Brief Articles

Synthesis and Antimycobacterial Activity of Pyrazine and Quinoxaline Derivatives

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A series of pyrazine and quinoxaline derivatives have been synthesized, and their activity against *M. tuberculosis* (Mtb) and *Mycobacterium avium* (MAC) are reported. The 4-acetoxybenzyl ester of pyrazinoic acid and 4′-acetoxybenzyl 2-quinoxalinecarboxylate showed excellent activity against Mtb (MIC ranges of less than 1−6.25 μg/mL) but only modest activity against MAC (MICs of 4−32 μg/mL).

Introduction

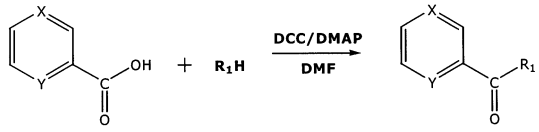
The worldwide appearance of strains of Mtb that are resistant to many if not all of the clinically used antitubercular agents has led to a call for new approaches to treatment of tuberculosis (Tb).[1] In addition, alteration of clinically used agents that might improve their initial activity or modify the susceptibility profile of clinically resistant disease is also a viable approach to developing more potent antitubercualr drugs. Researchers have reported pyrazinamide (PZA),[2,3] isoniazid,[4,5] and ethambutol[6] analogues with significant activity against Tb.

PZA is one of the front-line agents prescribed for the treatment of Mtb. The initial treatment regimen includes the use of four drugs: PZA, isoniazid, rifampin, and either ethambutol or streptomycin. Demonstration of PZA activity against Mtb in vitro requires an acidic environment (pH ≤ 5.6).[7,8] Although PZA has been used clinically since the 1950s, a proposed mechanism of action has only recently been reported to be inhibition of the eukaryotic-like fatty acid synthetase I (FASI) of Mtb.[9] The results of a more recent study, however, suggest that neither PZA nor pyrazinoic acid (POA) directly inhibit FASI.[10] PZA is considered to be a prodrug of POA, which is believed to be the active inhibitor of Mtb.[2] Activation of PZA to POA is regulated by a pyrazinamidase present in all PZA-sensitive strains of Mtb. PZA-resistant strains of Mtb and intrinsically resistant strains of *M. bovis* are defective in this amidase activity because of mutations in the associated gene and are therefore unaffected by PZA.[11]

In an effort to circumvent this mechanism of clinical resistance while maintaining the activity of this class of agent, pyrazinoic acid ester derivatives have been prepared and screened in vitro.[2,3] It was reasoned that the ester forms of POA might be activated by bacterial esterases, thus allowing production of POA even in clinically resistant forms of Mtb that are not sensitive to PZA through loss of the amidase activity. In fact, several POA esters show significant activity against PZA-susceptible and -resistant Mtb strains as well as several intrinsically PZA-resistant mycobacteria such as *M. bovis*, *M. kansasii*, and *M. avium* (MAC).[2,3]

Herein, several pyrazinoic acid ester derivatives are reported that were designed as potential prodrugs of POA through a "self-immolative"[12] process. A "self-immolative" prodrug is defined as generating an unstable intermediate that, following the activation process, will extrude the active drug in a number of subsequent steps.[13] For example, enzymatic deacylation of the 4-acetoxy group (**3**, **8**, **10**, **17**) generates a hydroxy group. Bioreduction (**1**, **2**, **6**, **7**, **9**, **15**, **16**) converts the nitro group into an amino group. Upon activation, additional electron density in the aromatic π system would lead to the extrusion of a substituted quinone methide and POA. Herein, several ester derivatives of POA, nicotinic acid, 2-quinoxalinecarboxylic acid (QOA), benzoic acid, and acetic acid were synthesized and screened for activity against Mtb and MAC in order to test this approach for inhibitor design.

Scheme 1. Preparation of POA Analogues



(1-10: see Table 1 for X, Y and R_1)

Chemistry

The POA esters (**1**−**10**) were synthesized according to reported procedures (Scheme 1).[14] Esterification of 2-pyrazinecarboxylic acid in the presence of DCC/DMAP and the appropriate alcohol gave the desired product upon purification. The QOA ester derivatives (**11**−**17**) were prepared by a method adapted from Cynamon et al. for the preparation of POA esters (Scheme 2).[2]

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Scheme 2. Preparation of QOA Analogues

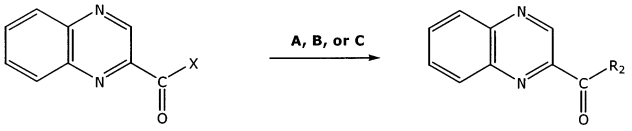
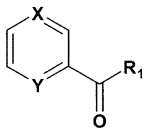
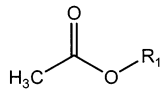


Table 1. Activity of Pyrazine and Acetic Acid Esters[a]

				MIC (μg/mL)			
compd	X	Y	R_1	Mtb H37Rv	Mtb H37Ra	MAC NJ211	MlogP
							
1	N	N	2-nitrobenzyloxy	>6.25	64	>128	0.99
2	N	N	4-nitrobenzyloxy	6.25	128	>128	0.99
3	N	N	4-acetoxybenzyloxy	6.25	<0.25	16	0.92
4	N	N	benzyloxy	>6.25	16	>128	0.94
5	N	N	4-CF_3-benzyloxy	>6.25	128	>128	1.87
6	CH	N	4-nitrobenzyloxy	>6.25	128	>128	2.29
7	CH	CH	4-nitrobenzyloxy	>6.25	ND	ND	3.45
8	CH	CH	4-acetoxybenzyloxy	>6.25	16	128	3.38
POA	N	N	OH	ND	64	>128	ND
							
9			4-nitrobenzyl	>6.25	>128	>128	2.02
10			4-acetoxybenzyl	ND	8	128	2.03

[a] Mtb H37Rv (TAACF using BACTEC 12B broth), Mtb H37Ra, and MAC were screened using Middlebrook 7H9 with glycerol and OADC enrichment, pH 6.6.

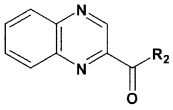
Nucleophilic displacement of 2-quinoxalinecarbonyl chloride by the appropriate alcohol afforded the desired ester. Ammonolysis of 2-methoxycarbonylquinoxaline (**11**) by standard methods[15] using 7 N methanolic ammonia produced 2-quinoxalinecarboxamide (**18**) (Scheme 2). 2-Quinoxalinecarbohydrazide (**19**) was also prepared from methyl 2-quinoxalinecarboxylate (**11**) as shown in Scheme 2 through reaction with hydrazine.

Results and Discussion

We synthesized several POA ester derivatives, one nicotinic acid ester derivative, two benzoic acid ester derivatives, and two acetic acid ester derivatives as putative inhibitors of Mtb and MAC (Table 1). The activities of three of the starting materials (4-acetoxybenzyl alcohol, 2-nitrobenzyl alcohol, and 4-nitrobenzyl alcohol) were determined to rule out the possibility of their participation in inhibition as ester hydrolysis products. Several novel QOA ester derivatives were also prepared and evaluated as PZA analogues (Table 2).

The derivatives were tested initially against Mtb H_{37}Rv through the Tuberculosis Acquisition and Coordinating Facility (TAACF: NIH, NIAID Contract AI45246). They were also assayed in our laboratory against Mtb H_{37}Ra and MAC. The results are presented in Tables 1 and 2. We also determined the effect of pH (6.6 vs 5.8) and medium enrichment (ADC vs OADC; see Supporting Information) on the activity of derivatives **2**, **3**, **17**, and PZA against Mtb. The lower pH reduced the MIC for the derivatives, as well as for PZA, similar to results

Table 2. Activity of Quinoxaline Derivatives[a]



		MIC (μg/mL)			
compd	R_2	Mtb H37Rv	Mtb H37Ra	MAC NJ211	MlogP
11	methoxy	>6.25	>128	>128	0.84
12	*n*-propoxy	6.25	64	128	1.41
13	*n*-octyloxy	>6.25	128	128	2.61
14	2-octyloxy	>6.25	64	128	2.70
15	4-nitrobenzyloxy	>6.25	>128	>128	2.27
16	2-nitrobenzyloxy	>6.25	>128	>128	2.27
17	4-acetoxybenzyloxy	6.25	0.5	16	2.15
18	NH_2	>6.25	>128	>128	0.13
19	$HNNH_2$	>6.25	64	>128	0.16
QOA	OH	ND	>128	>128	ND

[a] Mtb H37Rv (TAACF using BACTEC 12B broth), Mtb H37Ra, and MAC were screened using Middlebrook 7H9 with glycerol and OADC enrichment, pH 6.6.

reported by Cynamon et al.[2] for a series of POA ester derivatives. Also, medium with OADC at pH 6.6 lowered the MIC for **3** and **17**, but not for **2** and PZA, by 4- and 8-fold, respectively, compared to medium with ADC (data not shown). Ethambutol activity was not affected by the type of enrichment. The MIC of ethambutol for Mtb H_{37}Ra and MAC was in the range 2−8 μg/mL. The presence of OADC or ADC in the medium did not alter the MICs of the derivatives for MAC (data not shown).

Tables 1 and 2 present the MICs for Mtb H_{37}Ra and MAC determined at pH 6.6 and with OADC enrichment and for Mtb H_{37}Rv assayed in BACTEC 12B medium. Several derivatives (**2**, **3**, **12**, **17**) appeared to have good activity (MICs, 6.25 μg/mL) against Mtb H_{37}Rv. Two derivatives, the 4-acetoxybenzyl esters of POA (**3**) and 2-quinoxaline carboxylate (**17**), exhibited excellent activity against Mtb H_{37}Ra with MICs of 0.25−0.5 μg/mL. Derivatives **3** and **17** also had moderate activity (MIC, 16 μg/mL) against MAC NJ211. The MIC for two additional strains of MAC, NJ168 and NJ3404, was 32 μg/mL for **3**, and for **17**, the MIC was 4 and 8 μg/mL, respectively. Another derivative, 4-acetoxybenzyl acetate (**10**), had good activity against Mtb H_{37}Ra but not against MAC (Table 1). None of the starting materials (4-acetoxybenzyl alcohol, 2-nitrobenzyl alcohol, 4-nitrobenzyl alcohol) were active at 12.8 μg/mL, the highest amount tested (data not shown).

Derivatives **3** and **17** were the most active of the esters tested, and it is noteworthy that both of these derivatives contain the 4-acetoxybenzyl substituent discussed previously as a potential "self-immolative" prodrug. The promising in vitro activity of these two compounds gives credibility to the "self-immolative" mechanism. Although we cannot definitively state that this mechanism is operative, our data are suggestive that it may play a role in the activity of certain analogues. The reason for making prodrug forms of POA is to enhance the intracellular concentration of POA by passive diffusion of an uncharged species that is then hydrolyzed to yield the active drug. The relationship between hydrophobicity and activity in antitubercular drugs is well established.[16] POA shows only modest activity, but ester forms of the acid are known to significantly enhance antibacterial activity likely through

increased uptake and bioactivation. Theoretically, if passive diffusion and hydrolysis at the pyrazine (or quinoxaline) carboxylic acid ester were the primary mode of delivery and activation, presumably all of the benzyl esters (**1**−**5** and **11**−**17**) would show similar activity that positively correlates with the calculated log *P* (i.e., the more hydrophobic esters should more rapidly reach higher intracellular concentrations). Tables 1 and 2 both give log *P* values for the synthetic compounds as calculated by the method of Morgiuchi et al.[17] In fact, many of the analogues showed little or no activity including the more hydrophobic compounds **5** and **13**. The benzyl analogue **4** has virtually the same MlogP value but is at least 50 times less active than the acetoxybenzyl analogue **3**. The in vitro activity of **4** for Mtb and MAC at pH 5.8 has been reported.[3] Interestingly, the 2-nitro and the 4-nitrobenzyl analogues (**1** and **2**; **15** and **16**) are much less active than the acetoxybenzyl analogues (**3** and **17**), suggesting that the nitro function does not appear to be bioreduced under these conditions. This result may not be surprising considering the wealth of data regarding bioreducing groups as chemotherapeutic prodrugs.[18] However, under conditions of hypoxia[19] as in encased tuberculous lesions, bioreduction may be more favorable.[20] In vitro assays under hypoxic conditions are now available,[21] and we are pursuing screening in these assays.

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Supporting Information Available: Experimental procedures for the syntheses of compounds **1**−**19**, analytical data, and procedures for the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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