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Antimycobacterial Activity of 9-Sulfonylated/ Sulfenylated-6-mercaptopurine Derivatives

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Abstract—A series of 9-sulfonylated/sulfenylated-6-mercaptopurines has been prepared by reaction of 6-mercaptopurine with sulfonyl/sulfenyl halides. These compounds constitute a new class of potent antimycobacterial agents, possessing MIC values against *Mycobacterium tuberculosis* H37Rv in the range of 0.39–3.39 μg/mL, as well as appreciable activity against *Mycobacterium avium*. Furthermore, a compound of this small series exhibited good activity (MIC under 1 μg/mL) against several drug resistant strains of *M. tuberculosis*.

Introduction

Mycobacterium tuberculosis infection remains a major global health problem, principally due to the emergence of multidrug resistant bacterial strains, correlated with widespread mycobacterial infections in HIV-positive patients.[1–3] Furthermore, no new antimycobacterial drugs appeared in the last 30 years when the last such compound (rifampin) has been introduced, whereas mycobacteria other than tuberculosis [such as *Mycobacterium avium* complex (MAC)] frequently produce severe disseminated infections in a growing number of patients.[4–6] Although *M. tuberculosis* (two of its strains) was the first bacterial species to have the entire genome sequenced, this was of little help in identifying targets for the development of novel antimycobacterial drugs, mainly due to the complexity of this genoma (with at least 100 genes involved, e.g., in the synthesis of mycobacterial cell wall).[7] Progress has been made ultimately in understanding the role of several proteins involved in susceptibility of mycobacteria to drugs or in the emergence of drug resistance, such as KatG, a multifunctional heme enzyme possessing catalase-peroxidase and cytochrome P450-like oxidase activities (which is responsible for activation of the widely used drug isoniazid **1**);[8] InhA, an enoyl reductase which also seems to be a target for **1** and other antitubercular drugs;[9] DegP,[10] a widely conserved heat shock protein possessing both general molecular chaperone and protease activities (this widely spread bacterial protease plays a major role in the degradation of proteins exported beyond the cytoplasm; its main function being most likely the removal of misfolded membrane and periplasmic proteins or not properly processed proteins);[10] or β-ketoacyl synthetase (the enzyme involved in fatty acid synthesis and elongation)[11] among others. Considering all these data, many groups have recently reported[11–18] the synthesis and antimycobacterial activity of novel classes of compounds that hopefully will lead to new therapeutic agents useful in the fight against mycobacterial diseases. Among the new structures recently reported, of particular interest seem to be some 2,6-bis(alkylthio)-4-pyridine carboxamides **2**,[12] *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides **3**,[15] 6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones and -dithiones **4**,[18] 9-benzyl-purines **5** [13] or some sulfonyl-containing fatty acid derivatives (carboxamides and sulfonamides) of types **6**.[11]

Considering structural elements present in some of the antimycobacterial compounds **1**–**6** mentioned above, and our interest in the design of biologically active compounds incorporating sulfonyl moieties (such as sulfonamide carbonic anhydrase inhibitors,[19–21] sulfonylated amino acid hydroxamates as metalloprotease inhibitors,[22–25] or sulfonylated derivatives with antifungal[26–28] and anticancer[29–31] activity) we report here the serendipitous discovery of strong antimycobacterial

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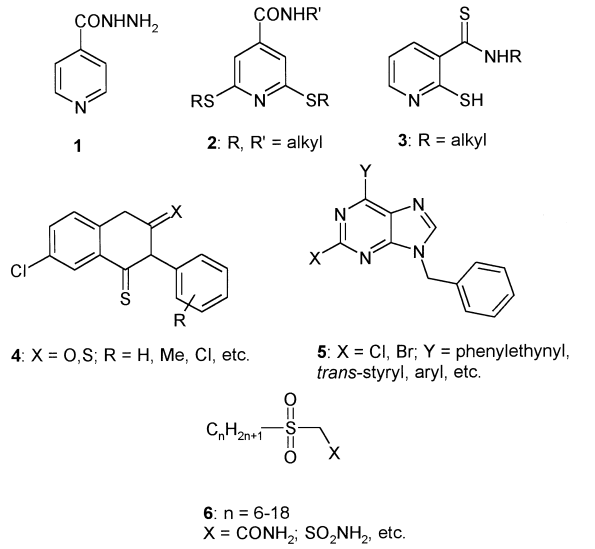
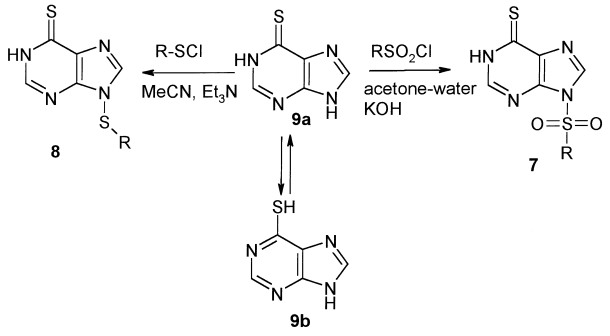


activity of 9-sulfonylated-6-mercaptopurines of type **7** as well as some structurally related sulfenyl derivatives **8**.

Compounds **7** and **8** were prepared from 6-mercapto purine **9** (with its two tautomeric forms, the thione **9a** and the thiol **9b** forms, Scheme 1) which was reacted with sulfonyl halides in Schotten–Baumann conditions, or sulfenyl halides, in acetonitrile and in the presence of triethylamine as base acceptor, as reported previously for other heterocyclic derivatives incorporating sulfonyl moieties (Scheme 1 and Table 1).[19–31]

The sulfonylation/sulfenylation reaction occurred only at the N-9 atom, without the concurrent N-1,7 or *S*-sulfonylation, as proved by the spectral data of the new derivatives **7** and **8** (the most important argument in favor of this assumption was the presence of the unchanged thioamide bands at 1045 (thioamide III), 1550 (thioamide I), and 3300 (NH) cm^{-1}, respectively, in the IR spectra of **9** and its derivatives **7** and **8**; in fact, it is well established that the thione form **9a** is the predominant one in this and related heterocycles containing SH moieties in their molecules).[33] For the 3-amino- and 4-aminophenylsulfonyl derivatives **7f**,**g** the corresponding acetamido sulfonyl chlorides were used in the synthesis, and the free amino derivatives were obtained by deacetylation in the presence of 10% HCl in ethanol.[22,23] The



Scheme 1.

new compounds **7** and **8** were easily purified by recrystallization from ethanol or ethanol/water 2:1 (v/v).[34]

The sulfonylated/sulfenylated-6-mercaptopurines reported here were tested for their antimycobacterial activity against *M. tuberculosis* strain H37Rv and *M. avium* by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Frederick, MD, USA (Table 1).

As seen from data of Table 1, all the sulfonylated/sulfenylated-6-mercaptopurines **7** and **8** possess very good antimycobacterial activity, with MIC values against *M. tuberculosis* strain H37Rv in the range of 0.39–3.39 μg/mL, although the parent compound **9** is devoid of such an activity (data not shown). It must be noted that compounds possessing an MIC < 1 μg/mL are considered as excellent leads (TAACF communication). Mention must be made at this point that 6-mercaptopurine **9** is a widely used antimetabolite drug, employed in chemotherapy combinations for the treatment of various leukemias and solid tumors,[35] as well as for the management of other conditions such as inflammatory bowel disease (Crohn's disease),[36] or as an immunosuppressive drug (the actual drug used is azathioprine, a prodrug of **9**).[37] The nature of the R moiety in derivatives **7** and **8**, and the oxidation state of the sulfur atom greatly influenced the antimycobacterial activity of the new compounds reported here. Best activity against *M. tuberculosis* H37Rv was observed for the sulfamide derivative **7a**, which is only slightly less potent than the standard drug rifampin (MIC of 0.125 μg/mL in the same conditions of the assay), followed by the acetamido-substituted derivative **7i**, the amino- and bromo-substituted sulfonamides **7e** and **7f**, as well as the sulfenylated derivatives **8a**,**b** (which all possess MIC values under 1 μg/mL). Slightly less active against *M. tuberculosis* H37Rv were the 4-methyl- and 2-nitro-substituted sulfonamides **7b** and **7h**, followed by the 4-chloro- and 3-amino-substituted derivatives **7d** and **7g**, which in turn were more active than the fluoro-substituted derivative **7c** (the least active in this small series, with an MIC of 3.39 μg/mL). For the 2-nitrophenyl-substituted derivatives **7h** and **8a**, best activity is observed for the sulfenamide derivative (twice as active as the corresponding sulfonamide derivative), whereas in the series of halogeno-substituted compounds, best activity was correlated with an increased atomic weight of the halogen atom, with the bromo-derivative **7e** more active than the chloro- and fluoro-substituted congeners **7c** and **7d**.

The biological activity is slightly different against *M. avium* (Table 1). Again, all compounds **7** and **8** possess important inhibitory properties at 12.5 μM concentrations in the assay system (this is the primary assay performed for all compounds).[32] Against this pathogen, best activity was observed for the bromo-sulfonamide **7e** and the 4-nitrophenylsulfenyl-derivative **8b**, followed by the other sulfenyl derivative **8a**, the 4-methyl-, 4-fluoro-, 3-amino-, 4-amino-, 4-acetamido and 4-chloro-substituted derivatives of type **7** (with % inhibition in the range of 57–81%). Less active were the 2-nitro-substituted sulfonamide **7h** (inhibition of 50%), and the

sulfamide **7a** (inhibition of 40%), which was in fact the most active compound against *M. tuberculosis*.

The cytotoxicity against VERO cells (IC_{50}) as well as the selectivity index (SI, defined as the IC_{50}/MIC ratio) for compounds **7** and **8** were also determined,[32] as these parameters are of great importance for the pharmacology of a potential antimycobacterial agent (Table 1). One must mention that compounds with an SI over 10 (but occasionally lower) are of interest for a potential development as antimycobacterial agents. Among the compounds reported here, six derivatives possess an SI value ≥ 40, with derivative **7a** having the highest such value, of >513, really comparable with that of the clinically used drug rifampin (SI $>$ 8000) (Table 1).

Some of the new derivatives **7** and **8** were also tested for their antimycobacterial activity against drug resistant *M. tuberculosis* strains (Table 2). Strains resistant to nine of the main antimycobacterial clinically used drugs [isoniazid **1** (INH); rifampin (RMP); pyrazinamide (PZA); ethambutol (EMB); kanamycin (KM); ethionamide (ETA); *para*-aminosalicylic acid (PAS); thiacetazone (TAC); cycloserine (CS)] were included in the assay. It may be observed from data of Table 2 that compounds **7** and **8** show a higher MIC against these drug resistant strains, as compared to the MIC value against the wild-type (WT) *M. tuberculosis* strain H37Rv, proving thus the existence of different degrees of cross-resistance between these new antimycobacterial agents and the clinically used drugs mentioned above. Worst cross-resistance was observed against ethionamide resistant strains. Less cross-resistance was seen, on the other hand, for **7a** against PZA-, EMB-, PAS-, TAC- and CS-resistant strains, but the most interesting observation regards compound **8b**, which exhibited the same activity against WT and RMP-, EMB- and KM-resistant

Table 1. Compounds **7** and **8** synthesized in the present study and their antimycobacterial activity data against *M. tuberculosis* H37Rv [minimum inhibitory concentration (MIC), in μg/mL] and *M. avium* (% inhibition at 12.5 μM)

Compd	R	*M. tuberculosis*			*M. avium*
		MIC (μg/mL)[a]	IC_{50} (μg/mL)[b]	SI[c]	% Inhibition (at 12.5 μM)[d]
7a	Me_2N	0.39	>200	>513	40
7b	4-MeC_6H_4	1.60	4	2.5	73
7c	4-FC_6H_4	3.39	1	0.30	70
7d	4-ClC_6H_4	3.13	1	0.32	60
7e	4-BrC_6H_4	0.78	2	2.6	81
7f	4-$H_2NC_6H_4$	0.78	>100	>128	63
7g	3-$H_2NC_6H_4$	3.13	468	150	68
7h	2-$O_2NC_6H_4$	1.56	64	40	50
7i	4-$AcNHC_6H_4$	0.78	>200	>256	57
8a	2-$O_2NC_6H_4$	0.78	5	6.4	75
8b	4-$O_2NC_6H_4$	0.78[e]	45	58	79
—	Rifampicin	0.125	>1000	>8000	—

[a]MIC against *M. tuberculosis* H37Rv was in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence (activity) were then tested in the BACTEC 460-radiometric system.[32] MIC was defined as the lowest concentration effecting a 90% reduction of fluorescence relative to controls.
[b]IC_{50} represents the cytotoxicity determined in VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC against *M. tuberculosis* H37Rv. After 72 h exposure to drug viability was assessed on the basis of cellular conversion of MTT into formazan, using the Promega Cell Titer 96 non-radioactive cell proliferation assay.[32]
[c]Selectivity index, SI = IC_{50}/MIC.
[d]The compounds were tested against *M. avium* in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence (activity) were then tested in the BACTEC 460-radiometric system.[32]
[e]MIC against *M. tuberculosis* Erdman was 0.78 μg/mL.

Table 2. Antimycobacterial activity data of some compounds **7** and **8** against *M. tuberculosis* drug resistant strains [minimum inhibitory concentration (MIC), in μg/mL]

Compd	R	*M. tuberculosis* resistant strain MIC (μg/mL)[a]									
		WT	INH	RMP	PZA	EMB	KM	ETA	PAS	TAC	CS[b]
7a	Me_2N	0.39	6.25	6.25	3.13	3.13	6.25	12.5	3.13	3.13	3.13
7d	4-ClC_6H_4	3.13	12.5	12.5	12.5	6.25	12.5	25	6.25	6.25	6.25
7g	3-$H_2NC_6H_4$	3.13	12.5	12.5	12.5	12.5	12.5	50	12.5	12.5	12.5
7i	4-$AcNHC_6H_4$	0.78	12.5	12.5	6.25	6.25	12.5	25	6.25	6.25	6.25
8b	4-$O_2NC_6H_4$	0.78[e]	6.25	0.78	—	0.78	1.56	—	—	—	—
—	Rifampicin	0.125	0.125	>8	0.125	0.125	0.125	0.125	0.125	0.125	0.125

[a]MIC against *M. tuberculosis* wild type (WT, H37Rv) and drug-resistant strains were determined in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence (activity) were then tested in the BACTEC 460-radiometric system.[32] MIC was defined as the lowest concentration effecting a 90% reduction of fluorescence relative to controls.
[b]The tested *M. tuberculosis* strains were resistant to: INH, isoniazid **1**; RMP, rifampin; PZA, pyrazinamide; EMB, ethambutol; KM, kanamycin; ETA, ethionamide; PAS, *para*-aminosalicilyc acid; TAC, thiacetazone; CS, cycloserine.

strains, being only slightly less effective against the isoniazid-resistant mycobacteria.

In conclusion, we report here a small series of 9-sulfenylated/sulfonylated-6-mercaptopurines that show very interesting antimycobacterial activity against *M. tuberculosis* strain H37Rv as well as *M. avium*. Furthermore, one of these new derivatives is also active against several drug-resistant strains of *M. tuberculosis*. These compounds thus constitute interesting leads for obtaining more efficient antimycobacterial agents. Work is in progress in our laboratory for the synthesis of a larger number of congeners in these series of derivatives, as well as for testing their antimycobacterial activity in vitro and in vivo.

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34. For example: An amount of 150 mg (1 mmol) of 6-mercaptopurine **9** was suspended in 10 mL acetone and 120 mg of KOH (2 mmol) dissolved in 5 mL of water were added. The obtained solution was treated dropwise with 144 mg (1 mmol) of *N*,*N*-dimethylaminosulfamoyl chloride, maintaining the temperature at 4 °C, under powerful magnetic stirring. After 2 h (TLC control), the pH was brought to 7 with 5% aqueous HCl, the acetone was evaporated in vacuo and the precipitated sulfonamide filtered and recrystallized from ethanol. Pale yellow needles, mp 246–247 °C (dec.). IR (KBr), cm^{-1}: 1045 (thioamide III), 1155 (SO_2^{sym}), 1340 (SO_2^{as}), 1550 (thioamide I), and 3300 (NH); ^1H NMR (DMSO-d_6–D_2O), 300 MHz, δ ppm: 4.53 (s, 6H, Me_2N); 8.45 (s, 1H, CH); 8.63 (s, 1H, CH); ^{13}C NMR (DMSO-d_6–D_2O), δ ppm: 53.5; 115.9; 145.2; 145.8; 150.7; 158.3. Anal. Found: C, 32.56; H, 3.69; N, 26.72; S, 24.54%. $C_7H_9N_5O_2S_2$ requires: C, 32.42; H, 3.50; N, 27.01; S, 24.73%.
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