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6-Oxo and 6-thio purine analogs as antimycobacterial agents

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ABSTRACT

6-Oxo and 6-thio analogs of purine were prepared based on the initial activity screening of a small, diverse purine library against *Mycobacterium tuberculosis* (Mtb). Certain 6-oxo and 6-thio-substituted purine analogs described herein showed moderate to good inhibitory activity. N^9-substitution apparently enhances the anti-mycobacterial activity in the purine series described herein. Several 2-amino and 2-chloro purine analogs were also synthesized that showed moderate inhibitory activity against Mtb.

1. Introduction

In spite of the availability of highly active anti-tubercular agents, tuberculosis has remained one of the primary causes of human death and suffering worldwide. It is estimated that approximately one third of the world's population is infected with the bacteria that causes tuberculosis, 2–3 million people die worldwide each year from the disease, and an additional 8 million people become ill with tuberculosis annually.[1] *Mycobacterium tuberculosis* (Mtb) is a facultative intracellular pathogen that persists primarily within macrophages in the human host, and these cells are involved in propagation of infection.[2] Intracellularly sequestered bacilli are considered more resistant to treatment and clearance due to limited access of drugs and the immune system to bacteria within macrophages, necessitating chronic treatment with high therapeutic doses for effective control and treatment of the disease.[3] Additionally, AIDS patients and others with compromised immune systems are susceptible to other opportunistic mycobacteria including *Mycobacterium avium* and *Mycobacterium kansaii*, resulting in further morbidity and a high mortality.[4] It is not surprising that drug resistance, from single drug resistant (SDR) up to totally drug resistant (TDR) strains,[5] is now becoming commonplace considering the fact that virtually the same drug regimens have been in place and poorly deployed worldwide for over half a century. Treatment of highly resistant forms of *Mtb* is both difficult and expensive, and for these more intractable forms, few treatment options are available. Although newer drugs are now in clinical trials, these issues critically underscore the need for continued emphasis on the discovery of newer drugs with novel mechanisms of action.[6]

Phenotypic screening of diverse drug-like compound libraries against Mtb has been more recently implemented in order to respond to this need and discover compounds that are active against whole Mtb bacilli,[7] potentially circumventing issues with antibacterial drug discovery using specific target based screens.[8]

Through similar screens of the Southern Research proprietary library, it has been found that several 9-benzylpurines with a variety of substitutions in the 2-, 6- and/or 8-positions exhibit inhibitory activity against Mtb.[9] High inhibitory activity was found for 9-benzylpurines containing a phenylethynyl-, *trans*-styryl or aryl substituent in the 6-position, and generally chlorine in the 2-position tends to increase activity (compounds **1**–**4**, Fig. 1). Several 6-arylpurines carrying a variety of substituents in the 9-position were prepared by Stille coupling between appropriately substituted 6-chloropurines and aryl(tributyl)tin, and the compounds were screened for antibacterial activity against Mtb $H_{37}Rv$.[10] One of the derivatives, 9-benzyl-2-chloro-6-(2-furyl)purine (**2**), showed a MIC value of 0.78 μg/mL and also showed relatively low cytotoxicity against several singly drug-resistant strains. A series of 9-sulfonylated/sulfenylated-6-mercapto purines (**3** and **4**) has been prepared by reaction of 6-mercaptopurine with sulfonyl/sulfenyl halides.[11] These compounds constitute a new class of potent antimycobacterial agents, possessing excellent MIC values against Mtb $H_{37}Rv$, as well as appreciable activity against *M. avium*. A few compounds in this series have exhibited activity against several drug resistant strains of *Mtb* (e.g., compound **4**). Currently, beyond the broad phenotypic activity, no target(s) has/have been identified.

We have also synthesized a small library of 6-thioalkyl/aryl/benzyl purine analogs to generate a modest structure–activity

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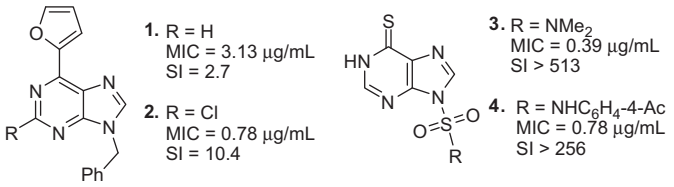
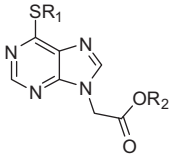


Figure. 1.

relationship (SAR). All the purine derivatives synthesized were screened for their activity against two strains of Mtb ($H_{37}Rv$ & $H_{37}Ra$) and three strains of *M. avium* (MAC NJ211, NJ168, NJ3404). Most of these derivatives were inactive against *M. avium*. In particular, (6-decylsulfanyl-purin-9-yl)-acetic acid ethyl ester **5** and the dodecyl derivative **6**, and (6-decylsulfanyl-purin-9-yl)-acetic acid *tert*-butyl ester **7** exhibited MIC values of 1.56, 0.78 and 3.13 μg/mL respectively against the Mtb $H_{37}Rv$ strain (Fig. 2).[12] Concurrent with the determination of MICs, analogs **5**–**7** were also tested for cytotoxicity (CC_{50}) in a cell proliferation assay for VERO cells at concentrations less than or equal to 62.5 μg/mL or 10 times the MIC for Mtb $H_{37}Rv$: selectivity is assigned by calculating the selectivity index (SI) ratio CC_{50}/MIC.

Very little is known about the mechanism of action of these purine analogs. Purine salvage pathways are predicted to be present from the genome sequence of Mtb, and the metabolism in mycobacteria is similar to that in humans and other organisms.[13] Information about the substrate preferences of the mycobacterial enzymes involved with purine metabolism is still unknown. The Mtb *deoD* gene encodes a presumptive purine nucleoside phosphorylase (PNP) and the gene was cloned, expressed, purified, and found to exhibit PNP activity.[14] Modest biochemical work has been pursued on purine nucleosides with anti-mycobacterial activity, especially 2-methyladenosine that showed potent activity (99% inhibition, MIC = 3 μg/mL, IC_{50} (VERO Cells = 1000 μg/mL, SI >1000).[15] 2-Methyladenosine has demonstrated selective activity against Mtb, suggesting differences in the substrate preferences between mycobacterial and human adenosine kinases that might be exploited to develop novel nucleoside-based drugs for the treatment of mycobacterial diseases. Beyond the purine salvage pathways, ATP binding proteins and kinases are also being interrogated as new drug targets in Mtb.[16]

Based upon our previously synthesized purine series,[12] we planned to generate more diversified 6-substituted mercaptopurines analogs with substitutions at C-2 position along with 6-oxo substituted series for antimycobacterial screening. Compounds were prepared from a diversity perspective in order to further probe specific active scaffolds, but also to explore other purines such as the 2-amino and 2-chloropurine scaffolds.



5. $R_1 = C_{10}H_{21}$, R_2 = Et; MIC = 1.56 μg/mL, CC_{50} VERO Cells = >62.5 μg/mL; SI = >40
6. $R_1 = C_{12}H_{25}$, R_2 = Et; MIC = 0.78 μg/mL, CC_{50} VERO Cells = >10 μg/mL; SI = >12.8
7. $R_1 = C_{10}H_{21}$, $R_2 = C(CH_3)_3$; MIC = 3.13 μg/mL, CC_{50} VERO Cells = >10 μg/mL; SI = >3.19

Figure 2.

2. Results and discussion

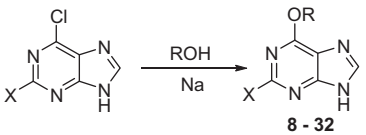
2.1. Chemistry

In the first target set, synthesis of several new 6-oxoaryl/benzyl/aryl purines and their 2-amino or 2-chloro purine analogs (**8**–**32**) was carried out as shown in Scheme 1 by reacting a suitable 6-chloropurine analog with alcohols in the presence of sodium metal. Neutralization with acetic acid followed by the standard workup and column chromatographic purification on silica gel G produced pure products. A total of six analogs of 6-oxopurine, 10 analogs of 2-amino-6-oxopurine and nine analogs of 2-chloro-6-oxopurine were prepared in the initial phase to determine their antibacterial activity. A total of nine analogs have shown >50% inhibitory activity against Mtb $H_{37}Rv$ and are discussed herein (Table 1).

As previously described by us,[12] substitution at the N^9-position enhances antimycobacterial activity, and we have synthesized and screened four analogs **33**–**36** starting from active 6-oxopurine analogs **9**, **10**, **15** and **16** (Scheme 2). The synthesis of these analogs was carried out by reacting the appropriate commercially available purine analog with ethylbromoacetate and K_2CO_3 in anhydrous DMSO at room temperature.

Further analoging of compounds **34** and **35** was achieved by treating these with different D- and L-amino acids. These compounds can utilize bacterial dipeptide transporters to enhance transport via membrane amino acids beyond the fact that adding amino acids at the end increases diversity for probing activity. It is sometimes the case that adding specific chirality can improve activity and only one stereoisomer shows activity. This result is, however, a gross generality as that is not always the case and sometimes both enantiomers are inactive or can show similar activity. Compounds **40**–**50** (Scheme 3) which contain D- or L-amino acid chains were synthesized from commercially available starting material, 2-amino-6-chloro-9*H*-purine-9-acetic acid (**37**). The 6-decyloxy derivative **38** was synthesized by reacting 37 with 1-decanol in the presence of sodium metal. The 6-decylthio derivative **39** was synthesized from **37** by the reaction with 1-decylthiol and $(CH_3)_3COK$ as previously described.[12] Analoging of **38** and **39** was achieved by treating with different D- or L-amino acids using the coupling reagent benzotriazole-1-yl-oxy-trispyrrolidino-phosphonium hexafluorophosphate (PyBOP) in the presence of base Et_3N.

Alternatively, for diversity point of view, compounds **51**–**69** were prepared from easily accessible starting materials. 6-Decylmercaptopurine analogs **51**–**54** were prepared starting from 6-chloropurine analogs by the reaction with 1-decylthiol and $(CH_3)_3COK$ under reflux as previously described (Scheme 4A).[12,17] However, 6-decylmercapto purine analogs **55**–**69** were prepared



8. X=H; R = $CH(CH_3)_2$
9. X=H; R = $(CH_2)_9CH_3$
10. X=H; R = $(CH_2)_{11}CH_3$
11. X=H; R = C_6H_4-4-Cl
12. X=H; R = CH_2-C_6H_{11}
13. X=H; R = CH_2-C_6H_3-3-Cl,4-Cl
14. X=NH_2; R = $CH(CH_3)_2$
15. X=NH_2; R = $(CH_2)_9CH_3$
16. X=NH_2; R = $(CH_2)_{11}CH_3$
17. X= NH_2; R = C_6H_5
18. X= NH_2; R = C_6H_4-4-Cl
19. X= NH_2; R = C_6H_4-4-CH_3
20. X= NH_2; R = C_6H_4-3-CH_3
21. X=NH_2; R = CH_2-C_6H_{11}
22. X=NH_2; R = CH_2-C_6H_5
23. X=NH_2; R = CH_2-C_6H_3-3-Cl,4-Cl
24. X=Cl; R = CH_3
25. X=Cl; R = $CH(CH_3)_2$
26. X=Cl; R = $(CH_2)_9CH_3$
27. X=Cl; R = $(CH_2)_{11}CH_3$
28. X=Cl; R = C_6H_4-4-Cl
29. X=Cl; R = C_6H_4-4-CH_3
30. X=Cl; R = C_6H_4-3-CH_3
31. X=Cl; R = CH_2-C_6H_{11}
32. X=Cl; R = CH_2-C_6H_3-3-Cl,4-Cl

Scheme 1.