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HOMOLOGATED AZA ANALOGS OF ARABINOSE AS ANTIMYCOBACTERIAL AGENTS[1]

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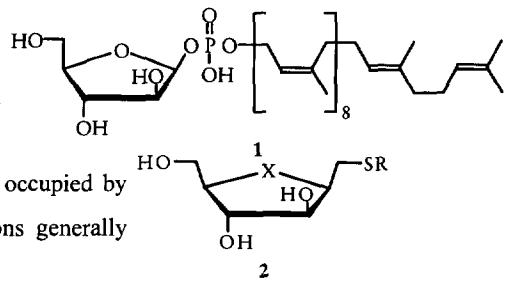
Abstract: A series of hydrolytically-stable aza analogs of arabinofuranose was prepared and evaluated against *Mycobacterium tuberculosis* and *M. avium*. The compounds were designed to mimic the putative arabinose donor involved in biogenesis of the essential cell wall polysaccharide, arabinogalactan. Though most compounds displayed little activity in cell culture, one compound showed significant activity in infected macrophage models.

Tuberculosis (TB) remains the most prevalent infectious disease worldwide, resulting in 2.9 million deaths annually.[2] Recently, TB has been resurgent in the United States, where outbreaks of pernicious, multiply drug resistant forms have made the need for new antimycobacterials acute.[3] Moreover, infection by *M. tuberculosis*, the etiologic agent of TB, and other opportunist mycobacteria such as *M. avium* Complex (MAC), is one of the leading sources of morbidity and mortality in AIDS patients.[4]

Among the unusual characteristics of mycobacteria that contribute to their resistance to mainstream antibacterials is a thick cellular envelope attached to the cell wall exterior.[5] This lipopolysaccharide barrier is thought to hinder uptake of some drugs, but conversely is also the site of action of other effective agents. One of the chief components of the mycobacterial envelope is an arabinogalactan that has been shown to be essential to the organism.[6] Since ethambutol, one of the chief antimycobacterial drugs despite its low potency, is thought to act by disruption of arabinan biosynthesis,[7] we embarked on a program to develop novel, selective, and more potent inhibitors of this pathway, and in particular, of arabinosyltransferases (ATs).

Figure 1 depicts the structure of 1-β-D-arabinofuranosyl decaprenol-1-phosphate **1**, the putative arabinose donor utilized by mycobacterial ATs.[8] We envisioned that non-ionic compounds based on this structure might be effective inhibitors of at least some of the several ATs necessary for elaboration of the various complex arabinan motifs. We further reasoned that the affinity of such analogs would be enhanced if (i) the decaprenol binding site were occupied by suitable hydrophobic groups, since hydrophobic interactions generally

FIGURE 1



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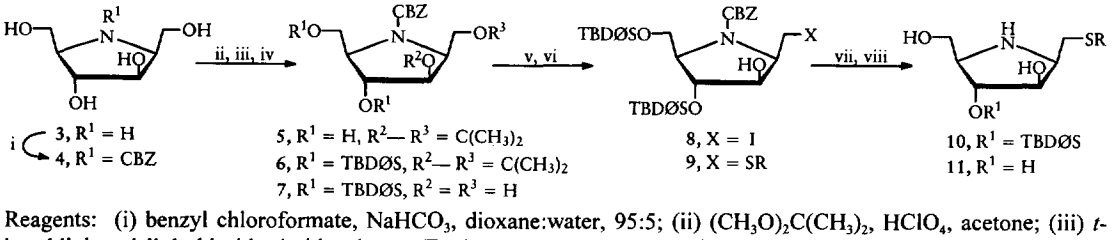
provide the major driving force for ligand binding,[9] and (ii) chelating moieties were incorporated into the bridging phosphate surrogate, to coordinate with the bivalent metal ion often found within glycosyltransferase active sites.[10] Figure 1 also shows the general structure of a series of compounds whose design is based on these principles (**2**). In this series, the arabinose substructure has been homologated at the 1-position to more appropriately position the chelating sulfur atom, and to remove any potential hydrolytic lability at the anomeric position; alternatively, **2** can be viewed as a transition state analog, since the anomeric bond of the native donor would be lengthened during S_N2-type sugar transfer to the elongating polysaccharide chain.[11] In the aza series (**X** = *NH*), the pyrrolidine nitrogen atom was also viewed as a potential chelator, depending upon the precise binding orientation within the transferase arabinose site.

Scheme 1 depicts our synthetic approach to the desired sulfides.[12] Pyrrolidine **3**, derived from 5-ketofructose[13] via the procedure of Reitz and Baxter,[14] was protected as benzyl carbamate **4**. Subsequently, conversion of the *cis*-1,3-diol to isopropylidene **5** was followed by masking of the remaining hydroxyls as the *t*-butyldiphenylsilyl ethers (**6**). Hydrolysis of the ketal afforded **7**, which was selectively transformed to iodide **8** using the general procedures of Garegg and Samuelsson.[15] Displacement of the iodine with the appropriate sodium sulfide, generated in situ from the corresponding thiol, produced **9**, which was deblocked in two steps to yield the desired arabinose analogs **11**. All target compounds and intermediates had acceptable spectroscopic and analytical data.[16]

SCHEME 1



Reagents: (i) benzyl chloroformate, $NaHCO_3$, dioxane:water, 95:5; (ii) $(CH_3O)_2C(CH_3)_2$, $HClO_4$, acetone; (iii) *t*-butyldiphenylsilyl chloride, imidazole, DMF; (iv) 80% HOAc, Δ; (v) ϕ_3P, I_2, imidazole, toluene; (vi) Na, RSH, methanol; (vii) 40% KOH, methanol; (viii) $Et_4N^{\oplus}F^{\ominus}$, CH_3CN.

Several additional compounds were prepared for comparative purposes (Figure 2). Oxidation of thioether **11e** [or **11b**] (Table 1) afforded sulfoxide **12a [12b]** and sulfone **13a [13b]**. Also, **14a** and **14b** (the β and α epimeric forms of the furan analog of **11e**) were synthesized from 2,5-anhydroglucitol[17] and 2,5-anhydromannitol,[18] respectively, using procedures essentially identical to those in the pyrrolidine series.

Compounds were evaluated in vitro against *M. tuberculosis* strain H37Ra and also against a panel of five clinical MAC isolates using a colorimetric broth microdilution assay.[19] Selected compounds were also evaluated for intracellular activity against MAC strain 101 in mouse and human monocyte cell lines,[20] and in a similar TB model.[21] Some compounds were evaluated against H37Rv using a standard BACTEC assay[22] as a check on the

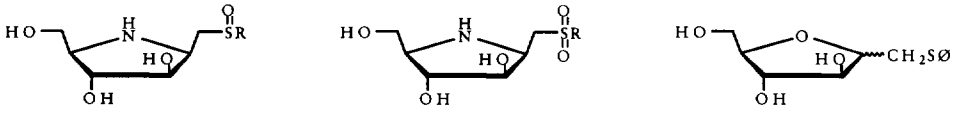
broth assays. Table 1 shows the structures of the compounds and summarizes the biological data.

Table 1
Structures and Biological Activities of Arabinose Analogs[a,b]

Compound	R	MIC[c] (μg/mL)	MBC[c] (μg/mL)	BACTEC % Inhibition @12.5 μg/mL	Macrophage (*M. avium*)
11a	$(CH_2)_9CH_3$	32-128	≥128	ND	ND
11b	$(CH_2)_{11}CH_3$	16-32	64->128	ND	C
11c	$(CH_2)_{15}CH_3$	16-64	64->128	ND	ND
11d	$[CH_2CH=C(CH_3)CH_2]_2H$ (geranyl)	64->128	ND	ND	C
11e	C_6H_5	>128	ND	0	4 μg/mL
11f	*p*-$CH_3OC_6H_5$	>128	ND	ND	ND
11g	*o*-$CH_3OC_6H_5$	>128	ND	ND	ND
11h	*p*-NCC_6H_5	>128	ND	5	ND
11i	4-pyridyl	>128	ND	6	ND
11j	$CH_2C_6H_5$	>128	ND	ND	ND
11k	β-naphthyl	>128[d]	ND	18	ND
12a	C_6H_5	>128	ND	18	I
12b	$(CH_2)_{11}CH_3$	32-64	≥128	ND	ND
13a	C_6H_5	>128	ND	6	I
13b	$(CH_2)_{11}CH_3$	32-128	≥128	ND	C
14a	(see Figure 2)	>128	ND	ND	>64 μg/mL
14b	(see Figure 2)	>128	ND	ND	>64 μg/mL
15	(α-epimer of **13b**)	>12.8-≤128	ND	ND	ND

[a]See Scheme 1 (**11**) and Figure 2 (**12**, **13**, **14**) for structures; [b]Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; ND, not determined; C, cytotoxic to macrophages; I, inactive in macrophages. [c]Numbers represent the range of values obtained with the various strains (specific strains used are listed in Ref. 19); **the following compounds were used as controls: ethambutol, 8-32; isoniazid, 0.03 (*M. tuberculosis*), 0.5->2 (MAC).** [d]Partial inhibition at 128 μg/mL.

FIGURE 2



12 13 14

a, R = Ø
b, R = $(CH_2)_{11}CH_3$

a, β-phenylthiomethyl
b, α-phenylthiomethyl

Most compounds showed lackluster activity against mycobacteria in the alamar Blue and BACTEC assays. However, the marginal activity of several agents in these assays prompted further evaluation of the leads in MAC

macrophage models. Though most compounds were not active at noncytotoxic levels, surprisingly **11e** was consistently and reproducibly found to be quite active against three strains of MAC (belonging to serovars 1, 4, and 8) at 4 μg/mL in infected macrophages. The activity was in part dependent on TNFα production by infected macrophages, triggered upon treatment with **11e** (uninfected control macrophages, 5 pg/mL of TNFα; infected, untreated macrophages, 89 ± 13 pg/mL; uninfected + **11e**, 16 ± 3 pg/mL; infected + **11e**, 196 ± 21 pg/mL). Furthermore, the activity could be abrogated by utilizing anti-TNFα antibody (data not shown). Interestingly, the oxygen counterpart of **11e** (compound **14a**) showed no activity in macrophages; nor did the potential metabolites **12a** and **13a**. Subsequently, **11e** was examined for antituberculosis activity in infected mouse macrophages. Though the compound showed no reproducible effects against *M. tuberculosis*, there was a similar elevation of TNFα production in treated, infected cells relative to untreated controls.

The mechanism for the activity of **11e** is unclear. While the inactivity of the compound against mycobacteria in broth assays may argue against inhibition of cell wall biogenesis, it is still possible that any putative disruption of wall integrity caused by **11e** is insufficient in itself to cause lethality, but nonetheless provokes an increased immune response in macrophages by, for example, exposing unusual wall structures or promoting increased shedding of wall components. Experiments to test these hypotheses are ongoing.

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12. Procedures for preparation of intermediates through iodide **8** will be published elsewhere.[23] Other representative procedures follow: *(a) Preparation of 1-deoxy-1-phenylthio-2,5-anhydroimino-D-glucitol* **11e**.

Sodium metal (480 mg, 21 mmol) was dissolved in 150 mL of degassed methanol at ambient temperature over 10 min with stirring under nitrogen atmosphere. Thiophenol (2.3 mL, 22.6 mmol) was added and stirring continued for 1 h. To this was added a solution of **8** (10.0 g, 11.3 mmol) in 100 mL degassed methanol, and the reaction mixture heated at 46 °C for 18 h. The solution was neutralized with Dowex 50W X8-100 ion exchange resin ($H^{\oplus}$ form), filtered, and the filtrate evaporated under reduced pressure. Silica gel chromatography yielded 1.5 g recovered **8**, and 6.3 g of sulfide **9** (**R** = C_6H_5; 76% based on recovered **8**). To a solution of 5.7 g (6.6 mmol) of **9** (**R** = C_6H_5) in methanol was added 200 mL of 40% aqueous KOH, and the mixture heated at 70 °C for 48 h. After cooling, the reaction was neutralized to pH 8 with conc. HCl, then evaporated under reduced pressure to remove methanol. Water was added, and the solution extracted with ethyl acetate (3 x 100 mL), the pooled organic layers dried ($MgSO_4$) and filtered to give crude **10** (**R** = C_6H_5), which was taken up in acetonitrile (50 mL). Tetraethylammonium fluoride (1.18 g, 7.92 mmol) was added, and the solution stirred for 3 days. After evaporation to dryness, chromatography over silica afforded 862 mg **11e** (51% from **9**).

(b) Preparation of sulfoxide **12a**. To a solution of **10** (**R** = C_6H_5; 352 mg, 0.71 mmol) in 6 mL acetic acid was added sodium perborate (124 mg, 0.8 mmol). After 2 h methanol (10mL) was added, and the solution neutralized with Amberlite IRA-400 resin ($OH^{\ominus}$ form). After filtration and concentration under reduced pressure, silica chromatography yielded 207 mg (57%) of the 4-(*t*-butyldiphenylsilyl-blocked) sulfoxide, which was deprotected with tetraethylammonium fluoride as for **10** to yield **12a**.

(c) Preparation of sulfone **13a**. To a solution of **9** (**R** = C_6H_5; 2.0 g, 2.3 mmol) in dry dichloromethane (20 mL) was added *m*-chloroperbenzoic acid (2.4 g, 13.8 mmol) and the reaction stirred at ambient temperature for 45 min. The solution was diluted with an additional 20 mL portion of dichloromethane, and washed with saturated aqueous sodium bicarbonate solution (2 x 40 mL). The organic phase was dried over Na_2SO_4 and concentrated to yield 1.53 g (74%) crude, protected sulfone. This material was deblocked in two steps as for **9** to afford **13a**.

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16. **11e**, **12a**, and **13a** are typical. **11e**: *m/e* (FAB), 256 $(M + H)^+$; Anal. ($C_{12}H_{17}NO_3S \cdot 0.1\ H_2O$) C,H,N; ^1H NMR (300 MHz, DMSO-d_6, δ) 2.83 (m, 1H, *H*-5), 2.94, 3.14 (m, 2H, CH_2-1), 3.24 (m, 1H, *H*-2), 3.36, 3.46 (m, 2H, CH_2-6), 3.63 (m, 1H, *H*-4), 3.73 (m, 1H, *H*-3), 4.64 (bs, 1H, O*H*-6), 4.83 (d, 1H, O*H*-3), 4.86 (d, 1H, O*H*-4), 7.18 (m, 1H, *H*-4′), 7.32 (m, 4H, *H*-2′,3′,5′,6′). **12a**: *m/e* (FAB) 272 $(M + H)^+$; Anal. ($C_{12}H_{17}NO_4S$) C,H,N; ^1H NMR (300 MHz, DMSO-d_6, δ) 2.80 (m, 1H, *H*-2), 3.02 (m, 2H, CH_2-1), 3.42 (m, 3H, *H*-5 & CH_2-6), 3.60 (m, 1H, *H*-3), 3.72 (m, 1H, *H*-4), 4.60 (bs, 1H, O*H*-6), 4.80 (d, 1H, O*H*-4), 4.90 (d, 1H, O*H*-3), 7.50-7.75 (m, 5H, aromatic *H*). **13a**: *m/e* (FAB), 288 $(M + H)^+$; Anal. (C12H17NO$_5$S) C,H,N; ^1H NMR (300 MHz, DMSO-d_6, δ) 2.28 (bs, 1H, N*H*), 2.80 (m, 1H, *H*-2), 3.12 (m, 1H, *H*-5), 3.20-3.50 (bm, 6H, CH_2-1, *H*-3, *H*-4, & CH_2-6), 4.66 (bs, 1H, O*H*-6), 4.98 (d, 1H, O*H*-3), 5.08 (d, 1H, O*H*-4), 7.65 (m, 2H, *H*-3′ & *H*-5′), 7.75 (m, 1H, *H*-4′), 7.90 (m, 2H, *H*-2′ & *H*-6′).
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19. Minimum inhibitory concentrations (MICs) were determined on *M. tuberculosis* strain H37Ra and on MAC strains NJ 168 (serovar 1), NJ 211 (serovar 4,6), NJ 1854 (serovar 8), NJ 3009 (serovar 4), and NJ 3404 (serovar 4) using a modification of a colorimetric microdilution broth assay.[22a,24] Stock cultures of the MAC strains were derived from colonies possessing the smooth, transparent morphology on 7H10 agar plates. Cultures were grown at 37 °C in 7H9 broth supplemented with ADC enrichment and 0.2% glycerol to a turbidity equivalent to a #1 McFarland Standard. After particulate settling, the upper layers of culture were removed and 2 mL portions dispensed into cryovials and stored at -70 °C. Prior to assay, a frozen culture was diluted in 7H9 broth to about 2 x 10^5 and 1 x 10^5 CFU/mL for MAC and H37Ra, respectively, and used as the inoculum. The assay used 96-well microtiter plates containing color, medium, and viability controls and formats designed to accommodate either seven test compounds in 4-$\log_{10}$ dilutions in the range of 0.128 and 128 μg/mL, or four compounds in a series of twofold dilutions. Each test compound was dissolved in DMSO,

then diluted into broth medium at twice the desired concentration, and 0.05 mL added to duplicate assay wells; the final concentration of DMSO was 1.25%. Each well was then inoculated with 0.05 mL of standardized culture and the plates incubated at 37 °C in polyethylene bags in a humidified incubator for 6 or 13 days, depending on strain. The redox indicator alamar blue (Accumed International, Inc., Westlake, OH) was then added to each well as a mixture with Tween 80 and the plates incubated for an additional 18-22 h. The plates were read in an optical reader programmed to subtract the absorbance at 600 nm from that at 570 nm to blank out turbidity and absorbance due to oxidized dye. Isoniazid and ethambutol were used as positive controls.

Minimal bactericidal concentrations (MBCs) were determined by plating samples obtained from the microtiter plates just prior to the addition of alamar Blue. The plates were examined visually using a magnifying mirror. The contents of those wells with no apparent growth were mixed by drawing liquid in and out of a microliter pipette followed by plating 10 μL from the duplicate wells onto each half of a 10 mm x 60 mm 7H10 agar plate. The viable counts obtained after incubation were compared to the initial inoculum count to determine percent survivors. The MBC was the lowest drug concentration that killed 99% of the starting inoculum.[25]

20. The anti-MAC activity of compounds was tested in vitro using the macrophage model as previously described.[26] Either mouse peritoneal macrophage or U937 human macrophage cell lines were infected with different MAC strains (AIDS patient-derived MAC 101, 100, and 109), and 4 h later extracellular bacteria were removed by washing and treatment was initiated with an experimental drug. Monolayers were treated for four days (medium and drug were replenished daily) and then macrophage monolayers were lysed and the viable intracellular bacteria (CFU/10^5 cells) quantitated. To measure tumor necrosis factor-alpha (TNFα) production, infected macrophage monolayers (MAC strain 101) were trated with different concentrations of **11e** for 18 h, and the supernate obtained. Concentration of TNFα in the supernate was measured using an ELISA kit (Biosource International, Camarillo, CA) as previously published.[27]
21. Bone marrow macrophages from C57BL/6 mice were cultured in RPMI 1640 tissue culture medium containing 10% L929 fibroblast conditioned medium as a source of M-CSF for ten days, then washed and infected with 10^6 Erdman. Four hours later monolayers were washed to remove extracellular bacteria, then incubated for eight days in the presence of a series of dilutions of **11e**. Monolayers were lysed and plated on 7H11 agar for three weeks. A TNF response was also performed.
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