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Synthesis and Antimycobacterial Activity of 3,5-Disubstituted Thiadiazine Thiones☆

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Abstract—A series of 3,5-disubstituted thiadiazine thiones (**4**–**24**) have been synthesized by reaction of primary amines with carbon disulphide followed by cyclocondensation of the resulting intermediate with formaldehyde and primary amines or amino acids. The compounds were screened for antitubercular activity in vitro against *Mycobacterium tuberculosis* H37Rv. Three compounds **4**, **12** and **18** showed antimycobacterial activity with MIC 12.5 μg/mL. Compound **4**, was tested in vitro against five multidrug resistant (MDR) strains of *M. tuberculosis* and was found to be active. Compound **4** also exhibited activity in vivo. While all the mice died in the untreated group, the mean survival time (MST) of the compound treated mice was enhanced, 33% mice were surviving in treated group and the load of bacilli in the lung was considerably less in the compound treated group than in the untreated control group.

Introduction

Tuberculosis, caused by single infectious agent *Mycobacterium tuberculosis*, remains on the top of the killing infectious diseases in spite of intensive funding and research, and is a priority area globally.[1,2] As per estimate of W.H.O there were 8.4 million new cases of tuberculosis in 1999 and the annual global rate of increase of TB incidence was 3%. Assuming this rate of increase in TB incidence, there will be over 10 million new cases of TB in 2005. Currently, one third of world's population is infected with this bacillus.[3] Development of drug resistance against the frontline drugs and synergy of this disease with HIV and mycotic infections in immunocompromised patients have aggravated the problem.[1–4] A number of antitubercular agents belonging to different class of molecules are known today but these are associated with one or more drawbacks.[5] In spite of enormous amount of work done in genetic engineering and structural biology of this bacterium no new chemical entity has appeared in clinics since the last 40 years. Hence, in view of the above there is an emergent need to develop new antitubercular agents having a new mode of action and at the same time possessing anti-HIV, antimycotic and immunomodulatory activities.

Thiadiazine thione nucleus has been reported for different biological activities[6] including antituberculostatic, antibacterial, antiviral, antifungal and anthelmenthic, where it has been postulated that biological activity in these molecules is dependent on isothiocyanates and dithiocarbamic acid species generated in the biosystem on hydrolysis.[7,8] Dithiocarbamates themselves are associated with antifungal, antiviral, immunomodulatory and many other bioactivities[9–11] which could be an added advantage in developing new drugs against tuberculosis. Diethyl dithio carbamic acid sodium salt (DEDTC **1**), a dithiocarbamic acid derivative, a well known immunopotentiator[12] has been a safe clinical candidate for the treatment of HIV infections.[13,14] Encouraged by the above and in continuation of our synthetic work on dithiocarbamates[15] and thiadiazine thiones,[16] we were prompted to develop thiadiazine thiones as a new class of antitubercular agents. Further, this study was undertaken keeping in mind our recent finding that amino acid derivatives[17] have antitubercular activity. The potential of this class of molecule to block mycobacterial cell wall biosynthesis has been documented.[18,19] It is envisaged if amino acids or

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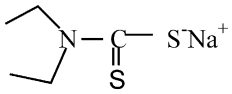
their isosteres are coupled with appropriate thiadiazine thione skeleton, a new class of molecules would emerge active against mycobacteria. The immunopotentiating, antimycotic and anti-HIV activities associated with thiocarbamic acid moiety would be beneficial in treating this disease in immunocompromised patients.



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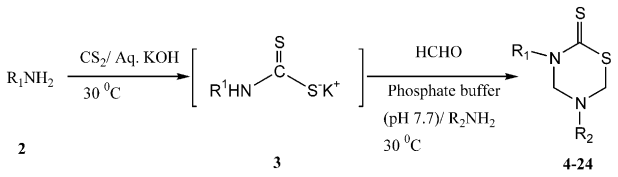
In this context, we were interested to synthesize a combinatorial library of thiadiazine thiones having amino acid and hetero aromatic acid components. Our attempt to synthesize a small library of 48 compounds on solid phase in a parallel format manner was unsuccessful as the products obtained after cleavage from resin were a complex mixture of several compounds including 3,5-disubstituted thiadiazine thiones (molecular ion peaks as evidenced by FAB MS) and none of them could be isolated in pure form. However, these compounds as such were screened against *M. tuberculosis* and few of them showed mild *in vitro* activity (unpublished result). We then decided to synthesize some of these compounds with thiadiazine thione skeleton in conventional manner and evaluate them for antitubercular activity.

Results and Discussion

Chemistry

The compounds **4**–**24** (Table 1) were prepared by a slight modification of the earlier reported method.[6] Thus reaction of the appropriate primary amines of the general formula **2**, including cyclopropyl amine, furfuryl amine, benzyl amine, cyclohexyl amine, butyl amine, octyl amine and dodecyl amine, with carbon disulphide



Scheme 1.

separately resulted in the formation of respective intermediate dithiocarbamate potassium salts **3** (which were not isolated). Further reaction of the intermediates (**3**) with formaldehyde and selected amino acids, amines and amino ester, including β-alanine, glycine, γ-amino butyric acid, phenylalanine, 6-amino caproic acid, cyclohexyl amine, cyclopropyl amine, hexadecyl amine and 3-amino ethyl propionate, afforded respective 3,5-disubstituted thiadiazine-2-thiones (**4**–**24**) in fair to good yields (Scheme 1). Structures of all the compounds were established on the basis of spectroscopic data and microanalysis. IR spectroscopy of the compounds showed absorption bands ranging from 3060 to 3450 cm^{-1} for (OH and NH), 1690 to 1720 cm^{-1} for (C=O) and from 1445 to 1508 cm^{-1} for (C=S). In ^1H NMR spectra, H-4 and H-6 protons of tetrahydro-2H-1,3,5-thiadiazine-2-thione ring appeared as two separate singlets around δ 4.50 and 4.40 respectively besides other usual signals of the substituents.

In ^{13}C NMR spectrum of compound **4**, characteristic signals for C=S, C-4 and C-6 appeared at δ 195.1, 71.8 and 57.3, respectively. The signals corresponding to these carbons appear at δ 195, 71.6, 57.3; 195.2, 71.6, 57.3; 195.3, 71.7, 57.3; 195.2, 71.3, 53.0; 193.4, 68.4, 58.6; 195.4, 67.4, 59.6; 193.4, 68.4, 58.6; 193.6, 67.4, 56.7; 190.9, 65.1, 57.1; 191.5, 70.6, 58.2; 191.5, 70.6, 58.2; 191.5, 70.6, 58.2; 193.9, 66.7, 56.2; 193, 65.9, 56.1; 193.5, 68.3, 59.1; 195.1, 71.8, 57.5; 195.8, 69.0, 55.4 and 195.7, 71.5, 58.1 in compounds **5**, **6**, **7**, **8**, **9**, **11**, **12**, **13**, **14**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23** and **24**, respectively. All

Table 1. In vitro activity of compounds **4**–**24** against *M. tuberculosis* H37Rv

Compd	R^1	R^2	ClogP	CMR	Water solubilities	MIC (μg/mL)
4	Cyclopropyl	$-CH_2CH_2COOH$	−1.29	6.66	Soluble	12.5
5	Cyclopropyl	$-CH_2COOH$	−0.73	6.19	Soluble	50
6	Cyclopropyl	$-CH_2CH_2CH_2COOH$	−1.03	7.12	Partially soluble	25
7	Cyclopropyl	$-CH_2(CH_2)_4COOH$	−0.46	8.05	Partially soluble	25
8	Furfuryl	$-CH_2CH_2COOH$	−0.68	7.59	Partially soluble	50
9	Furfuryl	$-CH_2CH_2CH_2COOH$	−0.41	8.05	Partially soluble	50
10	Furfuryl	$CH(CH_2C_6H_5)COOH$	2.29	10.01	Insoluble	50
11	Benzyl	$-CH_2CH_2COOH$	0.14	8.38	Partially soluble	50
12	Benzyl	$-CH_2CH_2CH_2COOH$	0.41	8.84	Insoluble	12.5
13	Benzyl	$CH(CH_2C_6H_5)COOH$	3.12	10.89	Insoluble	50
14	Cyclohexyl	$-CH_2CH_2COOH$	0.37	8.01	Partially soluble	100
15	Cyclohexyl	$-(CH_2C_6H_5)COOH$	3.39	9.63	Insoluble	50
16	Butyl	$-CH_2CH_2COOH$	−0.05	7.26	Partially soluble	25
17	Octyl	$-CH_2CH_2COOH$	2.06	9.12	Insoluble	25
18	Dodecyl	$-CH_2CH_2COOH$	4.18	10.97	Insoluble	12.5
19	Furfuryl	Cyclohexyl	3.54	7.72	Insoluble	50
20	Benzyl	Cyclohexyl	4.91	9.40	Insoluble	50
21	Benzyl	Hexadecyl	10.48	14.22	Insoluble	50
22	Cyclopropyl	$-CH_2CH_2COOEt$	1.96	7.58	Insoluble	12.5
23	Cyclopropyl	Cyclohexyl	2.93	7.68	Insoluble	100
24	Cyclopropyl	Cyclopropyl	1.25	6.33	Insoluble	25
Isoniazid	—	—	−0.668	3.257	Soluble	0.75
Ethambutol	—	—	0.1188	5.859	Soluble	3.25

other signals of the above compounds are given in the experimental section.

Biology

A search for new antitubercular compounds based on inhibition in the biosynthesis of cell wall components is an ideal approach and presents an attractive target as it is involved in the transportation of nutrients and drugs, forms a permeability barrier and protects the organism from the hostile environment of the host's immune system.[20,21] Compounds having thiourea (isoxyl) functionality are known to inhibit biosynthesis of mycolic acid,[22] an important constituent of the cell wall. Several amino acid analogues[18] are known to inhibit an enzyme D-alanine racemase[19] required in one of the steps during peptidoglycan biosynthesis. Therefore, it was thought to synthesize compounds with thioureidyl and amino acid functionalities, wherein the thione moiety may serve as a site for nucleophilic attack by the enzymes of *M. tuberculosis* and unnatural amino acids or its isosteres will inhibit the peptidoglycan biosynthesis by inhibiting the enzyme D-alanine racemase. The compounds have been designed keeping in mind the fact that in thiadiazine thiones N-3- and N-5-substitutions with hydrophobic and hydrophilic components respectively leads better activity and low toxicity to the host.[23]

The activity of the compounds **4**–**24** synthesized and reported here was determined against M. tuberculosis H37Rv. The *in vitro* activity of the compounds is shown in Table 1, and expressed as the minimal inhibitory concentration (MIC). The colony forming ability of *M. tuberculosis* H37Rv was completely inhibited at the reported MIC's. As evident out of 21 compounds synthesized, compounds **4**, **6**, **7**, **12**, **16**, **17**, **18**, **22** and **24** showed activity *in vitro* at one or the other concentrations. Careful examination of biological activity data reveals that compounds with N-3 cyclopropyl and N-5 β-alanyl (**4**) or its congener having 2-carbethoxy ethyl substituent at N-5 (**22**) showed *in vitro* activity at 12.5 μg/mL. Other compounds of this series having cyclopropyl substituent at N-3 and cyclopropyl, cyclohexyl or other substituent at N-5 always resulted in lower activity with higher MIC's. However, compounds with N-3 furfuryl substituent having any substituent at N-5 always resulted in poor activity as all the compounds of this series have MIC's 50 or 100 μg/mL. Compound **18** having a 12 carbon substituent at N-3 and 3-carboxy ethyl substituent at N-5 also exhibited antitubercular activity with MIC of 12.5 μg/mL. In the N-3-benzyl series of compounds only one compound with carboxypropyl substituent at N-5 (**12**) was found to be potent with an MIC of 12.5 μg/mL. All other compounds were inactive as they have MIC values >25 μg/mL.

The *in vitro* high MIC values in this series of compounds did not discourage us as it is known that *in vitro* activity often displays poor correlation with activity *in vivo* and the reason for this failure is most often conflicting balances between bioavailability and activity.[24] The bioavailability depends mainly on solubility, permeability and metabolic half-life. Compounds with thiocarbonyl and sulphide moiety functionalities are known to be activated *in vivo* by oxidation of S to sulphoxides and sulphones. MIC of the sulphides are much less *in vitro* in comparison to sulphone and there is no gain in giving the oxidized product to *in vivo* system as it will only increase the cost of the drug with no net gain in activity profile. An estimate of lipophilicity can easily be predicted by simply counting the NH and OH bonds and N and O atoms. Looking into the Log P and molar refractivities values, determined using chem office 6.0 programme, and water solubilities of these compounds, compound **4** was selected for further study.

The *in vitro* activity of compound **4** was determined against some selected multi-drug resistant (MDR) strains isolated from tuberculosis patients. Activity was tested against only one concentration (50 μg/mL) of compound **4** present in the media and was found to inhibit the growth of MDR strains (Table 2).

As compound **4** showed *in vitro* activity in MDR strains it was screened *in vivo* too in mouse model. Mice infected with *M. tuberculosis* H37Rv strain were treated with the compound **4** and it was found to be promising as compared to the untreated control group. Enhancement of mean survival time, reduction in the load of tubercule bacilli in the lungs and marginal increased survival of mice were observed in the treated group (Table 3). Compound **4** proved to be promising as the survival of treated mice was enhanced marginally and the burden of bacilli was reduced at least 10-fold as compared to untreated control.

Table 2. *In vitro* activity of compound **4** (50 μg/mL) against MDR strains of *M. tuberculosis* isolated from TB patients

Compd or drug	Growth of MDR strains after 6 weeks				
	BC-248 (1)	BC-243 (1)	VA-101 (2)	BC-426 (3)	BC-437 (3)
Compd **4**	−	−	−	+	−
Sparfloxacin	+	++	++	++	++
No drug control	++	++	++	++	++

−, no growth; +, 1–20 colonies; ++, heavy growth.

(1) Strains resistant to rifampicin, isoniazid, ofloxicin and ethambutol; (2) strains resistant to rifampicin, isoniazid and ethambutol; (3) strains resistant to rifampicin and isoniazid.

Table 3. Activity of compound **4** against *M. tuberculosis* H37Rv in mice model of tuberculosis

Group	MST (Days)	% Survival on day 35 post infection	Load of bacilli in lung (CFU/g)
Compd **4** treated	21.3	33	2.1×10^5
Untreated control	19	00	5×10^6
Sparfloxacin	>35	100	4.8×10^2

MST, Mean survival time = mortality rate each day/total no. of mice.

Results are mean values of two experiments. All the groups were infected with 10^7 CFU of *M. tuberculosis* H37Rv/mouse intravenously. Animals were kept under observation for 35 days post infection. Compound **4** (100 mg/kg)/Sparfloxacin 25 mg/kg was given intraperitoneally for 10 days consecutively.

Experimental

Chemistry

Melting points were determined on a Buchi 510 apparatus and are uncorrected. Elemental analysis for all new compounds were performed on a Carlo Erba Model EA-1108 elemental analyzer and data of C, H, and N is within ±0.4% of calculated values. Thin layer chromatography was used to monitor the reactions. IR(KBr) spectra were recorded using Perkin-Elmer 881 spectrophotometer and the values are expressed as ν_{max} cm^{-1}. Mass spectral data were recorded on a Jeol (Japan) SX 102/DA-6000 Mass Spectrometer/Data system. The ^1H NMR and ^{13}C NMR spectra were recorded on Brucker Spectrospin spectrometer at 200 and 50 MHz, respectively, using TMS as internal standard. The chemical shift values are on δ scale and the coupling constants (*J*) are in Hz.

General procedure for the synthesis of compounds 4–18

5-(3-Carboxyethyl)-3-cyclopropyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (4). To a magnetically stirred solution of cyclopropyl amine (1.39 mL, 20 mmol) in 50 mL of H_2O, KOH (1.12 g, as 20% aq solution, 20 mmol) and CS_2 (1.20 mL, 20 mmol) were added at 30 °C. The reaction mixture was stirred for 4 h more followed by addition of 37% formaldehyde solution (3.01 mL, 40 mmol) and stirring continued for 1 h. The reaction mixture was filtered and resulting filtrate was added drop wise to a suspension of β-alanine (1.78 g, 20 mmol) in 20 mL of phosphate buffer solution (pH 7.7) and stirred additional for 1 h. The reaction mixture filtered off and the filtrate extracted with diethyl ether. The aqueous solution was cooled in an ice bath and acidified with 15% HCl (pH 2.0). The precipitated solid filtered off and washed with cold water, dried and crystallized from ethanol to give the title compound **4** as colourless granules. Yield 80%, mp 92–95 °C. IR (KBr): 3422, 1703, 1458; MS (FAB): 247 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 4.41 (s, 2H, H-4), 4.34 (s, 2H, H-6), 3.09–3.04 (m, 3H, NCH_2 and NCH), 2.54 (t, 2H, $J=6.4$ Hz, CH_2COOH), 1.04–0.87 (m, 4H, cyclopropyl ring protons); ^{13}C NMR ($CDCl_3$): δ 195.1 (C=S), 176.7 (C=O), 71.8 (C-4), 57.3 (C-6), 46.3 (NCH_2), 36.2 (NCH), 33.3 ($\underline{C}H_2COOH$), 9.3 (cyclopropyl ring carbons). Anal. calcd for $C_9H_{14}N_2O_2S_2$: C, 43.90; H, 5.69; N, 11.38. Found: C, 43.90; H, 5.70; N, 11.29.

5-(2-Carboxymethyl)-3-cyclopropyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (5). This was obtained by cyclopropyl amine (1.39 mL, 20 mmol) and glycine (1.5 g, 20 mmol) as described above and isolated as colourless solid, yield 62%, mp 123–125 °C. IR (KBr): 3439, 1711, 1480; MS (FAB): 233 $(M+H)^+$; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 4.48 (s, 2H, H-4), 4.41 (s, 2H, H-6), 3.58 (s, 2H, $NC\underline{H}_2COOH$), 3.09 (m, 1H, NCH), 1.04–0.79 (m, 4H, cyclopropyl ring protons); ^{13}C NMR ($CDCl_3$): δ 195 (C=S), 175.3 (C=O), 71.6 (C-4), 57.3 (C-6), 54.8 (NCH_2), 36.3 (NCH), 9.3 (cyclopropyl ring carbons). Anal. calcd for $C_8H_{12}N_2O_2S_2$: C, 41.38; H, 5.17; N, 12.06. Found: C, 41.36; H, 5.17; N, 12.06.

5-(4-Carboxypropyl)-3-(cyclopropyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (6). This was obtained by cyclopropyl amine (1.39 mL, 20 mmol) and γ-amino butyric acid (2.06 g, 20 mmol) as described above and isolated as colourless solid, yield 65%, mp 112–115 °C. IR (KBr): 3439, 1699, 1475; MS (FAB): 261 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 4.36 (s, 2H, H-4), 4.30 (s, 2H, H-6), 3.08 (m, 1H, NCH), 2.81 (t, 2H, $J=6.9$ Hz, $NC\underline{H}_2$), 2.46 (t, 2H, $J=7.1$ Hz, $C\underline{H}_2COOH$), 1.88 (m, 2H, $NCH_2C\underline{H}_2$), 1.04–0.87 (m, 4H, cyclopropyl ring protons); ^{13}C NMR ($CDCl_3$): δ 195.2 (C=S), 176.3 (C=O), 71.6 (C-4), 57.3 (C-6), 49.8 (NCH_2), 36.3 (NCH), 31.7 ($\underline{C}H_2COOH$), 22.8 ($NCH_2\underline{C}H_2$), 9.3 (cyclopropyl ring carbons). Anal. calcd for $C_{10}H_{16}N_2O_2S_2$: C, 46.15; H, 6.15; N, 10.76. Found: C, 45.96; H, 6.15; N, 10.72.

5-(6-Carboxypentyl)-3-cyclopropyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (7). This was obtained by cyclopropyl amine (1.39 mL, 20 mmol) and 6-amino caproic acid (2.62 g, 20 mmol) as described above and isolated as yellow oil, yield 56%; IR (KBr): 2939, 1711, 1461; MS (FAB): 289 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 4.37 (s, 2H, H-4), 4.32 (s, 2H, H-6), 3.09–3.07 (m, 1H, NCH), 2.73 (t, 2H, $J=7.1$ Hz, $NC\underline{H}_2$), 2.37 (t, 2H, $J=7.2$, $C\underline{H}_2COOH$), 1.67–1.41 (m, 6H, $[CH_2(C\underline{H}_2)_3CH_2]$, 1.03–0.83 (m, 4H, cyclopropyl ring protons); ^{13}C NMR ($CDCl_3$): δ 195.3 (C=S), 179.8 (C=O), 71.7 (C-4), 57.3 (C-6), 50.3 (NCH_2), 36.3 (NCH), 34.3 ($\underline{C}H_2COOH$), 27.6, 26.8, 24.8 (CH_2's), 9.2 (cyclopropyl ring carbons). Anal. calcd for $C_{12}H_{20}N_2O_2S_2$: C, 50.0; H, 6.9; N, 9.72. Found: C, 49.9; H, 7.02; N, 9.72.

5-(3-carboxyethyl)-3-(2′-furfuryl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (8). This was obtained by furfuryl amine (1.76 mL, 20 mmol) and β-alanine (1.78 g, 20 mmol) as described above and isolated as colourless solid, yield 71%, mp 128–30 °C (lit.[25] mp 131–132 °C). IR (KBr): 3122, 1699, 1493; MS (FAB): 287 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 7.54 (d, 1H, $J=1.7$ Hz, furfuryl H-5′), 6.50 (d, 1H, $J=3.2$ Hz, furfuryl H-3′), 6.44 (dd, 1H, $J=3.2$ and 1.7 Hz, furfuryl H-4′), 5.41(s, 2H, furfuryl CH_2), 4.63 (s, 2H, H-4), 4.57 (s, 2H, H-6), 3.01 (t, 2H, $J=6.8$ Hz, $NC\underline{H}_2$), 2.45 (t, 2H, $J=6.7$, $C\underline{H}_2COOH$); ^{13}C NMR ($CD\underline{Cl}_3$): δ 195.2 (C=S), 177.9 (C=O), 152 (C-2′), 144.9 (C-5′), 115.0 (C-4′), 114.1 (C-3′), 71.3 (C-4), 61.8 (furfuryl CH_2), 53.0 (C-6), 51.0 (NCH_2), 34.6 ($\underline{C}H_2COOH$). Anal. calcd for $C_{11}H_{14}N_2O_3S_2$: C, 46.15; H, 4.89; N, 9.79. Found: C, 46.16; H, 4.89; N, 9.78.

5-(4-Carboxypropyl)-3-(2′-furfuryl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (9). This was obtained by furfuryl amine (1.76 mL, 20 mmol) and γ-amino butyric acid (2.06 g, 20 mmol) as described above and isolated as colourless solid, yield 62%, mp 115–118 °C. IR (KBr): 3101, 1709, 1480; MS (FAB): 301$(M+H)^+$; ^1H NMR ($CDCl_3$): δ 7.37 (d, 1H, $J=1.8$ Hz, furfuryl H-5′), 6.47 (d, 1H, $J=3.0$ Hz, furfuryl H-3′), 6.37 (dd, 1H, $J=3.0$ and 1.8 Hz, furfuryl H-4′), 5.30 (s, 2H, furfuryl CH_2), 4.56 (s, 2H, H-4), 4.36 (s, 2H, H-6), 2.70 (t, 2H, $J=6.9$ Hz, NCH_2), 2.37 (t, 2H, $J=7.1$ Hz, $C\underline{H}_2COOH$), 1.80–1.66 (m, 2H, $NCH_2C\underline{H}_2$); $^{13}CNMR$ ($CDCl_3$): δ 197.3 (C=S), 179.9 (C=O), 152.9 (C-2′), 146.9 (C-5′), 115.0 (C-4′), 114.7 (C-3′), 73.3 (C-4), 62.8 (furfuryl CH_2), 53.7

(C-6), 51.3 (NCH_2), 35.6 ($\underline{C}H_2COOH$), 26.6 ($NCH_2\underline{C}H_2$). Anal. calcd for $C_{12}H_{16}N_2O_3S_2$: C, 48.0; H, 5.33; N, 9.33. Found: C, 47.96; H, 5.33; N, 9.33.

5-[α-(Benzyl)carboxymethyl]-3-(furfuryl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (10). This was obtained by furfuryl amine (1.76 mL, 20mmol) and DL-phenyl alanine (3.3 g, 20 mmol) as described above as colourless oil and treated with cool ether to precipitate it, yield 50%, mp 167–169 °C (lit.[25] mp 166–168 °C). IR (KBr): 3062, 1719, 1597, 1490; MS (FAB): 363 $(M+H)^+$; ^1H NMR (DMSO-d_6): δ 7.37 (m, 6H, furfuryl H-5′ and ArH), 6.47 (d, 1H, $J=3.0$ Hz, furfuryl H-3′), 6.37 (dd, 1H, $J=3.0$ and 1.8 Hz, furfuryl H-4′), 5.32 and 5.10 (each d, each 1H, $J=9.3$ Hz, furfuryl CH_A and CH_B), 4.56 (s, 2H, H-4), 4.36 (s, 2H, H-6), 3.83–3.76 (m, 1H, NCHCOOH), 3.32–3.28 (m, 2H, $C\underline{H}_2C_6H_5$). Anal. calcd for $C_{17}H_{18}N_2O_3S_2$: C, 56.35; H, 4.97; N, 7.73. Found: C, 56.36; H, 4.82; N, 7.78.

5-(3-Carboxyethyl)-3-(phenylmethyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (11). This was obtained by benzyl amine (2.18 mL, 20 mmol) and β-alanine (1.78 g, 20 mmol) as described above and isolated as colourless solid, yield 72%, mp 134–136 °C. IR (KBr): 3386, 1699, 1489; MS (FAB): 297 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 7.41–7.26 (m, 5H, Ar-H), 5.35 (s, 2H, $C_6H_5CH_2$), 4.39 (s, 2H, H-4), 4.29 (s, 2H, H-6), 2.92 (t, 2H, $J=6.5$ Hz, NCH_2), 2.19 (t, 2H, $J=6.5$, CH_2COOH). ^{13}C NMR ($CDCl_3$): δ 195.4 (C=S), 175.2 (C=O), 135.2 (Ar-C), 129.2, 128.9 and 128.6 (ArCH), 67.4 (C-4), 59.6 (C-6), 53.2 ($\underline{C}H_2C_6H_5$), 48.9 (NCH_2), 32.5 ($\underline{C}H_2COOH$). Anal. calcd for $C_{13}H_{16}N_2O_2S_2$: C, 52.70; H, 5.4; N, 9.45. Found: C, 52.72; H, 5.4; N, 9.45.

5-(4-Carboxypropyl)-3-(phenylmethyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (12). This was obtained by benzyl amine (2.18 mL, 20 mmol) and γ-amino butyric acid (2.06 g, 20 mmol) as described above and isolated as colourless solid, yield 67%, mp 138–140 °C. IR (KBr): 3424, 1703, 1485; MS (FAB): 311$(M+H)^+$; ^1H NMR ($CDCl_3$): δ 7.49–7.32 (m, 5H, Ar-H), 5.36 (s, 2H, $C_6H_5C\underline{H}_2$), 4.40 (s, 2H, H-4), 4.29 (s, 2H, H-6), 2.64 (t, 2H, $J=6.9$ Hz, NCH_2), 2.20 (t, 2H, $J=7.1$ Hz, $C\underline{H}_2COOH$), 1.57–1.43 (m, 2H, $NCH_2C\underline{H}_2$); ^{13}C NMR($CDCl_3$): δ 193.4 (C=S), 176.2 (C=O), 135.6 (Ar-C), 129.2, 128.9 and 128.6 (ArCH), 68.4 (C-4), 58.6 (C-6), 54.2 ($\underline{C}H_2C_6H_5$), 49.6 (NCH_2), 31.5 ($\underline{C}H_2COOH$), 22.5 ($NCH_2\underline{C}H_2$). Anal. calcd for $C_{14}H_{18}N_2O_2S_2$: C, 54.19; H, 5.80; N, 9.03. Found: C, 54.24; H, 5.8; N, 8.98.

5-[α-(Benzyl)carboxymethyl]-3-(2-phenylmethyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (13). This was obtained by using benzyl amine (2.18 mL, 20 mmol) and DL-phenyl alanine (3.3 g, 20 mmol) as described above and isolated as colourless solid, yield 72%, mp 150–152 °C. IR (KBr): 3062, 1719, 1597, 1490; MS (FAB): 373 $(M+H)^+$; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 7.40–7.06 (m, 10H, Ar-H), 5.43 and 5.05 (each d, each 1H, $J=14.7$ Hz, $C_6H_5C\underline{H}_A$ and CH_B), 4.52 (s, 2H, H-4), 4.44 (s, 2H, H-6), 3.82 (t, 1H, $J=7.2$ Hz, NCH), 2.90 (d, 2H, $J=7.2$ Hz, $CHC\underline{H}_2C_6H_5$). ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 193.6 (C=S), 173.6(C=O), 136.5 and 135.4 (Ar-C), 129.4, 128.8 and 128.6 (ArCH), 67.4 (C-4), 64.2 (NCH), 56.7, 54.8, and 36.7 (CH_2's). Anal. calcd for $C_{19}H_{20}N_2O_2S_2$: C, 61.29; H, 5.37; N, 7.52. Found: C, 61.30; H, 5.4; N, 7.52.

5-(3-Carboxyethyl)-3-cyclohexyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (14). This was obtained by cyclohexyl amine (2.28 mL, 20 mmol) and β-alanine (2.06 g, 20 mmol) as described above and isolated as colourless solid, yield 67%, mp 148–50 °C (lit.[25] mp 145–146). IR (KBr): 3405, 1710, 1461; MS (FAB): 289$(M+H)^+$; ^1H NMR ($CDCl_3$): δ 4.34 (s, 2H, H-4), 4.32 (s, 2H, H-6), 3.07 (t, 2H, $J=6.5$ Hz, NCH_2), 2.53 (t, 2H, $J=6.4$, $C\underline{H}_2COOH$), 1.87–1.36 (m, 10H, cyclohexyl ring protons); ^{13}C NMR ($CDCl_3$): δ 190.9 (C=S), 173.5 (C=O), 65.1 (C-4), 57.9 (N–CH), 57.1 (C-6), 45.9, 33.3, 28.8, 25.6, 25.3 (CH_2's). Anal. calcd for $C_{12}H_{20}N_2O_2S_2$: C, 50.0; H, 6.94; N, 9.72. Found: C, 49.98; H, 7.0; N, 9.72.

5-[α-(Benzyl)carboxymethyl]-3-cyclohexyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (15). This was obtained by cyclohexyl amine (2.28 mL, 20 mmol) and DL-phenyl alanine (3.3 g, 20 mmol) as described above and isolated as colourless solid, yield 52%, mp 136–138 °C (lit.[25] mp 134–135 °C). IR (KBr): 3071, 2952, 1589, 1503; MS (FAB): 365 $(M+H)^+$; ^1H NMR (DMSO-d_6): δ 7.34–7.17 (m, 5H, ArH), 5.76 (m, 1H, NCH), 4.49 (s, 2H, H-4), 4.43 (s, 2H, H-6), 3.85–3.78 (m, 1H, NC$\underline{H}$COOH), 3.16 (d, 2H, $J=6.5$ Hz, $C\underline{H}_2C_6H_5$), 1.92–1.11 (m, 10H, cyclohexyl ring protons). Anal. calcd for $C_{18}H_{24}N_2O_2S_2$: C, 59.34; H, 6.59; N, 7.69. Found: C, 59.34; H, 6.61; N, 7.69.

5-(3-Carboxyethyl)-3-butyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (16). This was obtained by butyl amine (1.98 mL, 20 mmol) and β-alanine (1.78 g, 20 mmol) as described above and isolated as colourless solid, yield 75%, mp 125–127 °C; IR (KBr): 2956, 1718, 1507; MS (FAB): 263 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 4.38 (s, 2H, H-4), 4.37 (s, 2H, H-6), 3.99 (t, 2H, $J=7.7$ Hz, NCH_2CH_2COOH), 3.15 (t, 2H, $J=6.5$ Hz, $NC\underline{H}_2CH_2$), 2.61 (t, 2H, $J=6.4$ Hz, $C\underline{H}_2COOH$), 1.71–1.60 (m, 2H, $C\underline{H}_2CH_2CH_3$), 1.44–1.29 (m, 2H, $C\underline{H}_2CH_3$), 0.96 (t, 3H, $J=7.2$ Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 191.5 (C=S), 177.6 (C=O), 70.6 (C-4), 58.2 (C-6), 52.6 ($N\underline{C}H_2CH_2COOH$), 46.4 ($N\underline{C}H_2CH_2$), 33.4 ($\underline{C}H_2COOH$), 29.1 and 20.4 ($CH_2$'s), 14.2 ($CH_2\underline{C}H_3$). Anal. calcd for $C_{10}H_{18}N_2O_2S_2$: C, 45.80; H, 6.87; N, 10.68. Found: C, 45.80; H, 6.79; N, 10.87.

5-(3-Carboxyethyl)-3-octyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (17). It was obtained by octyl amine (3.30 mL, 20 mmol) and β-alanine (1.78 g, 20 mmol) as described above and isolated as colourless solid, yield 72%, mp 140–142 °C. IR (KBr): 3446, 1707, 1502; MS (FAB): 319 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 4.38 (s, 2H, H-4), 4.37 (s, 2H, H-6), 3.97 (t, 2H, $J=7.6$ Hz, NCH_2CH_2COOH), 3.15 (t, 2H, $J=6.4$, $NC\underline{H}_2CH_2$), 2.61 (t, 2H, $J=6.4$ Hz, $C\underline{H}_2COOH$), 1.30–127 (m, 12H, CH_2's), 0.88 (t, 3H, $J=7.1$ Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 191.5 (C=S), 177.5 (C=O), 70.6 (C-4), 58.2 (C-6), 52.8, 46.4, 33.4, 32.1, 29.6, 29.5, 27.2, 27.0, 22.9

(CH_2's), 14.4 ($CH_2\underline{C}H_3$). Anal. calcd for $C_{14}H_{26}N_2O_2S_2$: C, 52.83; H, 8.18; N, 8.80. Found: C, 52.85; H, 8.14; N, 8.61.

5-(3-Carboxyethyl)-3-dodecyl-tetrahydro-2H-1,3,5, thiadiazine-2-thione (18). This was obtained by dodecyl amine (4.62 mL, 20 mmol) and β-alanine (1.78 g, 20 mmol) as described above and isolated as colourless solid, yield 76%, mp 139–142 °C. IR (KBr): 3428, 1707, 1502; MS (FAB): 375 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): δ 4.38 (s, 2H, H-4), 4.36(s, 2H, H-6), 3.97 (t, 2H, $J=7.7$ Hz, $NC\underline{H}_2CH_2COOH$), 3.15 (t, 2H, $J=6.4$ Hz, $NC\underline{H}_2CH_2$), 2.61 (t, 2H, $J=6.5$ Hz, $C\underline{H}_2COOH$), 130–1.26 (m, 20H, CH_2's), 0.88 (t, 3H, $J=6.7$ Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 191.5 (C=S), 177 (C=O), 70.6 (C-4), 58.2 (C-6), 52.8, 46.4, 33.4, 32.3, 30.0, 29.9, 29.7, 29.6, 27.2, 27.0, 23.1 (CH_2's), 14.5 ($CH_2\underline{C}H_3$). Anal. calcd for $C_{18}H_{34}N_2O_2S_2$: C, 57.75; H, 9.09; N, 7.48. Found: C, 57.76; H, 9.09; N, 7.42.

General procedure for the synthesis of compounds 19–24

5-Cyclohexyl-3-(2′-furfuryl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (19). To a magnetically stirred solution of furfuryl amine (1.76 mL, 20 mmol) in 50 mL of H_2O, KOH (1.12 g, as 20% aq solution, 20 mmol) and CS_2 (1.20 mL, 20 mmol) were added at 30 °C. The reaction mixture was stirred for 4 h more followed by addition of 37% formaldehyde solution (3.1 mL, 40 mmol) and stirring continued for 1 h. The reaction mixture was filtered and resulting filtrate was added drop wise to a suspension of cyclohexyl amine (2.28 mL, 20 mmol) in 60 mL of phosphate buffer (pH 7.7)/toluene (1:2 mixture) and stirred again for 1 h. The reaction mixture filtered off and organic layer of the filtrate was separated, dried over sodium sulphate, concentrated and the product was allowed to solidify overnight which was further purified by crystallization with ethanol as colourless granules, yield 52%, mp 131–133 °C. IR (KBr): 2928, 1595, 1487; MS (FAB): 297 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): δ 7.37 (d, 1H, $J=1.8$ Hz, furfuryl H-5′), 6.47 (d, 1H, $J=3.2$ Hz, furfuryl H-3′), 6.44 (dd, 1H, $J=3.1$ and 1.8 Hz, furfuryl H-4′), 5.31 (s, 2H, furfuryl CH_2), 4.55 (s, 2H, H-4), 4.49 (s, 2H, H-6), 2.69 (m, 1H, NCH), 1.80–1.04 (m, 10H, cyclohexyl ring protons); ^{13}C NMR ($CDCl_3$): δ 193.9 (C=S), 149.2 (C-2′), 142.8 (C-5′), 111.2 (C-4′), 110.8 (C-3′), 66.7(C-4), 56.2(C-6), 55.6 (NCH), 47.0, 30.8, 25.9, 25.1 (CH_2's). Anal. calcd for $C_{14}H_{20}N_2OS_2$: C, 56.75; H, 6.75; N, 9.46. Found: C, 56.32; H, 6.79; N, 9.62.

5 - Cyclohexyl - 3 - (phenylmethyl) - tetrahydro - 2H - 1,3,5, thiadiazine-2-thione (20). This was obtained by benzyl amine (2.18 mL, 20 mmol) and cyclohexyl amine (2.28 mL, 20 mmol) as described above and isolated as colourless solid, yield 51%, mp 180–81 °C. IR (KBr): 2930, 1492, 1447; MS (FAB): 307 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): δ 7.45–7.26 (m, 5H, Ar-H), 5.34 (s, 2H, $C_6H_5C\underline{H}_2$), 4.50 (s, 2H, H-4), 4.39 (s, 2H, H-6), 2.61–2.58 (m, 1H, NCH), 1.58–0.93 (m, 10H, cyclohexyl ring protons); ^{13}C NMR ($CDCl_3$): δ 193 (C=S), 136 (Ar-C), 129.2, 129.1 and 128.5 (Ar-CH), 65.9 (C-4), 56.1 (C-6), 54.9 (NCH), 54.1, 30.7, 25.8, 25.0, (CH_2's). Anal. calcd for $C_{16}H_{22}N_2S_2$: C, 62.75; H, 7.19; N, 9.09. Found: C, 62.73; H, 7.19; N, 8.98.

5-Hexadecyl-3-(phenylmethyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (21). This was obtained by benzyl amine (2.18 mL, 20 mmol) and hexadecyl amine (4.82 g, 20 mmol) as described above and isolated as colourless solid, yield 64%, mp 78–81 °C. IR (KBr): 2918, 1595, 1489; MS (FAB): 449 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): δ 7.39–7.31 (m, 5H, Ar-H), 5.34 (s, 2H, $C_6H_5C\underline{H}_2$), 4.40 (s, 2H, H-4), 4.27(s, 2H, H-6), 2.55 (t, 2H, $J=7.6$ Hz, $NC\underline{H}_2CH_2$), 1.26–1.13 (m, 28H, CH_2's), 0.88 (t, 3H, $J=6.6$ Hz, $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$): δ 193.5 (C=S), 135.7 (Ar-C), 129.2, 128.9 and 128.5 (Ar-CH), 68.3 (C-4), 59.1 (C-6), 54.1 ($\underline{C}H_2C_6H_5$), 50.7, 32.3, 30.1, 29.9, 29.8, 29.7, 29.6, 27.5, 27.2, 23.1 (CH_2's), 14.5 ($CH_2\underline{C}H_3$). Anal. calcd for $C_{26}H_{44}N_2S_2$: C, 69.64; H, 9.82; N, 6.25. Found: C, 69.71, H, 9.82, N, 6.25.

5-Carbethoxyethyl-3-(cyclopropyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (22). This was obtained by cyclopropyl amine (1.39 mL, 20 mmol) and 2-amino ethyl propionate (2.34 g, 20 mmol) as described above and isolated as colourless solid, yield 55%, mp 80–81 °C. IR (KBr): 2970, 1731, 1470; MS (FAB): 275 $(M+H)^+$; ^{1}H NMR (CDCl3): δ 4.38 (s, 2H, H-4), 4.30 (s, 2H, H-6), 4.17 (q, 2H, $J=7.1$ Hz, $OC\underline{H}_2CH_3$), 3.13–3.04 (m, 3H, NCH_2 and NCH), 2.54 (t, 2H, $J=6.49$ Hz, CH_2COOH), 1.27 (t, 3H, $OCH_2C\underline{H}_3$), 1.08–0.81 (m, 4H, cyclopropyl ring protons); ^{13}C NMR ($CDCl_3$): δ 195.1 (C=S), 171.9 (C=O), 71.8 (C-4), 61.2 (OCH_2), 57.5 (C-6), 46.5 ($N\underline{C}H_2$), 36.2 ($N\underline{C}H$), 33.7 ($\underline{C}H_2COOEt$), 14.6 ($OCH_2\underline{C}H_3$), 9.3 (cyclopropyl ring carbons). Anal. calcd for $C_{11}H_{18}N_2O_2S_2$: C, 48.18; H, 6.56; N, 10.24. Found: C, 48.22; H, 6.52; N, 10.24.

5-Cyclohexyl-3-(cyclopropyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (23). This was obtained by cyclopropyl amine (1.39 mL, 20mmol) and cyclohexyl amine (2.28 mL, 20 mmol) as described above and isolated as yellow oil, yield 42%. IR (Neat): 2934, 1450; MS (FAB): 257 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): δ 4.46 (s, 2H, H-4), 4.42 (s, 2H, H-6), 3.12–3.06 (m, 1H, cyclohexyl NCH), 2.89 (m, 1H, cyclopropyl NCH), 1.97–1.27 (m, 10H, cyclohexyl ring protons), 1.02–0.85 (m, 4H, cyclopropyl ring protons); ^{13}C NMR ($CDCl_3$): δ 195.8 (C=S), 69.0 (C-4), 55.6 (NCH), 55.4 (C-6), 36.2 (NCH), 33.3, 31.5, 30.4, 25.9, 25.1, 9.5, 8.9 (CH_2's). Anal. calcd for $C_{12}H_{20}N_2S_2$: C, 56.25; H, 7.81; N, 10.93. Found: C, 56.26; H, 7.92; N, 11.01.

5-Cyclopropyl-3-(cyclopropyl)-tetrahydro-2H-1,3,5, thiadiazine-2-thione (24). It was obtained by cyclopropyl amine (1.39 mL, 20 mmol) and cyclopropyl amine (1.39 mL, 20 mmol) as described above and isolated as colourless solid, yield 45%, mp 92–93 °C; IR(KBr): 2999, 1449; MS (FAB): 215 $(M+H)^+$; ^{1}H NMR ($CDCl_3$): δ 4.40 (s, 2H, H-4), 4.35 (s, 2H, H-6), 3.15–3.10 (m, 1H, NCH), 2.46–2.40 (m, 1H, NCH), 1.05–0.89 (m, 4H, cyclopropyl ring protons), 0.66–0.58 (m, 4H, cyclopropyl ring protons); ^{13}C NMR ($CDCl_3$): δ 195.7 (C=S), 71.5 (C-4), 58.1 (C-6), 36.1 ($N\underline{C}H$), 32.2 ($N\underline{C}H$), 9.5 (cyclopropyl ring carbons), 7.4 (cyclopropyl ring

carbons). Anal. calcd for $C_9H_{14}N_2S_2$: C, 50.46; H, 6.54; N, 13.08. Found: C, 50.42; H, 6.51; N, 12.92.

Biology

Determination of antimycobacterial activity *in vitro*. The minimum inhibitory concentration (MIC) of the test compounds that inhibit the growth of *M. tuberculosis* H37Rv was determined by incorporating lowering concentrations of the test compound in Middlebrook 7H10 agar medium supplemented with OADC.[26] A culture of *M. tuberculosis* H37Rv growing on L-J medium[27] was harvested in 0.85% saline with 0.05% Tween-80. Approximately 5×10^4 colony forming unit bacilli (CFU) contained in 0.1 mL was plated on the Middlebrook medium with and without the test compounds and incubated at 37 °C for 4–6 weeks till the growth is visible to naked eyes.

Determination of antimycobacterial activity *in vivo*. Thirty inbred female AKR mice, weighing 18–20 g and bred in the animal house of this institute, were infected iv via *lateral* vein with 10^7 CFU of *M. tuberculosis* H37Rv. They were divided into three groups of 10 mice each after 2 days. One group received daily for 10 days the aqueous suspension of the test compounds by intra peritoneal route (ip) at the dose of 100 mg/kg body weight. The second group received sparfloxacin at 25 mg/kg body weight (ED_{90}) for 10 days by ip route, whereas the third group served as the control receiving no drug. Antitubercular activity was assessed by comparing mean survival time and the load of bacilli in the lungs and survival of treated and untreated mice.

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