Discovery of New Antitubercular Oxazolyl Thiosemicarbazones

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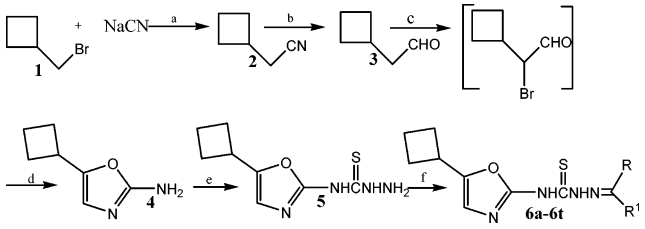
Abstract: Twenty 4-(5-cyclobutyloxazol-2-yl)thiosemicarbazones were synthesized and evaluated for preliminary in vitro and in vivo activity against *Mycobacterium tuberculosis* H37Rv (MTB) and multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB). Among them, (4-bromophenyl)(phenyl)methanone *N*-(5-cyclobutyl-1,3-oxazol-2-yl)-thiosemicarbazone **6q** was found to be the most active compound in vitro with minimum inhibitory concentration of 0.05 μg/mL against MTB and MDR-TB. In the in vivo animal model **6q** decreased the bacterial load in lung and spleen tissues with 2.1 log 10 and 3.72 log 10 protections, respectively, at 50 mg/kg body weight dose.

Tuberculosis (TB) remains the leading cause of mortality because of a bacterial pathogen, *Mycobacterium tuberculosis*. It is estimated that 8.2 million new TB cases occurred worldwide in the year 2000, with approximately 1.8 million deaths in the same year, and more than 95% of those were in developing countries.[1] The incidence of TB infection has steadily risen in the past decade, and this increase can be attributed to a similar increase in human immunodeficiency virus (HIV) infection.[2] The association of TB and HIV infections is so dramatic that in some cases nearly two-thirds of the patients diagnosed with TB are also HIV-1 seropositive.[3] Furthermore, numerous studies have shown that TB is a cofactor in the progression of HIV infection.[4] The reemergence of TB infection is further complicated by an increase in cases that are resistant to conventional antitubercular drug therapy. Not only does the increasing rate of multidrug-resistant TB create problems for the treatment but also the costs are exploding. Thus, new drugs are necessary to overcome the current problems of therapy. Earlier we reported antitubercular N^1-(4-acetamido phenyl)-N^4-(2-nitrobenzylidene)-semicarbazone, which inhibited in vitro *Mycobacterium tuberculosis* H37Rv (MTB) and was more potent than the commonly used antitubercular agents.[5] In the course of screening to discover new compounds employed in the chemotherapy of tuberculosis, we identified oxazolylthiosemicarbazones derivatives that inhibited in vitro MTB and multidrug resistant *Mycobacterium tuberculosis* (MDR-TB). We present the preliminary results concerning the synthesis and the in vitro and in vivo antituberculous activity of the first representative compound of this family.

Bromomethylcyclobutane (**1**) was reacted with sodium cyanide in dimethylsulfoxide medium at 70 °C for 3 h to afford 2-cyclobutylacetonitrile (**2**) in 75% yield.[6] 2-Cyclobutylacetonitrile was dissolved in dry dichloromethane and was reduced with diisobutylaluminum hydride (20% weight by solution in toluene) at −75 °C under nitrogen atmosphere to yield 2-cyclobutylacetaldehyde (**3**) in 70% yield.[7] Cyclobutylacetaldehyde, on treatment with bromine in dichloromethane, yielded 2-bromo-2-cyclobutylacetaldehyde, which on heating with urea in dimethyl formamide at 90 °C for 3 h yielded 5-cyclobutyloxazol-2-amine[8] (**4**) in 48% yield. The synthesis of thiosemicarbazone derivatives was carried out in three steps,[9] as shown in Scheme 1. First, to a solution of 5-cyclobutyloxazol-2-amine (0.01 mol) in THF (10 mL) was added potassium hydroxide (0.01 mol) and carbon disulfide (0.75 mL), and the mixture was stirred at 15−20 °C for 1 h to form a potassium salt of dithiocarbamate. To the stirred mixture was added hydrazine hydrate (0.01 mol), and the stirring was continued at 60 °C for 1 h to obtain 4-(5-cyclobutyloxazol-2-yl)thiosemicarbazide (**5**) in 90% yield. Thiosemicarbazide derivative on condensation with various carbonyl compounds in the presence of glacial acetic acid afforded various thiosemicarbazones (**6a**−**t**) (Table 1) in 62−86% yields. The purity of the compounds was checked by TLC and elemental analyses, and the compounds of this study were identified by spectral data. In the ^{1}H NMR spectra the signals of the respective protons of the prepared derivatives were verified on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of all the compounds showed a singlet at δ 6.68 ppm corresponding to fourth-position proton of the oxazole ring, a broad multiplet in the region of 1.76−2.28 corresponding to six cyclobutyl protons, a quintet with $J = 7.2$ corresponding to a single proton of the cyclobutyl ring, and a D_2O exchangeable singlet at δ 7.26 ppm corresponding to NH protons. The elemental analysis results were within ±0.4% of the theoretical values.

Scheme 1[a]



[a] Reagents: (a) DMSO; (b) DIBAL-H, CH_2Cl_2; (c) Br_2, CH_2Cl_2; (d) NH_2CONH_2, DMF; (e) CS_2, NaOH, NH_2NH_2, C_2H_5OH, HCl; (f) CH_3COONa, O=CRR^1, C_2H_5OH.

All compounds were screened for their in vitro antimycobacterial activity against MTB and MDR-TB by an agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards[10] for the determination of minimum inhibitory concentration (MIC) in duplicate. The MDR-TB clinical isolate was obtained from Tuberculosis Research Center, Chennai, India, and was resistant to isoniazid (INH), rifampicin, ethambutol, and ciprofloxacin. The MIC is defined as the minimum concentration of compound required to give 99% inhibition of bacterial growth, and MIC values of the synthesized compounds along with the standard drugs for comparison were reported (Table 1). Among the synthesized compounds, **6q** (MIC = 0.05 μg/mL) was equally active as INH and was more potent than rifampicin against MTB. Compared to ethambutol (MIC = 1.56 μg/mL), eight compounds were more potent against MTB. Against MDR-TB, when compared to INH (MIC = 1.56 μg/mL), eight compounds were more active with MIC values ranging from 0.05 to 0.78 μg/mL. Compound **6q** was found to be the most potent (MIC = 0.05 μg/mL) and was 31 times more potent against MDR-TB when compared to the standard drug INH. Compared to rifampicin (MIC = 3.12 μg/mL), nine compounds were more potent and five compounds were equipotent against MTB. All the compounds were more potent than ethambutol against MDR-TB.

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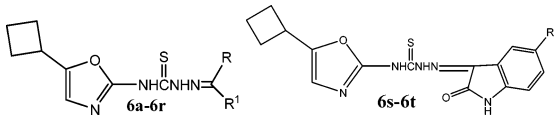
Table 1. Physical Constants and in Vitro Antimycobacterial and Cytotoxicity of the Synthesized Compounds



compd	R	R^1	yield (%)	mp (°C)	IC_{50} [a] (μg/mL)	MIC in (μg/mL) MTB[b]	MIC in (μg/mL) MDR-TB[c]
6a	H	2-hydroxyphenyl	62	152	NT	6.25	6.25
6b	H	2-nitrophenyl	76	205	NT	0.78	0.78
6c	H	3-nitrophenyl	73	183	<62.5	0.78	0.78
6d	H	4-nitrophenyl	81	237	<62.5	0.39	0.39
6e	H	4-methylphenyl	71	155	<62.5	3.12	3.12
6f	H	4-chlorophenyl	67	207	NT	1.56	1.56
6g	H	4-dimethylaminophenyl	86	256	NT	3.12	3.12
6h	H	4-methoxyphenyl	64	166	NT	6.25	6.25
6i	H	4-hydroxy-3-methoxyphenyl	67	172	NT	12.5	12.5
6j	CH_3	phenyl	70	126	NT	3.12	3.12
6k	CH_3	2-hydroxyphenyl	69	111	NT	3.12	3.12
6l	CH_3	4-hydroxyphenyl	64	131	NT	3.12	3.12
6m	CH_3	4-methylphenyl	70	57	NT	0.78	0.78
6n	CH_3	4-aminophenyl	63	107	62.5	0.78	0.78
6o	CH_3	4-nitrophenyl	68	188	62.5	0.20	0.20
6p	C_6H_5	phenyl	60	41	>62.5	0.10	0.10
6q	C_6H_5	4-bromophenyl	62	65	>62.5	0.05	0.05
6r	$C_6H_5CH_2$-	benzyl	66	91	NT	6.25	6.25
6s	H		78	194	NT	25.00	25.00
6t	F		72	168	NT	12.5	12.5
Ison					>62.5	0.05	1.56
Rifa					>62.5	0.1	3.12
Gati					>62.5	0.2	6.25
Etha					>62.5	1.56	25.00

[a] NT: not tested. [b] *Mycobacterium tuberculosis* H37Rv. [c] Multidrug-resistant *Mycobacterium tuberculosis*.

Table 2. In Vivo Activity Data of **6q**, Isoniazid, and Gatifloxacin against *M. tuberculosis* in Mice

compd	log CFU ± SEM lung	log CFU ± SEM spleen
control	7.88 ± 0.22	8.84 ± 0.21
6q (50 mg/kg)	5.78 ± 0.12	5.12 ± 0.10
gatifloxacin (50 mg/kg)	6.02 ± 0.18	6.24 ± 0.12
isoniazid (25 mg/kg)	5.12 ± 0.10	4.11 ± 0.17

Compound (4-bromophenyl)(phenyl)methanone *N*-(5-cyclobutyl-1,3-oxazol-2-yl)thiosemicarbazone (**6q**) was found to be the most active compound in vitro with an MIC of 0.05 μg/mL against MTB and MDR-TB. Among the benzaldehyde and actophenone derived thiosemicarbazones (**6a**−**r**), substituents with electron-withdrawing groups such as nitro (**6b**−**d**,**o**) and chloro (**6f**) enhanced the activity. With respect to the carbimino terminal, the order of activity was found to be (sub)dipheylbenzophenone > (sub)acetophenone > (sub)benzaldehyde > dibenzyl ketone > isatin. The intermediate 4-(5-cyclobutyloxazol-2-yl)thiosemicarbazide (**5**) did not show any inhibition to 25 μg/mL.

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at 62.5 μg/mL. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 nonradioactive cell proliferation assay.[11] Compounds **6p** and **6q** were found to be nontoxic to 62.5 μg/mL. Compound **6p** showed selectivity index (IC_{50}/ MIC) of more than 1250.

Subsequently, **6q** was tested for efficacy against MTB at a dose of 50 mg/kg (Table 2) in 6-week-old female CD-1 mice. In this model,[12] the mice were infected intravenously through the caudal vein with approximately 10^7 viable *M. tuberculosis* ATCC 35801. Drug treatment began after inoculation of the animal with microorganism for 10 days by the intraperitoneal route. Thirty-five days after infection, the spleens and right lungs were aseptically removed and ground in a tissue homogenizer and the number of viable organisms was determined by serial 10-fold dilutions and subsequent inoculation onto 7H10 agar plates. Cultures were incubated at 37 °C in ambient air for 4 weeks prior to counting. Bacterial counts were measured and compared with the counts from negative (untreated) controls (mean culture forming units (CFU) in lung, 7.88; mean CFU in spleen, 8.84). Compound **6q** decreased the bacterial load in lung and spleen tissues with 2.1 log 10 and 3.72 log 10 protections, respectively, and was considered to be promising in reducing bacterial count in lung and spleen tissues. When compared to gatifloxacin at the same dose level, **6q** decreases the bacterial load with 0.24 log 10 and 1.12 log 10 protections in lung and spleen tissues, respectively. Compound **6q** was found to be less active than INH in the in vivo study. The reason for this less in vivo activity might be due to the instability of the compounds, as it gets hydrolyzed to the inactive intermediate 4-(5-cyclobutyloxazol-2-yl)thiosemicarbazide (**5**).

Screening of the antimycobacterial activity of these novel series identified oxazolylthiosemicarbazones as a new lead endowed with antitubercular activity, exhibiting MIC values between 0.05 and 12.5 μg/mL. In conclusion, it has been shown that the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity. Further structure−activity and mechanistic studies should prove to be fruitful.

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Supporting Information Available: Experimental procedures for synthesis and analytical data of representative compounds are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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