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Pyridine and nitro-phenyl linked 1,3,4-thiadiazoles as MDR-TB inhibitors

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Graphical Abstract

Pyridine and Nitro-phenyl linked 1,3,4-thiadiazoles as MDR-TB Inhibitors

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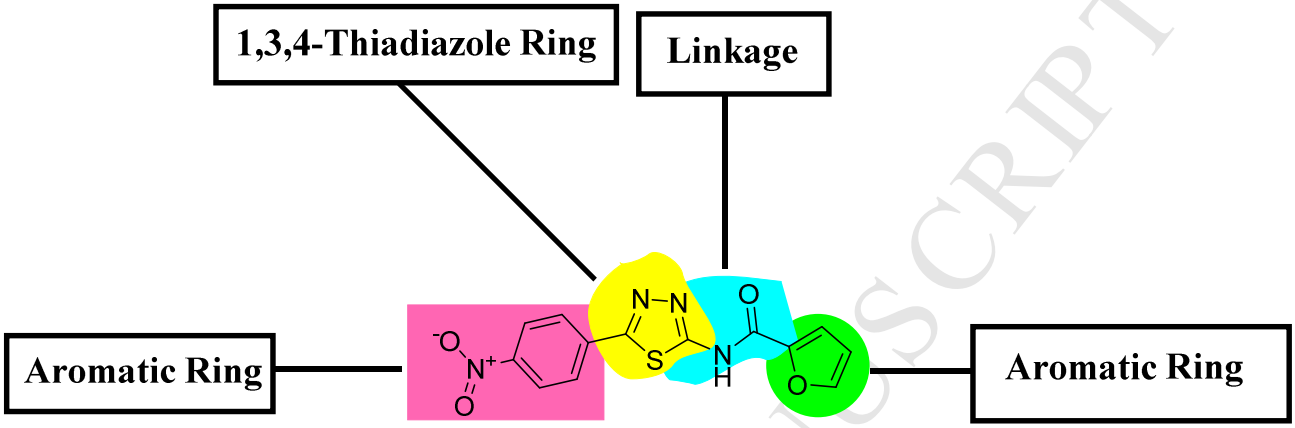

Compound 4H

H37rv and MDR-TB (MIC) = 9.87 µM

Pyridine and Nitro-phenyl linked 1,3,4-thiadiazoles as MDR-TB Inhibitors

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Abstract

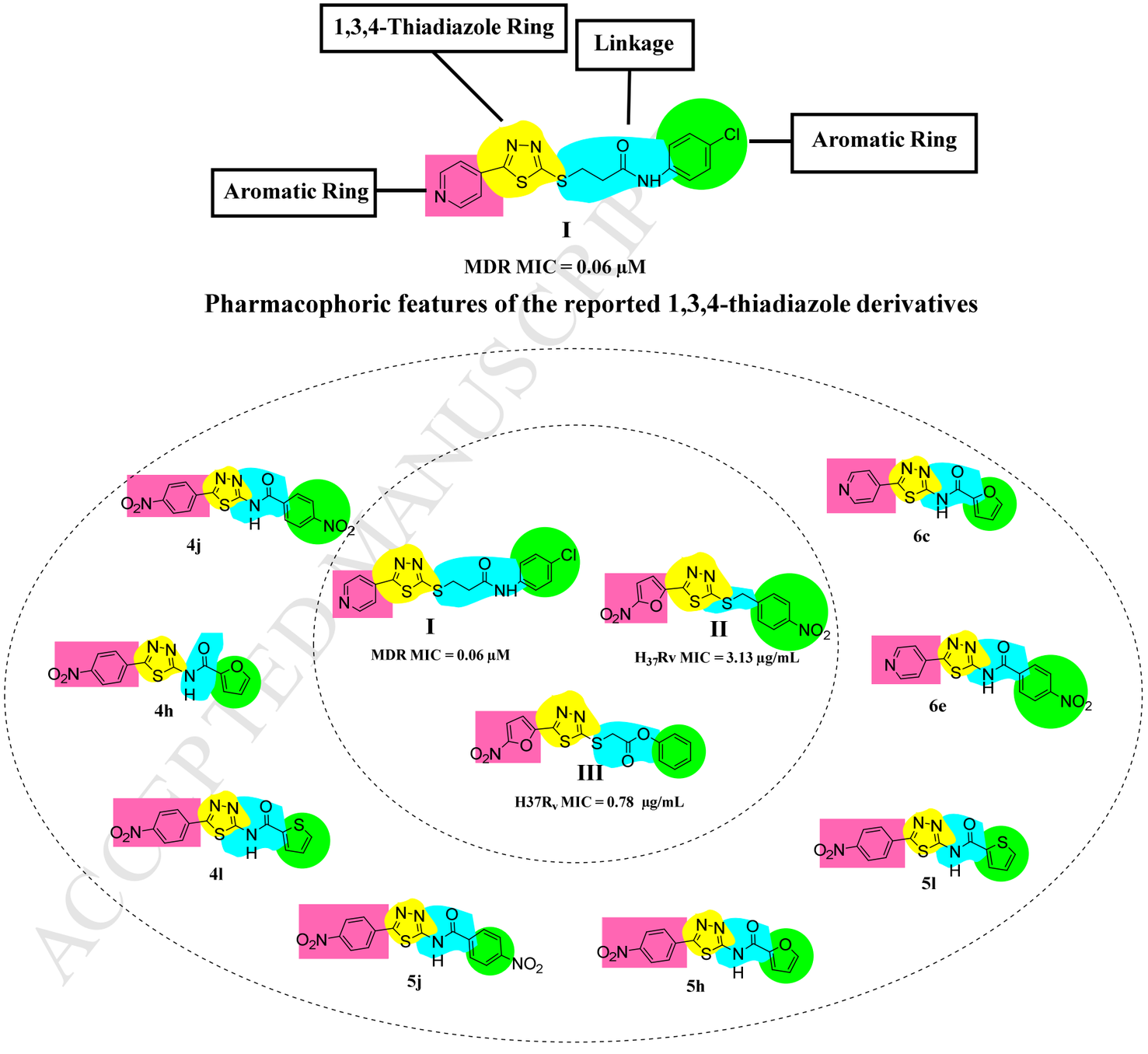
In the present study, a series of substituted 1,3,4-thiadiazole derivatives **4(a–o), 5(a-m)** and **6(a-j)** were synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic technique. The synthesized compounds were evaluated for their *in vitro* anti-mycobacterial activity against the *Mycobacterium tuberculosis* H37Rv and resistance MDR-TB strain. Among the compounds tested *N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)furan-2-carboxamide* (**4h**) showed significant inhibitory activity with MIC of 9.87 μM (H37Rv strain) and 9.87 μM (MDR-TB strain) compared to isoniazide [MIC of 3.64 μM (H37Rv) and > 200 μM (MDR-TB strain)] and rifampin [MIC of 0.152 μM (H37Rv) and 128 μM (MDR-TB strain)]. In addition, these compounds have also been assessed for their cyto-toxicity to a mammalian Vero cell line using the MTT assay. The result shows that these compounds exhibit anti-tubercular activity at non-cytototoxic concentrations.

Keywords: Synthesis;1,3,4-thiadiazole; MDR-TB; Cyto-toxicity

1. Introduction

Multi-drug-resistant tuberculosis (MDR-TB) is a tuberculosis infection caused by bacteria that is resistant to the most capable first-line anti-TB drugs, *i.e.* isoniazide and rifampin [1]. MDR-TB has been an issue of growing significance in the most recent decade. Regardless of its global significance, the problem has not been addressed effectively [2-5]. According to the WHO, about 33 percent of the 40 million people with HIV/AIDS are co-infected with *Mycobacterium tuberculosis* [6]. About 80 percent of TB cases are found in 23 countries; the highest occurrence rates are found in Africa and South-East Asia [7,8]. After the presentation of RIF in 1967, bedaquiline is the merely novel chemical compound developed for the treatment of TB that has reached the market, yet confined to the treatment of MDR and XDR-TB [9-11]. Shockingly, after its presentation in the clinical practice, the number of abnormal deaths has been accounted due to cardiac arrhythmia [12-13]. A nitroimidazole derivative delamanid: the mycolic acid biosynthesis inhibitor is another novel drug approved for MDR-TB by the European Medicines Agency (EMA) in 2014 [14]. When delamanid was used in combination with INH or fluoroquinolones, serious side effects such as cardiac arrhythmia and CNS toxicity were shown [15]. These findings have settled down the preliminary excitement as a result of the arrival of this novel anti-TB agent in therapy. Similarly, mutations inside Mtb genome causing resistance to bedaquiline and delamanide were recently reported [16]. Hence efforts are still needed to develop new anti-TB therapeutic alternatives, which are safe and effective for drug-resistant Mtb. The 1,3,4-thiadiazole and their derivatives are extensively recognized for their antimicrobial profile because of the occurrence of toxophoric N-C-S moiety, which reveals a wide range of biological activities [17-23]. Currently, there are numerous reports referring thiadiazoles as capacity anti-tubercular agent [24-31]. Roused by the references and in continuation of our research, we herein converse the synthesis and MDR TB-inhibitory report of 1,3,4-thiadiazoles.

According to the updated guidelines of the world health organization, the medications effective against MDR-TB are confined because only a limited selection of drugs are available; therefore, the development of novel or re-purposed drugs with activity against MDR is strongly desired [32]. The emergence of multidrug-resistant tuberculosis coupled with the increasing overlap of the AIDS and tuberculosis pandemics has brought tuberculosis to the forefront as a major global health concern. Number of 1,3,4-thiadiazoles have been reported for the anti-mycobacterial activity [18-25]. Mahajan et al., identified and reported a substituted pyridinyl-thiadiazole (**I**) to have MDR inhibitory activity of 0.06 µM [33]. Foroumadi et al., synthesized 2-(5-nitro-2-furyl)-and 2-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole derivatives and screened for their *in vitro* anti-mycobacterial activity against *Mycobacterium tuberculosis* H37Rv using alamar-blue susceptibility test [34]. Compounds with 5-nitro-2-furyl (**II**) have shown the highest activity against *M. tuberculosis* (MIC =3.13 µg/mL). Among nitrofuran derivatives, substitution of the thioester group at C-5 of 1,3,4-thiadiazole ring with a benzyl analog (**III**) was found to be the most active (MIC = 0.78 µg/mL) [35]. Among nitrofuran derivatives, thioester substitution at C-5 of 1,3,4-thiadiazole ring with benzyl analog (III) was found to be the most active (MIC=0,78 µg / mL).We deduce the common pharmacophore after studying these three potent compounds (**I**, **II** and **III**). The pharmacophoric features indicate that 1,3,4-thiadiazole should be linked with aromatic ring *via* a linker (in case of **Compound I**; its amidal linker). Other aromatic ring should be directly attached to the 5^{th} position of 1,3,4-thiadiazole. Presence of nitro group on either of aromatic ring is favourable for the anti-mycobacterial activity. Based upon this assumption, we designed our compounds as shown in **Fig.1**, where we incorporated all the pharmacophoric features.



Pharmacophoric features of the reported 1,3,4-thiadiazole derivatives

Fig. 1 Pharmacophoric features of the reported compounds and structural correlation between the reported and proposed compounds

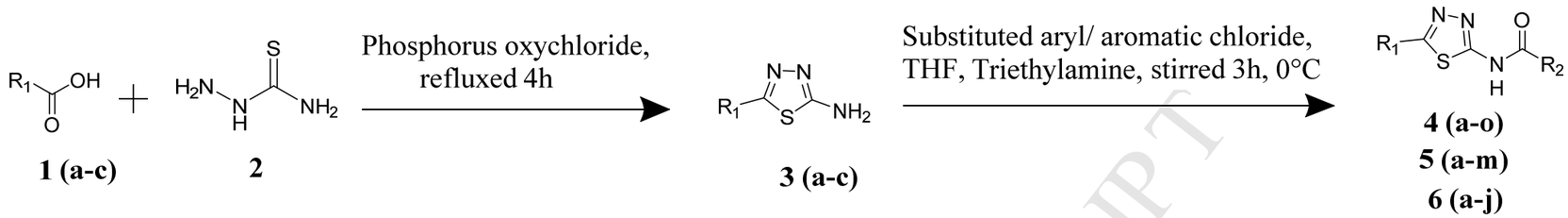
2. Result and Discussion

The desired compounds **4(a-o), 5(a-m)** and **6(a–j)** were synthesized in significant yield as given in **Scheme 1-3.** 5-substituted-1,3,4-thiadiazol-2-amine **3(a-c)** were synthesized according to the procedure specified in our earlier research work by the Oxidative cyclization mechanism [17]. These substituted 5-substituted-1,3,4-thiadiazol-2-amines **3(a-c)** were further reacted with different aryl and aliphatic carbonyl chlorides in THF with prolong stirring for 3 hrs at 0°C to get the preferred compounds by the Schotten-Baumann reaction mechanism.

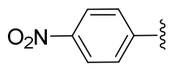
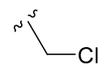
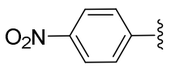
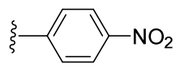
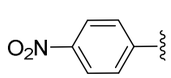
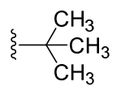
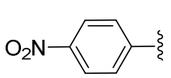
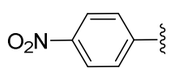
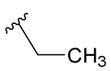
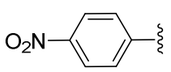
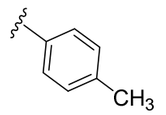
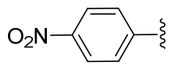
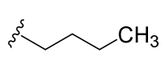
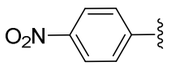
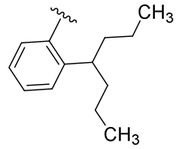
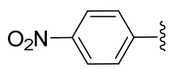
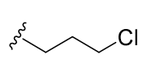
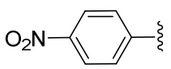
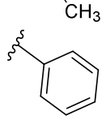
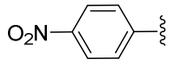
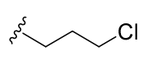
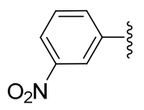
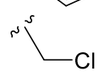
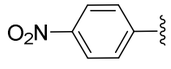
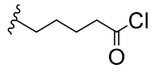
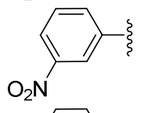
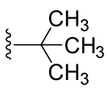
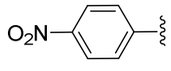
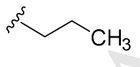
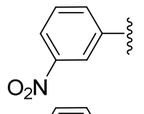
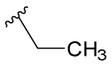
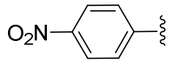
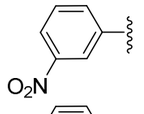
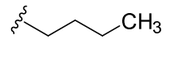
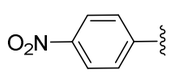
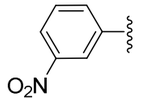
A series of nitro-phenyl and pyridine clubbed 1,3,4-thiadiazole derivatives **4(a–o), 5(a-m)** and **6(a-j)** have been synthesized in better yields using the synthetic route outlined in **Scheme 1-3**. Substituted 1,3,4-thiadiazol-2-amine (**3**) was prepared by reacting aromatic carboxylic acid (**1**) with thiosemicarbazide (**2**) in the presence of excess phosphorus oxychloride ($POCl_3$) by oxidative cyclization. Unreacted phosphorus oxychloride was eliminated by neutralizing with saturated NaOH solution. Substituted 1,3,4-thiadiazol-2-amine (**3**) was further reacted with different aryl and aliphatic carbonyl chlorides in tetrahydrofuran (THF) to obtain the preferred compounds **4(a–o), 5(a-m)** and **6(a-j)**. The structures of the synthesized final compounds were confirmed based on IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy data.

The IR spectrum of title compounds was confirmed by a single lobed absorption band at ~3400 cm^{-1} due to secondary amine (-NH-), which was further substantiated by the ^{1}H-NMR singlet at approximately δ 12.00 ppm. In ^{13}C NMR, the amidal carbonyl peak was observed at~170 ppm and it was further validated by the IR spectrum peak of the carbonyl group at ~1700 cm^{-1}, indicating the substitution of different aromatic/aliphatic carbonyl chloride on title compound.

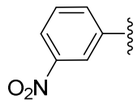
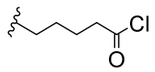
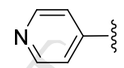
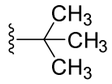
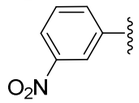
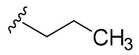
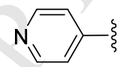
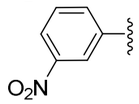
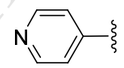
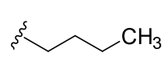
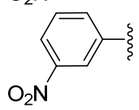
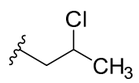
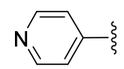
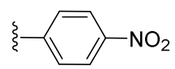
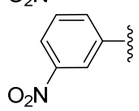
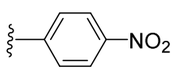
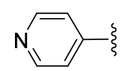
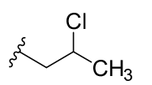
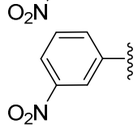
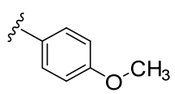
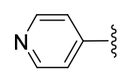
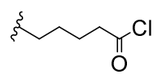
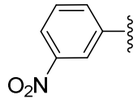
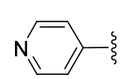
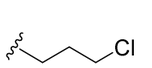
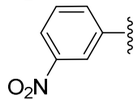
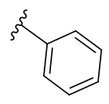
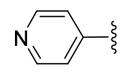
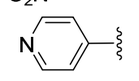
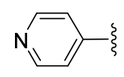
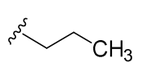
Scheme 1. Synthesis of compounds 4(a-o), 5(a-m) and 6(a-j)



1 (a-c) **2** **3 (a-c)** **4 (a-o)** **5 (a-m)** **6 (a-j)**

Compound Code	R_1	R_2	Yield	Compound Code	R_1	R_2	Yield
4a			64%	**4k**			52%
4b			42%	**4l**			72%
4c			80%	**4m**			78%
4d			81%	**4n**			65%
4e			81%	**4o**			60%
4f			81%	**5a**			64%
4g			75%	**5b**			50%
4h			50%	**5c**			78%
4i			50%	**5d**			70%
4j			36%	**5e**			87%

6

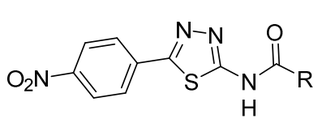
Compound Code	R_1	R_2	Yield	Compound Code	R_1	R_2	Yield
5f			75%	**6b**			75%
5g			50%	**6c**			55%
5h			76%	**6d**			64%
5i			81%	**6e**			75%
5j			62%	**6f**			51%
5k			69%	**6g**			54%
5l			65%	**6h**			74%
5m			75%	**6i**			68%
6a			75%	**6j**			76%

In this study a new series of pyridine and nitro phenyl linked 1,3,4-thiadiazole derivatives **4(a-o), 5(a-m)** and **6(a–j)** were synthesized and evaluated against *M. tuberculosis* and resistant phenotypic MDR-TB strain. The result of anti-mycobacterial and MDR-TB inhibitory activity is presented in **Table 1**. It is observed that the activity is significantly influenced by various substituents at the 2nd position of 1,3,4-thiadiazole. The tested compounds **4d, 4g, 4h, 4j, 4k, 4m, 5d, 5h, 5j** and **6e** displayed significant MDR inhibitory activity as shown in **Table 1**. Compound **4h** was found to be the potent compound of the series having MIC of 9.87 µM against the MDR-TB strain as compared to the standard isoniazid (> 200 µM).

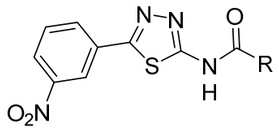
A concise analysis of the structure–activity reveals that the anti-mycobacterial activity is significantly influenced by different substituents on the thiadiazole ring. Electron withdrawing group on aliphatic side chain at 2nd position of 1,3,4-thiadiazole has diminishing effect on anti-mycobacterial and MDR inhibitory activity (**4i**, **5e**, **5f**, **5i**, **6g**, **6h**, **6i**) compared to the unsubstituted aliphatic side chain (**4c**, **4d**, **4g**, **5d**, **6d**). In case of aromatic substitution on 1,3,4-thiadiazole; nitro substitution favors activity followed by the methoxy group at para position (**4j**, **4k**, **5j**, **5k**, **6e**). In addition, we have seen that the heterocyclic furan ring has a significant contribution to anti-mycobacterial activity compared to the other compounds (**4h**, **5h**). The synthesized potent compounds of the series were further screened for cyto-toxicity (IC_{50}) in a mammalian Vero cell line (**Table 1**). All the tested derivatives showed lower toxicity with IC_{50} values >250 µM and none of the synthesized compounds displayed significant activity against the mammalian Vero cell line at concentrations <100 mM.

These outcomes are vital, as compounds with increased cytoliability are appealing in the development of new chemical entities for the treatment of tuberculosis. This is mainly because the management of tuberculosis requires extensive course of drug treatment, leading to a number of side-effects with a high margin of safety.

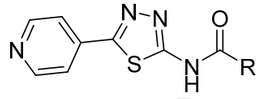
Table 1. Anti-mycobacterial and MDR inhibitory profile of synthesized compound



4(a-o)



7(a-m)



10(a-j)

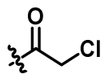
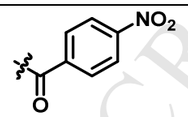
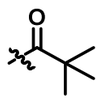
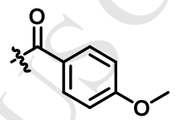
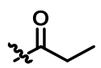
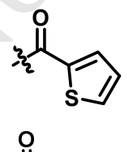
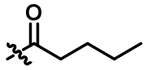
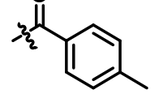
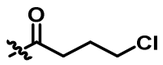
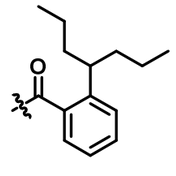
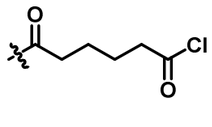
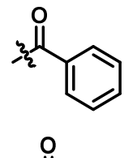
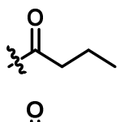
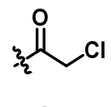
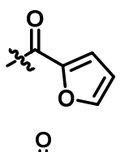
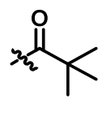
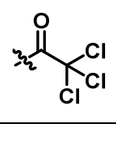
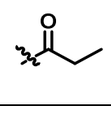
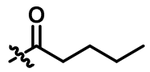
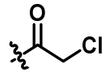
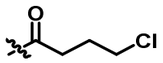
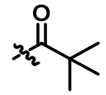
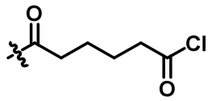
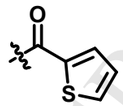
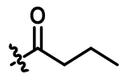
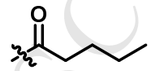
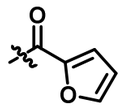
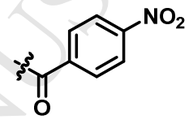
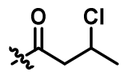
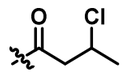
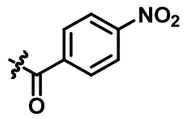
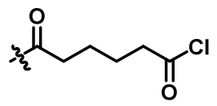
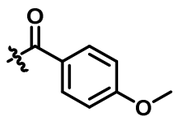
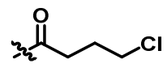
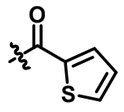
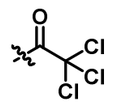
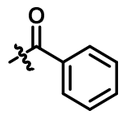
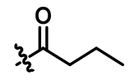
Compound Code	R	H37rv MIC (µM)	MDR-TB MIC (µM)	Cyto-toxicity IC_{50} (µM)	LogP	Compound Code	R	H37rv MIC (µM)	MDR-TB MIC (µM)	Cyto-toxicity IC_{50} (µM)	LogP
4a		168 ± 6.24	> 200	---	2.32	**4j**		16.84 ± 2.67	67 ± 3.12	290 ± 6.34	1.76
4b		204 ± 5.23	> 200	---	3.41	**4k**		35 ± 2.98	140 ± 4.12	278 ± 5.74	2.22
4c		89 ± 3.23	> 200	---	2.59	**4l**		> 200	> 200	---	3.32
4d		40 ± 3.45	163 ± 6.34	278 ± 6.64	3.65	**4m**		18.38 ± 3.78	147 ± 4.76	310 ± 6.24	3.87
4e		153 ± 3.86	> 200	---	2.86	**4n**		117 ± 4.34	> 200	---	4.46
4f		135 ± 3.75	271 ± 5.23	---	3.75	**4o**		38 ± 3.86	306 ± 7.12	---	3.42
4g		42 ± 3.45	171 ± 4.23	301 ± 5.89	3.15	**5a**		21 ± 3.65	> 200	---	2.29
4h		9.87 ± 1.56	9.87 ± 1.12	306 ± 7.21	2.68	**5b**		204 ± 6.23	> 200	---	3.39
4i		273 ± 5.34	> 200	---	3.56	**5c**		89 ± 4.12	> 200	---	2.56

Table 1. Continue….

Compound Code	R	H37rv MIC (µM)	MDR-TB MIC (µM)	Cyto-toxicity IC_{50} (µM)	LogP	Compound Code	R	H37rv MIC (µM)	MDR-TB MIC (µM)	Cyto-toxicity IC_{50} (µM)	LogP
5d		40 ± 2.86	163 ± 3.23	289 ± 5.34	3.65	**6a**		196 ± 4.12	> 200	---	1.07
5e		153 ± 4.32	> 200	---	2.82	**6b**		238 ± 3.15	> 200	---	2.17
5f		135 ± 3.68	> 200	---	3.72	**6c**		21 ± 1.76	> 200	---	1.43
5g		42 ± 4.76	> 200	---	3.12	**6d**		95 ± 2.10	381 ± 5.21	---	2.40
5h		19 ± 2.87	39 ± 2.43	312 ± 6.23	2.66	**6e**		9.93 ± 1.12	76 ± 2.10	369 ± 6.23	2.13
5i		> 200	> 200	---	2.92	**6f**		> 200	> 200	---	1.70
5j		33 ± 2.87	134 ± 2.86	268 ± 7.23	3.36	**6g**		192 ± 3.78	> 200	---	2.50
5k		35 ± 2.98	280 ± 4.75	---	3.46	**6h**		221 ± 3.86	> 200	---	1.61
5l		301 ± 6.87	> 200	---	3.30	**6i**		> 200	> 200	---	2.31
5m		38 ± 2.76	> 200	---	3.40	**6j**		201 ± 4.65	> 200	---	1.90
Isoniazid	---	3.64 ± 0.68	> 200	---	-0.7	**Staurosporine**	---	---	---	0.028 ± 0.008	---
Rifampin	---	0.152 ± 0.09	128 ± 2.86	---	2.7						
Staurosporine	---	---	---	0.028 ± 0.008							

The inhibitory activity (MIC_{50}) was determined against *M. tuberculosis* H37Rv and clinically isolated phenotype MDR-TB strain. Assay were carried out at least three times n = 3

LogP value is calculated using ChemBioDraw 14

3. Conclusion

In the current research paper, we detailed synthesis and anti-mycobacterial activity of a new series of sustituted-1,3,4-thiadiazoles. The *in vitro* results of the anti-mycobacterial activities against resistant MDR strain suggest that the presence of furoyl substitution on 1,3,4-thiadiazole *N*-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)furan-2-carboxamide (**4h**) has demonstrated the significant inhibitory activity with MIC of 9.87 µM against MTB-H_{37}Rv and MDR-TB respectively as compared to the isoniazid and rifampin. Structure–activity reveals that the electron withdrawing group on aliphatic side chain at 2^{nd} position of 1,3,4-thiadiazole has diminishing effect on the anti-mycobacterial and MDR inhibitory activity (**4i**, **5e**, **5f**, **5i**, **6g**, **6h**, **6i**) as compared to the unsubstituted aliphatic side chain (**4c**, **4d**, **4g**, **5d**, **6d**). In case of aromatic substitution on 1,3,4-thiadiazole; nitro substitution favors the activity followed by the methoxy groups at para position (**4j**, **4k**, **5j**, **5k**, **6e**). We additionally have seen that among the heterocyclic substitution furan ring has the significant contribution on anti-mycobacterial activity as compared to the others (**4h**, **5h**). The synthesized compounds, which demonstrated significant anti-mycobacterial activity, additionally evaluated for their cyto-toxicity (IC_{50}) against the mammalian Vero cell line, utilizing the MTT assay. The outcomes demonstrated that these compounds confirmed anti-mycobacterial activity at non-cytotoxic level. In conclusion, compound **4h** was found to be the most potent compound of the series and could be exploited further for developing safe antimicrobial agents by slight modifications at second position of 1,3,4-thiadiazole ring and/or extensive additional functionalization that necessitate further investigations.

4. Experimental

All the solvents and chemicals have been provided by Spectrochem and Sigma-Aldrich. The reactions were supervised with the aid of pre-coated silica gel TLC aluminium sheets. Melting points were established using an Analab Scientific Melting point apparatus. FTIR spectrum was documented utilizing FTIR-8400S Shimadzu spectrometer. ^{1}HNMR (DMSO/$CDCl_3$) spectra of

the compounds have been determined by Bruker Avance-II spectrometer at 400 MHz (Punjab University-Chandigarh). Chemical shift were assessed relative to the internal standard TMS and are reported in δ ppm. Mass spectra of the compounds were determined at Oxygen Heath Care Pvt.Ltd.at Ahmadabad, Gujarat.

4.1. Preparation of 5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (3a)

This compound is prepared as per the method given by Patel et al [17].

4.2. General procedure for the synthesis of (5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)substituted carbonyl chloride 4(a-o)

An equimolar amount of 5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine **(3a)** dissolve in 10-15 mL of tetrahydrofuran (THF), add 2-3 drops of triethylamine, to this solution add 1.5 mole different aryl chlorides gradually, continuous stirring for 3 hrs on ice bath (0°C). After completion of reaction solid will precipitate out, washed with sodium bicarbonate solution to remove excess of chloride and recrystallized from ethanol to get pure compound.

4.2.1. 2-Chloro-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide (4a)

64% yield; mp 184-186°C; IR (KBr) ν_{max}: 3283 (N-H stretch), 3045 (Arom.CH strech), 2956 (Alip.CH strech), 1698 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.51(s, 1H, -NH), 7.79 (d, 2H, J = 8.8 Hz, Ar-H), 8.27 (d, 2H, J = 8.8 Hz, Ar-H), 4.29 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 170.9, 169.2, 153.1, 148.5, 136.0, 124.1, 125.8, 45.7; HRMS (ESI) m/z calcd. $C_{10}H_7ClN_4O_3S$: 297.9927; found: 297.9923 [Mass Fragments: 223, 297].

4.2.2. N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)pivalamide (4b)

42% yield; mp 156-158 °C; IR (KBr) ν_{max}: 3296 (N-H stretch), 2924 (Arom.CH strech), 2856 (Alip.CH strech), 1658 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.58(s, 1H, -NH), 7.69 (d, 2H, J = 8.8 Hz, Ar-H), 8.36 (d, 2H, J = 8.8 Hz, Ar-H), 1.29 (s, 9H, CH_3); ^{13}C NMR (DMSO-d_6) δ 177.3, 170.2, 154.2, 148.4, 135.9, 128.7, 125.1, 45.7, 26.9; HRMS (EI) m/z calcd. $C_{13}H_{14}N_4O_3S$: 306.0787; found: 329.0660 [M+Na^+] [Mass Fragments: 223, 329].

4.2.3. N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)propionamide (4c)

80% yield; mp 219-221°C; IR (KBr) ν_{max}: 3352 (N-H stretch), 3008 (Arom.CH strech), 2956 (Alip.CH strech), 1698 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.55(s, 1H, -NH), 7.76 (d, 2H, J = 8.8 Hz, Ar-H), 8.21 (d, 2H, J = 8.8 Hz, Ar-H), 2.29 (q, 2H, J = 7.4, CH_2), 1.45 (t, 3H, J = 7.4 CH_3); ^{13}C NMR (DMSO-d_6) δ 169.8, 178.4, 154.2, 149.7, 137.9, 128.9, 123.2, 31.7, 10.0; HRMS (ESI) m/z calcd. $C_{11}H_{10}N_4O_3S$: 278.0474; found: 278.0479 [Mass Fragments: 223, 278].

4.2.4. N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)pentanamide (4d)

81% yield; mp 172-174°C; IR (KBr) ν_{max}: 3292 (N-H stretch), 3139 (Arom.CH strech), 2946 (Alip.CH strech), 1664 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.40(s, 1H, -NH), 7.70 (d, 2H, J = 8.8 Hz, Ar-H), 8.39 (d, 2H, J = 8.8 Hz, Ar-H), 2.29 (m, 2H, CH_2), 1.38-1.55 (m, 4H, CH_2 Aliphatic chain), 1.05 (t, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 171.7, 170.03, 153.1, 147.8, 140.2, 137.9,130.2, 38.3, 31.7, 21.0, 14.0; HRMS (ESI) m/z calcd. $C_{13}H_{14}N_4O_3S$: 306.0787; found: 306.0795 (86.0732, 223.0133, 306.0795) [Mass Fragments: 223, 306].

4.2.5. 4-Chloro-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)butanamide (4e)

81% yield; mp 164-166°C; IR (KBr) ν_{max} : 3251 (N-H stretch), 3125 (Arom.CH strech), 2986 (Alip.CH strech), 1674 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.40(s, 1H, -NH), 7.56 (d, 2H, J = 8.8 Hz, Ar-H), 8.40 (d, 2H, J = 8.8 Hz, Ar-H), 3.50 (t, 2H, J = 7.4 Hz, CH_2), 2.38 (t, 2H, J = 7.4 Hz, CH_2) 1.55 (m, 2H, CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 174.0, 173.0, 153.1, 147.8, 137.9, 128.9, 124.7, 47.1, 33.7, 26.0; HRMS (ESI) m/z calcd. $C_{12}H_{11}ClN_4O_3S$: 326.0240; found: 326.0248 [Mass Fragments: 223, 326].

4.2.6. 6-((5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl)amino)-6-oxohexanoyl chloride (4f)

75% yield; mp 159-161°C; IR (KBr) ν_{max} : 3321 (N-H stretch), 3078 (Arom.CH strech), 2956 (Alip.CH strech), 1697 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.45(s, 1H, -NH), 7.90 (d, 2H, J = 8.8 Hz, Ar-H), 8.40 (d, 2H, J = 8.8 Hz, Ar-H), 2.82 (t, 2H, J = 7.4 Hz, CH_2), 2.38 (t, 2H, J =

7.4 Hz, CH_2) 1.55 (t, 2H, *J* = 7.4 Hz, CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 172.8, 171.9, 152.7, 147.5, 137.9, 128.0, 126.2, 124.9, 46.8, 35.9, 26.9, 24.8; HRMS (ESI) m/z calcd. $C_{14}H_{13}ClN_4O_3S$: 368.0346; found: 368.0353 [Mass Fragments: 223, 368].

4.2.7. N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)butyramide (4g)

50% yield; mp 239-241°C; IR (KBr) ν_{max} : 3464 (N-H stretch), 3115 (Arom.CH strech), 2992 (Alip.CH strech), 1664 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.40(s, 1H, -NH), 7.70 (d, 2H, *J* = 8.8 Hz, Ar-H), 8.30 (d, 2H, *J* = 8.8 Hz, Ar-H), 2.29 (t, 2H, *J* = 7.4 Hz, CH_2), 1.45 (m, 2H, CH_3), 1.01 (t, 3H, *J* = 7.4 Hz, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.8, 178.4, 154.2, 149.7, 137.9, 128.9, 128.5, 123.2, 31.7, 10.0; HRMS (ESI) m/z calcd. $C_{12}H_{12}N_4O_3S$: 292.0630; found: 292.0638 [Mass Fragments: 223, 292].

4.2.8. N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)furan-2-carboxamide (4h)

50% yield; mp 220-222°C; IR (KBr) ν_{max} : 3153 (N-H stretch), 2920 (C-H Strech), 1701 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.92 (s, 1H, NH), 6.74 (1H, t, *J* = 7.5 Hz, Furoyl), 7.37 (1H, d, *J* = 7.5, 0.9 Hz, Furoyl), 8.03 (1H, d, *J* = 7.8, 0.9 Hz, Furoyl), 8.23 (d, 2H, *J* = 8.8 Hz, Ar-H), 8.24 (d, 2H, *J* = 8.8 Hz, Ar-H); ^{13}C NMR (DMSO-d_6) δ 171.3, 160.1, 149.8, 148.6, 143.3, 140.1, 128.3, 124.9, 116.3, 111.3; HRMS (ESI) m/z $C_{13}H_8N_40_4S$: 316.0266; found: 317.0272 [M+1] [Mass Fragments: 223, 291, 317].

4.2.9. 2,2,2-Trichloro-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide (4i)

36% yield; mp 151-153°C; IR (KBr) ν_{max} : 3367 (N-H stretch), 3018 (Arom.CH strech), 2909 (Alip.CH strech), 1697 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.70(s, 1H, -NH), 7.84 (d, 2H, *J* = 8.8 Hz, Ar-H), 8.34 (d, 2H, *J* = 8.8 Hz, Ar-H); ^{13}C NMR (DMSO-d_6) δ 175.4, 160.2, 154.29, 148.4, 135.9, 128.0, 125.9, 94.2; HRMS (EI) m/z calcd. $C_{10}H_5Cl_3N_4O_3S$: 365.9148; found: 365.9154 [Mass Fragments: 223, 365]

4.2.10. 4-Nitro-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (4j)

52% yield; mp 240-242°C; IR (KBr) ν_{max} : 3354 (N-H stretch), 3034 (Arom.CH strech), 2911 (Alip.CH strech), 1707 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.50 (s, 1H, -NH), 8.24-8.39 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ 174.5, 164.2, 152.7, 147.2, 138.5, 134.3, 132.67, 130.5, 129.8, 128.4, 124.3; HRMS (ESI) m/z calcd. $C_{15}H_9N_5O_5S$: 371.0324; found: 371.0330 [Mass Fragments: 223, 371]

4.2.11. 4-Methoxy-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (4k)

65% yield; mp 224-226°C; IR (KBr) ν_{max} : 3287 (N-H stretch), 3134 (Arom.CH strech), 2957 (Alip.CH strech), 1687 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.69 (s, 1H, -NH), 7.08-8.27 (m, 8H, Ar-H), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 174.0, 165.2, 164.3, 151.3, 148.9, 134.2, 132.5, 130.8, 129.8, 124.3, 114.2, 55.6 ; HRMS (ESI) m/z calcd. $C_{18}H_{12}N_4O_4S$: 356.0579; found: 356.0584 [Mass Fragments: 223, 356]

4.2.12. N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)thiophene-2-carboxamide (4l)

72% yield; mp 247-249°C; IR (KBr) ν_{max} : 3291 (N-H stretch), 2971 (C-H Strech),1691 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.64 (s, 1H, NH), 8.01-8.40 (m, 4H, Ar-CH), 7.20-7.98 (m, 3H, Ar-CH thiophene); ^{13}C NMR (DMSO-d_6) δ 174.3, 161.0, 152.7, 148.6, 150.3, 146.7, 139.5, 138.9, 132.9, 131.2, 130.3, 128.4, 124.9; HRMS (ESI) m/z $C_{13}H_8N_4O_3S_2$: 332.0038; found: 332.0038 [Mass Fragments: 223, 332]

4.2.13. 4-Methyl-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (4m)

78% yield; mp 169-171°C; IR (KBr) ν_{max} : 3297 (N-H stretch), 3084 (Arom.CH strech), 2948 (Alip.CH strech), 1690 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.55 (s, 1H, -NH), 7.24-8.39 (m, 8H, Ar-H), 2.41 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 174.0, 165.2, 151.3, 148.9, 139.5, 131.7, 130.2, 129.7, 128.3, 129.8, 124.3, 21.5; HRMS (ESI) m/z calcd. $C_{18}H_{12}N_4O_3S$: 340.0630; found: 340.0638 [Mass Fragments: 223, 340]

4.2.14. 2-(Heptan-4-yl)-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (4n)

65% yield; mp 163-165°C; IR (KBr) ν_{max} : 3185 (N-H stretch), 3014 (Arom.CH strech), 2950 (Alip.CH strech), 1690 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.73 (s, 1H, -NH), 8.24-7.11 (m, 8H, Ar-H), 1.54-2.58 (m, 15H, CH aliphatic branch); ^{13}C NMR (DMSO-d_6) δ 174.0, 165.5, 152.2, 148.2, 147.0, 145.9, 138.5, 139.7, 137.7, 130.2,128.3, 129.1, 124.9, 41.8, 38.9, 20.9, 14.3; HRMS (ESI) m/z calcd. $C_{22}H_{24}N_4O_3S$: 424.1569; found: 424.1574 [Mass Fragments: 223, 424]

4.2.15. N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (4o)

60% yield; mp 129-131°C; IR (KBr) ν_{max} : 3227.92 (N-H stretch), 3024.01 (Arom.CH strech), 2961.59 (Alip.CH strech), 1695.96 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.78 (s, 1H, -NH), 7.54-8.27 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ 174.1, 165.7, 152.4, 147.0, 142.3, 139.2, 132.3, 130.2, 129.6, 128.5, 129.9; HRMS (ESI) m/z calcd. $C_{15}H_{10}N_4O_3S$: 326.0474; found: 326.0480 [Mass Fragments: 223, 326]

4.3. Preparation of 5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine (3b)

This compound is prepared as per the method given by Patel et al [17].

4.4. General procedure for the synthesis of N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl) substituted carbonyl chloride 5(a-m)

An equimolar amount of 5-(3-nitrophenyl)-1,3,4-thiadiazol-2-amine **(3b)** dissolve in 10-15 mL of tetrahydrofuran (THF), add 2-3 drops of triethylamine, to this solution add 1.5 mole different aryl chlorides gradually, continuous stirring for 3 hrs on ice bath(0°C). After completion of reaction solid will precipitate out, washed with sodium bicarbonate solution to remove excess of chloride and recrystallized from ethanol to get pure compound

4.4.1. 2-Chloro-N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide (5a)

64% yield; mp 192-194°C; IR (KBr) ν_{max} : 3283 (N-H stretch), 3038 (Arom.CH strech), 2956 (Alip.CH strech), 1698 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.51(s, 1H, -NH), 7.59 (t, 1H, J = 8.3 Hz, Ar-H), 8.05 (d, 1H, J = 8.3 Hz, Ar-H), 8.19 (d, 1H, J = 8.3 Hz, Ar-H), 8.52 (s, 1H,

Ar-H), 4.29 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 170.9, 169.2, 153.1, 148.5, 136.0, 129.7, 128.5, 124.1, 125.8, 45.7; HRMS (ESI) m/z calcd. $C_{10}H_7ClN_4O_3S$: 297.9927; found: 297.9933 [Mass Fragments: 223, 297].

4.4.2. N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)pivalamide (5b)

50% yield; mp 162-164°C; IR (KBr) ν_{max} : 3253 (N-H stretch), 3038 (Arom.CH strech), 2909 (Alip.CH strech), 1658 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.51(s, 1H, -NH), 7.50 (t, 1H, J = 8.3 Hz, Ar-H), 8.10 (d, 1H, J = 8.3 Hz, Ar-H), 8.21 (d, 1H, J = 8.3 Hz, Ar-H), 8.42 (s, 1H, Ar-H), 1.29 (s, 9H, CH_3); ^{13}C NMR (DMSO-d_6) δ 177.3, 169.2, 154.2, 148.5, 135.9, 128.8, 128.0, 125.9, 125.9, 39.7, 26.9; HRMS (EI) m/z calcd. $C_{13}H_{14}N_4O_3S$: 306.0787; found: 306.0794 [Mass Fragments: 223, 306].

4.4.3. N-(5-(3-Nitrophenyl)-1,3,4-thiadiazol-2-yl)propionamide (5c)

78% yield; mp 168-170°C; IR (KBr) ν_{max} : 3275 (N-H stretch), 3022 (Arom.CH strech), 2922 (Ali C-H Strech),1691 (amide C=O); ^{1}H NMR (DMSO-d_6) 12.58 (s, 1H, NH), 8.04 (t, 1H, J = 8.3 Hz, Ar-H), 8.21 (d, 1H, J = 8.3 Hz, Ar-H), 8.24 (s, 1H, Ar-H), 8.31 (d, 1H, J = 8.3 Hz, Ar-H), 3.07(q, 2H, J = 7.4 Hz,CH_2), 1.29 (t, 3H, J = 7.4 Hz,CH_3); ^{13}C NMR (DMSO-d_6) δ 170.4, 154.5, 148.4, 132.9, 133.3, 131.6, 124.0, 121.9, 120.9, 30.5, 10.8; HRMS (EI) m/z calcd. $C_{11}H_{10}N_4O_3S$: 278.0474; found 279.0480 [Mass Fragments: 223, 278].

4.4.4. N-(5-(3-Nitrophenyl)-1,3,4-thiadiazol-2-yl)pentanamide (5d)

70% yield; mp 202-204 °C; $C_{13}H_{14}N_4O_3S$ °C; IR (KBr) ν_{max} : 3290 (N-H stretch), 3045 (Arom.CH strech), 2974 (Alip.CH strech), 1694 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.40 (s, 1H, -NH), 7.60 (t, 1H, J = 8.3 Hz, Ar-H), 7.90 (d, 1H, J = 8.3 Hz, Ar-H), 8.01 (d, 1H, J = 8.3 Hz, Ar-H), 8.31 (s, 1H, Ar-H), 1.40-2.30 (m, 9H, CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 171.7, 169.0, 153.1, 147.8, 137.9,133.2, 132.6, 130.8, 128.9, 31.7, 27.2, 21.0, 14.0; HRMS (ESI) m/z calcd. $C_{13}H_{14}N_4O_3S$: 306.0787; found: 306.0794 [Mass Fragments: 223, 306].

4.4.5. 4-Chloro-N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)butanamide (5e)

87% yield; mp 218-220°C; IR (KBr) ν_{max} : 3261 (N-H stretch), 3035 (Arom.CH strech), 2966 (Alip.CH strech), 1676 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.75 (s, 1H, -NH), 7.62 (t, 1H, *J* = 8.3 Hz, Ar-H), 8.05 (d, 1H, *J* = 8.3 Hz, Ar-H), 8.18 (d, 1H, *J* = 8.3 Hz, Ar-H), 8.40 (s, 1H, Ar-H), 3.50 (t, 2H, *J* = 7.4 Hz, CH_2 Aliphatic chain), 2.38 (t, 2H, *J* = 7.4 Hz, CH_2 Aliphatic chain) 1.55 (p, 2H, *J* = 7.4 Hz, CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 174.8, 172.0, 153.4, 147.8, 135.9, 132.6, 130.7, 128.9, 124.7, 47.1, 33.8, 26.7 ; HRMS (ESI) m/z calcd. $C_{12}H_{11}ClN_4O_3S$: 326.0240; found: 326.0248 [Mass Fragments: 223, 326].

4.4.6. 6-((5-(3-Nitrophenyl)-1,3,4-thiadiazol-2-yl)amino)-6-oxohexanoyl chloride (5f)

75% yield; mp 132-134°C; IR (KBr) ν_{max} : 3221 (N-H stretch), 3082 (Arom.CH strech), 2982 (Alip.CH strech), 1687 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.44 (s, 1H, -NH), 7.70 (t, 1H, *J* = 8.3 Hz, Ar-H), 7.98 (d, 1H, *J* = 8.3 Hz, Ar-H), 8.14 (d, 1H, *J* = 8.3 Hz, Ar-H), 8.28 (s, 1H, Ar-H), 1.55-2.38 (m, 8H, CH_2-CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 173.8, 172.9, 152.2, 147.7, 137.4,134.8, 132.8, 131.9, 130.1, 128.1, 125.1, 46.7, 35.3, 26.8, 24.3; HRMS (ESI) m/z calcd. $C_{14}H_{13}ClN_4O_3S$: 368.0346; found: 368.0354 [Mass Fragments: 223, 368].

4.4.7. N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)butyramide (5g)

50% yield; mp 144-146°C; IR (KBr) ν_{max} : 3495 (N-H stretch), 3078 (Arom.CH strech), 2996 (Alip.CH strech), 1669 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.40(s, 1H, -NH), 7.69 (t, 1H, *J* = 8.3 Hz, Ar-H), 7.92 (d, 1H, *J* = 8.3 Hz, Ar-H), 8.12 (d, 1H, *J* = 8.3 Hz, Ar-H), 8.38 (s, 1H, Ar-H), 3.83 (t, 2H, 7.4 Hz, CH_2), 1.45-2.13 (m, 5H, CH_3-CH_2) ; ^{13}C NMR (DMSO-d_6) δ 169.8, 177.6, 155.2, 149.7, 137.9,132.8, 128.9, 128.5, 123.2, 31.7, 20.1, 10.0; HRMS (ESI) m/z calcd. $C_{12}H_{12}N_4O_3S$: 292.0630; found: 292.0638 [Mass Fragments: 223, 292].

4.4.8. N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)furan-2-carboxamide (5h)

76% yield; mp 268-270°C; IR (KBr) ν_{max} : 3263 (N-H stretch), 2996 (C-H Strech),1761 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.75 (s, 1H, NH), 7.37-8.11 (m, 7H, Ar-CH); ^{13}C NMR (DMSO-d_6) δ 170.5, 164.2, 159.1, 152.1, 148.8, 148.6, 137.1, 130.1, 128.3, 126.2, 124.9, 116.3, 111.3 ; HRMS (ESI) m/z $C_{13}H_8N_40_4S$: 316.0266; found: 316.0274 [Mass Fragments: 223, 316].

4.4.9. 3-Chloro-N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)butanamide (5i)

81% yield; mp 139-141°C; IR (KBr) ν_{max} : 3394 (N-H stretch), 3045 (Arom.CH strech), 2985 (Alip.CH strech), 1694 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.40(s, 1H, -NH), 7.72 (t, 1H, J = 8.3 Hz, Ar-H), 8.12 (d, 1H, J = 8.3 Hz, Ar-H), 8.28 (d, 1H, J = 8.3 Hz, Ar-H), 8.35 (s, 1H, Ar-H), 1.45-3.01 (m, 6H, CH_2) ; ^{13}C NMR (DMSO-d_6) δ 178.4, 169.8, 154.2, 149.7, 137.9,130.2, 129.8, 128.9, 128.5, 123.2, 40.1, 31.7, 10.0; HRMS (ESI) m/z calcd. $C_{12}H_{12}N_4O_3S$: 326.0240; found: 326.0248 [Mass Fragments: 223, 326].

4.4.10. 4-Nitro-N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (5j)

62% yield; mp 222-224°C; IR (KBr) ν_{max} : 3394 (N-H stretch), 3035 (Arom.CH strech), 2911 (Alip.CH strech), 1684 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.50 (s, 1H, -NH), 7.23-8.29 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ 174.5, 166.2, 164.2, 158.1, 152.7, 148.2, 147.2, 140.2, 138.5,136.7,129.8,124.3,122.1;HRMS (ESI) m/z calcd. $C_{15}H_9N_5O_5S$: 371.0324; found: 371.0332 [Mass Fragments: 223, 371].

4.4.11. 4-Methoxy-N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (5k)

69% yield; mp 217-219°C; IR (KBr) ν_{max} : 3287(N-H stretch), 3034 (Arom.CH strech), 2957 (Alip.CH strech), 1687 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.69 (s, 1H, -NH), 7.08-8.27 (m, 8H, Ar-H), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 174.0, 165.2, 164.3, 151.3,149.8, 148.9,146.2,143.1,140.3,132.7,129.8, 124.3, 114.2, 55.6; HRMS (ESI) m/z calcd. $C_{18}H_{12}N_4O_4S$: 356.0579; found: 356.0584 [Mass Fragments: 223, 356].

4.4.12. N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)thiophene-2-carboxamide (5l)

65% yield; mp 298-300°C; IR (KBr) ν_{max} : 3281 (N-H stretch), 2991 (C-H Strech),1709 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.64 (s, 1H, NH), 7.98-8.40 (m, 7H, Ar-CH); ^{13}C NMR (DMSO-d_6) δ 174.3, 161.0, 152.7, 148.6, 139.5, 138.2, 136.1, 135.7, 134.1, 132.7, 131.2, 128.4, 124.9; HRMS (ESI) m/z $C_{13}H_8N_4O_3S_2$: 332.0038; found: 332.0046 [Mass Fragments: 223, 332].

4.4.13. N-(5-(3-nitrophenyl)-1,3,4-thiadiazol-2-yl)benzamide (5m)

75% yield; mp 263-265°C; IR (KBr) ν_{max} : 3395 (N-H stretch), 3093(Arom.CH strech), 2955 (Alip.CH strech), 1662 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.55 (s, 1H, -NH), 7.28-8.42 (m, 9H, Ar-H), ^{13}C NMR (DMSO-d_6) δ 174.0, 165.2, 151.3, 148.9, 144.1, 142.6, 140.1, 139.5, 136.8, 134.2, 131.7, 128.3, 129.8; HRMS (ESI) m/z calcd. $C_{18}H_{12}N_4O_3S$: 326.0474; found: 326.0480 [Mass Fragments: 223, 326].

4.5. Preparation of 5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine (3c)

This compound is prepared as per the method given by Patel et al [17].

4.6. General procedure for the synthesis of N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl) substituted carbonyl choloride 6(a-j)

An equimolar amount of of 5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine (***3c)*** dissolve in 10-15 mL of tetrahydrofuran (THF), add 2-3 drops of triethylamine, to this solution add 1.5 mole different aryl chlorides gradually, continuous stirring for 3 hrs on ice bath(0°C). After completion of reaction solid will precipitate out, washed with sodium bicarbonate solution to remove excess of chloride and recrystallized from ethanol to get pure compound

4.6.1. 2-Chloro-N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)acetamide (6a)

75% yield; mp 138-140°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max} : 3345 (N-H stretch), 3053 (Arom.CH strech), 2945 (Alip.CH strech), 1692 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.80 (s, 1H, NH), 8.14 (d, 2H, J = 5.2 Hz, Ar-H), 8.63 (d, 2H, J = 5.2, Hz, Ar-H), 4.18 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 174.1, 173.2, 152.4, 149.9, 131.5, 121.2, 42.7; HRMS (ESI) m/z calcd. $C_9H_7ClN_4OS$: 254.0029; found: 254.0035 [Mass Fragments: 179, 254].

4.6.2. N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)pivalamide (6b)

75% yield; mp 264-266°C; 59% yield; mp 148-151°C; IR (KBr) ν_{max} : 3384 (N-H stretch), 3083 (Arom.CH strech), 2956 (Alip.CH strech), 1680 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.72 (s, 1H, NH), 8.10 (d, 2H, J = 5.2 Hz, Ar-H), 8.39 (d, 2H, J = 5.2, Hz, Ar-H), 1.38 (s, 9H, CH_3);

^{13}C NMR (DMSO-d_6) δ 174.5, 173.6, 152.4, 149.9, 131.4, 121.2, 45.7, 26.9; HRMS (ESI) m/z calcd. $C_{12}H_{14}N_4OS$: 262.0888; found: 262.0896 [Mass Fragments: 179, 262].

4.6.3. N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)thiophene-2-carboxamide (6c)

55% yield; 158-160°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max} : 3394 (N-H stretch), 3083 (Arom.CH strech), 2945 (Alip.CH strech), 1662 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.82 (s, 1H, NH), 8.09 (d, 2H, J = 5.2 Hz, Ar-H), 8.43 (d, 2H, J = 5.2, Hz, Ar-H), 7.77 (d, 2H, J = 5 Hz, thiophene), 7.21 (t, 2H, J = 5 Hz, thiophene), 7.95 (d, 2H, J = 5 Hz, thiophene); ^{13}C NMR (DMSO-d_6) δ 174.8, 173.3, 152.4, 149.9, 148.2, 146.3, 140.1, 121.2, 116.3, 111.3 HRMS (ESI) m/z calcd. $C_{12}H_{14}N_4OS$: 288.0140; found: 288.0149 [Mass Fragments: 179, 288].

4.6.4. N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)pentanamide (6d)

64% yield; mp 154-156°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max} : 3375 (N-H stretch), 3083 (Arom.CH strech), 2945 (Alip.CH strech), 1672 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.89 (s, 1H, NH), 8.14 (d, 2H, J = 5.2 Hz, Ar-H), 8.32 (d, 2H, J = 5.2, Hz, Ar-H), 1.05-2.29 (m, 9H, CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 174.6, 173.2, 152.3, 149.9, 146.1, 140.1, 121.2, 31.7, 21.0, 14.0 HRMS (ESI) m/z calcd. $C_{12}H_{14}N_4OS$: 262.0888; found: 262.0894 [Mass Fragments: 179, 262].

4.6.5. 4-Nitro-N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)benzamide (6e)

75% yield; mp 254-256°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max} : 3375 (N-H stretch), 3093(Arom.CH strech), 2945 (Alip.CH strech), 1662 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.92 (s, 1H, NH), 7.45-8.50 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6) δ 174.0, 173.2, 152.3, 149.9, 146.2, 140.2, 136.7, 132.1, 130.2, 121.2 ; HRMS (ESI) m/z calcd. $C_{14}H_9N_5O_3S$: 327.0426; found: 328.0432 [M+1] [Mass Fragments: 179, 328].

4.6.6. 3-Chloro-N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)butanamide (6f)

51% yield; mp 187-189°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max} : 3495 (N-H stretch), 3093 (Arom.CH strech), 2975 (Alip.CH strech), 1692 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.52

(s, 1H, NH), 8.12 (d, 2H, *J* = 5.2 Hz, Ar-H), 8.42 (d, 2H, *J* = 5.2, Hz, Ar-H), 1.45-3.29 (m, 6H, CH_2 aliphatic side chain); ^{13}C NMR (DMSO-d_6) δ 173.9, 173.5, 152.9, 148.9, 130.1, 121.2, 51.5, 31.7, 10.1; HRMS (ESI) m/z calcd. $C_{14}H_9N_5O_3S$: 282.0342; found: 282.0350 [Mass Fragments: 179, 282].

4.6.7. 6-Oxo-6-((5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)amino)hexanoyl chloride (6g)

54% yield; mp 153-155°C; IR (KBr) ν_{max}: 3395 (N-H stretch), 3043 (Arom.CH strech), 2955 (Alip.CH strech), 1692 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.80 (s, 1H, NH), 8.09 (d, 2H, *J* = 5.2 Hz, Ar-H), 8.14 (d, 2H, *J* = 5.2, Hz, Ar-H), 1.55-2.82 (m, 8H, CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 174.7, 173.2, 152.4,149.9,132.1,130.4, 121.9, 46.8, 35.9, 26.9, 24.8; HRMS (ESI) m/z $C_{13}H_{13}ClN_4O_2S$: 324.0448; found: 324.0454 [Mass Fragments: 179, 324].

4.6.8. 4-Chloro-N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)butanamide (6h)

74% yield; mp 148-150°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max}: 3345 (N-H stretch), 3043 (Arom.CH strech), 2963 (Alip.CH strech), 1692 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.81 (s, 1H, NH), 8.10 (d, 2H, *J* = 5.2 Hz, Ar-H), 8.43 (d, 2H, *J* = 5.2, Hz, Ar-H), 1.45-3.56 (m, 6H, CH_2 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 174.2, 173.6, 153.9, 149.9,130.1, 121.2, 47.1, 33.7, 26.1 ; HRMS (ESI) m/z $C_{11}H_{11}ClN_4OS$: 282.0342; found: 282.0350 [Mass Fragments: 179, 282].

4.6.9. 2,2,2-Trichloro-N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)acetamide (6i)

68% yield; mp 258-260°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max} : 3485 (N-H stretch), 3043 (Arom.CH strech), 2956 (Alip.CH strech), 1692 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.56 (s, 1H, NH), 8.10 (d, 2H, *J* = 5.2 Hz, Ar-H), 8.31 (d, 2H, *J* = 5.2, Hz, Ar-H), ^{13}C NMR (DMSO-d_6) δ 174.2, 173.9, 152.6, 149.9,125.9, 95.2 HRMS (ESI) m/z $C_9H_5Cl_3N_4OS$: 321.9250; found: 321.9258 [Mass Fragments: 179, 321].

4.6.10. N-(5-(pyridin-4-yl)-1,3,4-thiadiazol-2-yl)butyramide (6j)

76% yield; mp 287-290°C; 75% yield; mp 258-261°C; IR (KBr) ν_{max} : 3405 (N-H stretch), 3003 (Arom.CH strech), 2855 (Alip.CH strech), 1692 (amide C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) 12.62 (s, 1H, NH), 8.26 (d, 2H, *J* = 5.2 Hz, Ar-H), 8.77 (d, 2H, *J* = 5.2, Hz, Ar-H), 2.79 (t, 2H, *J* =7.3 Hz, CH_2 Aliphatic chain), 1.79 (h, 2H, *J* = 7.3 Hz, CH_2 Aliphatic chain), 0.94 (d, 3H, *J* =7.3 Hz, CH_3 Aliphatic chain); ^{13}C NMR (DMSO-d_6) δ 174.2, 176.2, 152.4, 149.5,125.3, 94.2, 45.1, 31.7, 10.0; HRMS (ESI) m/ $C_{11}H_{12}N_4OS$: 248.0732; found 249.0738 [M+1] [Mass Fragments: 188, 249].

4.7. Anti-mycobacterial activity

In vitro evaluation of the anti-mycobacterial activity of the newly synthesized compounds **6 (a-o)**, **5 (a-m)** and **6 (a–j)** was carried out at the Micro Care Laboratory and Tuberculosis Research Center Surat, India screening program. The antitubercular screening for test compounds was conducted using *M. Tuberculosis* H37Rv strain and the clinically isolated phenotype MDR-TB strain by adopting L. J. (conventional Lowenstein and Jensen) agar dilution method for the measurement of MIC, and is defined as the lowest concentration of drug, which inhibits ≥ 99 % of bacterial population present at the beginning of the assay. Stock solutions of 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.12, and 1.56 μg /mL dilutions of each test compound in DMSO were added in the liquid L. J. Medium, and then media were sterilized by inspissations method. A culture of *M. tuberculosis (H37Rv strain and MDR-TB strain)* growing on L. J. Medium was harvested in 0.85 % saline in bijou bottles. These tubes were then incubated at 37 ºC for 24 h followed by streaking of *M. tuberculosis (H37Rv strain and MDR-TB strain)* [(5 x 10^{4}) bacilli per tube]. These tubes were then incubated at 37 ºC. Growth of bacilli was seen after 12 days, 22 days, and finally at 28th day of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis (H37Rv strain and MDR-TB strain)*. The concentration at which no development of colonies occurred or < 20 colonies was taken as MIC concentration of test compound. The standard strain *M. tuberculosis (H37Rv strain*

and MDR-TB strain) was tested with known drugs Isoniazid and Rifampin. MIC values µg /mL are converted to µM/mL [36].

4.8. Cyto-toxicity

The selected set of compounds, which showed potent activity against MTB (H37Rv) and MDR-TB strains were also evaluated for their cyto-toxicity on VERO cell Lines by MTT assay. Mammalian VERO cells were cultured in Dulbecco Modified Eagle Medium (DMEM) containing 2 mM Na_2CO_3 supplemented with 10% (v/v) fetal bovine serum (FBS). The cells were incubated at 37 °C under 5% CO_2 and 95% air in a humidified atmosphere until confluent and then diluted with phosphate-buffered saline to 106 cells/ml. Stock solutions were prepared in dimethyl sulfoxide (DMSO) and further dilutions were made with fresh culture medium. The concentration of DMSO in the final culture medium was 1%, which had no effect on the cell viability. In a transparent 96-well plate, serially diluted stock solutions were placed at 37 °C for 72 h then the medium was removed and monolayer was washed twice with 100 µL of warm Hanks' balanced salt solution (HBSS). 100 µL of warm medium and 20 µL of freshly made MTS-PMS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and phenylmethasulfazone] (Promega) were added to each well, plates were incubated for 3 h and absorbance was determined at 490 nm using a plate reader. The same experimental conditions were provided for all compounds and analysis was repeated three times for the cell line tested using the standard compound Staurosporine.

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Supplementary Information

Pyridine and Nitro-phenyl linked 1,3,4-thiadiazoles as MDR-TB Inhibitors

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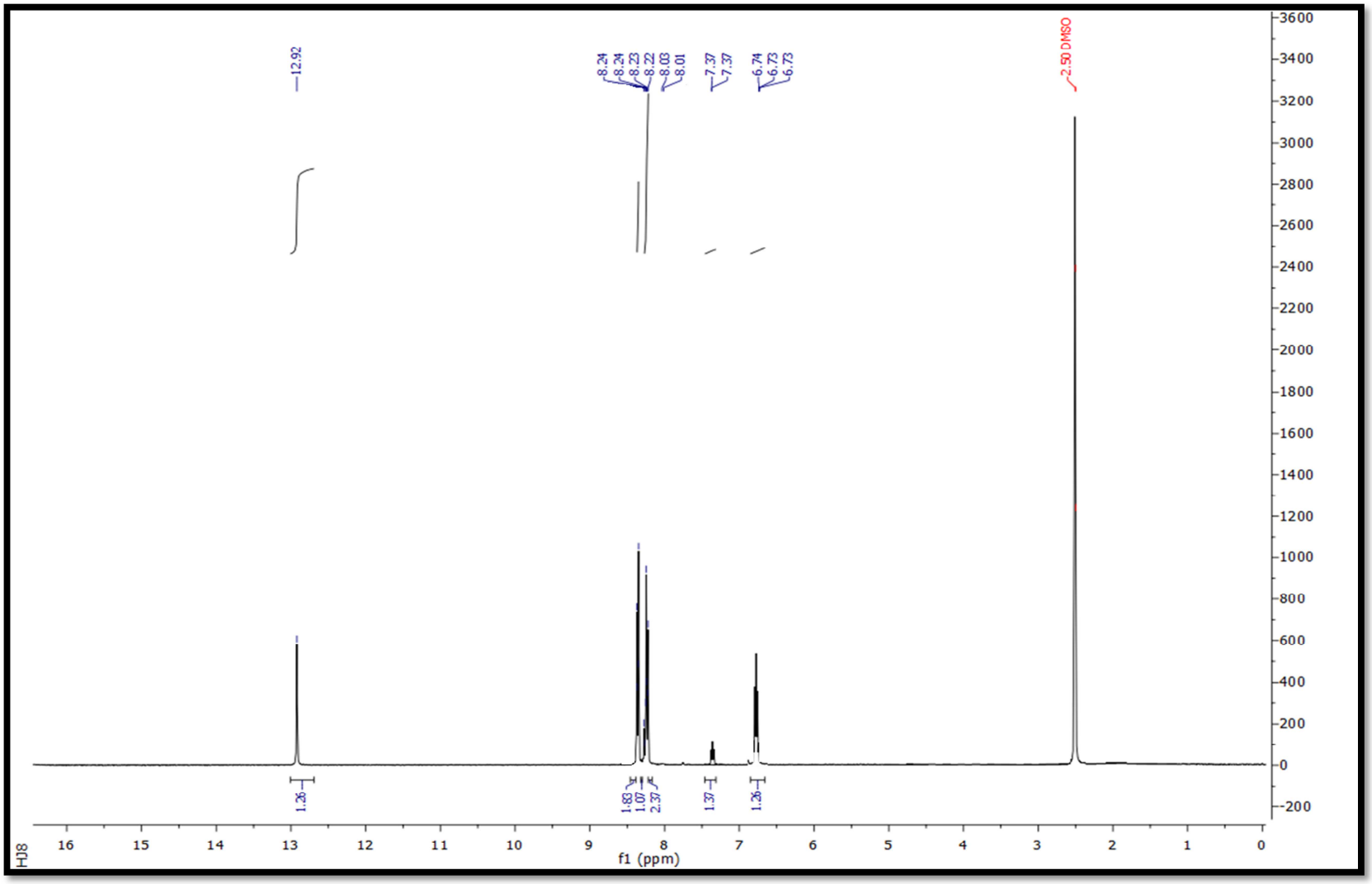
^{1}H NMR of Compound 4H



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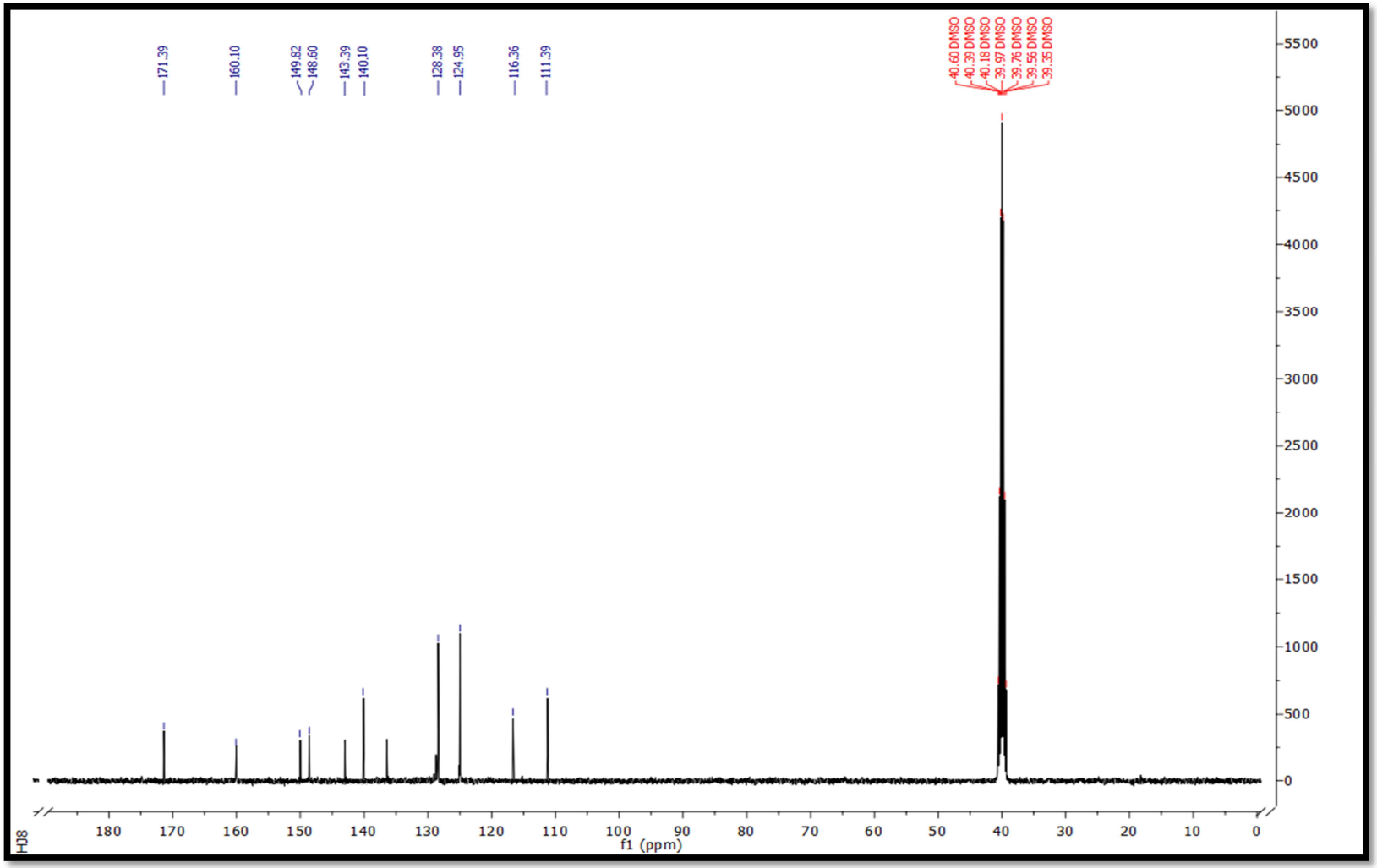

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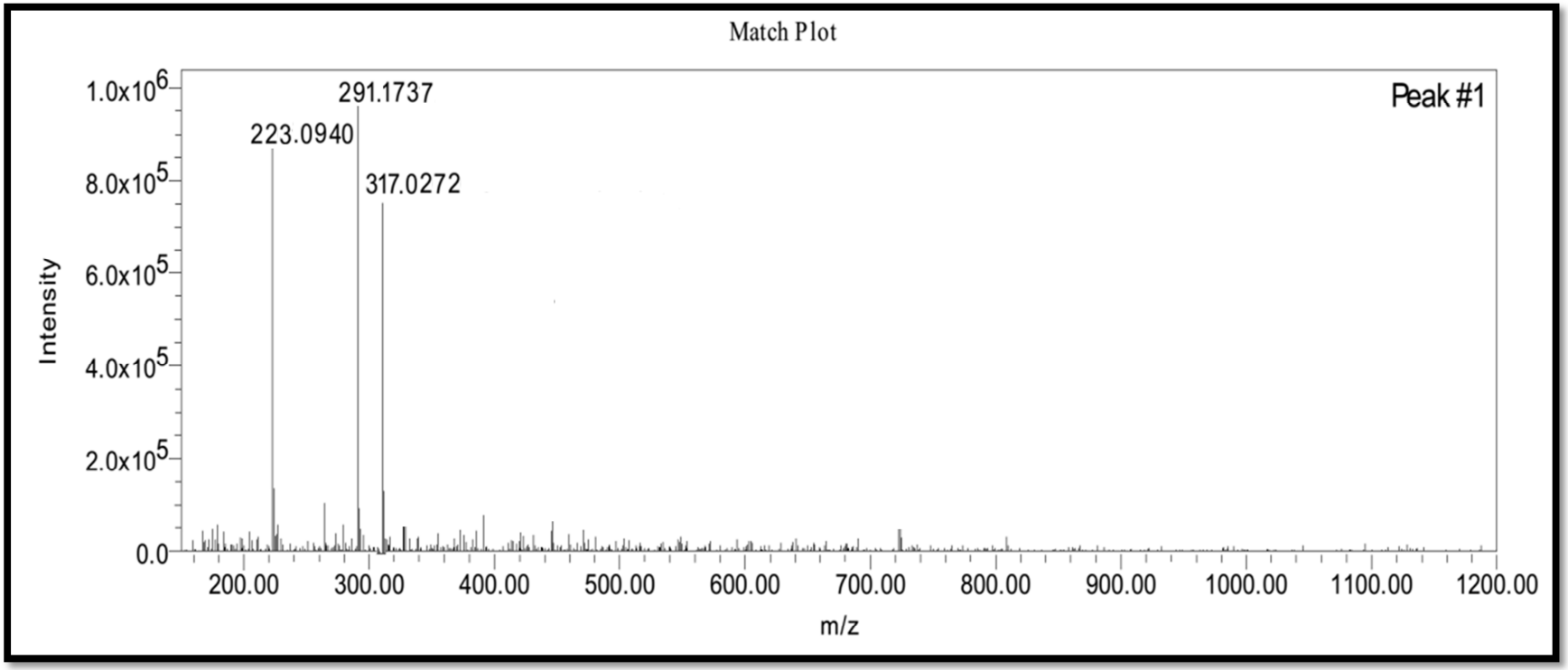
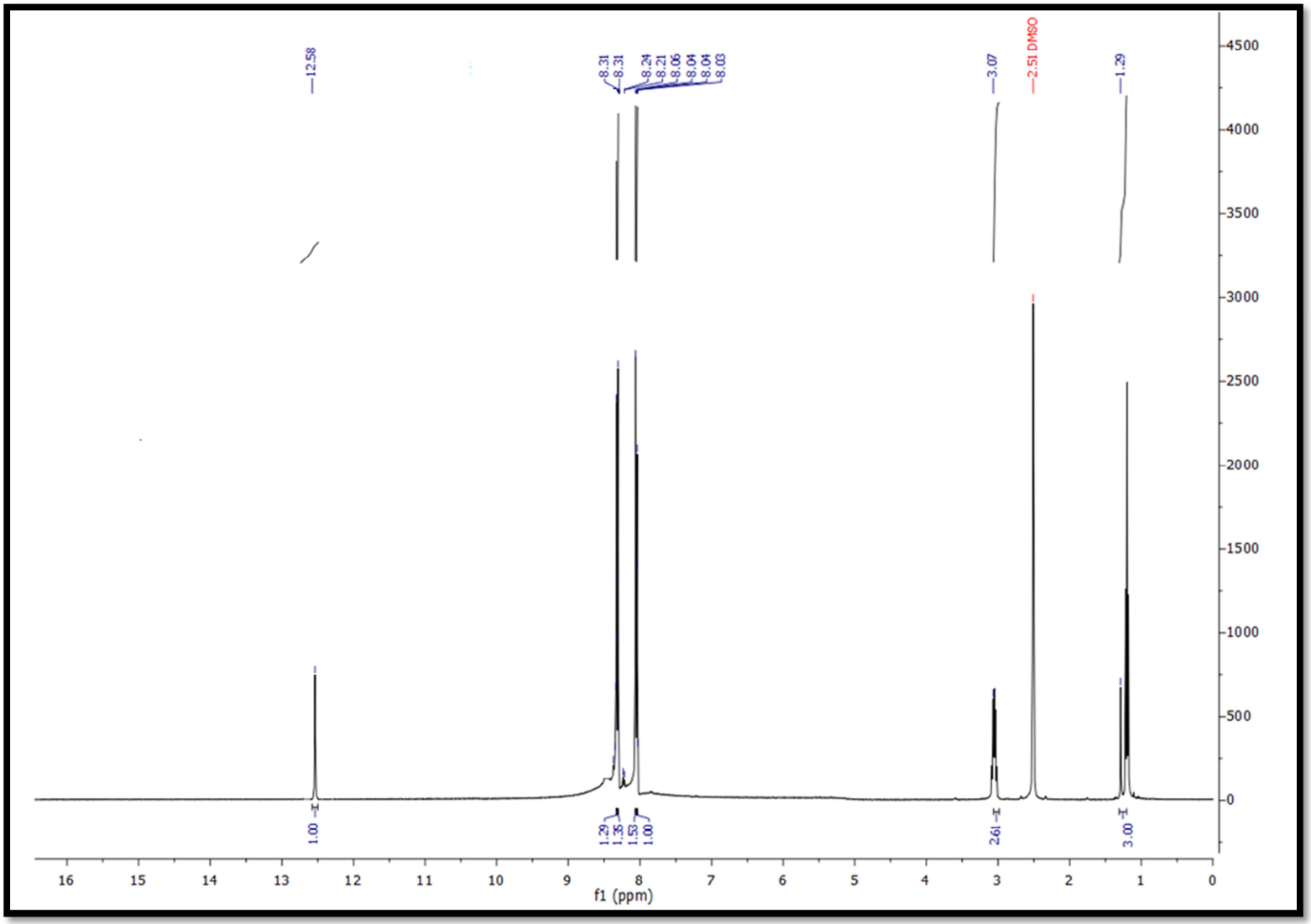


^{1}H NMR of Compound 7c



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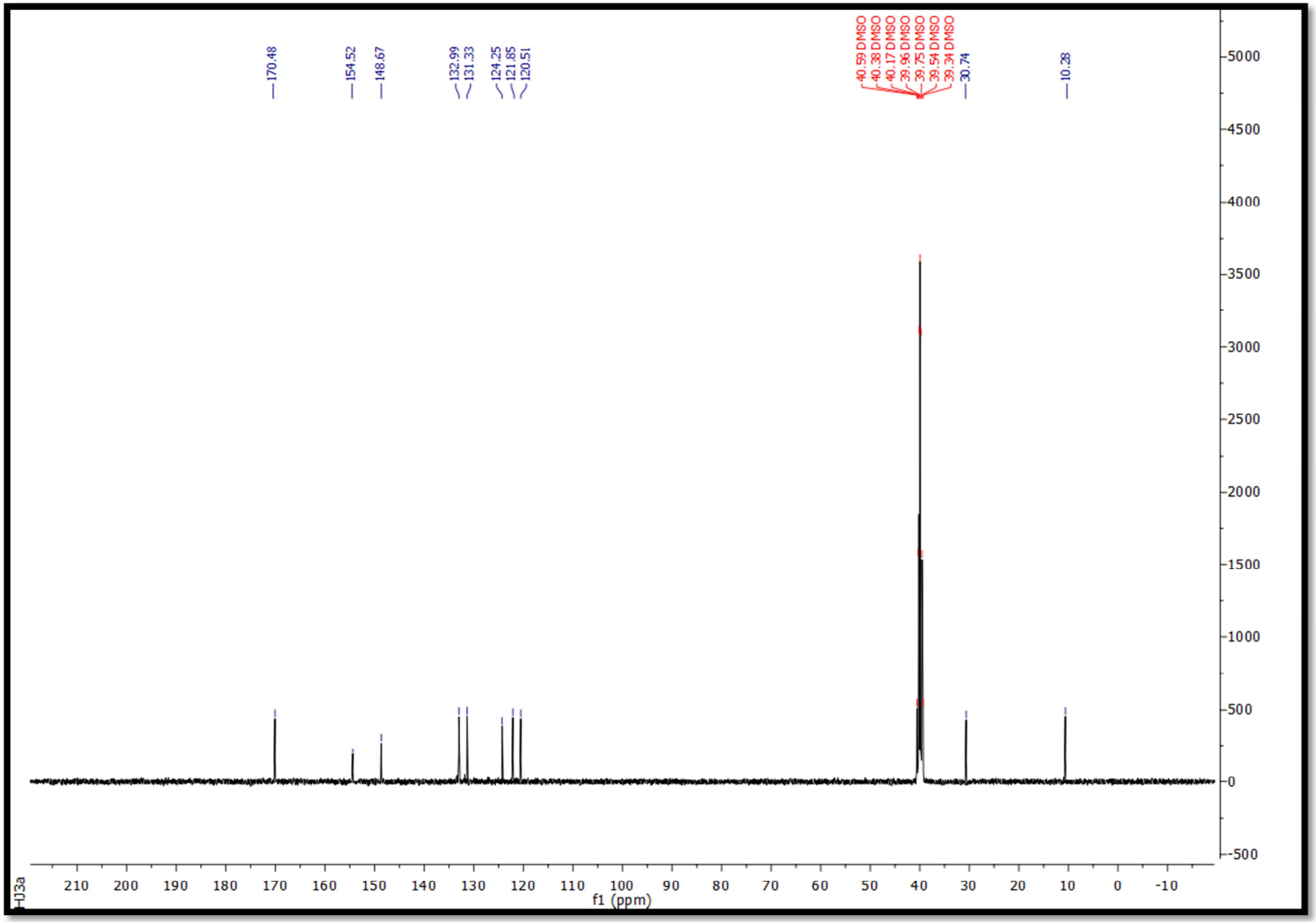
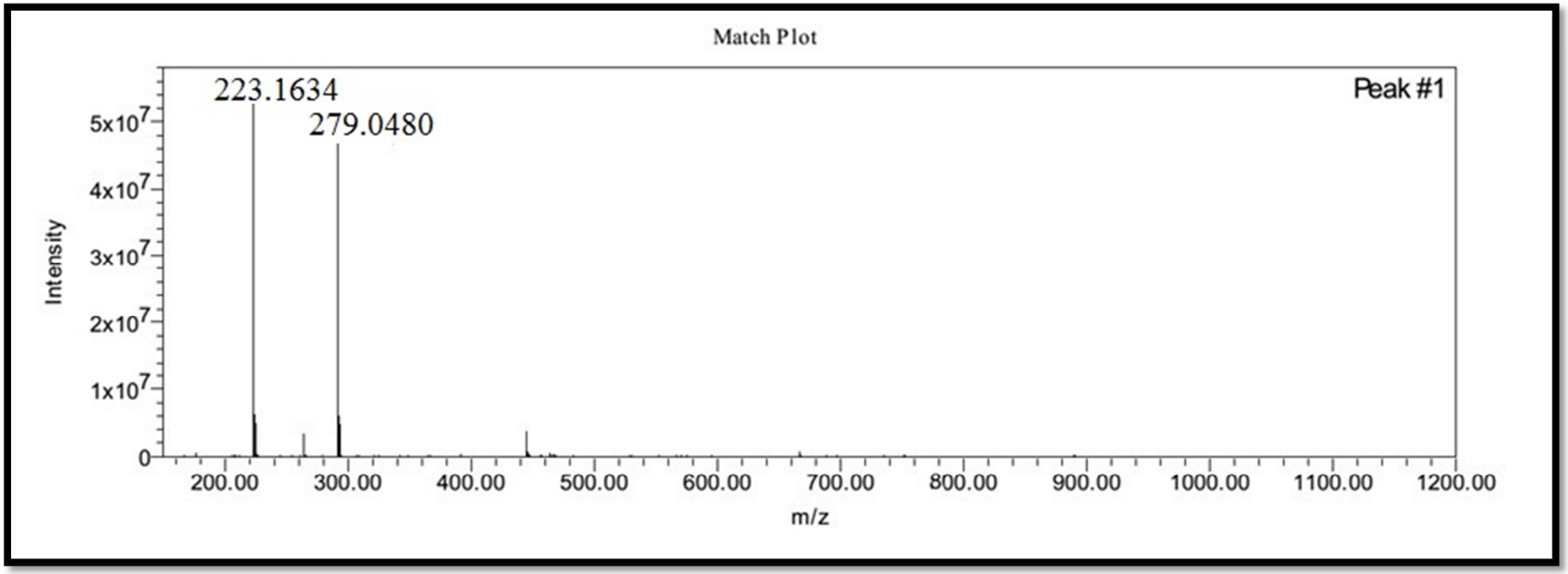


Mass of Compound 7c



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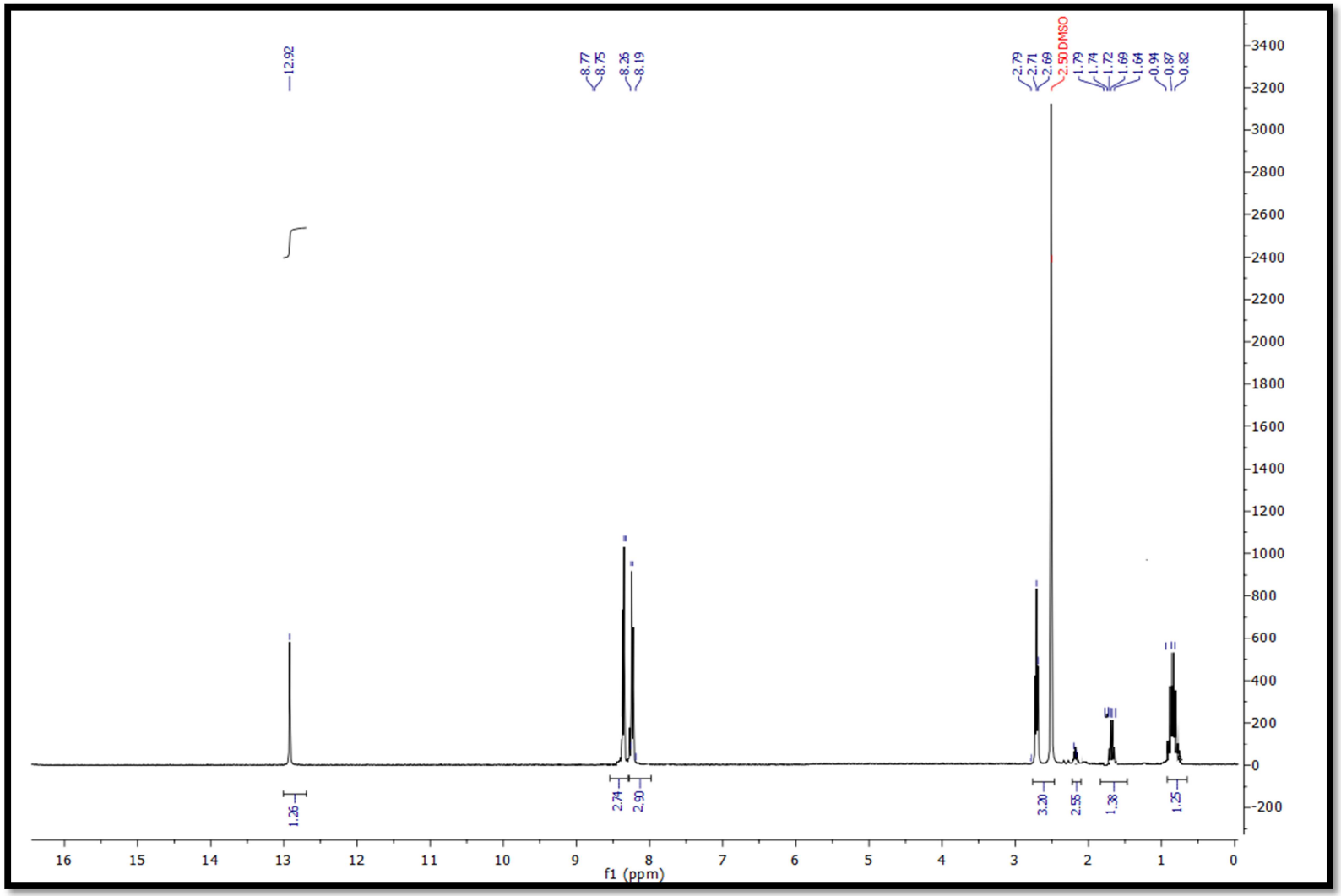

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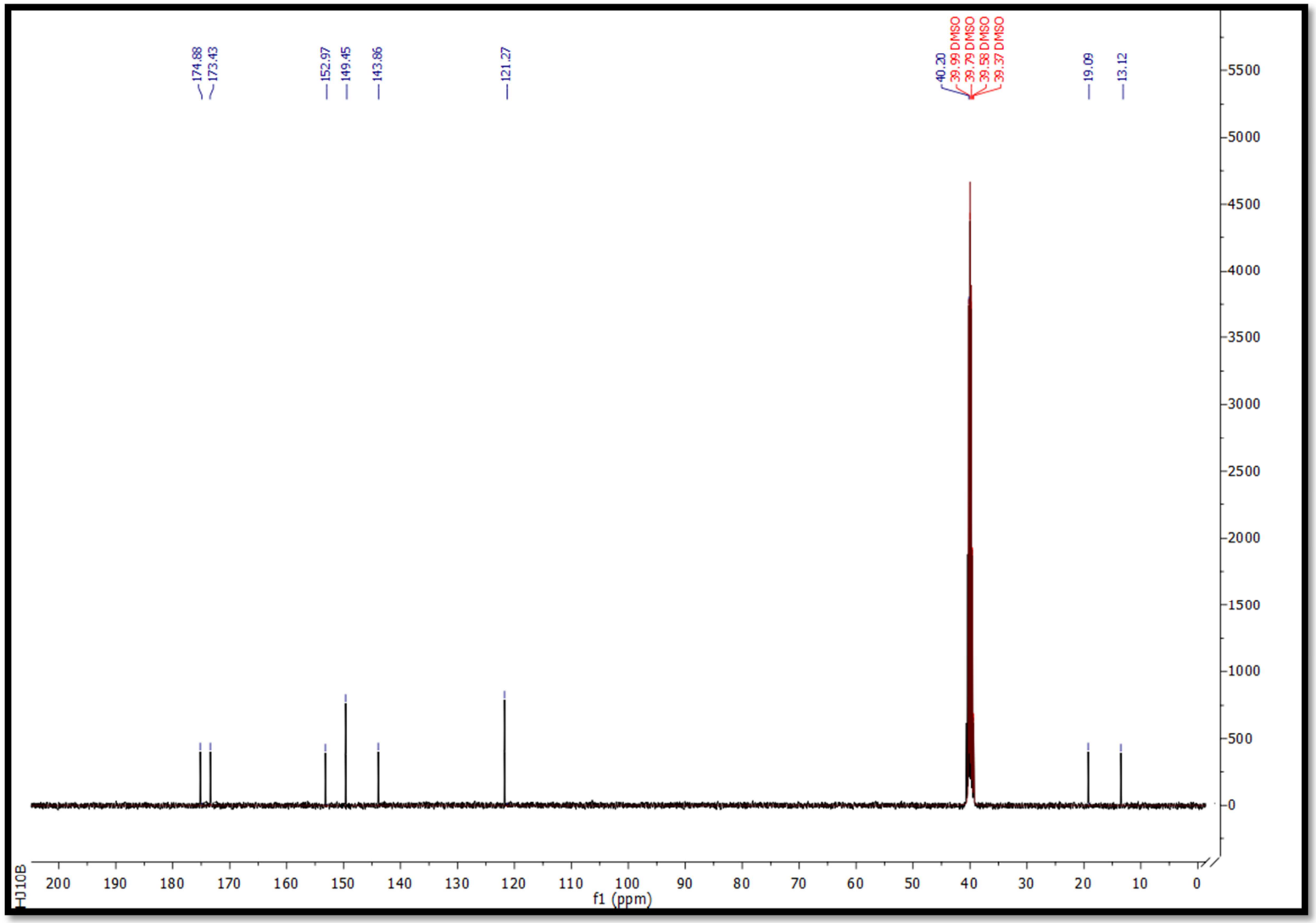

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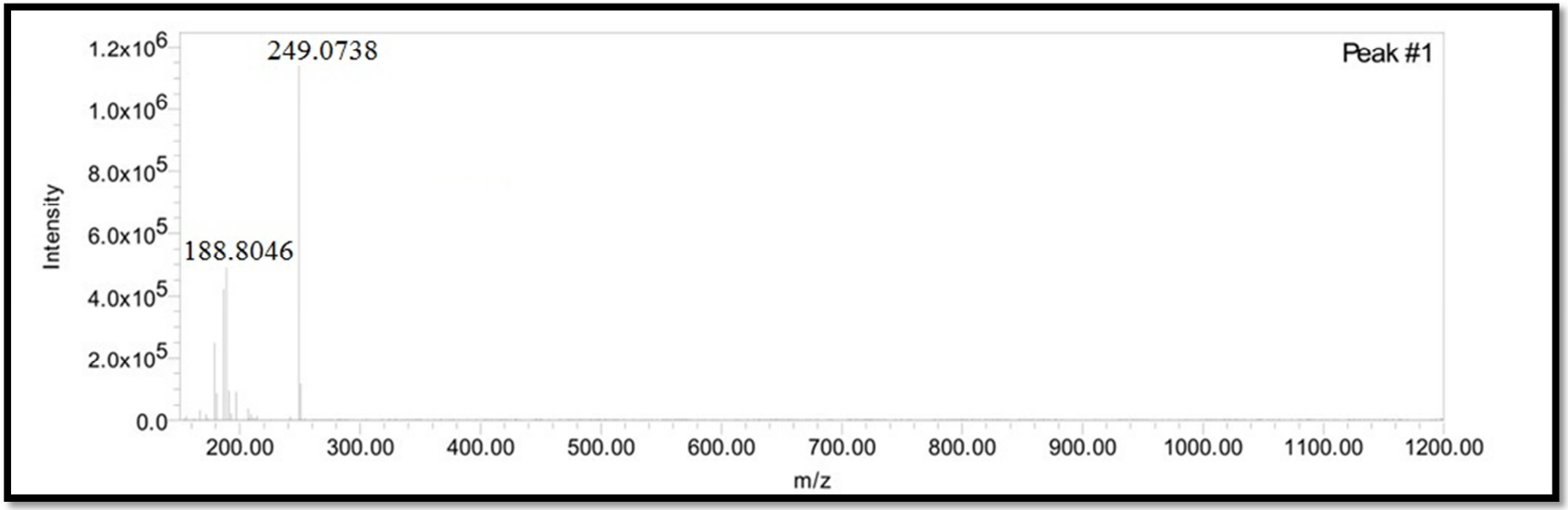

Highlights

► Substituted [1,3,4]thiadiazole derivatives **4(a–o), 7(a-m)** and **10(a-j)** were synthesized

► Characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral technique

► Evaluated *in vitro* against the H37Rv and resistance MDR-TB strain