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Some 3-Thioxo/Alkylthio-1,2,4-triazoles with a Substituted Thiourea Moiety as Possible Antimycobacterials

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Abstract—A series of novel *N*-alkyl/aryl-*N*′-[4-(4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione-5-yl)phenyl]thioureas **1**–**19** and three *S*-alkylated representatives of the former, *N*-alkyl/aryl-*N*′-[4-(3-aralkylthio-4-alkyl/aryl-4*H*-1,2,4-triazole-5-yl)phenyl]thioureas **20**–**22**, were synthesized and tested for antimycobacterial activity against *Mycobacterium tuberculosis H37Rv* as well as *Mycobacterium fortuitum ATCC 6841* which is a rapid growing opportunistic pathogen. Compounds **4** and **9**–**11** were found to possess the same MIC value with that of Tobramycin against *M. fortuitum ATCC 6841* whereas **1**–**3** and **21** had positive response against *M. tuberculosis H37Rv* at varying degrees. Compound **21** was identified as the most potent derivative of the **1**–**22** series by an MIC value of 6.25 μg/mL and selectivity index of 1.6.

Introduction

There has been an increasing demand for new antituberculosis agents, as the fast development of mycobacterial resistance to conventional drugs is one of the major difficulties in the treatment of tuberculosis. Among azoles, 1,2,4-triazoles have been reported to exhibit antibacterial,[1–5] antifungal,[3–7] antiviral[8] and tuberculostatic[9] activities. Earlier reports also indicated similar biological properties such as antibacterial[10] and anti-HIV[11,12] effects associated with substituted thioureas. Thiacetazone [**A**] which possesses a thiosemicarbazone structure, has been reported as a tuberculostatic agent. In a previous work, Doub and co-workers revealed antituberculosis properties of a wide number of phenylthioureas [**B**].[13] Glasser and Dougthy also reported similarly the same type of activity associated with a number of thiourea derivatives substituted with a heterocyclic ring at one nitrogen.[14] Based on these observations we have prepared a number of *N*-substituted-*N*′-[4-(4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione-5-yl)phenyl]thioureas **1**–**19** as well as three *S*-alkylated derivatives **20**–**22**. The similarity of pharmacophores present in our structures with those of reported antituberculosis agents led us to investigate the antimycobacterial effects of these compounds (Fig. 1).

Chemistry

Synthesis of **1**–**22** required stepwise reactions including benzoylation of benzocaine[15] at the first step which provided protection of the amino function during the following two reactions (Scheme 1) and allowed to introduce different groups at R_1 and R_2 positions. Hydrazinolysis of **I** then gave 4-(benzoylamino)-benzoylhydrazine **II**[15] which was then reacted with alkyl/aryl isothiocyanates to give 1-aroyl-4-alkyl/aryl thiosemicarbazides **III**[16] as described. Cyclocondensation of the latter to give 5-(4-aminophenyl)-4-alkyl/aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **IV** was performed by refluxing **III** in alkaline medium.[6] 3-Benzylthio-4-alkyl/aryl-5-(4-aminophenyl)-4*H*-1,2,4-triazoles **V** were obtained as previously described by the reactions of **IV** with appropriate benzylchlorides in ethanolic sodium hydroxide.[17,18]

The final step comprised of the reaction of the amines **IV** or **V** with various alkyl or aryl isothiocyanates yielding the corresponding title compounds **1**–**22**. This kind of reaction was reported to be performed in certain dry solvents or mixtures.[10,17,19–22] In the present study,

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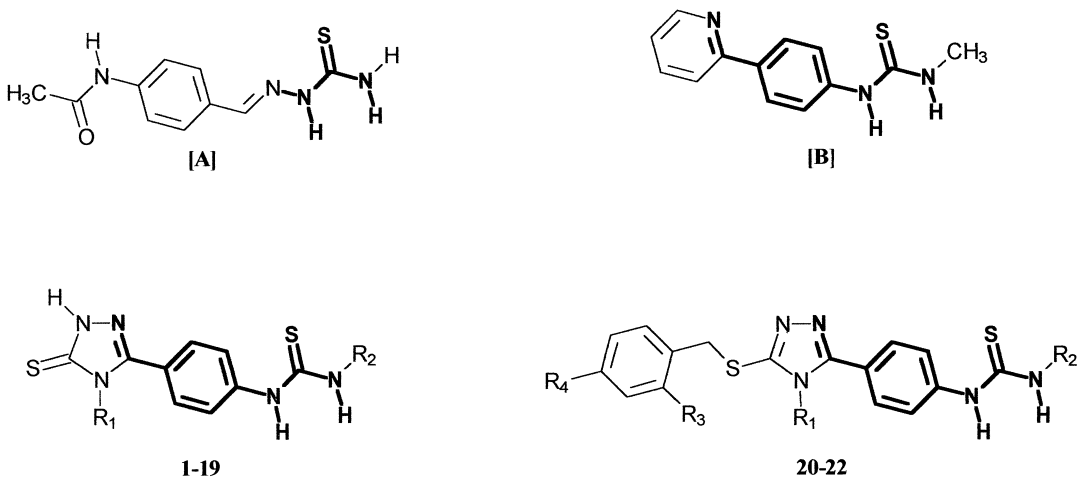
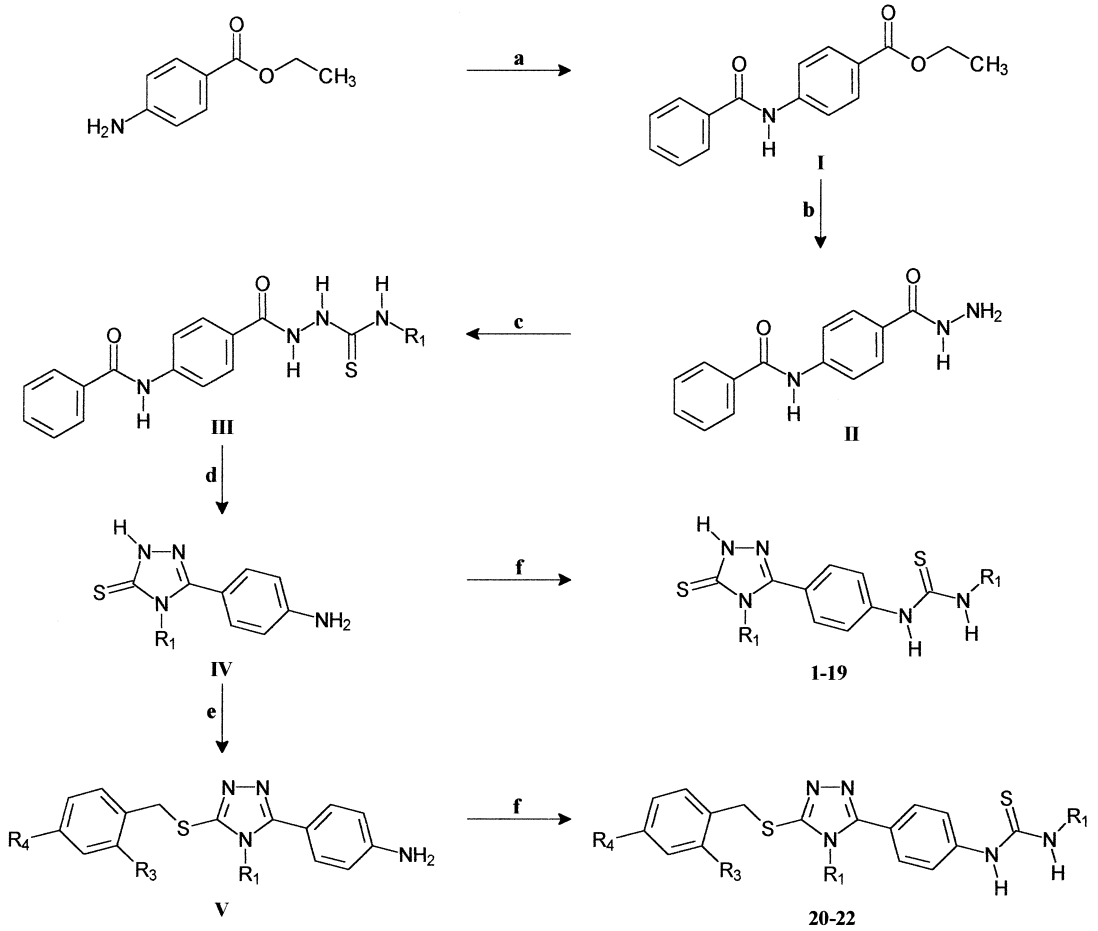


Figure 1.



Scheme 1. Synthetic route to **1**–**22**. Reagents and conditions: (a) $C_6H_5COCl/(C_2H_5)_2O$; (b) $H_2N–NH_2·H_2O$/EtOH, reflux; (c) R_1-NCS/EtOH, reflux; (d) NaOH (2 N), reflux; (e) Ar-CH_2Cl/NaOH–EtOH; (f) R_2-NCS/dioxane–MeOH (2:1, v/v), reflux.

dioxane–methanol (1:2, v/v) was tried and found to be useful.[23,24] The yields of title compounds seemed, however, to be affected by the possible formation of several byproducts such as urethans.[13] Another yield-limiting factor might be the side reaction of isothiocyanates with heterocyclic nitrogen at the second position of 1,2,4-triazoline ring present in **IV** series.[22] In spite of these probabilities, spectral findings such as the ^{1}H NMR resonances at 13–14 ppm due to heterocyclic NH–CS function and the lack of resonances attributable to NH_2 function supports the regioselectivity of this reaction. Of these compounds, **8**,[25] **20**,[26] **21**[27] and **22**[28] were originally synthesized in the present study and were characterized by UV, IR and ^{1}H NMR spectroscopy data as well as elemental analysis.

Results and Discussion

Antimycobacterial activity of the synthesized compounds were investigated against *M. fortuitum ATCC 6841*, a rapidly growing strain, by the use of microdilution broth susceptibility method.[29–32] As shown in Table 1, most of the thiourea derivatives showed activity against *M. fortuitum ATCC 6841* whilst six compounds, **8**, **12**, **18** and **20**–**22** were found completely inactive. Of the screened compounds, **4**, **9**, **10** and **11** were observed to have the same MIC with that of tobramycin, the standard used, by 32 μg/mL. However, it was also observed that the substituents at the terminal nitrogen of thiourea function had no determining influence on the antimycobacterial activity towards *M. fortuitum ATCC 6841*.

Compounds **1**–**22** were also tested for in vitro antituberculosis activity against *M. tuberculosis H37Rv* using the BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[33,34] Rifampicin was used as the standard in the antimycobacterial assays. As shown in Table 1, compounds **8** and **10** were completely inactive against *M. tuberculosis H37Rv* at 12.5 μg/mL, whereas remaining compounds exhibited varying degrees of inhibition in the primary screen. Of these compounds, the ones which exhibited < 90% inhibition in the primary screen (MIC > 12.5 μg/mL) were not considered for further evaluation.

Compounds **1**–**3** effecting ≥90% inhibition in the primary screen at 12.5 μg/mL were re-tested at lower concentrations against *M. tuberculosis H37Rv* to determine the actual MIC in a broth microdilution assay using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Only the re-test of **21** was performed using the BACTEC 460 radiometric system as it could not be achieved using MABA because of the fluorescent nature of this compound.[33]

As illustrated in Table 2, only one 3-alklythio derivative (**21**) of **1**–**22** demonstrated potent inhibitory activity against *M. tuberculosis H37Rv* by an MIC value of 6.25 μg/mL, whereas remaining compounds **1**–**3**, which were selected for level 2 screening, did not possess an MIC less than 12.5 μg/mL.

Compound **21** was also screened to assess toxicity to a VERO cell line at concentrations equal to and greater than the MIC for *M. tuberculosis H37Rv*.[34] The IC_{50} value was found at a concentration level of > 10 μg/mL for compound **21** and the resulting selectivity index ($SI = IC_{50}/MIC$) was calculated as > 1.6 (Table 2). Having the SI < 10, this compound was not considered to be evaluated further due to its remarkable cytotoxicity.

Three representatives of **1**–**19** series, namely compounds **1**–**3** were tested in vitro for killing of *M. tuberculosis Erdman* (ATCC 35801) in monolayers of mouse bone marrow macrophages by determining EC_{90} and EC_{99}, lowest concentration effecting a 90 and 99% reduction, respectively, in colony forming units at 7 days compared to drug-free controls.[35] As shown in Table 2, compounds **1**–**3** were observed to effect 90% reduction in mycobacterial growth at concentration levels 18–34-fold lower than those of their MIC values whilst EC_{99} of the above were extremely high to compare to that of standard used.

In many cases, it has been demonstrated that substituted thioureas may be of interest as antibacterial,

Table 1. Antimycobacterial activity versus *M. fortuitum ATCC 6841* and primary antituberculosis activity screening results of **1**–**22**

Compound	R_1	R_2	R_3	R_4	MIC[a] (μg/mL)	MIC[b] (μg/mL)	Inhibition[c] (%)
1	CH_3	CH_3	—	—	64	< 12.5	93
2	CH_3	CH_2CH_3	—	—	64	< 12.5	90
3	CH_3	$CH_2CH{=}CH_2$	—	—	64	< 12.5	94
4	CH_3	C_6H_{11}	—	—	32	> 12.5	10
5	CH_3	C_6H_5	—	—	64	> 12.5	29
6	CH_2CH_3	$CH_2CH{=}CH_2$	—	—	64	> 12.5	89
7	CH_2CH_3	C_6H_{11}	—	—	64	> 12.5	10
8	$CH_2CH{=}CH_2$	CH_3	—	—	> 128	> 12.5	0
9	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	—	—	32	> 12.5	71
10	$CH_2CH{=}CH_2$	C_6H_5	—	—	32	> 12.5	0
11	C_6H_{11}	$CH_2CH{=}CH_2$	—	—	32	> 12.5	35
12	C_6H_{11}	C_6H_{11}	—	—	> 128	> 12.5	12
13	$CH_2CH_2C_6H_5$	$CH_2CH{=}CH_2$	—	—	64	> 12.5	6
14	C_6H_5	CH_3	—	—	64	> 12.5	27
15	C_6H_5	CH_2CH_3	—	—	64	> 12.5	17
16	C_6H_5	$CH_2CH{=}CH_2$	—	—	64	> 12.5	12
17	C_6H_5	C_6H_{11}	—	—	64	> 12.5	13
18	C_6H_5	C_6H_5	—	—	> 128	> 12.5	13
19	C_6H_5	$C_6H_4Cl(4)$	—	—	64	> 12.5	11
20	CH_3	CH_3	H	H	> 128	> 12.5	85
21	CH_3	$CH_2CH{=}CH_2$	Cl	Cl	> 128	< 12.5	94
22	C_6H_5	$CH_2CH{=}CH_2$	Cl	Cl	> 128	> 12.5	63

[a]MIC effecting *M. fortuitum ATCC6841*; MIC of tobramycin = 32 μg/mL.
[b]MIC effecting *M. tuberculosis H37Rv*; MIC of rifampicin = 0.25 μg/mL.
[c]Reduction of mycobacterial growth using *M. tuberculosis H37Rv* at 12.5 μg/mL.

Table 2. Level 2 antituberculosis activity assay results of **1**–**3** and **21**

Compound	Level 2 assay			Level 3 assay		
	MIC (μg/mL)	IC_{50}[a] (μg/mL)	SI	EC_{90}[b]	EC_{99}[c]	EC_{90}/MIC
1	>12.5	—	—	0.371	>100	<0.02968
2	>12.5	—	—	0.303	48.65	<0.02424
3	>12.5	—	—	0.698	>100	<0.05584
21	6.25[d]	>10	>1.6	—	—	—

[a]IC_{50} of RMP = 147.
[b]EC_{90} of RMP = 0.007.
[c]EC_{99} of RMP = 0.451.
[d]The assay was performed using BACTEC in which MIC of RMP was found as 0.125 μg/mL.

antiviral or antituberculosis agents. As 1,2,4-triazoles have also been reported as possible antimicrobials, incorporation of these two moieties in a single molecule was aimed to enhance possible antituberculosis activity which might arise from each side.

In contrast to the results obtained from *M. fortuitum ATCC 6841*, in vitro antituberculosis assay results of **1**–**22** using *M. tuberculosis H37Rv* revealed that either substituents at the N-4 position of the triazole ring (R_1) or the ones at the terminal nitrogen of thioureas (R_2) influenced the antituberculosis activity. As shown in Tables 1 and 2, optimal activity was achieved with the derivatives possessing a methyl group at the R_1 position whilst a linear decrease in antituberculosis activity was observed in the direction of methyl > ethyl > allyl > heavier groups. On the other hand, functionalities bulkier than an allyl group at the R_2 position also gave rise to almost complete loss of inhibition of mycobacterial growth. The fact that highest antituberculosis activity in phenylthioureas could be reached by derivatives with the terminal nitrogen substituted by a single short chain alkyl group, was also in accordance with previous reports.[13,14]

From the above data one can conclude that both 1,2,4-triazole or thiourea components of the structures **1**–**22** contributed to antituberculosis activity. Moreover, bulky groups introduced at either R_1 and R_2 positions led to a dramatic decrease in activity most probably due to a steric hindrance which does not allow the compounds to reach the active site. In addition, *S*-alkylation products of three derivatives selected from the **1**–**19** series have been prepared not only to mask the –**NH**–**CS**– function present in 1,2,4-triazoline-3-thiones but also to provide them an increased lipophilicity. This modification led to the most active derivative, compound **21**, which still needs to be improved from the toxicological point of view.

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25. *N*-Methyl-*N*′-[4-(4-allyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione-5-yl)phenyl]thiourea **8**: M_r 305.42; mp 197–199 °C; Yield 45%; UV λ_{max} (ε) 260 (28832), 285 (26724) nm; IR ν_{max} 3436, 3342, 3107, 2931, 1601, 1578, 1513, 1443, 1319, 1313, 1262, 1190 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.94 (d, 3H, CH_3), 4.64–4.71 (d, 2H, N-CH_2), 4.86 (d, 1H, J = 17.3 Hz, CH=CH_2 *trans*), 5.15 (d, 1H, J = 10.5 Hz, CH=CH_2 *cis*), 5.81–5.89 (m, 1H, –CH=), 7.58–7.64 (m, 4H), 7.78–8.16 (b, 1H, NH-CH_3), 9.08–10.31 (b, 1H, Ar–NH), 13.75 (b, 1H, NH–triazoline); anal. for $C_{13}H_{15}N_5S_2 \cdot 1/2\ H_2O$ (%, calcd/found): 49.68/50.33 (C), 5.09/4.89 (H), 22.29/21.49 (N).

26. *N*-Methyl-*N*′-[4-(3-benzylthio-4-methyl-4*H*-1,2,4-triazole-5-yl)phenyl]thiourea **20**: M_r 369.51; mp 200–202 °C; yield 74%; UV λ_{max} (ε) 231.3 (18549), 256.4 (24794), 289.4 (28822) nm; IR ν_{max} 3283, 3248, 3060, 1607, 1548, 1478, 1419, 1319, 1313, 1272, 714 cm^{-1} ; ^1H NMR (DMSO-d_6) δ 2.95 (d, 3H, J = 4 Hz, CS–NH–CH_3), 3.41 (s, 3H, >N–CH_3), 4.36 (s, 2H, S-CH_2), 7.26–7.61 (m, 9H, Ar-H), 7.92 (b, 1H, NH-CH_3), 9.84 (b, 1H, Ar-NH). Anal. for $C_{18}H_{19}N_5S_2$ (%, calcd/found): 58.51/58.16 (C), 5.18/4.96 (H), 18.95/18.68 (N).

27. *N*-Allyl-*N*′-{4-[3-[(2,4-dichlorobenzyl)thio]-4-methyl-4*H*-1,2,4-triazole-5-yl]phenyl}thiourea **21**: M_r 464.43; mp 186–189 °C; yield 65%; UV λ_{max} (ε) 234 (33857), 257 (37108), 291 (37805) nm; IR ν_{max} 3260, 3060, 1608, 1590, 1543, 1472, 1320, 1261, 732 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.52 (s, 3H, >N–CH_3), 4.16 (s, 2H, N-CH_2), 4.41 (s, 2H, S-CH_2), 5.11–5.14 (d, 1H, J = 9.3Hz, CH=CH_2 *cis*), 5.19–5.24 (d, 1H, J = 9.3 Hz, CH=CH_2 *trans*), 5.88–5.92 (m, 1H, –CH=), 7.37–7.68 (m, 7H, Ar–H), 7.86–8.31 (b, 1H, NH–CH_2), 9.41–10.01 (b, 1H, Ar–NH). Anal. for $C_{20}H_{19}Cl_2N_5S_2$ (%, calcd/found): 51.72/51.81 (C), 4.12/4.01 (H), 15.08/14.92 (N).

28. *N*-Allyl-*N*′-{4-[3-[(2,4-dichlorobenzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-5-yl]phenyl}thiourea **22**: M_r 526.51; mp. 195–198 °C; yield 60%; UV λ_{max} (ε) 232 (14264), 262 (11966), 298 (15699) nm; IR ν_{max} 3648, 3237, 1595, 1560, 1543, 1319, 1272, 726 cm^{-1} ; ^1H NMR (DMSO-d_6) δ 4.11 (s, 2H, NH–CH_2), 4.43 (s, 2H, S-CH_2), 5.08–5.11 (d, 1H, J = 10.3 Hz, CH=CH_2 *cis*), 5.15–5.20 (d, 1H, J = 17.3 Hz, CH=CH_2 *trans*), 5.84–5.91 (m, 1H, –CH=), 7.26–7.61 (m, 12H, Ar–H), 8.01 (b, 1H, NH–CH_2), 9.67 (b, 1H, Ar–NH). Anal. for $C_{25}H_{21}Cl_2N_5S_2$ (%, calcd/found): 57.03/57.45 (C), 4.02/4.09 (H), 13.30/13.14 (N).

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