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Antimycobacterial activity of novel 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(sub)phenyl/pyridylthiourea compounds endowed with high activity toward multidrug-resistant *Mycobacterium tuberculosis*

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***Objectives*: The objective of this work was to synthesize 15 new 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(sub)-phenyl/pyridylthiourea compounds and evaluate their *in vitro* and *in vivo* antimycobacterial activities.**

***Methods*: 5-Cyclobutyloxazol-2-amine was reacted with 1,1′-thiocarbonyldiimidazole, followed by various substituted anilines and 2-amino pyridines to yield the 15 compounds, which were subjected to *in vitro* and *in vivo* evaluation against *Mycobacterium tuberculosis* H37Rv (MTB) and a clinical isolate of multidrug-resistant *M. tuberculosis* (MDR-TB).**

***Results*: Among the 15 compounds screened, 7 compounds inhibited both MTB and MDR-TB *in vitro* with MICs of <1 μM. In the *in vivo* screening, compound 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(2′-trifluoromethyl)phenylthiourea (compound 8) was equally active as isoniazid at the same dose level.**

***Conclusions*: Compound 8 was found to be the most active, with an *in vitro* MIC of 0.14 μM and was 2.5 and 80 times more active than isoniazid against MTB and MDR-TB, respectively. Compound 8 was non-toxic to Vero cells up to 183 μM, with a selectivity index of >1307. In the *in vivo* animal model, compound 8 decreased the mycobacterium load in lung and spleen tissues with 2.8 and 3.94 $\log_{10}$ reductions, respectively.**

Key words: antimycobacterial activity, antitubercular activity, thiourea derivatives, oxazole derivatives

Introduction

The emergence of drug-resistant *Mycobacterium tuberculosis* H37Rv (MTB) and particularly multidrug-resistant *M. tuberculosis* (MDR-TB) poses a serious threat to treatment and control of disease. Among the new classes of compounds under investigation, the oxazolyl thiosemicarbazone derivatives were first reported by us in 2006[1] as antimycobacterial agents. The most potent derivative (4-bromophenyl)(phenyl)methanone *N*-(5-cyclobutyl-1,3-oxazol-2-yl)thiosemicarbazone (**6q**) showed an MIC of 0.05 mg/L against MTB and MDR-TB. Compound **6q** was found to be 50% less active than isoniazid in the *in vivo* animal study model at the dose level tested. The reduced *in vivo* activity might be due to the instability of the compound, as it is hydrolysed into the inactive intermediate 4-(5-cyclobutyloxazol-2-yl)thiosemicarbazide.[1] In an effort to search for novel derivatives of **6q** endowed with a better biological profile and *in vivo* stability, we synthesized 15 new 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(sub)phenyl/pyridyl thioureas and evaluated their *in vitro* and *in vivo* antimycobacterial activities.

Materials and methods

Compounds

All the compounds were synthesized from 5-cyclobutyloxazol-2-amine by reacting with 1,1′-thiocarbonyl diimidazole, followed by appropriate substituted anilines or 2-amino pyridines by a previously reported procedure.[2]

MIC determination

All compounds were screened for their *in vitro* antimycobacterial activity against MTB and MDR-TB by an agar dilution method

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similar to that recommended by the CLSI (formerly NCCLS)[3] for the determination of MIC in duplicate. The MDR-TB clinical isolate was obtained from the Tuberculosis Research Centre (Chennai, India) and was resistant to isoniazid, rifampicin, ethambutol and ofloxacin. MIC is defined as the minimum concentration of a compound required to give complete inhibition of bacterial growth.

Cytotoxicity

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at concentrations of 62.5 mg/L. After 72 h of exposure, viability was assessed on the basis of cellular conversion of [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.[4]

In vivo studies

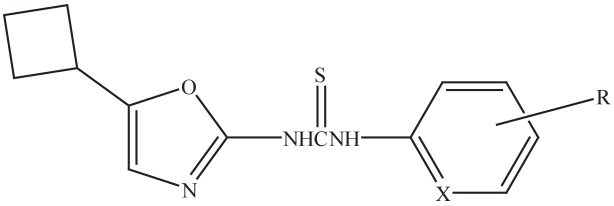
The experiments described were approved by the Institutional Animal Ethics Committee (IAEC; Protocol no. IAEC/RES/11). Compound **8**, which showed good activity in the *in vitro* study, was tested for efficacy against MTB at a dose of 25 mg/kg in 6-week-old female CD-1 mice, six per group. In this model,[5] the mice were infected intravenously through the caudal vein with ~10^7 viable *M. tuberculosis* ATCC 35801. Drug treatment by the intraperitoneal route began after 10 days of inoculation of the animal with the microorganism and continued for 10 days. Thirty-five days post-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 controls (vehicle-treated) in lungs and spleen.

Results and discussion

Fifteen novel 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(sub)phenyl/pyridylthioureas were prepared from 5-cyclobutyloxazol-2-amine by one pot synthesis via an unisolated thiocarbamoyl intermediate. The structures were elucidated with the help of 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 6.62 ppm corresponding to the fourth-position proton of the oxazole ring, a broad multiplet in the region of 1.68–2.2 corresponding to six cyclobutyl protons, a quintet with $J = 7.2$ corresponding to the single proton of the cyclobutyl ring and D_2O exchangeable bi-singlets at δ 7.75 ppm corresponding to NH protons. The elemental analysis results were within ±0.4% of the theoretical values. All the newly synthesized compounds were screened for their *in vitro* antimycobacterial activity against MTB and MDR-TB by an agar dilution method and MICs of the synthesized compounds along with the standard drugs for comparison are reported (Table 1). All the compounds showed excellent activity against both MTB and MDR-TB with MICs of <12 μM. Seven compounds (**4**, **7**, **8** and **12**–**15**) inhibited both MTB and MDR-TB *in vitro* with MICs of <1 μM.

Among the synthesized compounds, 4 compounds (**4**, **8**, **13** and **15**) were more active than isoniazid (MIC 0.36 μM), 1 compound (**8**) was more active than rifampicin (MIC 0.15 μM), 7 compounds (**4**, **7**, **8** and **12**–**15**) were more active than gatifloxacin (MIC 0.99 μM) and 10 compounds (**4**–**8** and **11**–**15**) were more active than ethambutol (MIC 5.62 μM) against MTB. Against MDR-TB, all 15 compounds were more potent than standard isoniazid, ethambutol and gatifloxacin, and 10 compounds (**4**–**8** and **11**–**15**) were more active than rifampicin (MIC 3.79 μM). Compound 1-(5-cyclobutyl-1,3-oxazol-2-yl)-3-(2′-trifluoromethyl)phenylthiourea (**8**) was found to be the most active compound *in vitro*, with an MIC of 0.14 μM for MTB and MDR-TB. When compared with isoniazid, compound **8** was 2.5 and 80 times more active, when compared with rifampicin, it was equally and 27 times more active, when

Table 1. Structure, *in vitro* antimycobacterial activity and cytotoxicity of the compounds



Number	X	R	MIC (μM) MTB[a]	MIC (μM) MDR-TB[b]	IC_{50} (μM)[c]	SI[d] MTB/MDR-TB
1	CH	H	11.46	5.73	NT	–
2	CH	2-OH	10.81	10.81	NT	–
3	CH	4-CH_3	6.12	6.12	NT	–
4	CH	4-NO_2	0.31	0.62	196.32	633/316
5	CH	4-$N(CH_3)_2$	5.56	2.78	NT	–
6	CH	4-Cl	1.23	1.23	>203.05	>165/165
7	CH	4-F	0.68	0.68	>214.52	>315/315
8	CH	2-CF_3	0.14	0.14	>183.09	>1307/1307
9	N	H	6.42	6.42	NT	–
10	N	3-OH	10.78	5.39	NT	–
11	N	3-CH_3	1.35	2.70	>216.73	>160/80
12	N	4,5-$(CH_3)_2$	0.62	0.62	>206.68	>333/333
13	N	5-NO_2	0.15	0.3	195.71	1304/652
14	N	5-Cl	0.64	0.64	>202.40	316/316
15	N	3,5-$(Br)_2$	0.23	0.23	>144.63	>628/628
INH	–	–	0.36	11.37	>455.78	>1266/40
RIF	–	–	0.15	3.79	>75.94	>506/20
GAT	–	–	0.99	15.53	>155.31	>156/10
ETH	–	–	5.62	90.17	>225.44	>40/2

NT, not tested; SI, selectivity index; INH, isoniazid; RIF, rifampicin; GAT, gatifloxacin; ETH, ethambutol.
[a]*Mycobacterium tuberculosis* H37Rv.
[b]Multidrug-resistant *Mycobacterium tuberculosis.*
[c]Cytotoxicity in VERO cell line.
[d]IC_{50}/MIC.

Table 2. *In vivo* activity data for compound **8** and isoniazid against *M. tuberculosis* ATCC 35801 in mice

Compound	Lungs (log cfu ± SEM)	Spleen (log cfu ± SEM)
Control	7.88 ± 0.22	8.84 ± 0.21
8 (25 mg/kg)	5.08 ± 0.12	4.90 ± 0.10
Isoniazid (25 mg/kg)	4.98 ± 0.12	5.11 ± 0.09

compared with gatifloxacin, it was 7 and 110 times more active and when compared with ethambutol, it was 40 and 644 times more active against MTB and MDR-TB, respectively.

With respect to structure–activity relationships, pyridylthioureas were found to be more active than phenylthioureas. Among the phenyl or pyridyl ring substituents, electron-withdrawing groups like nitro (**4** and **13**), halogen (**6**, **7** and **15**) and trifluoromethyl (**8**) enhanced the activity. Compounds with electron-donating groups decreased the activity considerably (**2**, **3**, **5** and **10**–**12**). Among the pyridylthiourea derivatives, the di-substituted compound (**12**) showed enhanced activity compared with the mono-substituted derivative (**11**).

Some compounds were further examined for toxicity (IC_{50}) in a mammalian Vero cell line at concentrations of 62.5 mg/L. Most of the tested compounds were non-toxic (15% to 31% cytotoxic) to Vero cells; compounds with nitro substitution showed toxicity at 62.5 mg/L (68%). Compound **8** showed the maximum selectivity index (IC_{50}/MIC) of >1307.

Subsequently, compound **8** was tested for efficacy against MTB at a dose of 25 mg/kg (Table 2) in 6-week-old female CD-1 mice. Compound **8** decreased the bacterial load in lung and spleen tissues with 2.8 and 3.94 $\log_{10}$ reductions, respectively, and was considered to be promising in reducing bacterial count in lung and spleen tissues. When compared with isoniazid at the same dose level, compound **8** decreased the bacterial load with 0.1 and 0.21 $\log_{10}$ reductions in lung and spleen tissues, respectively. Compound **8** was found to be equally active as isoniazid in the *in vivo* animal model, and this study indicated that compound **8** was stable in biological fluids, unlike our previous results.[5]

Conclusions

Screening of the antimycobacterial activity of this novel series identified oxazolyl thiourea as a new lead compound endowed with high activity towards MDR-TB, exhibiting MIC values between 0.14 and 10.81 μM. 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 fruitful.

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Transparency declarations

None to declare.

References

1. Sriram D, Yogeeswari P, Thirumurugan R *et al.* Discovery of new antitubercular oxazolyl thiosemicarbazones. *J Med Chem* 2006; **49**: 3448–50.

2. Venkatachalam TK, Sudbeck EA, Uckun FM. Regiospecific synthesis, X-ray crystal structure and biological activities of 5-bromothiophenethyl thioureas. *Tetrahed Lett* 2001; **42**: 6629–32.

3. National Committee for Clinical Laboratory Standards. *Antimycobacterial Susceptibility Testing for Mycobacterium tuberculosis: Proposed Standard M24-T*. NCCLS, Villanova, PA, USA, 1995.

4. Gundersen LL, Meyer NJ, Spilsberg B. Synthesis and antimycobacterial activity of 6-arylpurines: the requirements for the N-9 substituent in active antimycobacterial purines. *J Med Chem* 2002; **45**: 1383–6.

5. Sriram D, Yogeeswari P, Basha SJ *et al.* Synthesis and antimycobacterial evaluation of various 7-substituted ciprofloxacin derivatives. *Bioorg Med Chem* 2005; **13**: 5774–8.