>50	>50
8d	$-CH_2$-4-ClC_6H_4	1.68 ± 0.68	n.d.	n.d.	n.d.
8e	- 2-ClC_6H_5	2.05 ± 1.06	5-10	>50	>50
8f	-4-ClC_6H_4	0.22 ± 0.5	1.25-2.5	>50	>50
8g	-2,5-$Cl_2C_6H_3$	1.55 ± 0.64	5-10	>50	>50
8h	-2,4-$Cl_2C_6H_3$	1.48 ± 0.66	5-10	>50	>50
Isoniazid	--	0.03 ± 0.01	n.d.	n.d.	n.d.

Table 1: Preliminary Structure Activity Relationship of compounds 5a-h, 6a-h, 7a-h and 8a-h.

The inhibitory activity (MIC_{50}) was determined against *M. tuberculosis* $H_{37}Rv$. 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.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-phenylacetamide(**5a**).*

Yield 76%; mp 187°C;^{1}H NMR (500 MHz, $CDCl_3$): δ 3.83 (s, 2H, CH_2), 7.02-7.51 (m, 5H, ArH), 7.60-7.63 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.10 (br, 1H, NH), 8.67-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 313.41 (M^+, 100); Anal. Calcd. for $C_{15}H_{12}N_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-benzylacetamide(**5b**).*

Yield 68%; mp 189°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.87 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 7.01-7.15 (m, 5H, ArH), 7.62-7.65 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.13 (br, 1H, NH), 8.62-8.65 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 327.48 (M^+, 100); Anal. Calcd. for $C_{16}H_{14}N_4O_2S$.

*N-(2-Chlorobenzyl)-2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio) acetamide (**5c**).*

Yield 82%; mp 212°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.89 (s, 2H, CH_2), 4.48 (s, 2H, CH_2), 7.05-7.17 (m, 4H, ArH), 7.61-7.64 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.15 (br, 1H, NH), 8.60-8.64 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 362.88 (M^+, 100); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$.

*N-(4-Chlorobenzyl)-2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio) acetamide (**5d**).*

Yield 68%; mp 208°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.84 (s, 2H, CH_2), 4.43 (s, 2H, CH_2), 7.03-7.05 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.12-7.14 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.63-7.66 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.13 (br, 1H, NH), 8.63-8.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 362.88 (M^+, 100); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2-chlorophenyl) acetamide (**5e**).*

Yield 82%; mp 112°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.82 (s, 2H, CH_2), 6.96-7.53 (m, 4H, ArH), 7.64-7.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.17 (br, 1H, NH), 8.68-8.71 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 348.90 (M^+, 100); Anal. Calcd. for $C_{15}H_{11}ClN_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(4-chlorophenyl) acetamide (**5f**).*

Yield 69%; mp 198°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.85 (s, 2H, CH_2), 7.24-7.27 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.55-7.58 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.62-7.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.19 (br, 1H, NH), 8.62-8.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 348.90 (M^+, 100); Anal. Calcd. for $C_{15}H_{11}ClN_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,6-dichlorophenyl) acetamide (**5g**).*

Yield 73%; mp 216°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.86 (s, 2H, CH_2), 6.94-7.24 (m, 3H, ArH), 7.64-7.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.12 (br, 1H, NH), 8.69-8.72 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 383.3 (M^+, 100); Anal. Calcd. for $C_{15}H_{10}C_{l2}N_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,4-dichlorophenyl) acetamide (**5h**).*

Yield 81%; mp 227°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.87 (s, 2H, CH_2), 7.12-7.15 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.24 (s, 1H, ArH), 7.51-7.53 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.64-7.69 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.13 (br, 1H, NH), 8.67-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 383.3 (M^+, 100); Anal. Calcd.for $C_{15}H_{10}C_{l2}N_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-phenylpropanamide (**6a**).*

Yield 53%; mp 248°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.53 (d, 3H, CH_3), 3.60-3.64 (q, 1H, CH), 7.02-7.51 (m, 5H, ArH), 7.62-7.64 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.14 (br, 1H, NH), 8.62-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 327.5 (M^+, 100); Anal. Calcd. for $C_{16}H_{14}N_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-benzylpropanamide (**6b**).*

Yield 60%; mp 237°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.50 (d, 3H, CH_3), 3.61-3.65 (q, 1H, CH), 4.45 (s, 2H, CH_2), 7.01-7.15 (m, 5H, ArH), 7.61-7.64 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.13 (br, 1H, NH), 8.63-8.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 341.5 (M^+, 100); Anal. Calcd. for $C_{17}H_{16}N_4O_2S$.

*N-(2-Chlorobenzyl)-2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio) propanamide (**6c**).*

Yield 68%; mp 227°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.52 (d, 3H, CH_3), 3.65-3.69 (q, 1H, CH), 4.48 (s, 2H, CH_2), 7.05-7.17 (m, 4H, ArH), 7.63-7.66 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.16 (br, 1H, NH), 8.62-8.66 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 376.9 (M^+, 100); Anal. Calcd. for $C_{17}H_{15}ClN_4O_2S$.

*N-(4-Chlorobenzyl)-2-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio) propanamide (**6d**).*

Yield 56%; mp 208°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.54 (d, 3H, CH_3), 3.63-3.67 (q, 1H, CH), 4.43 (s, 2H, CH_2), 7.03-7.05 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.12-7.14 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.64-7.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.12 (br, 1H, NH), 8.65-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 376.9 (M^+, 100); Anal. Calcd. for $C_{17}H_{15}ClN_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2-chlorophenyl) propanamide(**6e**).*

Yield 63%; mp 215°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.55 (d, 3H, CH_3), 3.63-3.66 (q, 1H, CH), 6.96-7.53 (m, 4H, ArH), 7.62-7.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.14 (br, 1H, NH), 8.68-8.73 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 362.9 (M^+, 100); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(4-chlorophenyl) propanamide (**6f**).*

Yield 84%; mp 235°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.52 (d, 3H, CH_3), 3.62-3.65 (q, 1H, CH),7.24-7.27 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.55-7.58 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.65-7.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.10 (br, 1H, NH), 8.67-8.71 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 362.9 (M^+, 100); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,6-dichlorophenyl) propanamide (**6g**).*

Yield 80%; mp 242°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.57 (d, 3H, CH_3), 3.63-3.67 (q, 1H, CH), 6.94-7.24 (m, 3H, ArH), 7.63-7.66 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.11 (br, 1H, NH), 8.65-8.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 397.3 (M^+, 100); Anal. Calcd. for $C_{16}H_{12}Cl_2N_4O_2S$.

*2-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,4-dichlorophenyl) propanamide (**6h**).*

Yield 64%; mp 236°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.56 (d, 3H, CH_3), 3.60-3.63 (q, 1H, CH), 7.12-7.15 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.24 (s, 1H, ArH), 7.51-7.53 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.60-7.63 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.10 (br, 1H, NH), 8.63-8.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 397.3 (M^+, 100); Anal. Calcd.for $C_{16}H_{12}Cl_2N_4O_2S$.

*3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-phenylpropanamide **(7a).***

Yield 68%; mp 203°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.63-2.67 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.32-3.36 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 7.02-7.51 (m, 5H, ArH), 7.61-7.64 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.15 (br, 1H, NH), 8.60-8.76 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 327.4 (M^+, 100); Anal. Calcd. for $C_{16}H_{14}N_4O_2S$.

*3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-benzylpropanamide **(7b).***

Yield 63%; mp 218°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.61-2.64 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.31-3.36 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 4.45 (s, 2H, CH_2), 7.01-7.15 (m, 5H, ArH), 7.62-7.66 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.16 (br, 1H, NH), 8.67-8.73 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 341.5 (M^+, 100); Anal. Calcd. for $C_{17}H_{16}N_4O_2S$.

*N-(2-Chlorobenzyl)-3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio) propanamide **(7c).***

Yield 60%; mp 213°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.62-2.65 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.30-3.35 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 4.48 (s, 2H, CH_2), 7.05-7.17 (m, 4H, ArH), 7.62-7.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.12 (br, 1H, NH), 8.63-8.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 376.9 (M^+, 100); Anal. Calcd. for $C_{17}H_{15}ClN_4O_2S$.

*N-(4-Chlorobenzyl)-3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio) propanamide **(7d).***

Yield 59%; mp 217°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.64-2.68 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.34-3.38 (tt, 2H, $J_{CH=CH}$=2.7, ArH),4.43 (s, 2H, CH_2), 7.03-7.05 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.12-7.14 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.62-7.69 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.19 (br, 1H, NH), 8.67-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 376.9 (M^+, 100); Anal. Calcd. for $C_{17}H_{15}ClN_4O_2S$.

*3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2-chlorophenyl) propanamide **(7e).***

Yield 63%; mp 203°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.62-2.65 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.33-3.37 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 6.96-7.53 (m, 4H, ArH), 7.66-7.73 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.12 (br, 1H, NH), 8.63-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 362.9 (M^{+1}, 100); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$.

*3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(4-chlorophenyl) propanamide**(7f).***

Yield 64%; mp 211°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.62-2.66 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.35-3.38 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 7.24-7.27 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.55-7.58 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.65-7.69 (dd, 2H,

$J_{CH=CH}$=1.8, ArH), 8.18 (br, 1H, NH), 8.66-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 362.9 (M^{+1}, 100); Anal. Calcd. for $C_{16}H_{13}ClN_4O_2S$.

3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,6-dichlorophenyl) propanamide ***(7g).***

Yield 60%; mp 207°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.61-2.66 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.32-3.35 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 6.94-7.24 (m, 3H, ArH), 7.64-7.69 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.12 (br, 1H, NH), 8.65-8.72 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 397.3 (M^{+1}, 100); Anal. Calcd. for $C_{16}H_{12}Cl_2N_4O_2S$.

*3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,4-dichlorophenyl) propanamide****(7h).***

Yield 61%; mp 209°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.64-2.67 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 3.30-3.33 (tt, 2H, $J_{CH=CH}$=2.7, ArH), 7.12-7.15 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.24 (s, 1H, ArH), 7.51-7.53 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.60-7.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.17 (br, 1H, NH), 8.68-8.73 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 397.3 (M^{+1}, 100); Anal. Calcd. for $C_{16}H_{12}Cl_2N_4O_2S$.

3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-2-methyl-N-phenylpropanamide ***(8a).***

Yield 62%; mp 205°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25 (d, 3H, CH_3), 2.88-2.92 (m, 1H, CH), 3.21-3.34 (d, 2H, CH_2), 7.02-7.51 (m, 5H, ArH), 7.63-7.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.12 (br, 1H, NH), 8.68-8.77 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 341.4 (M^+, 100); Anal. Calcd. for $C_{17}H_{16}N_4O_2S$.

3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-benzyl-2-methylpropanamide ***(8b).***

Yield 71%; mp187°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (d, 3H, CH_3), 2.81-2.85 (m, 1H, CH), 3.20-3.31 (d, 2H, CH_2), 4.45 (s, 2H, CH_2), 7.01-7.15 (m, 5H, ArH), 7.66-7.75 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.10 (br, 1H, NH), 8.67-8.76 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 355.5 (M^+, 100); Anal. Calcd. for $C_{18}H_{18}N_4O_2S$.

N-(2-Chlorobenzyl)-3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-2-methylpropanamide ***(8c).***

Yield 72%; mp186°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.26 (d, 3H, CH_3), 2.84-2.89 (m, 1H, CH), 3.21-3.30 (d, 2H, CH_2), 4.48 (s, 2H, CH_2), 7.05-7.17 (m, 4H, ArH), 7.63-7.69 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.17 (br, 1H, NH), 8.64-8.71 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 390.9 (M^{+1}, 100); Anal. Calcd. for $C_{18}H_{17}ClN_4O_2S$.

N-(4-Chlorobenzyl)-3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-2-methylpropanamide ***(8d).***

Yield 73%; mp176°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22 (d, 3H, CH_3), 2.83-2.88 (m, 1H, CH), 3.22-3.31 (d, 2H, CH_2), 4.43 (s, 2H, CH_2), 7.03-7.05 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.12-7.14 (dd, 2H, $J_{CH=CH}$=2.4, ArH), 7.62-7.67 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.16 (br, 1H, NH), 8.63-8.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 390.9 (M^{+1}, 100); Anal. Calcd. for $C_{18}H_{17}ClN_4O_2S$.

3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2-chlorophenyl)-2-methylpropanamide ***(8e).***

Yield 77%; mp 208°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.24 (d, 3H, CH_3), 2.84-2.87 (m, 1H, CH), 3.23-3.35 (d, 2H, CH_2), 6.96-7.53 (m, 4H, ArH), 7.62-7.69 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.13 (br, 1H, NH), 8.65-8.71 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 376.9 (M^{+1}, 100); Anal. Calcd. for $C_{17}H_{15}ClN_4O_2S$.

3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(4-chlorophenyl)-2-methylpropanamide ***(8f).***

Yield 73%; mp185°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.27 (d, 3H, CH_3), 2.82-2.86 (m, 1H, CH), 3.24-3.36 (d, 2H, CH_2), 7.24-7.27 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.55-7.58 (dd, 2H, $J_{CH=CH}$=2.6, ArH), 7.61-7.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.14 (br, 1H, NH), 8.64-8.73 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 376.9 (M^{+1}, 100); Anal. Calcd. for $C_{17}H_{15}ClN_4O_2S$.

3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,6-dichlorophenyl)-2-methylpropanamide ***(8g).***

Yield 71%; mp 174°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.20 (d, 3H, CH_3), 2.83-2.90 (m, 1H, CH), 3.23-3.37 (d, 2H, CH_2), 6.94-7.24 (m, 3H, ArH), 7.62-7.68 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.17 (br, 1H, NH), 8.63-8.75 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 411.3 (M^{+1}, 100); Anal. Calcd. for $C_{17}H_{14}C_{l2}N_4O_2S$.

3-(5-(Pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-N-(2,4-dichlorophenyl)-2-methylpropanamide ***(8h).***

Yield 78%; mp180°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.23 (d, 3H, CH_3), 2.80-2.85 (m, 1H, CH), 3.24-3.38 (d, 2H, CH_2), 7.12-7.15 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.24 (s, 1H, ArH), 7.51-7.53 (dd, 2H, $J_{CH=CH}$=2.2, ArH), 7.62-7.70 (dd, 2H, $J_{CH=CH}$=1.8, ArH), 8.18 (br, 1H, NH), 8.61-8.73 (dd, 2H, $J_{CH=CH}$=1.8, ArH); MS m/z (%) 411.3 (M^{+1}, 100); Anal. Calcd. for $C_{17}H_{14}C_{l2}N_4O_2S$.

Antimycobacterial activity

Strains and growth conditions: *M. tuberculosis* $H_{37}Rv$ (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.

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.

MIC_{50} determination: MIC_{50} were determined as previously described, with slight modifications [17]. 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 [18].

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).

Determination of intracellular ATP levels: The intracellular ATP level was quantified as previously described [19]. 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 Safire2 plate reader.

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

Chemistry

In our attempt to synthesise cost effective drug, oxadiazole was identified as better target, easy and cheaper to synthesis. The synthetic route was followed as reported in (Figure-1)[16]. In brief, the ester to hydrazide chemotransformation was carried out by using hydrazine hydrate in ethanol at reflux condition. The hydrazide was then transformed to thiosemicarbazate by usual CS_2, KOH and ethanol method. The resultant solid was then cyclised using proton donar (sulphuric acid) at temperature ranging from 0 to 5°C. The pyridinyl-oxadiazole "parent" then reacted with various chloro-substituted compounds for further analog synthesis. These reactions led us to the thio substituted array of compounds **5, 6, 7 and 8**.

Antitubercular activity

A cellular screen was developed to identify mycobacterial growth inhibitors. The screen was carried out against *Mycobacterium bovis (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 pyridinyl-oxadiazole compound. One of the lead **4**, (synthesized at our laboratory for another program, unpublished) with an MIC_{50} ranging from 0.21 µM (Scheme 1). The compound was bactericidal with cytotoxic profile within acceptable range. Therefore, a lead optimization programme was initiated with the goal of achieving potent antitubercular activity.

The program of chemo-transformation initiated with compound **5a**. An increase in the length of alkyl chain with amide linkage intact (**5a**) has shown improved potency. Another substitution with Ar =Benzyl, **5b** showed similar pharmacological activity. To achieve better activity, we used phenyl and benzyl group with chloro substitution. In case of **5e** and **5f**, improved activity was observed as reported. Similar attempt with the benzyl substitution (**5c** and **5d**) was disappointing with no further increase in activity. Assuming, chlorine has vital role to play in the orientation and receptor bindings, **5g** and **5h** were synthesised but failed to reflect potentiation.

Close look at SAR revealed that "small changes make big difference", thus wondering if one carbon elongation or branching will embark any betterment in inhibition, further set of series **6a-h, 7a-h** and **8a-h** were synthesized and tested. In case of branched series **6a-h** it was not surprising to note either equipotent or diminished action. This may owe to the addition of bulk to the 3D structure of molecules. Only compound **6f** has shown slight improved activity. The attempt of chain elongation, **7a-h** disclosed different SAR. Except for dichloro compounds **7g** and **7h,** all showed increased potency. Molecule **7f** has emerged as hit with almost equipotent to isoniazide.

When synthesised branched analogs of **7** series i.e. **8a-h** series, on contrary to our expectations, none of the compounds was as promising as **7f**. Thus we decided to further evaluate **7f** to compare with standard drug isoniazid (INH).

First we compared **7f** with INH for their susceptibilities on 18 clinical isolates table-2 of MTB (*M. tuberculosis)*, out of which 16 were pan-

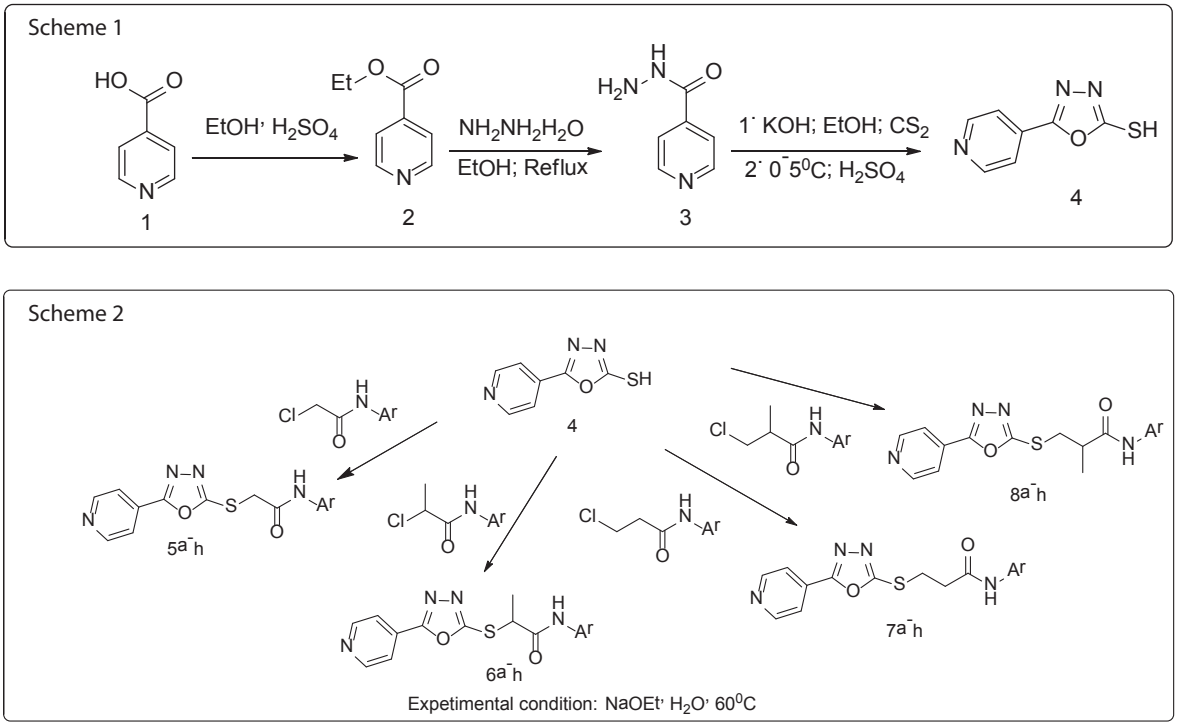

Figure 1: Route of synthesis for the compounds **5a-h, 6a-h, 7a-h** and **8a-h**.

susceptible and 2 were mono-rifampin resistant isolates. We are happy to report that our compound **7f** have been shown almost equipotent to that of INH. Having seen its potential, we decided to evaluate **7f** against 9 multi drug resistant (MDR) and 2 poly-drug resistant MTB strains (Table-3). Gladly, compound has shown promising activity against almost all the resistant strains. The compound **7f** is now under further evaluation stage, which shall be shortly communicated.

Conclusion

Keeping a widespread use of future antimycobacterials, we aimed to synthesis a cheaper but better agent for today's XDR and MDR tuberculosis. In order to do so, we have zeroed at pyridinyl-oxadiazole, which gave us really tractable small molecules. The evaluation of the synthesised series revealed a potent compound **7f** which was comparable with Isoniazid against $H_{37}Rv$. The next step of evaluation surprised us with the effectiveness of **7f** against 25 different isolates. The newer compound has shown promising anti-XDR and anti-MDR tuberculosis activity. Further attempts to study the toxophore of the compound are on and communicated short as the outcomes are available.

SN	Strain	MIC (μgml^{-1})	
		Isoniazid	7f
1	$H_{37}Rv$	0.03	0.015
2	TN675*	0.03	0.013
3	TN913	0.03	0.013
4	TN994*	0.03	0.03
5	TN1008	0.06	0.06
6	TN1037	0.03	0.06
7	TN1040	0.03	0.03
8	TN1051	0.03	0.06
9	TN1082	0.03	0.06
10	TN2351	0.06	0.25
11	TN2524	0.06	0.25
12	TN3183	0.03	0.06
13	TN3979	0.06	0.13
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

Table 2: **7f** drug susceptibilities for MTB (pan-susceptible and mono-rifampin resistant) clinical isolates.

Compound **7f** and Isoniazid drug susceptibilities were determined on 16 pan-susceptible and 2 mono-rifampin resistant (asterisk) clinical isolates.

SN	Strain	Drug Resistance	7fMIC (μgml^{-1})
1	TN565	R,S,EM,ET,K.Cl	0.06
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	0.06
6	TN772	I,R,EM	0.03
7	TN1195	I,S,EM	0.25
8	TN1314	I,R	0.03
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	0.13

Table 3: **7f** drug susceptibilities for MTB (MDR and poly-resistant) clinical isolates.

The **7f** 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.

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