Brief Articles

Antimycobacterial Agents. 1. Thio Analogues of Purine†

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Thio analogues of purine, pyridine, and pyrimidine were prepared based on the initial activity screening of several analogues of these heterocycles against *Mycobacterium tuberculosis* (Mtb). Certain 6-thio-substituted purine analogues described herein showed moderate to good inhibitory activity. In particular, two purine analogues 9-(ethylcarboxymethyl)-6-(decylthio)-9*H*-purine (**20**) and 9-(ethylcarboxymethyl)-6-(dodecylthio)-9*H*-purine (**21**) exhibited MIC values of 1.56 and 0.78 μg/mL respectively against the Mtb H_{37}Rv strain. N^9-Substitution apparently enhances the antimycobacterial activity in the purine series described herein.

Introduction

Despite the availability of highly active antitubercular agents, tuberculosis (TB) remains one of the primary causes of human death and suffering worldwide.[1] Of even greater concern is the development of highly drug-resistant forms of the disease, particularly multiple drug-resistant tuberculosis (MDR-TB) that is both difficult and expensive to treat.[2] TB treatment is also complicated in AIDS patients undergoing chronic, combined therapies for HIV and attendant opportunistic diseases.[3] The sequencing of the Mtb genome has greatly facilitated the identification of potential new and unique targets within this organism.[4]

Another approach to identification of new antitubercular agents is based on broad screening of chemical libraries against the tuberculosis bacillus. An extensive library of heterocycles, developed over the last five decades at Southern Research Institute (SRI), was screened through the Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF).[5] Due to the paucity of biochemical data concerning these heterocycles in bacteria and the interesting preliminary activity shown against mycobacteria, a modest number of analogues in these series were pursued. It was also deemed appropriate due to the fact that both purines and pyrimidines are not represented in the current clinical antitubercular regimens, suggesting that active compounds in these series may target new biochemical mechanisms, potentially allowing treatment of MDR-TB. The purine ring system is a key structural element of substrates and ligands of many biosynthetic, regulatory, and signal transduction proteins including cellular kinases, G proteins, and polymerases.[6,7] Very little work has been done to explore the potential of purine analogues as antitubercular agents. Recently, syntheses of purine analogues possessing antitubercular activity have been published.[8]

On the basis of reasonable activity shown by several purine analogues (data not shown) in initial screening by the TAACF, we prepared a small 6-thiopurine library for antimycobacterial screening. This library can be further subdivided into 2-H, 2-Cl, and 2-OH analogues of the primary 6-thiopurine grouping. On the basis of the activity screening for our initial library by the TAACF, we further prepared certain 6-thio-N^9-substituted purine analogues. Promising activity suggested the importance of N^9-substitution. Additionally, a disconnection approach recommended preparation of small sets of 2-thio-substituted pyrimidine/pyridine and 4-thio-substituted pyridine analogues as portions of the active 6-thiopurine nucleus.

Chemistry. We carried out the synthesis of several diverse 6-thioaryl/alkyl purines, 2-thioaryl/alkyl pyrimidine and 2- and 4-thioaryl/alkyl pyridine analogues for screening against Mtb and *Mycobacterium avium* complex (MAC) strains. A total of 28 analogues of 6-thiopurine, 26 analogues of 2-chloro-6-thiopurine, 32 analogues of 2-hydroxy-6-thiopurine, 16 analogues of 2-thiopyrimidine, 16 analogues of 4-thiopyridine, and 7 analogues of 2-thiopyridine were prepared in the initial phase to determine antibacterial inhibitor activity and only purine analogues have shown promising activity (see Supporting Information). A total of 19 purine analogues have shown ≥50% inhibitory activity against Mtb H37Rv (Chart 1) and are discussed herein.

The purine analogues **1** and **2** were prepared by the reactions of 6-mercaptopurine with the corresponding alkyl/benzyl halides in the presence of K_2CO_3 in DMAc at room temperature. The compounds **3**−**11** were obtained by reactions of 6-chloropurine or 2,6-dichloropurine with the corresponding alkyl/aryl thiols in 2-propanol using $(CH_3)_3COK$ while heating at 50 °C. The 2-hydroxy purine analogues **12**−**19** were prepared by the treatment of 6-thioxanthine with alkyl/benzyl halides in 0.1 N NaOH.

To evaluate the effect of N^9-substitution on inhibitory activity, N^9-alkylation of a small number of active 6-thio-substituted purines was performed. The alkyla-

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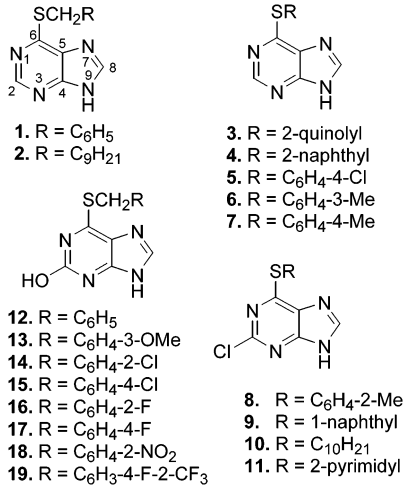
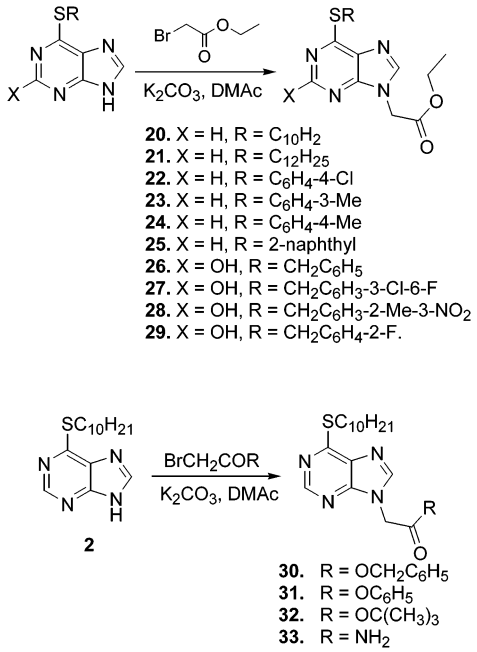
Chart 1



Scheme 1



tion was carried out with ethyl bromoacetate in the presence of K_2CO_3 using DMAc as solvent and resulted in 6,9-disubstituted purine analogues **20**−**29** (Scheme 1A). Upon evaluation of the antimycobacterial activity of **20** that has shown promising antimycobacterial activity, four N^9-alkylated analogues of **2** were also synthesized (**30**−**33**) as shown in Scheme 1B. All the new analogues were characterized using NMR, FABMS/MS-ESI spectroscopic, and CHN analysis before evaluating for antimycobacterial activity. Compound **29** was found to contain 7% of the N^7-substituted analogue (by NMR analysis) even after several column chromatographic purifications and was used as such for antimycobacterial screening.

Antimycobacterial Activity. All synthesized purine, pyrimidine, and pyridine analogues were screened for their antimycobacterial activity against two strains of Mtb $H_{37}Rv$ and $H_{37}Ra$, and three strains of MAC (NJ211, NJ168, and NJ3404). Pyrimidine and pyridine analogues were found inactive. The 6-thio-substituted purine analogues, having more than 50% inhibition against $H_{37}Rv$, are presented in Table 1, and activity results of 6,9-substituted purine analogues are presented in Table 2.

Table 1. Antimycobacterial Activity against Mtb of 6-Thio-Substituted Purine Analogues

compd no.	% inhib Mtb $H_{37}Rv$ 6.25 μg/mL	MIC_{90} Mtb $H_{37}Rv$	MIC[a] Mtb$H_{37}Ra$
1	52[b]	>12.5	>12.8
2[d]	98	3.13	>12.8
3	69[b]	>12.5	>12.8
4	69[b]	>12.5	>12.8[c]
5	78[b]	>12.5	>12.8
6	99[b]	>12.5	>12.8
7	65[b]	>12.5	>12.8
8	72	>6.25	>12.8
9	56	>6.25	>12.8
10	86	>6.25	>12.8
11	53	>6.25	>12.8
12	94	>6.25	>12.8
13	59	>6.25	16
14	62	>6.25	16
15	55	>6.25	>12.8[c]
16	50	>6.25	8
17	52	>6.25	>12.8[c]
18	61	>6.25	16
19	50	>6.25	16
EMB	-	-	2−4

[a] MIC are given in μg/mL. [b] Determined at 12.5 μg/mL. [c] Indicated partial inhibition of growth representing an estimated 50% or more. [d] IC_{50} against VERO cells >62.5, selectivity index (SI) > 20 (SI = IC_{50}/MIC) and activity against Mtb Erdman in monolayers of mouse bone marrow macrophages EC_{90} = 2.54 μg/mL. EMB = ethambutol.

Table 2. Antimycobacterial Activity of N^9-Substituted Purines against Mtb

compd. no.	% inhib Mtb $H_{37}Rv$ 6.25 μg/mL	MIC_{90}[a] against Mtb $H_{37}Rv$	MIC[a] against Mtb $H_{37}Ra$	IC_{50} VERO cells	selectivity index (SI)[b]
20	100	1.56	>12.8[c]	>62.5	>40
21	98	0.78	8	>10	>12.8
22	99	>6.25	32	n.d	n.d
23	0	>6.25	>12.8[c]	n.d	n.d
24	99	>6.25	>12.8	n.d.	n.d.
25	100	>6.25	>12.8[c]	n.d.	n.d.
26	25	>6.25	4	n.d	n.d
27	17	>6.25	4	n.d	n.d
28	0	>6.25	16	n.d	n.d
29	22	>6.25	4	n.d	n.d
30	0	>6.25	>12.8	n.d	n.d
31	0	>6.25	>12.8	n.d	n.d
32	99	3.13	16	>10	>3.19
33	0	>6.25	32	n.d	n.d

[a] MIC are given in μg/mL. [b] SI = IC_{50}/MIC. [c] Indicated partial inhibition of growth representing an estimated 50% or more. Positive control, ethambutol, MIC 2−4 μg/mL against Mtb $H_{37}Ra$. "n.d." indicates not determined.

Activity against Mtb $H_{37}Ra$ and MAC Strains. The compounds were tested against Mtb $H_{37}Ra$ and MAC strains using a colorimetric microdilution broth assay at two concentration 1.28 and 12.8 μg/mL.[9] Most of the analogues (**1**−**19**) were found to be inactive against $H_{37}Ra$, and none of the compounds were active (MIC >12.8 μg/mL) against MAC. However, N^9-alkylated 2-hydroxy-6-thio-substituted purines analogues **26**, **27**, and **29** showed improved MICs (4 μg/mL) against Mtb $H_{37}Ra$.

Activity against Mtb $H_{37}Rv$ Strain. All compounds were initially screened for their antimycobacterial activity at 6.25 μg/mL (few at 12.5 μg/mL) against $H_{37}Rv$ strain by the TAACF. Compounds exhibiting ≥90%

inhibition in the initial screen were retested at and below 6.25 mg/mL using 2-fold dilution to determine the MIC_{90}. In particular, 6-(decylthio)purine (**2**) had the most promising activity with a MIC_{90} of 3.13 μg/mL. To evaluate the effect of N^9-alkylation on the antimycobacterial activity, compound **20** was synthesized from compound **2** and tested. The alkylated analogue **20** possessed a MIC_{90} of 1.56 μg/mL. On the basis of this observation, we synthesized other N^9-alkylated purine analogues (**21**−**29**) of a few active samples for activity screening (Table 2). Compound **21** showed excellent activity with an MIC_{90} of 0.78 μg/mL, making it the most active analogue against Mtb H_{37}Rv in the present series. Meanwhile, to investigate the effect of different N^9-alkylating substitutions on antimycobacterial activity, compound **2** was also derivatized to compounds **30**−**33**, but none of these purine analogues showed enhanced activity as compared to compound **20**. The N^9-ethylcarboxymethyl substitution in compound **2** to give compound **20** showed a 2-fold increase in inhibitory activity, clearly indicating the value of N^9-alkylation for enhanced antimycobacterial activity in purines. A more lipophilic analogue, **32**, possessed a MIC_{90} of 3.13 μg/mL, which is 2-fold less active compared to compound **20** that contained an N^9-ethyl ester. The results described herein show that 6-thio-substituted purines exhibit good antimycobacterial activity, in particular, more hydrophobic analogues at both the 6- and 9 positions.

Compounds **2**, **20**, **21**, and **32** exhibited antimycobacterial activity in the preliminary screening against Mtb H_{37}Rv and were further examined for toxicity (IC_{50}) in a mammalian cell line, VERO cells by the TAACF (Tables 1 and 2). Compound **20** exhibited a better IC_{50} and selectivity index (SI) as compared to compound **21**. Compounds **2** and **20** were tested against Mtb Erdman in monolayers of a mouse bone marrow macrophages model,[10] and the concentrations effecting 90% reduction in the viable cell count after 7 days, compared to untreated controls (EC_{90}), were 2.53 μg/mL and 1.65 μg/mL, respectively. These data demonstrated that certain purine analogues exhibit high activity against Mtb inside macrophages since the EC_{90}:MIC_{90} (Mtb H_{37}Rv) ratios were calculated as 0.81 and 1.06, respectively. The present study has demonstrated the future potential for development of 6-thio-substituted purine analogues as antimycobacterial agents.

Experimental Section

Anhydrous solvents and reagents from Aldrich were used without further drying. Reactions were monitored by thin-layer chromatography (TLC) on precoated E. Merck silica gel ($60F_{254}$) plates (0.25 mm) and visualized using UV light (254 nm). Flash chromatography was carried out on Fischer silica gel G 60 (230−400 mesh). Melting points, determined with a Mel-Temp II capillary melting points apparatus, are uncorrected. ^{1}H NMR spectra were recorded on a Nicolet NT 300NB instrument at 300 MHz. The coupling constants (J) are reported in hertz, and chemical shifts are reported in ppm (δ) relative to residual solvent peak or internal standard. Microanalyses were performed on a Perkin-Elmer 2400 CHN analyzer. FABMS were recorded on a Varian/MAT 311A double-focusing mass spectrometer either by adding NBA or LiCl and MS-ESI on a BioTof-2 time-of-flight mass spectrometer.

Synthesis of 6-(Benzylthio)purine (1) and 6-(Decylthio)purine (2). 6-Mercaptopurine in DMAc was reacted at room temperature for 2−6 h with benzyl/decyl chloride (1.2 equiv) in the presence of dry K_2CO_3 (1.2 equiv) under argon atmosphere. Deionized water was added, and the reaction was neutralized by addition of acetic acid. The solid obtained was filtered, washed with deionized water followed by diethyl ether, and dried overnight in vacuo over P_2O_5 at 25 °C.

General Procedure for Synthesis of 6-Thioaryl Purines 3−7 and 2-Chloro-6- thioalkyl/aryl Purines 9−11. The starting chloro compound (6-chloropurine or 2,6-dichloropurine) in dry *i*-PrOH was heated under reflux for 3−5 h with mercaptoaryl/alkyl (1.2 equiv) in the presence of $(CH_3)_3$-COK (1.2 equiv) under argon atmosphere. Deionized water was added, and the resulting solid was filtered, washed with deionized water followed by diethyl ether, and dried overnight in vacuo over P_2O_5 at 25 °C.

General Procedure for Synthesis of 2-Hydroxy-6-thiobenzyl/alkyl Purines 12−19. 2-Hydroxy-6-mercaptopurine in 0.1 N NaOH was reacted with the appropriate benzyl/alkyl halide (1.2 equiv) at room temperature for 4−6 h. Deionized water was added followed by neutralization with acetic acid. The solid obtained was filtered, washed with deionized water followed by diethyl ether, and dried overnight in vacuo over P_2O_5 at 25 °C.

General Procedure for the Synthesis of N^9-Alkylated Purines 20−33. The 6-thio-substituted purine in DMAc and the appropriate alkyl- or aryl-bromoacetyl derivative (1.5 equiv) were reacted in the presence of K_2CO_3 (1.2 equiv) at room temperature. It was poured into deionized water and neutralized with acetic acid. The crude product was extracted with diethyl ether and dried over Na_2SO_4. The final compounds were purified by column chromatography over silica gel G.

Activity against Mtb H_{37}Ra Strain, MAC. All compounds were tested for their inhibitory activity against Mtb H_{37}Ra (ATCC 25177) and MAC NJ211, NJ168, and NJ3404 strains. The screening was performed at 1.28 and 12.8 μg/mL in Middlebrook 7H9 broth supplemented with 0.2% glycerol and ADC enrichment using a colorimetric (Alamar blue) microdilution broth assay.[9] The active compounds ($\leq$12.8 μg/mL) were retested using 2-fold dilutions to obtain the actual MIC. In this particular assay, the MIC was recorded as the lowest drug concentration that inhibited the growth completely.

Activity against Mtb H_{37}Rv Strain at the TAACF.[5] The primary screen was conducted at either 6.25 or 12.5 μg/mL against Mtb H_{37}Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA).[11] Compounds demonstrating at least 90% inhibition at 6.25 or $\leq$12.5 μg/mL were retested to determine the MIC_{90}, defined as the lowest concentrating inhibiting growth by 90% or higher.

Cytotoxicity against VERO cell Lines and Selectivity Index (SI).[5] Concurrent with the determination of MIC_{90}'s, compounds **2**, **20**, **21**, and **32** were tested for cytotoxicity (IC_{50}) in VERO cells. The selectivity index is defined as the ratio of the measured IC_{50} in VERO cells to the MIC_{90}.

Activity against Mtb Erdman in Macrophages. Compounds **2** and **20** were tested for killing of Mtb Erdman (ATCC 35801) in monolayers of mouse bone marrow macrophages.[10] EC_{90} is defined as the concentrations effecting 90% reduction in the viable cell counts after 7 days, compared to untreated controls.

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Supporting Information Available: Detailed information on synthetic methods, analytical, spectroscopic, and antimycobacterial activity of purine, pyrimidine, and pyridine compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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