Pyrazinoic Acid Esters with Broad Spectrum *in Vitro* Antimycobacterial Activity

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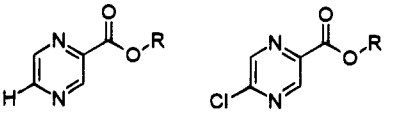
A series of substituted pyrazinoic acid esters has been prepared and examined for their *in vitro* activity against *Mycobacterium avium* and *Mycobacterium kansasii* as well as *Mycobacterium tuberculosis*. Modification of both the pyrazine nucleus and the ester functionality have been very successful in expanding the activity of pyrazinamide to include *M. avium* and *M. kansasii*, organisms normally not susceptible to pyrazinamide. Several of these compounds have activities 100-1000-fold greater than that of pyrazinamide against *M. tuberculosis*.

Introduction

The progressive immunological deterioration seen in AIDS is often accompanied by opportunistic infections causing tuberculosis (*Mycobacterium tuberculosis*) and disseminated nontuberculous mycobacterial disease (*Mycobacterium avium*). Treatment of these infections, along with other opportunistic infections which cause the majority of all AIDS-related deaths, is often complicated by patient intolerance of the drugs employed or pathogen resistance. The antibiotic therapies required for treatment of these infections are lengthy, and in the absence of effective patient compliance may result in the appearance of drug-resistant forms of the organisms. Pyrazinamide (PZA) is a first-line agent for the treatment of tuberculosis[1] and is an essential element of experimental preventive therapy regimens.[2]

The use of nicotinamide-related compounds for the therapy of tuberculosis followed the demonstration that nicotinamide was effective for the treatment of murine tuberculosis.[3,4] Of the many nicotinamide analogs that were subsequently synthesized and evaluated for antituberculosis activity,[5,6] PZA was the most active. PZA is unusual because of its narrow spectrum of activity. Although PZA is inhibitory against most isolates of *M. tuberculosis*, *M. bovis* (a closely related organism) and nontuberculous mycobacteria are usually resistant.[7] It has also been reported that esters of pyrazinoic acid and pyrazine-2,3-dicarboxylic acid have *in vitro* activity against *M. tuberculosis* H37Rv.[8-10] Since nontuberculous mycobacteria are usually resistant to PZA, the activity of PZA is normally discussed in relation to its activity against *M. tuberculosis*. There are conflicting data regarding the activity of analogs of PZA against *M. tuberculosis*, resulting from the fact that *in vivo* studies in mice have been done in the absence of preliminary *in vitro* studies.[4] Pyrazinoic acid and thiopyrazinamide were reported to be inactive in the murine tuberculosis model.[9] Subsequent studies demonstrated that pyrazinoic acid is active *in vitro* against *M. tuberculosis* and *M. bovis*.[11]

The mechanism of action of PZA is unknown; however, it is known that the majority of *M. tuberculosis* isolates resistant to PZA have low levels of pyrazinamidase activity as do *M. bovis* isolates.[12] We postulated that PZA was a prodrug that was converted to pyrazinoic acid (the active agent) by an intracellular amidase.[13] Recently it has been shown that the resistance of a number of *M. tuberculosis* isolates to PZA correlates well with the failure of those isolates to express the amidase.[14] Pyrazinoic acid esters (PAE) (**1-66**) which could be hydrolyzed by an esterase could serve as potential prodrugs, which would circumvent the requirement for activation by an amidase. A series of pyrazinoate esters was demonstrated to have substantially better *in vitro* activity than PZA against susceptible isolates of *M. tuberculosis*. The pyrazinoate esters were also active against PZA-resistant *M. tuberculosis* isolates, *M. bovis* and *M. kansasii*.[14] The esters prepared in this earlier study were not active against *M. avium complex* isolates.



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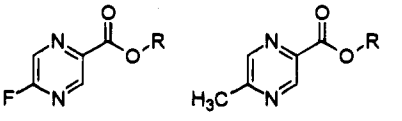

58-61 **62-66**

R= alkyl, aryl

The present study evaluated the *in vitro* activity of pyrazinoate esters that were further modified on the pyrazine nucleus and/or the ester moiety itself.

Results

The results are presented in Tables 1-5 where the effect of modifications of the pyrazine nucleus can be observed. Table 1 establishes the relative activities of pyrazinamide, pyrazinoic acid, 5-chloropyrazinoic acid, and 5-methylpyrazinoic acid against *M. avium*, *M. kansasii*, and *M. tuberculosis*. The activity of these compounds and the PAE against the highly pathogenic Erdman strain of *M. tuberculosis* (ATCC 35801) and Baldwin's 1905 human lung isolate, H37Rv (ATCC

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Table 1. Minimum Inhibitory Concentrations (MIC)[a] of Pyrazinoic Acids or Amides against Various Mycobacteria

	M. avium complex		*M. kansasii*	*M. tuberculosis*		
pyrazinoic acid (amide)	101[b]	ATCC 49601	S[c]	ATCC 35801	ATCC 27294	ATCC 35828[d]
pyrazinoic acid	>1024	>1024	256	32	32	32
pyrazinamide	>2048	>2048	2048	32	16	2048
5-chloropyrazinoic acid	1024	>1024	64	64	64	64
5-methylpyrazinoic acid	1024	>1024	1024	64	64	>64

[a] MIC is the minimum inhibitory concentration (μg/mL). [b] Clinical isolate from Lowell Young, Kuzell Institute for Arthritis and Infectious Disease. [c] Clinical isolate Veterans Administration Medical Center, Syracuse, NY. [d] Resistant to PZA.

Table 2. Minimum Inhibitory Concentrations (MIC) of Esters of Pyrazinoic Acid against Various Mycobacteria[a]

		M. avium complex		*M. kansasii*	*M. tuberculosis*		
compd	pyrazinoates	101	ATCC 49601	S	ATCC 35801	ATCC 27294	ATCC 35828
1	methyl	>256	>256	>64	16	≤8	64
2	isobutyl	256	256	16	4	2	4
3	*n*-decyl	32	8	0.25	0.25	0.5	0.25
4	*n*-pentadecyl	32	8	1	0.12	0.06	1
5	benzyl	64	64	1	1	≤1	2
6	2′,6′-di-*tert*-butyl-4′-methylphenyl	>1024	>1024	>16	ND[a]	128	128
7	4′-fluorophenyl	128	128	64	32	32	16
8	2′,4′,6′-tribromophenyl	128	128	64	32	32	32
9	naphthyl	128	64	>16	ND	8	8

[a] See footnotes to Table 1. [b] ND = not determined.

Table 3. Minimum Inhibitory Concentrations (MIC) of Esters of 5-Chloropyrazinoic Acid against Various Mycobacteria[a]

		M. avium complex		*M. kansasii*	*M. tuberculosis*		
compd	5-chloropyrazinoate	101	ATCC 49601	S	ATCC 35801	ATCC 27294	ATCC 35828
10	methyl	256	64	2	8	4	16
11	ethyl	128	128	2	2	1	>16
12	*n*-propyl	16	16	0.25	≤0.03	0.06	0.25
13	*n*-butyl	64	128	≤1	0.5	0.25	0.5
14	*n*-pentyl	32	32	≤0.03	0.25	0.06	0.12
15	*n*-hexyl	32	16	0.015	0.125	0.125	0.5
16	*n*-heptyl	8	16	≤0.03	≤0.03	0.06	≤0.03
17	*n*-octyl	16	16	≤0.03	≤0.03	0.06	≤0.03
18	*n*-nonyl	32	16	≤0.125	1	≤0.03	≤0.03
19	*n*-decyl	32	16	0.5	0.25	0.5	0.5
20	*n*-undecyl	16	8	0.06	0.5	0.25	0.5
21	allyl	64	64	≤1	1	1	2
22	isobutyl	64	64	≤1	0.25	2	2
23	*tert*-butyl	128	128	≤1	0.5	2	4
24	benzyl	8	4	1	0.5	1	1
25	2′-heptyl	32	32	≤0.125	0.5	0.06	0.06
26	2′-octyl	8	16	≤0.015	0.25	0.06	0.5
27	2′-nonyl	32	32	≤0.03	0.5	1	2
28	2′-decyl	32	32	≤0.12	0.5	0.06	≤0.03
29	2′-undecyl	16	16	0.25	0.125	0.25	1
30	2′-tridecyl	256	32	0.06	0.5	2	0.5
31	3′-octyl	>256	256	0.125	1	0.25	1
32	3′-undecyl	64	32	≤0.03	0.25	0.5	0.25
33	7′-tridecyl	16	64	0.5	0.5	0.25	0.5
34	2′-methyl-3′-decyl	>256	256	0.25	2	0.5	2
35	2′,2′-dimethyl-3′-decyl	>256	>256	2	8	4	8
36	2′,2′-dimethyl-3′-tridecyl	>256	256	4	8	16	16
37	2′,2′-dimethyl-1′-phenpropyl	128	256	2	4	4	16
38	2′-methyl-2′-octyl	64	64	1	4	0.25	0.25
39	2′-methyl-2′-decyl	64	64	1	1	0.125	0.25
40	2′-methyl-2′-undecyl	256	256	0.5	1	8	16
41	3′-methyl-3′-pentyl	>256	256	2	8	8	4
42	5′-methyl-5′-decyl	256	256	16	8	4	16
43	5′-methyl-5′-undecyl	>256	>256	4	8	4	16
44	5′-methyl-5′-dodecyl	>256	>256	16	4	8	16
45	5′-methyl-5′-tridecyl	>64	>64	>16	16	16	8
46	6′-methyl-6′-undecyl	>64	>64	8	8	8	16
47	6′-methyl-6′-dodecyl	>64	>64	8	8	2	16
48	6′-methyl-6′-tridecyl	>64	>64	ND[b]	4	2	8
49	7′-methyl-7′-tridecyl	>64	16	2	0.5	0.25	0.5
50	diphenylmethyl	>64	>64	8	16	16	16
51	2′-phenethyl	32	8	0.25	1	1	4
52	*p*-bromobenzyl	32	64	0.125	1	1	>16
53	1′-(*p*-bromophenyl)ethyl	32	16	0.25	8	2	16
54	2′-(*p*-chlorophenyl)ethyl	16	8	0.5	32	2	32
55	1′-(4-tolyl)ethyl	256	16	1	1	1	2
56	1′-phenpentyl	128	64	1	2	4	8
57	1′-methyl-1′-phenethyl	256	>256	32	16	16	>16

[a] See footnotes to Table 1. [b] ND = not determined.

Table 4. Minimum Inhibitory Concentrations (MIC) of Esters of 5-Fluoropyrazinoic Acid against Various Mycobacteria[a]

		M. avium complex		M. kansasii	M. tuberculosis		
compd	5-fluoropyrazinoate	101	ATCC 49601	S	ATCC 35801	ATCC 27294	ATCC 35828
58	methyl	64	64	8	8	4	8
59	*n*-hexyl	64	64	8	4	ND[b]	8
60	*n*-decyl	32	32	1	4	2	4
61	2′-octyl	32	64	≤0.03	4	2	4

[a] See footnotes to Table 1. [b] ND = not determined.

Table 5. Minimum Inhibitory Concentrations (MIC) of Esters of 5-Methylpyrazinoic Acid against Various Mycobacteria[a]

		M. avium complex		M. kansasii	M. tuberculosis		
compd	5-methylpyrazinoate	101	ATCC 49601	S	ATCC 35801	ATCC 27294	ATCC 35828
62	methyl	>256	>256	32	8	16	>16
63	*n*-propyl	>64	64	8	4	2	8
64	*n*-heptyl	32	32	4	0.25	0.5	1
65	*n*-nonyl	64	32	2	1	1	2
66	3′-octyl	64	64	4	0.5	1	1

[a] See footnotes to Table 1.

27294), are compared with the activity of the subject compounds against a PZA resistant strain of *M. tuberculosis* (ATCC 35828). Tables 2–5, containing data from unsubstituted pyrazinoates and from substitutions at the 5-position of the pyrazine nucleus, demonstrate convincingly the activity of PAE against nontuberculous mycobacteria.

Discussion

Structural modifications of the ester side chain rather than substitutions of the pyrazine nucleus have been very successful in expanding the activity of PAE to include *M. avium* and *M. kansasii*, organisms not normally susceptible to PZA. In addition, these PAE demonstrate very similar activity against *M. tuberculosis* ATCC 35828, a PZA-resistant organism, and the two susceptible *M. tuberculosis* strains. The *in vitro* activity of several of these compounds (such as 2′-octyl 5-chloropyrazinoate (**26**), *n*-octyl 5-chloropyrazinoate (**17**), and *n*-propyl 5-chloropyrazinoate (**12**)) are 100–1000-fold greater than that of PZA. A number of PAE have *in vitro* activity against the two *M. avium* isolates (organisms that are resistant to PZA) that is better than that of PZA against *M. tuberculosis* isolates. The *M. kansasii* isolate is susceptible to the PAE at approximately the same level as the *M. tuberculosis* isolates. Halogenation with chlorine at the 5-position on the pyrazine nucleus enhances *in vitro* activity against *M. tuberculosis*. Substitution of fluorine at the 5-position on the pyrazine ring or the use of 5-methyl pyrazinoates decreases the *in vitro* activity relative to chlorination.

These compounds have attractive *in vitro* activity; however, their bioavailability and pharmacology have to be further studied prior to *in vivo* evaluation in a murine tuberculosis model. The ester is likely to be inherently more labile than an amide prodrug. Initial studies of serum stability suggest that the PAE may prove to be labile with regard to the ester linkage. This instability can be addressed by increasing the steric demand of the alcohol residue of the ester. It is not known whether *in vivo* activity can be achieved by the appropriate manipulations of the alcohol moiety. A quantitative structure–activity study of these findings which will be used to guide further synthesis is in preparation.

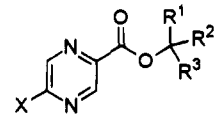
Experimental Section

5-Chloropyrazinoyl chloride and 5-methylpyrazinoic acid were gifts of the Lonza Co., Visp, Switzerland. ^{1}H NMR spectra were recorded at 300 MHz on a Varian XL-300 NMR spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) or residual chloroform as the internal standard. ^{13}C NMR spectra were recorded at 75.429 MHz on a Varian XL-300 NMR spectrometer with $CDCl_3$ as solvent and tetramethylsilane (TMS) or residual chloroform as the internal standard. ^{19}F NMR spectra were determined at 282.203 MHz on a Varian XL-300 NMR spectrometer with $CDCl_3$ as solvent and chlorotrifluoromethane ($CFCl_3$) as the internal standard. Melting points were determined in open glass capillaries utilizing a Mel-temp apparatus. Boiling points were reported uncorrected. Solvents were freshly distilled prior to use: dichloromethane (CH_2Cl_2) was distilled from anhydrous potassium carbonate; hexanes and pyridine were distilled from calcium hydride; and tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under a nitrogen atmosphere. Elemental microanalyses for carbon and hydrogen were determined by M.-H.-W. Laboratories, Phoenix, AZ, and are within ±0.4% of the theory for the formula given.

Method A. Preparation of Pyrazinoates 1–9. Benzyl Pyrazinoate (5). Pyrazine-2-carboxylic acid (Aldrich Chemical Co., 3.7 g, 0.030 mol) was dissolved in benzene (25 mL) and thionyl chloride (15 mL), and this mixture was refluxed for 2 h, after which benzene and excess thionyl chloride were distilled as an azeotrope. The dark red pyrazinoyl chloride was purified by sublimation *in vacuo* at 50–60 °C to give the pure product as white needles (3.2 g, 74%). The purified pyrazinoyl chloride (ca. 3.2 g, 0.022 mol) was rapidly transferred to a flame-dried flask containing 40 mL of dichloromethane and 2 mL of pyridine under nitrogen. The solution was cooled to 0 °C, and benzyl alcohol (2.59 g, 0.024 mol) was added. The reaction mixture was stirred at 0 °C, for 1 h, allowed to warm to room temperature, and stirred overnight. The mixture was washed with aqueous $CuSO_4$ solution (2 × 20 mL), followed by H_2O (20 mL) and brine (2 × 20 mL). The organic phase was dried over anhydrous $MgSO_4$. The solvent was then evaporated *in vacuo* to give the crude product. The crude product was purified by Kugelrohr distillation to yield 3.96 g (84%) of **5**: bp 119–121 °C/0.05 mm. This liquid solidifies on standing to a low-melting solid: mp 38–40 °C; IR (CH_2Cl_2) 3068, 3034, 2958, 1724, 1560, 1522, 1456, 1382, 1022 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.30 (d, J = 1.5 Hz, 1H), 8.72 (m, 2H), 7.46 (m, 2H), 7.35 (m, 3H), 5.46 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 163.63, 147.58, 146.22, 144.36, 143.29, 134.95, 128.59, 128.54, 67.77. Anal. ($C_{12}H_{10}N_2O_2$) C, H.

Method B. Preparation of 5-Chloropyrazinoates 10–36, 50–57. 2′-Decyl 5-Chloropyrazinoate (28). To a flame-dried flask cooled under a nitrogen atmosphere was added 15 mL of methylene chloride. 5-Chloro-2-pyrazinoyl chloride (Lonza Co., 0.89 g, 0.005 mol) was quickly weighed and

Table 6. Physical Data for Pyrazinoate Esters



compd	X	R^1	R^2	R^3	molecular formula[a]	mp or bp/mm (°C)	method of preparation[b]
1	H	H	H	H	$C_6H_6N_2O_2$		A
2	H	$CH(CH_3)_2$	H	H	$C_9H_{12}N_2O_2$	83–95/0.1	A
3	H	C_9H_{19}	H	H	$C_{15}H_{24}N_2O_2$	147–149/0.1	A
4	H	$C_{14}H_{29}$	H	H	$C_{20}H_{34}N_2O_2$	46–48	A
5	H	C_6H_5	H	H	$C_{12}H_{10}N_2O_2$	38–40	A
6	H	2′,6′-di-*tert*-butyl-4′-methylphenyl			$C_{20}H_{26}N_2O_2$	117–119	A
7	H	4′-fluorophenyl			$C_{11}H_7FN_2O_2$	104–106	A
8	H	2′,4′,6′-tribromophenyl			$C_{11}H_5BrN_2O_2$	156–160	A
9	H	napthyl			$C_{15}H_{10}N_2O_2$	154–156	A
10	Cl	H	H	H	$C_6H_5ClN_2O_2$	91–92	B
11	Cl	CH_3	H	H	$C_7H_7ClN_2O_2$	42–44	B
12	Cl	C_2H_5	H	H	$C_8H_9ClN_2O_2$	93–95/0.05	B
13	Cl	C_3H_7	H	H	$C_9H_{11}ClN_2O_2$	87–89/0.175	B
14	Cl	C_4H_9	H	H	$C_{10}H_{13}ClN_2O_2$	105/0.1	B
15	Cl	C_5H_{11}	H	H	$C_{11}H_{15}ClN_2O_2$	125/0.015	B
16	Cl	C_6H_{13}	H	H	$C_{12}H_{17}ClN_2O_2$	140/0.2	B
17	Cl	C_7H_{15}	H	H	$C_{13}H_{19}ClN_2O_2$	158–160/0.025	B
18	Cl	C_8H_{17}	H	H	$C_{14}H_{21}ClN_2O_2$	30–31	B
19	Cl	C_9H_{19}	H	H	$C_{15}H_{23}ClN_2O_2$	42–43	B
20	Cl	$C_{10}H_{21}$	H	H	$C_{16}H_{25}ClN_2O_2$	41–42	B
21	Cl	$CH{=}CH_2$	H	H	$C_8H_7ClN_2O_2$	88–90/0.1	B
22	Cl	$CH(CH_3)_2$	H	H	$C_9H_{11}ClN_2O_2$	88–90/0.175	B
23	Cl	CH_3	CH_3	CH_3	$C_9H_{11}ClN_2O_2$	95/0.10	B
24	Cl	C_6H_5	H	H	$C_{12}H_9ClN_2O_2$	133–135/0.2	B
25	Cl	C_5H_{11}	CH_3	H	$C_{12}H_{17}ClN_2O_2$	130/01	B
26	Cl	C_6H_{13}	CH_3	H	$C_{13}H_{19}ClN_2O_2$	115/0.01	B
27	Cl	C_7H_{15}	CH_3	H	$C_{14}H_{21}ClN_2O_2$	NA	B
28	Cl	C_8H_{17}	CH_3	H	$C_{15}H_{23}ClN_2O_2$	150/0.1	B
29	Cl	C_9H_{19}	CH_3	H	$C_{16}H_{25}ClN_2O_2$	NA	B
30	Cl	$C_{11}H_{23}$	CH_3	H	$C_{18}H_{29}ClN_2O_2$	37–38	B
31	Cl	C_8H_{17}	C_2H_5	H	$C_{16}H_{25}ClN_2O_2$	112–114/0.075	B
32	Cl	C_5H_{11}	C_2H_5	H	$C_{13}H_{19}ClN_2O_2$	NA	B
33	Cl	C_6H_{13}	C_6H_{13}	H	$C_{18}H_{29}ClN_2O_2$	162/0.25	B
34	Cl	C_7H_{15}	$CH(CH_3)_2$	H	$C_{16}H_{25}ClN_2O_2$	115–120/0.075	B
35	Cl	C_7H_{15}	$C(CH_3)_3$	H	$C_{17}H_{27}ClN_2O_2$	170–180/0.1	B
36	Cl	$C_{10}H_{21}$	$C(CH_3)_3$	H	$C_{20}H_{33}ClN_2O_2$	210–220/0.1	B
37	Cl	C_6H_5	$C(CH_3)_3$	H	$C_{16}H_{17}ClN_2O_2$	98–100	C
38	Cl	C_6H_{13}	CH_3	CH_3	$C_{14}H_{21}ClN_2O_2$	155/0.025	C
39	Cl	C_8H_{13}	CH_3	CH_3	$C_{16}H_{25}ClN_2O_2$	NA	C
40	Cl	C_9H_{19}	CH_3	CH_3	$C_{17}H_{27}ClN_2O_2$	NA	C
41	Cl	C_2H_5	C_2H_5	CH_3	$C_{11}H_{15}ClN_2O_2$	75–83/0.025	C
42	Cl	C_5H_{11}	C_4H_9	CH_3	$C_{16}H_{25}ClN_2O_2$	NA	C
43	Cl	C_6H_{13}	C_4H_9	CH_3	$C_{17}H_{27}ClN_2O_2$	NA	C
44	Cl	C_7H_{15}	C_4H_9	CH_3	$C_{18}H_{29}ClN_2O_2$	NA	C
45	Cl	C_8H_{17}	C_4H_9	CH_3	$C_{19}H_{31}ClN_2O_2$	120–122/0.25	C
46	Cl	C_5H_{11}	C_5H_{11}	CH_3	$C_{17}H_{27}ClN_2O_2$	NA	C
47	Cl	C_6H_{13}	C_5H_{11}	CH_3	$C_{18}H_{29}ClN_2O_2$	NA	C
48	Cl	C_7H_{15}	C_5H_{11}	CH_3	$C_{19}H_{31}ClN_2O_2$	NA	C
49	Cl	C_6H_{13}	C_6H_{13}	CH_3	$C_{19}H_{31}ClN_2O_2$	100/0.1	C
50	Cl	C_6H_5	C_6H_5	H	$C_{18}H_{13}ClN_2O_2$	104–106	B
51	Cl	$C_6H_5CH_2$	H	H	$C_{13}H_{11}ClN_2O_2$	60–62	B
52	Cl	p-BrC_6H_4	H	H	$C_{12}H_8BrClN_2O_2$	138–141	B
53	Cl	p-BrC_6H_4	CH_3	H	$C_{13}H_{10}BrClN_2O_2$	67–71	B
54	Cl	p-$ClC_6H_4CH_2$	H	H	$C_{13}H_{10}Cl_2N_2O_2$	88–92	B
55	Cl	p-$CH_3C_6H_4$	H	H	$C_{13}H_{11}ClN_2O_2$	82–88	B
56	Cl	C_6H_5	C_4H_9	H	$C_{16}H_{17}ClN_2O_2$	38–39	B
57	Cl	C_6H_5	CH_3	CH_3	$C_{16}H_{17}ClN_2O_2$	NA	B
58	F	H	H	H	$C_6H_5FN_2O_2$	52–54	D
59	F	C_5H_{11}	H	H	$C_{11}H_{15}FN_2O_2$	96/0.05	D
60	F	C_9H_{19}	H	H	$C_{15}H_{23}FN_2O_2$	47–49	D
61	F	C_6H_{13}	CH_3	H	$C_{13}H_{19}FN_2O_2$	96/0.1	D
62	CH_3	H	H	H	$C_7H_8N_2O_2$	92–95	E
63	CH_3	C_2H_5	H	H	$C_9H_{12}N_2O_2$	91/0.25	E
64	CH_3	C_6H_{13}	H	H	$C_{13}H_{20}N_2O_2$	135/0.35	E
65	CH_3	C_8H_{17}	H	H	$C_{15}H_{24}N_2O_2$	147/0.25	E
66	CH_3	C_5H_{11}	C_2H_5	H	$C_{14}H_{22}N_2O_2$	NA	E

[a] All compounds were analyzed for C, H: the results agreed to within ±0.4% of the theoretical values. [b] A general preparation for each type of synthesis is given in the Experimental Section.

transferred to the flask. The solution was cooled to 0 °C and was allowed to stir 10 min. Pyridine (0.45 mL, 0.0055 mol) was added dropwise as a solution in 5 mL of methylene chloride at 0 °C. After stirring an additional 10 min at 0 °C, 2-decanol (0.88 g, 0.0055 mol) dissolved in 5 mL of methylene chloride was added to the reaction mixture. On warming to

room temperature, it was allowed to stir for 48 h. Methylene chloride (20 mL) was added to the reaction mixture, and the solution was washed successively with saturated cupric sulfate to remove excess pyridine and brine and then was dried over anhydrous magnesium sulfate. Following concentration *in vacuo*, the crude oil was purified by Kugelrohr distillation (oven temperature 150 °C at 0.1 mmHg) to give 1.1 g (74% yield) of the product: IR (neat) 1746, 1721 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.00 (s, 1H), 8.65 (s, 1H), 5.20 (m, 1H), 1.67 (m, 2H), 1.33 (d, $^3J_{H-H}$ = 6.3 Hz, 3H), 1.18 (m, 12H), 0.79 (t, $^3J_{H-H}$ = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 162.67, 152.26, 145.49, 144.28, 141.35, 73.83, 35.73, 31.71, 29.33, 29.27, 29.09, 25.36, 22.53, 19.32, 13.97. Anal. ($C_{15}H_{23}ClN_2O_2$) C, H.

Method C. Preparation of 5-Chloropyrazinoates 37–49. 7′-Methyl-7′-tridecyl 5-Chloropyrazinoate (46). To a flame-dried flask cooled under a nitrogen atmosphere containing 7-methyl-7-tridecanol (1.1 g, 0.0057 mol) was added 6 mL of anhydrous tetrahydrofuran. To this solution with stirring at room temperature was added 2.3 mL of *n*-butyllithium (2.5 M in hexanes, 0.0057 mol). After 30 min at room temperature, 5-chloro-2-pyrazinoyl chloride (Lonza Co., 1.0 g, 0.0057 mol) dissolved in 6 mL of anhydrous tetrahydrofuran was added cautiously to the solution of the alkoxide. The reaction mixture was then heated under reflux for 1 h. On cooling to room temperature, the reaction mixture was poured onto 12 mL of water and the organic phase were separated. The aqueous phase was extracted with diethyl ether (three times, 10 mL). The combined organic phases were dried over anhydrous magnesium sulfate and then were concentrated *in vacuo*. The residue was purified by Kugelrohr distillation (oven temperature 100 °C at 0.1 mmHg) to yield the desired product (1.15 g, 63% yield): IR (neat) 1744, 1716 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.92 (s, 1H), 3.04 (s, 1H), 1.90 (m, HH), 1.53 (s, 3H), 1.24 (m, 16H), 0.82 (t, $^3J_{H-H}$ = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 161.59, 151.82, 145.03, 144.19, 141.66, 88.54, 38.09, 31.55, 29.39, 23.52, 22.42, 13.85. Anal. ($C_{19}H_{31}ClN_2O_2$) C, H.

Method D. Preparation of 5-Fluoropyrazinoates 58–61. Decyl 5-Fluoropyrazinoate (60). To 2-decyl 5-chloropyrazinoate (0.45 g, 0.0015 mol), prepared as described above, dissolved in 15 mL of anhydrous acetonitrile was added silver(I) fluoride (0.58 g, 0.0045 mol). The mixture was protected from moisture and was heated under reflux for 48 h. After filtration and concentration *in vacuo*, the residue was purified by Kugelrohr distillation (oven temperature 105 °C at 0.05 mmHg) to yield the desired fluorinated product (0.38 g, 91% yield): mp 47–49 °C; IR (CH_2Cl_2) 1736, 1726 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.95 (s, 1H), 8.54 (d, $^3J_{H-H}$ = 8.3 Hz, 1H), 4.43 (t, $^3J_{H-H}$ = 6.8 Hz, 2H), 1.81 (m, 2H), 1.25 (br, s, 14H), 0.86 (t, $^3J_{H-H}$ = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 162.89, 161.03 (d, $^1J_{C-F}$ = 247 Hz), 144.17 (d, $^3J_{C-F}$ = 11.3 Hz), 141.27 (d, $^1J_{C-F}$ = 5.2 Hz), 133.3 (d, $^2J_{C-F}$ = 38.6 Hz), 66.49, 31.74, 29.35, 29.15, 29.09, 28.45, 25.73, 22.53, 13.96; ^{19}F NMR ($CFCl_3$) δ −74.21 (d, $^3J_{F-H}$ = 8.1 Hz). Anal. ($C_{15}H_{23}FN_2O_2$) C, H.

Method E. Preparation of 5-Methylpyrazinoates 63–66. Nonyl 5-Methylpyrazinoate (66). To 5-methylpyrazinoic acid (0.79 g, 0.005 mol) dissolved in 10.5 mL of nonanol (8.64 g, 0.05 mol) was added 6.5 mL of chlorotrimethylsilane (5.56 g, 0.51 mol). The resultant mixture was heated under reflux for 2 h, becoming homogeneous during this period. After the reaction mixture was diluted with dichloromethane, the excess chlorotrimethylsilane and dichloromethane were removed *in vacuo*. The residue was purified by Kugelrohr distillation (oven temperature 105 °C at 0.25 mmHg). The distillate was further purified by column chromatography on silica gel, eluting with 14% ethyl acetate in hexane to yield **66** (0.5 g, 40% yield): IR (neat) 1745, 1722 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.04 (s, 1H), 8.45 (s, 1H), 4.29 (t, $^3J_{H-H}$ = 6.8 Hz, 2H), 2.53 (s, 3H), 1.67 (m, 2H), 1.13 (m, 12H), 0.73 (t, $^3J_{H-H}$ = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 163.90, 157.34, 145.00, 144.00, 140.53, 65.92, 31.57, 29.19, 28.98, 28.95, 28.38, 25.63, 22.38, 21.62, 13.82. Anal. ($C_{15}H_{24}N_2O_2$) C, H.

Mycobacterial Isolates. *M. tuberculosis* ATCC 27294 (H37Rv), ATCC 35801 (Erdman), and ATCC 35828 (PZA-resistant) were obtained from the American Type Culture Collection, Rockville, MD. *M. avium* ATCC 49601 (serotype 1) is a clinical isolate from a patient with AIDS at State University of New York Health Science Center, Syracuse, NY. This isolate has been used previously in beige mouse studies in our laboratory. *M. avium* strain 101 (serotype 1) was provided by Dr. Lowell Young, Kuzell Institute for Arthritis and Infectious Diseases, San Francisco, CA. *M. kansasii* strain S was a clinical isolate from a patient at the Veterans Affairs Medical Center, Syracuse, NY.

Medium. The organisms were grown in modified Middlebrook 7H10 broth (7H10 agar formulation with agar and malachite green omitted) with 10% OADC enrichment (Difco Laboratories, Detroit, MI) and 0.05% Tween 80 [5] on a rotary shaker at 37 °C for 3 days. The culture suspensions were diluted with 7H10 broth to yield 1 Klett unit/mL of *M. tuberculosis* and 0.1 Klett unit/mL of *M. avium complex*. (Klett–Summerson colorimeter, Klett Manufacturing, Brooklyn, NY) approximately 5×10^5 viable organisms/mL. The final concentration of mycobacteria used for susceptibility testing was approximately 2.5×10^4 viable organisms/mL.

Susceptibility Testing. Stock solutions of PZA, pyrazinoic acid, and PAE were prepared by hydrating a known weight of agent in water or DMSO. The stock solutions were sterilized by passage through a 0.2 μm nylon membrane filter. Serial 2-fold dilutions of the compounds in 7H10 broth at pH 5.8 (testing at pH 5.6 would yield a lower MIC for PZA; however, many of the organisms grow poorly at this pH) were prepared. The tubes were incubated at 37 °C on a rotary shaker for 7–10 days. A control tube without any drug was included in each experiment. The MIC was defined as the lowest concentration of drug that yielded an absence of visual turbidity.

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