bitory activity. On the other hand, it is interesting that the phloroglucinol derivatives show good activity by introducing the dialkyl chains on the same carbon of the six-membered ring. Marked inhibitory activity against both TxA_2 and LTD_4 was found alike in the natural phloroglucinol derivatives and synthesized analogues which possess disubstituted alkyl chains on the same carbon of the six-membered ring.

Experimental Section

NMR spectra were measured with a JEOL GX-270 spectrometer in $CDCl_3$ solution containing tetramethylsilane as an internal standard. IR and UV spectra measured on JASCO IR-810 spectrometer and a JASCO UVIDEC-460 UV-vis spectrophotometer.

Synthesis of 2-Acylphloroglucinols. 2-Methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, 2-methylpentanoic acid, or hexanoic acid (8.0 mmol) was dissolved in BF_3-Et_2O complex (5.0 mL) at room temperature. Anhydrous phloroglucinol (4.0 mmol) was added to this complex, and the mixture was heated on a steam-bath for 24 h. After cooling, the reaction mixture was cooled, it was added dropwise to aqueous potassium acetate (2.6 g/50 mL). After filtration, the filtrate was dissolved with AcOEt and dried over $MgSO_4$. Evaporation of the dried AcOEt and purification with silica-gel column chromatography (hexane–AcOEt) gave **5–9** respectively (yield: 14–40%). Diacylphloroglucinol was also isolated, and the reaction conditions was not optimized. Compounds **5–9** were identified with ^1H- and ^{13}C-NMR, IR, and UV spectra.

Synthesis of 2,4-Diacylphloroglucinols. Acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, hexanoic acid, or octanoic acid (12 mmol) was dissolved in BF_3-Et_2O complex (5.0 mL) at room temperature. Anhydrous phloroglucinol (4.0 mmol) was added to this complex. The reaction and separation was accomplished by the procedure described earlier to yield **10–17** (yield: 7.7–96.5%). Monoacylphloroglucinol was also isolated and the reaction condition was not optimized. Compounds **10–17** were identified with ^1H-NMR, ^{13}C-NMR, IR, and UV spectra.

Alkylation of 2,6-Diisobutyrylphloroglucinol. Anhydrous diisobutyrylphloroglucinol (7.5 mmol) was dissolved in a solution of sodium (1.0 g) in methanol (33 mL) followed by slow addition of methyl, ethyl, propyl, butyl, or pentyl iodide (185 mmol). After the addition was complete, stirring was continued for 15 min at room temperature. Then 2 M hydrochloric acid was added, and the reaction mixture was extracted with AcOEt. The combined AcOEt extracts were washed with water, dried over $MgSO_4$, and concentrated. Purification by column chromatography (over silica-gel, hexane–AcOEt–AcOH 5–20:1:0.1) gave **18–22**, respectively (yield: 17.9–34.1%). The monoalkyl derivative was also isolated, and the reaction condition was not optimized.

Registry No. 1, 110383-37-4; **2**, 110383-38-5; **3**, 137251-97-9; **4**, 137201-18-4; **5**, 35458-21-0; **6**, 2437-62-9; **7**, 26103-97-9; (±)-**8**, 98498-56-7; **9**, 5665-89-4; **10**, 2161-86-6; 11, 3145-11-7; **12**, 3133-29-7; **13**, 3098-40-6; **14**, 2999-10-2; **15**, 139409-36-2; **16**, 3118-34-1; **17**, 3118-46-5; **18**, 35932-10-6; **19**, 139409-37-3; **20**, 139426-54-3; **21**, 139409-38-4; **22**, 139409-39-5; TxA_2, 57576-52-0; LTD4, 73836-78-9; $HO_2CCH(CH_3)_2$, 79-31-2; $HO_2C(CH_2)_3H$, 107-92-6; $HO_2CCH_2CH(CH_3)_2$, 503-74-2; $HO_2CCH(CH_3)(CH_2)_3H$, 97-61-0; $HO_2C(CH_2)_5H$, 142-62-1; CH_3CO_2H, 64-19-7; $HO_2C(CH_2)_2H$, 79-09-4; $HO_2C(CH_2)_7H$, 124-07-2; MeI, 74-88-4; EtI, 75-03-6; PrI, 107-08-4; BuI, 542-69-8; pentyl iodide, 628-17-1; phloroglucinol, 108-73-6.

Antimycobacterial Activity of a Series of Pyrazinoic Acid Esters

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A series of pyrazinoic acid esters has been prepared and evaluated for in vitro antimycobacterial activity. Several of the pyrazinoate esters have substantially better activity than the first-line antituberculous agent pyrazinamide against susceptible isolates of *Mycobacterium turberculosis* as well as activity against pyrazinamide-resistant isolates. The minimal inhibitory concentrations (MICs) were lower for each organism and at each pH than the MICs for pyrazinamide. The esters have activity against *Mycobacterium bovis* and *Mycobacterium kansasii*, two species resistant to pyrazinamide, but not against *Mycobacterium avium* complex.

The use of nicotinamide-related compounds for the therapy of tuberculosis followed the demonstration by Chorine[1] and confirmation by McKenzie[2] that nicotinamide was effective for the treatment of murine tuberculosis. Many nicotinamide analogues, including pyrazinamide, were subsequently synthesized and tested for antituberculous activity.[3,4] Pyrazinamide was the most active of the analogues. Although it is active in vitro against most isolates of *Mycobacterium tuberculosis* at concentrations below 50 μg/mL, pyrazinamide is unusual because of its narrow spectrum of activity. *Mycobacterium bovis* and nontuberculous mycobacteria are usually resistant.[5] Other interesting features of this agent are its requirement for a low pH for activity[6,7] and its unique in vivo sterilizing activity.[8] The mode of action of pyrazinamide is not known. Although the mechanism of resistance has not

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0022-2623/92/1835-1212$03.00/0

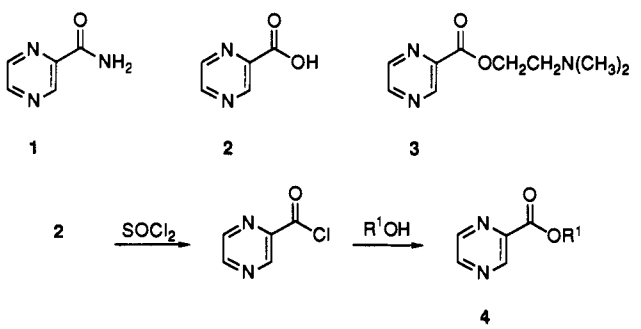
Table I. In Vitro Activity of Several Pyrazinoate Esters 4[a]

	4a	4c	4d	4h	3	1
			M. tuberculosis			
BUR	25	6.25	≤3.12	12.5	25	25
CES[b]	12.5	≤3.12	≤3.12	12.5	25	>200
DOL	25	≤3.12	≤3.12	6.25	12.5	25
SMA	25	3.12	≤3.12	25	25	50
DUB	25	6.25	≤3.12	25	25	50
GLA	12.5	6.25	6.25	6.25	25	12.5
ING	25	3.12	≤3.12	25	25	12.5
LEL[b]	50	3.12	6.25	12.5	25	>200
MCQ	25	≤3.12	≤3.12	6.25	25	25
WOO	25	6.25		25	25	50
BAK	25	3.12	≤3.12	12.5	25	50
DOU	100	3.12	≤3.12	12.5	25	25
ATCC 35828[b]	25	6.25	6.25	12.5	50	>200
			M. kansasii			
SWK[b]	200	6.25	6.25	12.5	100	>200
SCH[b]	25	6.25	12.5	12.5	50	>200

[a] MIC in μg/mL determined at pH 5.8. [b] Pyrazinamide resistant isolates.

been well characterized, it has been reported that *M. tuberculosis* isolates resistant to pyrazinamide have low levels of nicotinamidase activity,[9] an enzyme involved in the production of nicotinic acid from nicotinamide.

We postulated that pyrazinamide (1) was converted by an amidase into pyrazinoic acid (2) which was the active antimicrobial agent intracellularly. Pyrazinoic esters,



which could be hydrolyzed by a variety of enzymes, might also serve as prodrugs and would circumvent the requirement for activation by an amidase. Kushner[10] evaluated thiopyrazinoates and noted that isopropyl thiopyrazinoate was modestly active in a murine tuberculosis model. This activity was attributed to the release of ethyl mercaptan, not to the pyrazinoyl residue. Earlier it had been reported that ethylthio compounds had antituberculous activity,[11] thus supporting the concept that the activity of ethyl thiopyrazinoate was due to ethyl mercaptan and not the pyrazinoyl residue. In the course of evaluating esters of pyrazinoic acid and pyrazine-2,3-dicarboxylic acids as local anesthetics, Solomons and Spoerri[12] found several esters that had in vitro activity against *M. tuberculosis* H37Rv, including 2-(dimethylamino)ethyl pyrazinoate (3). To follow up on these early reports and to test our hypothesis, we synthesized a series of pyrazinoic acid esters and evaluated their in vitro antimycobacterial activity.

Results

Some of the pyrazinoic acid esters had better in vitro activity against isolates of *M. tuberculosis* than pyrazinamide. Pyrazinoate esters **4c** and **4d** were the most active of the esters tested (Table I). Several of the pyrazinoate esters were active against pyrazinamide-resistant isolates of *M. tuberculosis* (ATCC 35828, CES and LEL). In addition, the pyrazinoate esters had activity against *M. bovis* (ATCC 27289) and two isolates of *M. kansasii* (Table II). Disappointingly, these agents were not active against isolates of *M. avium* complex (data not shown).

The in vitro activity of the pyrazinoate esters is greater at pH 5.8 compared to pH 6.6 (Table II). The former pH was utilized rather than pH 5.6 or lower because *M. bovis* does not grow well at the lower pH. There was a less dramatic decrease in the activity of the pyrazinoate esters at pH 6.6 compared to pH 5.8 than is the case for pyrazinamide or pyrazinoic acid. It is noteworthy that the MICs for the pyrazinoate esters were lower for each organism and at each pH than the MICs for pyrazinamide or pyrazinoic acid. Pyrazinamide is more active than pyrazinoic acid in vitro, perhaps due to better entry of the amide compared to the charged acid moiety. Pyrazinoate esters **4c** and **4d** were active at ≤3.12 μg/mL against the pyrazinamide-susceptible isolates of *M. tuberculosis* and at ≤6.25 μg/mL for the pyrazinamide-resistant strains of *M. tuberculosis* and the isolates of *M. kansasii* and *M. bovis* that were evaluated.

Pyrazinoate esters **4a–c** were toxic in mice at 750 mg/kg for the former two compounds and 150 mg/kg for the latter (Table III). Pyrazinoate esters **4d, 4g–k** did not produce acute toxicity in single dose studies up to 900 mg/kg. Pyrazinoate esters **4d** and **4i** were well tolerated when given orally daily at 450 mg/kg for 10 days.

Discussion

Since 1985, there has been a resurgence of tuberculosis in the United States.[13] Several factors may be responsible for the increase in case rates, including infection with human immune deficiency virus, changing economic and social circumstances, and decline in tuberculosis control programs.[14,15] In addition, outbreaks of multi-drug resistant tuberculosis have been identified.[16] Currently available first-line antituberculous agents are highly effective and generally well tolerated. Second-line agents are less effective and more toxic, therefore new antimycobacterial agents are necessary to improve therapy for multi-drug resistant cases. Promising new agents may be developed by modification of existing antimycobacterial agents or by development of new classes of drugs.

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Table II. In Vitro Activity of Pyrazinoates 4 against Various Mycobacteria[a]

organism compd	*M. tuberculosis* H37Rv	*M. tuberculosis* BUR	*M. tuberculosis* ATCC 35828	*M. bovis* ATCC 27289	*M. kansasii* SWK
1	>200/12.5	200/25	>200/>200	>200/>200	>200/>200
2	200/50	>200/50	>200/100	>200/100	>200/>200
3	100/12.5	-/25	>200/25	>200/12.5	>200/200
4a	100/25	-/25	>200/25	>200/25	>200/100
4b	-	-/100	-/200	-/>200	-
4c	200/≤3.12	-/6.25	200/6.25	200/≤3.12	12.5/≤3.12
4d	25/≤3.12	-/≤3.12	25/6.25	25/6.25	12.5/≤3.12
4e	-	>200/-	>200/-	>200/-	>200/-
4f	-	-/6.25	-/25	-/6.25	-/25
4g	50/12.5	-/12.5	200/25	100/3.12	200/25
4h	25/12.5	100/12.5	200/12.5	50/6.25	200/12.5
4i	-	50/25	25/25	200/25	-/25
4j	100/12.5	-	100/12.5	100/12.5	>200/25
4k	-/6.25	50/-	100/25	100/25	100/25

[a] MIC in μg/mL determined at pH 6.6/pH 5.8.

Table III. Single Dose Toxicity in Mice of Pyrazinoate Esters 4

ester	outcome
4a	died at 750 mg/kg
4b	died at 750 mg/kg
4c	died at 150 mg/kg
4d	tolerated 900 mg/kg
4g	tolerated 900 mg/kg
4h	tolerated 900 mg/kg
4i	tolerated 900 mg/kg
4j	tolerated 900 mg/kg
4k	tolerated 900 mg/kg
3	tolerated 900 mg/kg

Pyrazinamide is a first-line agent for the treatment of tuberculosis[17] and a key part of "short course" preventive therapy regimens.[18] Pyrazinamide and pyrazinoate esters appear to be prodrugs with pyrazinoic acid being the more immediately active compound.[19] Several of the pyrazinoate esters have been found to have substantially better in vitro activity than pyrazinamide against susceptible isolates of *M. tuberculosis* as well as activity against pyrazinamide-resistant isolates of *M. tuberculosis*. In addition, these esters have in vitro activity against *M. bovis* and *M. kansasii* (organisms resistant to pyrazinamide). The esters are not active against the *M. avium* complex.

In vitro evaluation of these esters against a larger group of isolates is necessary to confirm our results. Pyrazinoate esters should be evaluated in a murine model of tuberculosis to determine whether their promising in vitro activity will be paralleled by comparable in vivo activity.

Experimental Section

General. ^{1}H NMR spectra were recorded at either 60 or 300 MHz. ^{13}C NMR spectra were recorded at either 22.63 or 75.429 MHz. ^{19}F NMR spectra were determined in $CDCl_3$ solution at 282.203 MHz. Chemical shifts are reported in parts per million (ppm) upfield from external CCl_3F. Analytical TLC was routinely used to monitor reactions on plates precoated with silica gel 60 F_{254} of 0.20-mm thickness. The chromatograms were visualized under UV light or by staining with iodine. Melting points and boiling points are 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.

General Method for the Preparation of Pyrazinoates. Pyrazinecarboxylic acid (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 off 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%).[1] The purified pyrazinoyl chloride (ca 3.2 g, 0.022 mol) was transferred to a flask containing 40 mL of dichloromethane and 2 mL of pyridine. The solution was cooled to 0 °C and the desired alcohol (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.

2,2,2-Trifluoroethyl Pyrazinoate (4a). Crude product was purified by recrystallization from hexanes to yield 3.6 g (79%) of **4a**: mp 46–48 °C; ^{19}F NMR ($CDCl_3$) δ −73.93 (t, $J_{H,F}$ = 8.1 Hz, 3 F); ^{1}H NMR ($CDCl_3$) δ 9.36 (d, J = 2.50 Hz, 1 H), 8.84 (d, J = 2.50 Hz, 1 H), 8.80 (t, J = 2.50 Hz, 1 H), 4.85 (q, $J_{H,F}$ = 8.35 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 162.61 (s), 148.592 (s), 146.72 (s), 144.96 (s), 142.52 (s), 122.94 (q, $J_{C,F}$ = 277.3 Hz), 61.56 (q, $J_{C,F}$ = 37.3 Hz). Anal. ($C_7H_5F_3N_2O_2$) C, H.

Bis(trifluoromethyl)methyl Pyrazinoate (4b). Crude product was purified by recrystallization from hexanes to yield 1.8 g (30%) of **4b**: mp 70–71 °C; ^{19}F NMR ($CDCl_3$) δ −73.27 (d, $J_{H,F}$ = 5.8 Hz, 6 F); ^{1}H NMR ($CDCl_3$) δ 9.35 (d, J = 1.46 Hz, 1 H), 8.85 (d, J = 2.50 Hz, 1 H), 8.80 (t, J = 2.50, 1.5 Hz, 1 H), 6.07 (septet, $J_{H,F}$ = 5.8 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 161.0 (s), 148.9 (s), 146.9 (s), 145.1 (s), 140.9 (s), 120.3 (q, $J_{C,F}$ = 279 Hz), 67.6 (septet, $J_{C,F}$ = 35 Hz). Anal. ($C_8H_4F_6N_2O_2$) C, H, N.

Allyl Pyrazinoate (4c). To 4.0 g (0.028 mol) of freshly purified pyrazinoyl chloride dissolved in 20 mL of dichloromethane and 2 mL of pyridine was added 2.6 g (0.045 mol) of allyl alcohol. After reaction and isolation in the usual manner, the crude product was purified by distillation to yield 2.66 g (58%) of **4c** as a colorless oil: bp 72–74 °C (0.25 mm); ^{1}H NMR ($CDCl_3$) δ 9.366 (br s, 1 H), 8.808 (br s, 1 H), 8.769 (br s, 1 H), 6.08 (ddt, J = 17.3 Hz, J = 10.0 Hz, 1 H), 5.476 (ddt, J = 1.68 Hz, J = 3.1 Hz, 1 H), 5.357 (ddt, J = 1.12 Hz, 1 H), 4.959 (ddd, 2H); ^{13}C NMR ($CDCl_3$) δ 163.487 (s), 147.670 (s), 146.208 (s), 144.399 (s), 143.291 (s), 131.249 (s), 119.539 (s), 66.687 (s). Anal. ($C_8H_8N_2O_2$) C, H.

***n*-Propyl Pyrazinoate (4d).** To 6.4 g (0.045 mol) of freshly purified pyrazinoyl chloride dissolved in 30 mL of dichloromethane and 4.1 mL of pyridine was added 2.95 g (0.0492 mol) of *n*-propyl alcohol. After reaction and isolation in the usual manner, the crude product was purified by distillation to yield 5.96 g (46%) of **4d** as a colorless oil: bp 46–48 °C (0.4 mm); ^{1}H NMR ($CDCl_3$) δ 9.321 (br s, 1 H), 8.783 (d, J = 1.68 Hz, 1 H), 8.752 (br s, 1 H), 4.425 (t, J = 6.83 Hz, 2 H), 1.869 (sextet, J = 7.25 Hz, 2 H), 1.049 (t, J = 7.15 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 163.979 (s), 147.592 (s), 146.274 (s), 144.470 (s), 143.675 (s), 67.853 (s), 22.023 (s), 10.375 (s). Anal. ($C_8H_{10}N_2O_2$) C, H.

2-Bromoethyl Pyrazinoate (4e). Crude product was purified by distillation to yield 3.15 g (62%) of **4e**: bp 98–100 °C (0.05

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mm); ^{1}H NMR ($CDCl_3$) δ 9.16 (d, J = 1.4 Hz, 1 H), 8.64 (d, J = 2.40 Hz, 1 H), 8.60 (t, J = 1.4, 2.50 Hz, 1 H), 4.58 (t, J = 6.0 Hz, 2 H), 3.54 (t, J = 6.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 163.0 (s), 147.6 (s), 146.0 (s), 144.2 (s), 142.5 (s), 64.8 (s), 27.9 (s). Anal. ($C_7H_7BrN_2O_2$) C, H, N.

2-Chloroethyl Pyrazinoate (4f). Crude product was purified by distillation to yield 2.33 g (57%) of **4f**: bp 83–85 °C (0.05 mm); ^{1}H NMR ($CDCl_3$) δ 9.17 (d, J = 1.4 Hz, 1 H), 8.65 (d, J = 2.50 Hz, 1 H), 8.61 (t, J = 1.4, 2.50 Hz, 1 H), 4.55 (t, J = 6.0 Hz, 2 H), 3.73 (t, J = 6.0 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 163.2 (s), 147.7 (s), 146.1 (s), 144.3 (s), 142.6 (s), 65.1 (s), 40.9 (s). Anal. ($C_7H_7ClN_2O_2$) C, H, N.

4-Nitrophenyl Pyrazinoate (4g). Crude product was purified by recrystallization from hexanes to yield 2.05 g (38%) of **4g**: mp 185–187 °C; ^{1}H NMR ($CDCl_3$) δ 9.47 (s, 1 H), 8.884 (d, J = 1.5 Hz, 1 H), 8.829 (d, J = 3 Hz, 1 H), 7.477 (d, J = 8.1 Hz, 2 H), 8.353 (d, J = 8.1 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 161.7 (s), 155.0 (s), 148.6 (s), 147.0 (s), 145.9 (s), 144.8 (s), 142.2 (s), 125.4 (s), 122.5 (s). Anal. ($C_{11}H_7N_3O_4$) C, H, N.

4-Tolyl Pyrazinoate (4h). To 3.5 g (0.025 mol) of freshly purified pyrazinoyl chloride dissolved in 20 mL of dichloromethane and 2 mL of pyridine was added 3.2 g (0.030 mol) of 4-tolyl alcohol. After reaction and isolation in the usual manner, the crude product was purified by recrystallization from hexanes to yield 1.51 g (24%) of **4h**: mp 120–123 °C; ^{1}H NMR ($CDCl_3$) δ 9.429 (m, 1 H), 8.794 (m, 1 H), 8.772 (d, J = 1.52 Hz, 1 H), 7.214 (dd J = 8.36 Hz, J = 2.01 Hz, 2 H), 7.123 (dd, J = 8.52 Hz, J = 2.28 Hz, 2 H), 2.35 (d, J = 2.44 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 162.601 (s), 148.120 (s), 147.965 (s), 146.606 (s), 144.483 (s), 142.965 (s), 136.041 (s), 130.012 (s), 120.957 (s), 20.774 (s). Anal. ($C_{12}H_{10}N_2O_2$) C, H.

4-*tert*-Butylphenyl Pyrazinoate (4i). To 1.62 g (0.011 mol) of freshly purified pyrazinoyl chloride dissolved in 20 mL of dichloromethane and 2 mL of pyridine was added 2.25 g (0.015 mol) of 4-*tert*-butylphyl alcohol. After reaction and isolation in the usual manner, the crude product was purified by recrystallization from hexanes to yield 2.1 g (72%) of **4i**: mp 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 9.47 (d, J = 2.55 Hz, 1 H), 8.82 (m, 1 H), 8.81 (m, 1 H), 7.46 (d, J = 8.79 Hz, 2 H), 7.20 (d, J = 8.79 Hz, 2 H), 1.35 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 162.52 (s), 149.19 (s), 148.00 (s), 147.94 (s), 146.61 (s), 144.50 (s), 142.99 (s), 126.38 (s), 120.59 (s), 34.42 (s), 31.28 (s). Anal. ($C_{15}H_{16}N_2O_2$) C, H.

Pentafluorophenyl Pyrazinoate (4j). Crude product was purified by recrystallization from hexanes to yield 2.04 g (32%) of **4j**: mp 62–64 °C; ^{19}F NMR ($CDCl_3$) δ −161.758 (t, 2 F), −156.805 (t, 1 F), −152.230 (d, 2 F); ^{1}H NMR ($CDCl_3$) δ 9.44 (d, J = 1.5 Hz, 1 H), 8.88 (d, J = 2.5 Hz, 1 H), 8.82 (dd, 1 H); ^{13}C NMR ($CDCl_3$) δ 160.11 (s), 149.0 (s), 147.2 (s), 144.9 (s), 141.0 (s), 145–135 (br m). Anal. ($C_{11}H_3F_5N_2O_2$) C, H, N.

4-Biphenylyl Pyrazinoate (4k). To 3.5 g (0.025 mol) of freshly purified pyrazinoyl chloride dissolved in 20 mL of dichloromethane and 2 mL of pyridine was added 5.0 g (0.030 mol) of 4-Biphenyl alcohol. After reaction and isolation in the usual manner, the crude product was purified by recrystallization from hexanes to yield 3.15 g (39%) of **4k**: mp 95–98 °C; ^{1}H NMR ($CDCl_3$) δ 9.94 (br s, 1 H), 8.848 (br s, 1 H), 8.827 (br s, 1 H), 7.62 (d, J = 7.32 Hz, 2 H), 7.592 (d, J = 7.60 Hz, 2 H), 7.451 (t, J = 7.60 Hz, 2 H), 7.366 (d, J = 6.56 Hz, 1 H), 7.354 (d, J = 7.54 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 162.89 (s), 150.136 (s), 148.453 (s), 147.105 (s), 144.941 (s), 143.259 (s), 140.441 (s), 139.920 (s), 129.134 (s), 128.634 (s), 127.798 (s), 127.416 (s), 122.002 (s). Anal. ($C_{17}H_{12}N_2O_2$) C, H.

Bioassay Procedures. Clinical isolates of *M. tuberculosis* and *M. kansasii* were obtained from patients at the Veterans Affairs Medical Center and the State University of New York Health Science Center at Syracuse, New York. *Mycobacterium bovis* (BCG) ATCC 27289, *M. tuberculosis* (H37Rv) ATCC 25618, and *M. tuberculosis* ATCC 35828 (pyrazinamide-resistant) were obtained from the American Type Culture Collection.

A stock solution of each compound was prepared by dissolving in dimethyl sulfoxide (DMSO) or absolute ethanol with subsequent dilution in distilled water. The stock solutions were sterilized by passage through a 0.22-mm membrane filter. Compartmented plates were prepared with serial 2-fold dilutions (200–3.12 μg/mL) of the various compounds in Middlebrook 7H10 agar with 10% OADC enrichment at pH 5.8[20] or pH 6.6. The plates were prepared 24–48 h prior to each experiment and stored at 4 °C.

Mycobacteria were grown in Middlebrook 7H10 broth with OADC enrichment and 0.05% Tween 80 at pH 6.6. The cell suspensions were standardized using a Klett-Summerson colorimeter with 7H10 broth as the diluent. Ten microliters of each cell suspension (1k and 0.01k) yielding approximately 5×10^3 and 5×10^2 CFU, respectively, were spotted on each compartment. A control compartment without any drug was included for each isolate. All plates were incubated at 37 °C for 4 weeks. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug which produced a 99% (2 log) inhibition of growth.

The relative toxicity of these materials was determined when the compounds were dissolved in water and given by gavage in escalating doses from 150 to 900 mg/kg (using 150 mg/kg increments). The compounds were given as single doses in 0.2 mL to two mice at each dose. Pyrazinoate ester **4d** and pyrazinoate ester **4i** were subsequently tested at 450 mg/kg daily for 10 days.

Acknowledgment. Financial support of this work by the National Science Foundation, Grant number CHE-8901986 (J.T.W.), and the Department of Veterans Affairs Merit Review Grant (M.H.C.) is gratefully acknowledged.

Registry No. 1, 98-96-4; **2**, 98-97-5; **3**, 139244-45-4; **4a**, 139244-46-5; **4b**, 139244-47-6; **4c**, 132172-97-5; **4d**, 73763-87-8; **4e**, 139244-48-7; **4f**, 139244-49-8; **4g**, 20088-23-7; **4h**, 132172-96-4; **4i**, 132172-94-2; **4j**, 139244-50-1; **4k**, 132172-95-3; F_3CCH_2OH, 75-89-8; $(F_3C)_2CHOH$, 920-66-1; $H_2C{=}CHCH_2OH$, 107-18-6; PrOH, 71-23-8; $Br(CH_2)_2OH$, 540-51-2; $Cl(CH_2)_2OH$, 107-07-3; $4\text{-}NO_2C_6H_4OH$, 100-02-7; $4\text{-}MeC_6H_4OH$, 106-44-5; $4\text{-}Me_3CC_6H_4OH$, 98-54-4; C_6F_5OH, 771-61-9; $4\text{-}PhC_6H_4OH$, 92-69-3; pyrazinoyl chloride, 19847-10-0.

(20) Vestal, A. L. Procedures for the Isolation and Identification of Mycobacterium. Public Health Service publication No. 1995; Laboratory Division, National Communicable Disease Center: Atlanta, GA, 1969.