Antimycobacterial Activity of Substituted Isosteres of Pyridine- and Pyrazinecarboxylic Acids

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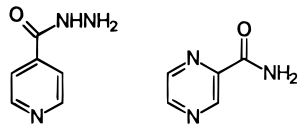
Pyrazines and pyridines substituted with alkylated tetrazoles, esterified vinylogous carboxylic acids, and ketosulfides were synthesized as precursors of antimycobacterial agents which, after penetration of the mycobacterial cell wall, could be biotransformed by esterases or peroxidase-catalases. The expected products are tetrazoles, a vinylogous carboxylic acid, and CH-acidic ketosulfoxides, isosteres of pyrazinoic and nicotinic acids, which should inhibit mycobacterial growth when released inside the bacterial cell. The growth inhibitory activity of the synthesized compounds against the H_{37}Rv strain of *Mycobacterium tuberculosis* was determined to assess the viability of this concept. It was shown that all of the compounds designed as lipophilic precursors were more active than the unmodified polar isosteres of pyrazinoic and nicotinic acids.

Introduction

In 1985 the incidence of tuberculosis in the United States increased for the first time since national recording began in 1953.[1] This fact and frequently observed resistance to drugs used in the treatment of tuberculosis make the development of new antitubercular drugs an important task. In analogy to the antitubercular drugs isoniazid (INH) and pyrazinamide (PZA), we assumed that precursors of isosteres of isonicotinic and pyrazinoic

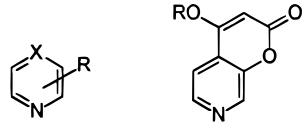


Isoniazid (INH) Pyrazinamide (PZA)

acids could possess antimycobacterial activity. Esters of pyrazinoic acid have been shown to be more active than the parent acid.[2−5,6] These esters are presumably activated by an esterase which we expected to also activate esters of isosteres of pyrazinoic and isonicotinic acids in a comparable way. We chose tetrazole analogues of these acids and 4-hydroxy-7-azacoumarin and attached groups which could be cleaved by esterases within the mycobacterium. These compounds could also overcome the observed drug resistance of certain strains of *Mycobacterium tuberculosis* against PZA and INH which has been attributed to a deficiency of nicotinamidase or catalase-peroxidase necessary for their activation.[7,8] Indeed, esters of pyrazinoic acid have been shown to possess activity against pyrazinamide-resistant isolates.[2−5] In addition, we chose to synthesize sulfides which could possibly be transformed to sulfoxides by the catalase-peroxidase enzyme system of *M. tuberculosis*, a reaction that has been observed with horseradish peroxidase.[9] The methylene group of the resulting β-ketosulfoxide would be acidic enough to imitate an acid function. These sulfides could, however, not overcome drug resistance which is due to the lack of a catalase-peroxidase. We here describe the syntheses of these compounds and their activity against the H_{37}Rv strain of *M. tuberculosis*.

Chemistry

The pivaloyloxymethyl (POM) esters of the known tetrazoles **1**−**3**[10,11] (Table 1) were prepared as mixtures of N(1)- and N(2)-alkylated isomers which were separated by column chromatography on silica gel. Comparison of compounds **4**, **6**, and **8** with their respective isomers **5**, **7**, and **9** showed that the former were the major products, their N−CH_2 protons showed resonances at lower field, and their melting points were lower. These characteristics are known to indicate substitution at N(2).[12] The reduction of the known sulfoxides **10** and **11**[13] with a large excess of sodium metabisulfite yielded the sulfides **13** and **14**, while the sulfoxide **12**[14] could not be reduced to its corresponding sulfide **15** with this method.[15] Instead, **15** was prepared from bromoacetylpyrazine and sodium methyl sulfide.[16] The 4-hydroxy group of the known 4-hydroxy-7-azacoumarin[17] was acetylated with acetyl chloride, benzoyl chloride, and ethyl chloroformate to give compounds **17**−**19**.



1 - 15 **16 - 19**

Results and Discussion

The MIC values in BACTEC 6A media[18] (pH = 6) of the tetrazoles **4**−**9** ranged from 13 to 105 μg/mL, the MICs of the sulfides **13**, **14**, and **15** were 105, 105, and 210 μg/mL, and the MIC of the benzoyl-substituted

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Table 1. Substituted Pyridines and Pyrazines **1**−**15** and Azacoumarins **16**−**19**

compd	X	R	mp (°C)	formula[a]
1[c,10]	CH	3-(5-tetrazolyl)	251−253	$C_6H_5N_5$
2[c,10]	CH	4-(5-tetrazolyl)	259−261	C_6[b]H_5N_5
3[c,11]	N	2-(5-tetrazolyl)	203−205	$C_5H_4N_6$
4	CH	3-[2-POM-(5-tetrazolyl)]	81−82	$C_{12}H_{15}N_5O_2$
5	CH	3-[1-POM-(5-tetrazolyl)]	100−101	$C_{12}H_{15}N_5O_2$
6	CH	4-[2-POM-(5-tetrazolyl)]	80−81	$C_{12}H_{15}N_5O_2$
7	CH	4-[1-POM-(5-tetrazolyl)]	90−91	$C_{12}H_{15}N_5O_2$
8	N	2-[2-POM-(5-tetrazolyl)]	86−87	$C_{11}H_{14}N_6O_2$
9	N	2-[1-POM-(5-tetrazolyl)]	109−110	$C_{11}H_{14}N_6O_2$
10[c,13]	CH	3-$COCH_2SOCH_3$	70−82	$C_8H_9NO_2S$
11[c,13]	CH	4-$COCH_2SOCH_3$	84−87	$C_8H_9NO_2S$
12[c,14]	N	2-$COCH_2SOCH_3$	119−120	$C_7H_8N_2O_2S$
13	CH	3-$COCH_2SCH_3$	liquid	$C_8H_9NOS\cdot HCl$
14	CH	4-$COCH_2SCH_3$	liquid	$C_8H_9NOS\cdot HCl$
15	N	2-$COCH_2SCH_3$	59−61	$C_7H_8N_2OS$
16[c,17]		H	235−237	$C_8H_5NO_3\cdot H_2O$
17		$COCH_3$	153−154	$C_{10}H_7NO_4$
18		COPh	124−126	$C_{15}H_9NO_4$
19		CO_2Et	73−76	$C_{11}H_9NO_5$

[a] Analyses were within 0.4% of the calculated values except where noted (footnote b). [b] C: calcd, 49.0; found, 48.4. [c] Reference for known compound.

Table 2. MIC Values of Compounds **1**−**19**

compd	MIC,[a] (μg/mL)/(μM)	cmpd	MIC,[a] (μg/mL)/(μM)
pyrazinamide[b]	52/400	isoniazid[b]	<0.02/0.15
1	>210/1400	**10**	>210/1100
2	>210/1400	**11**	>210/1100
3	>210/1400	**12**	>210/1100
4	13/50	**13**	210/1000
5	105/400	**14**	210/1000
6	26/100	**15**	105/500
7	52/200	**16**	>210/1200
8	26/100	**17**	>210/1000
9	13/50	**18**	210/800
		19	>210/900

[a] Determined in BACTEC 6A; see the Experimental Section for description of MIC determination. [b] For comparison.

coumarin **19** was 210 μg/mL (Table 2). The MIC values of all other compounds were greater than 210 μg/mL, limited solubility prohibiting an accurate determination. All values were determined using 2-fold dilutions and vary within one dilution. For compounds whose MICs were above 210 μg/mL, the percentage of inhibition of *M. tuberculosis* at 1600 μM—4 times the MIC of pyrazinamide—was determined to allow for comparison of their activities. The unsubstituted tetrazoles **1**−**3**, as already reported by others,[11,19] exhibited only minimal activity (33−50% inhibition of growth indices relative to drug-free controls). The sulfoxides **10**−**12** were devoid of any activity. In the azacoumarin series the acetate **17** and the ethyl carbonate **18** (82% and 83%) showed activity only slightly above that of the weakly active unsubstituted 4-hydroxy-7-azacoumarin (**16**) (39%). Pyrazinoic acid showed 71% inhibition under these conditions.

As expected, the tetrazoles **1**−**3**, the sulfoxides **10**−**12**, and the 4-hydroxy-7-azacoumarin show only insignificant or no inhibition of *M. tuberculosis* at all, which, we believe, is due to their structural relationship to the polar heteroaromatic acids isonicotinic and pyrazinoic acids which impedes penetration of the mycobacterial cell wall. The results also show that modification of these compounds to more lipophilic precursors can indeed increase their activity. However, only the transformation of **1**−**3** to the N-substituted tetrazoles **4**−**9**, which makes ionization impossible and may facilitate penetration of the cell wall, leads to compounds of interesting activity. The tetrazoles **4**−**9** show up to 4 times higher in vitro activity than PZA which is currently used in the therapy of tuberculosis. Different rates of hydrolysis may contribute to the observed differences in activity of the alkyated tetrazoles **4**−**9**. These results show that the modification to less polar precursors can increase the activity of isosteres of pyrazinoic and pyridinecarboxylic acids. Although we have not tested the described compounds against pyrazinamide-resistant strains of *M. tuberculosis*, it is reasonable to expect that they should be effective against certain strains whose resistance is due to the lack of pyrazinamidase.

Experimental Section

Chemistry. Melting points were determined on an Electrothermal capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini spectrometer at 200 MHz in deuterated solvents with TMS as internal standard. APCIMS were measured with a Finnegan TSQ 7000 and EIMS with a Hewlett-Packard 5988A at 200 °C, at 75 eV. Elemental analyses were performed at Desert Analytics, Tucson, AZ, and are within 0.4% of the calculated values except where stated otherwise (Table 1). For column chromatography silica gel 60, 63−200 μm, Macherey & Nagel, Düren, Germany, was used.

Caution: The preparation of tetrazoles **1**−**3** according to the cited literature involves the formation of potentially explosive hydrazoic acid!

General Procedure for the Preparation of the (Pivaloyloxymethyl)tetrazoles 4−**9.** A 10.0-mmol portion of the respective tetrazole **1**, **2**,[10] or **3**[11] and 10.0 mmol of chloromethyl pivalate were heated in 50 mL of dry DMF at 95 °C for 3 h. The mixture was poured on water and extracted three times with EtOAc. The combined extracts were washed with water, dried with anhydrous Na_2SO_4, and evaporated in vacuo. The residue was crystallized from petroleum ether at −20 °C. The mother liquors were concentrated to give a second crop. The combined materials were chromatographed on silica gel with EtOAc or EtOAc−petroleum ether mixtures to obtain the pure isomers which were crystallized from petroleum ether (PE) or petroleum ether/ethyl ether mixtures (PE/DEE). **4**: yield 73% (PE); ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 9H), 6.52 (s, 2H), 7.43−7.50 (m, 1H), 8.46 (dt, 1H, J = 2.0/7.8 Hz), 8.75 (dd, 1H, J = 1.5/4.8 Hz), 9.42 (d, 1H, J = 2.1 Hz). **5**: yield 7% (PE/DEE); ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 6.35 (s, 2H), 7.57 (dd, 1H, J = 4.8/8.0 Hz), 8.20 (dd, 1H, J = 2.0/8.0 Hz), 8.88 (dd, 1H, J = 1.6/4.8 Hz), 9.06 (d, 1H, J = 2.2 Hz). **6**: yield 71% (PE); ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 9H), 6.56 (s, 2H), 8.06 and 8.81 (AA′BB′, 4H, J = 6.0 Hz). **7**: yield 7% (PE/DEE); ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H), 6.38 (s, 2H), 7.75 and 8.90 (AA′BB′, 4H, J = 5.8 Hz). **8**: yield 41% (PE/DEE); ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 9H), 6.82 (s, 2H), 8.71 (dd, 1H, J = 1.5/2.5 Hz), 8.82 (m, 1H), 9.63 (d, 1H, J = 1.5 Hz). **9**: yield 29% (PE/DEE); ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 9H), 6.60 (s, 2H), 8.74−8.80 (m, 2H), 9.51 (d, 1H, J = 1.5 Hz).

General Procedure for the Preparation of the Sulfides 13 and 14. A 6.0-g (32.8 mmol) portion of the respective sulfoxide **10** or **11**[13] was dissolved in 150 mL of water and heated to 70 °C; 47 g of $Na_2S_2O_5$ (250 mmol) was added with vigorous stirring. After 30 min the reaction mixture was cooled to ambient temperature, diluted with 300 mL of water, and extracted three times with 100-mL portions of CH_2Cl_2. The combined extracts were dried with $MgSO_4$. The solvent was evaporated and the liquid residue purified by distillation at reduced pressure. Hydrochlorides were prepared by addition of HCl to a solution of the free bases in dry diethyl ether. **13**: yield 37%; ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H), 3.77 (s, 2H), 7,45 (t, 1H, J = 5.8 Hz), 8.29 (d, 1H, J = 5.9 Hz), 8.80 (d, 1H,

J = 5.6 Hz), 9.21 (s, 1H). **14**: yield 44%; ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H), 3.69 (s, 2H), 7.75 and 8.82 (AA′BB′, 4H, J = 5.7 Hz).

2-(Methylthioacetyl)pyrazine, 15. α-Bromoacetylpyrazine was prepared from acetylpyrazine by bromination in glacial acetic acid at 50 °C. The crude product was chromatographed on silica gel with a CH_2Cl_2/EtOAc mixture; 1.18 g (5.9 mmol) of this material was dissolved in 4 mL of MeOH, and a solution of 413 mg (5.9 mmol) of sodium methylthiolate in 4 mL of MeOH was added at room temperature. After 1 h 10 mL of water was added, and the product was extracted with CH_2Cl_2. The crude product was chromatographed on silica gel with a CH_2Cl_2/MeOH mixture. Recrystallization from hexanes gave the product as pale-yellow crystals: yield 25%; ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3H), 3.90 (s, 2H), 8.65 (s, 1H), 8.75 (s, 1H), 9.32 (s, 1H).

General Procedure for the Preparation of the 4-Substituted 7-Azacoumarins 17−19. 4-Hydroxy-7-azacoumarin was obtained according to Dejardin and Lapiere;[18] 3.3 mmol of triethylamine was added to a suspension of 3.0 mmol of **16** in 15 mL of CH_2Cl_2. After the starting material was dissolved completely, 10 mL of petroleum ether was added, and the mixture was cooled on an ice bath; 3.6 mmol of acetyl chloride or benzoyl chloride in 3 mL of cold petroleum ether was added slowly. The resulting slurry was stirred for 30 min, then the ice bath was removed, and stirring was continued for another 30 min. The solvents were removed in vacuo, and the residue was stirred with 50 mL of ice water. The crude product was obtained by filtering. It was dried over $CaCl_2$ and recrystallized from EtOAc or EtOAc/petroleum ether. For the synthesis of **19**: The reaction with ethyl chloroformate was carried out at − 20 °C; 100 mL of cold diethyl ether was added to the mixture which was then slowly brought to room temperature. After filtration the residue was treated with water and extracted with diethyl ether. The combined ethereal solutions were dried, concentrated to 40 mL, and left in the freezer for 15 h. The solution was filtered; evaporation of the filtrate gave the slightly yellow product. **17**: yield 74% (EtOAc); ^{1}H NMR ($CDCl_3$) δ 2.48 (s, 3H), 6.80 (s, 1H), 7.52 (d, 1H, J = 5.1 Hz), 8.57 (d, 1H, J = 5.1 Hz), 8.78 (s, 1H). **18**: yield 86% (EtOAc/PE); ^{1}H NMR ($CDCl_3$) δ 6.90 (s, 1H), 7.57−7.65 (m, 4H), 7.73−7.77 (m, 1H), 8.20−8.26 (m, 1H), 8.59 (d, 1H, J = 5.2 Hz), 8.83 (s, 1H). **19**: yield 38%; ^{1}H NMR ($CDCl_3$) δ 1.46 (t, 3H, J = 7.2 Hz), 4.44 (q, 2H, J = 7.2 Hz), 6.94 (s, 1H), 7.63 (d, 1H, J = 5.2 Hz), 8.58 (d, 1H, J = 5.2 Hz), 8.78 (s, 1H).

Determination of Biological Activity. The compounds were tested for inhibition of *M. tuberculosis* $H_{37}Rv$ ATCC 27294 using the BACTEC 460 system as previously described.[18] Percent inhibition was calculated as 1 − (growth index of test sample/growth index of control) × 100. The minimum inhibitory concentration is defined as the lowest concentration which inhibited 99% of the inoculum.

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