Antimycobacterial Activity of Substituted Isosteres of Pyridine- and Pyrazinecarboxylic Acids. 2.[1]

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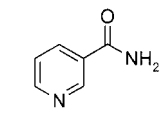
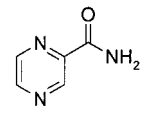
Pyridines and pyrazines substituted with 1,2,4-oxadiazole-5-ones, 1,2,4-oxadiazole-5-thiones, and 1,3,4-oxathiazoline-2-ones were synthesized and tested against *Mycobacterium tuberculosis*. The two former ring systems were documented in the literature to act as carboxylic acid isosteres. The latter series was synthesized as possible synthetic intermediates to 1,2,4-thiadiazole-3-ones and was included in this study due to their interesting activity. Pivaloyloxymethyl derivatives of the isosteres were also prepared in order to increase their lipophilicity and therefore improve their cellular permeability. The derivatized isosteres were expected to be biotransformed by esterases to the active species after penetration of the mycobacterial cell wall. Biological properties of the compounds were compared with the unmodified polar isosteres of pyrazinoic and nicotinic acids. The majority of the compounds exhibited activities ranging from 0.5 to 16 times the potency of pyrazinamide.

Introduction

In[1] the 1940s, Chorine[2] and McKenzie[3] independently demonstrated that nicotinamide was effective for the treatment of murine tuberculosis. The synthesis of many nicotinamide analogues including the pyrazine isostere pyrazinamide followed this discovery. It was soon shown that an enzyme called nicotinamidase inside the mycobacterium hydrolyzed nicotinamide and pyrazinamide to the corresponding carboxylic acids, and these carboxylic acids were the actual active compounds.[4] However, nicotinic and pyrazinoic acids did not demonstrate activity due to their poor penetration into mycobacterial cells.

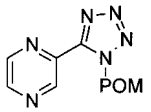


nicotinamide



pyrazinamide

One of the effective methods that can lead to new drug discoveries is the bioisosteric replacement of a functional group. Numerous functional groups have been reported as bioisosteric replacements for the carboxylic acid functionality.[5] Among those, the most commonly encountered one is probably the tetrazole ring. In the area of nonpeptidic angiotensin II (AII) receptor antagonists, especially successful results were obtained when the carboxylic acid group was replaced by a tetrazole ring.[6] These findings led our group to synthesize tetrazole isosteres of pyridine- and pyrazinecarboxylic acids.[1] The most active compounds in this series included **1** and its nicotinyl analogue, 3-(2-pivaloyloxymethy-5-tetrazolyl)-pyridine, and they had the MIC value of 50 μM (Table 2).



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Several other acidic heterocycles or functional groups have been used to replace carboxyl groups or the tetrazole ring itself.[7−11]

Now we are reporting the synthesis and antitubercular activity of 1,2,4-oxadiazole-5-one and 1,2,4-oxadiazole-5-thione isosteres of pyridine- and pyrazinecarboxylic acids. In addition to those, three 1,3,4-oxathiazoline-2-ones were synthesized (as potential synthetic intermediates in an unsuccessful attempt to prepare 1,2,4-thiadiazole-5-ones) and tested for activity. All of the synthesized compounds, except for the nonacidic oxathiazolinones, demonstrated very polar characteristics and poor solubility in organic solvents. It was reported that tetrazole derivatives that contain additional basic groups elsewhere in the structure existed in zwitterionic form and therefore exhibited poor absorption properties.[12] Although the heterocycles synthesized by us have not been studied in this way, it can be assumed that they also act as zwitterions due to their similar pK_a values to tetrazoles.[13] To overcome this problem, a general prodrug approach was adopted to improve the biomembrane transport. Thus, the heterocycles were alkylated in an attempt to bioreversibly modify them by an agent with a metabolic weak point for easy breakdown into the parent compound by enzymatic means under physiological conditions. Chloromethyl pivalate was chosen as the alkylating agent to serve this purpose. The resulting pivaloyloxymethyl (POM) derivatives are expected to be hydrolyzed by esterases to the hydroxymethyl derivatives, which spontaneously decompose to the parent compounds releasing a formaldehyde molecule.[12] The in vitro antitubercular activity of the resulting compounds are

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Table 1. Oxadiazolones **2**−**7**, Oxadiazolethiones **8**−**13**, and Oxathiazolinones **14**−**16**

compd	X	R	mp (°C)	formula[a]
2	C	H	253−5	$C_7H_5N_3O_2$
3	CH	H	233−5	$C_7H_5N_3O_2$
4[b]	N	H	229−31	$C_6H_4N_4O_2$
5	C	POM	64−6	$C_{13}H_{15}N_3O_4$
6	CH	POM	88−90	$C_{13}H_{15}N_3O_4$
7	N	POM	99−101	$C_{12}H_{14}N_4O_4$
8	C		228−9	$C_7H_5N_3OS$
9	CH		223−5	$C_7H_5N_3OS$
10	N		220−2	$C_6H_4N_4OS$
11	C	POM	73−6	$C_{13}H_{15}N_3O_3S$
12	CH	POM	45−8	$C_{13}H_{15}N_3O_3S$
13	N	POM	61−3	$C_{12}H_{14}N_4O_3S$
14	C		191−3	$C_7H_4N_2O_2S$
15	CH		115−7	$C_7H_4N_2O_2S$
16	N		130−2	$C_6H_3N_3O_2S$

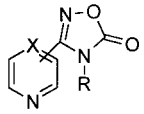
[a] Analyses were within 0.4% of the calculated values except where noted (footnote b). [b] N: calcd, 34.14; found, 33.57.

discussed and compared with the unmodified polar isosteres of nicotinic and pyrazinoic acids.

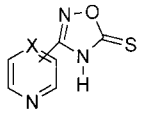
Chemistry

Pyridine-3-[14] and 4-carboxamidoximes[15] that served as starting materials were synthesized according to a published procedure with some improvement.[16] Pyrazine carboxamidoxime was synthesized using a similar procedure.[17]

1,2,4-Oxadiazole-5(4*H*)-ones **2**−**4** (Table 1) were synthesized by reacting the corresponding amidoxime with methyl chloroformate and then thermally cyclizing the resulting acylated intermediates. The heterocycles were alkylated with chloromethyl pivalate in the presence of KI to give POM derivatives **5**−**7**. 1,2,4-Oxadiazole-5(4*H*)-thiones **8**−**10** were synthesized using two different methodologies. In the first, the acetylated carbox-

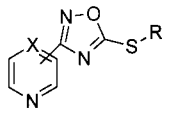


2-7

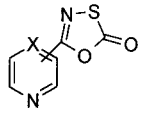


8-10

amidoximes were reacted with carbon disulfide in the presence of a base to yield **8** and **9**. However, the pyrazine derivate **10** decomposed during workup. Therefore, another methodology was applied to synthesize all three compounds, which involved thiocarbonyldiimidazole (TCDI) and a base. The alkylation of the thione derivatives **8**−**10** went smoothly to afford the corresponding POM derivatives **11**−**13**. 1,3,4-Oxathiazoline-2-ones **14**−**16**[18] were prepared by the action of chlorocarbonylsulfenyl chloride on the corresponding carboxamides. Attempted (3 + 2) cycloadditions of **14**−**16** with tosyl cyanide failed to give the desired 1,2,4-thiadiazole-3-ones, yielding elemental sulfur and the corresponding nitriles instead.



11-13



14-16

Table 2. MIC Values of Compounds **1**−**16**

compd	MIC[a] (μM)	MIC (μg/mL)	compd	MIC[a] (μM)	MIC (μg/mL)
pyrazinamide[b]	400	49	isoniazid[b]	0.15	0.018
1[1]	50	13	**9**	>1600	>286
2	>1600	>261	**10**	1600	288
3	>1600	>261	**11**	200	59
4	800	131	**12**	200	59
5	50	14	**13**	400	118
6	100	28	**14**	50	9
7	200	56	**15**	25	4.5
8	>1600	>286	**16**	25	4.5

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

Results and Discussion

Compounds **2**−**16** were tested against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) with the results shown in Table 2. The MIC value in BACTEC 6A[19] medium of the oxadiazole derivative **4** was 800 μM. MICs of the POM derivatives of the oxadiazoles **5**−**7** were 50, 100, and 200 μM, respectively. The POM derivatives of the oxadiazolethiones **11**−**13** exhibited MIC values of 200−400 μM, and the oxathiazoline-2-ones **14**−**16** exhibited MIC values of 25−50 μM. The MIC values of all the other compounds tested were greater than or equal to 1600 μM, limited solubility prohibiting an accurate determination. The unprotected polar heterocycles exhibited only minimal activity with the exception of 3-pyrazinyl-1,2,4-oxadiazole-5(4*H*)-one (**4**), which was half as potent as pyrazinamide. The oxadiazolethione series (**8**−**10**) was devoid of activity at the concentrations tested except for the 3-pyrazinyl-1,2,4-oxadiazole-5(4*H*)-thione (**10**), which was 4 times less potent than pyrazinamide.

As expected, the polar, unprotected isosteres of pyridine- and pyrazinecarboxylic acids **2**−**4** and **8**−**10** showed little or no inhibition of *M. tuberculosis*. We believe that this phenomenon is due to their structural similarity to nicotinic, isonicotinic, and pyrazinoic acids, which themselves are devoid of antitubercular activity, while derivatives thereof such as pyrazinamide are effective inhibitors. Pyrazinamide and nicotinamide are known to be converted by a nicotinamidase into pyrazinoic and nicotinic acids, which are thought to be the actual active species in the mycobacteria.[3] However, the highly polar nature of the acids prevents their efficient penetration of the mycobacterial cell wall, rendering the compounds inactive. Polarity appeared to be the only determinant of the activity at this stage of the experiments as demonstrated by the fact that slightly more lipophilic pyrazine derivatives were the only compounds with any activity.

The results also showed that derivatization of these compounds to more lipophilic precursors, which made ionization impossible and presumably facilitated penetration of the cell wall, was a successful method to improve their physicochemical properties. Modification of inactive oxadiazole **2** with the POM group produced compound **5**, which was 8 times as potent as the currently used drug pyrazinamide. Likewise, protection of inactive **3** resulted in **6**, which is 4 times as potent as pyrazinamide. Derivatization of compound **4** increased 4 times its already existing potency. The protected derivatives of the oxadiazolethiones **8** and **9** (**11** and **12**) were twice as potent as pyrazinamide,

whereas **10**, which was 4 times less potent than pyrazinamide, achieved equal potency with the drug when it was protected. To our surprise, the 1,3,4-oxathiazoline-2-ones **14**−**16** were the most active compounds in the series, having activities ranging from 8 to 16 times as potent as pyrazinamide. Interestingly enough, a member of this series (**15**) was recently reported as a microbicide in a patent application.[18]

In summary, the results show that bioreversible modification of pyridine- and pyrazinecarboxylic acid bioisosteres to more lipophilic precursors can increase their activity. Although the compounds were not tested against pyrazinamide-resistant strains of *M. tuberculosis*, they can be expected to be effective against most of these strains because of the knowledge that most pyrazinamide resistance results from lack of pyrazinamidase (nicotinamidase) enzyme in the bacteria.[20] Since the compounds do not contain an amide bond that has to be hydrolyzed for activity, pyrazinamidase would not be necessary for the activation of the synthesized compounds. In the case of the 1,3,4-oxathiazoline-2-ones **14**−**16**, the species responsible for the antimycobacterial activity is unknown, so it is uncertain if these compounds would be active against pyrazinamide-resistant strains.

Experimental Section

General. Melting points were determined with an electrothermal capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained using a Varian Gemini 200 spectrometer at 200 and 50 MHz, respectively. Chemical shifts are reported in ppm downfield (δ) from internal TMS. Elemental analyses were performed by Desert Analytics of Tucson, Arizona, and were within 0.4% of the calculated values except where stated otherwise (Table 1). Column chromatography was performed using silica gel purchased from Aldrich Chemical Co. (60 A, 70−230 mesh).

General Procedure for the Preparation of Oxadiazolones 2−4. The respective amidoxime[14,15,17] (10 mmol) and a magnetic stirring bar were placed in a 100 mL round-bottomed flask, and the flask was sealed with a rubber septum. N_2 was introduced, and 15 mL of anhydrous DMF was added via syringe. After the addition of 12 mmol pyridine, the mixture was stirred at room temperature until dissolution and then cooled to 0 °C in an ice−water bath. Methyl chloroformate (11 mmol) was added, and the mixture was stirred for 3 h while warming to room temperature. The mixture was diluted with 30 mL of H_2O, and the resulting solution was extracted with 3 × 30 mL of EtOAc. The organic phases were combined and dried over anhydrous Na_2SO_4. Upon filtration and evaporation of the solvent, a yellow oil (in the case of **2** and **3**) was obtained. The pyrazine derivative **4** gave, upon dilution of the reaction mixture with H_2O, a solid, which was washed with H_2O and air-dried. The oils were dissolved in 60 mL of toluene and refluxed for 24 h. The solid pyrazine derivative was suspended in 60 mL of xylenes and refluxed (140 °C) for 24 h. Then the mixtures were allowed to cool to room temperature, and the volatiles were removed under vacuum (**2** and **3**) or let stand overnight (**4**). The solids were recrystallized twice from EtOH (**2** and **4**) or MeCN (**3**) to give **2**−**4**. **2**: yield 48%. ^{1}H NMR (DMSO-d_6): δ 8.83 (d, 2H), 7.77 (d, 2H), 3.50 (br s, 1H). **3**: yield 52%. ^{1}H NMR (DMSO-d_6): δ 8.94 (br s, 1H), 8.75 (br s, 1H), 8.15 (d, 1H), 7.58 (q, 1H), 3.81 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 159.92, 155.86, 152.85, 146.91, 134.06, 124.46, 120.01. **4**: yield: 57%. ^{1}H NMR (DMSO-d_6): δ 9.13 (d, 1H), 8.81 (m, 2H), 3.61 (br s, 1H). ^{13}C NMR (DMSO-d_6): δ 159.69, 156.41, 147.57, 144.91, 142.60, 139.11.

General Procedure for the Alkylation of Oxadiazolones 2−4 (5−7). Chloromethyl pivalate (6 mmol) was added dropwise to a solution of 5 mmol of the respective oxadiazolone and 5 mmol of AcOK in 10 mL of DMF. NaI (5 mmol) was added, and the mixture was stirred at room temperature for 72 h. The reaction mixture was diluted with 20 mL of H_2O and extracted with 3 × 40 mL of EtOAc. The organic layers were combined and washed with 120 mL of 1% Na_2SO_3 solution, 120 mL of H_2O, and 120 mL of brine. The solution was dried over Na_2SO_4, and the solvent was evaporated. A yellow oil was obtained, which was chromatographed on silica gel using hexanes/EtOAc (7:3 for **5** and **6**, and 8:2 for **7**) as eluent. Colorless to yellow oils were obtained, which slowly crystallized. Recrystallization from a mixture of hexanes and EtOAc gave **5**−**7**. **5**: yield 33%. ^{1}H NMR ($CDCl_3$): δ 8.88 (d, 2H), 7.59 (d, 2H), 5.65 (s, 2H), 1.18 (s, 9H). **6**: yield 37%. ^{1}H NMR ($CDCl_3$): δ 8.90 (m, 2H), 7.99 (m, 1H), 7.55 (m, 1H), 5.63 (s, 2H), 1.19 (s, 9H). **7**: yield 51%. ^{1}H NMR ($CDCl_3$): δ 9.29 (d, 1H), 8.81 (d, 1H), 8.66 (d, 1H), 6.09 (s, 2H), 1.08 (s, 9H).

General Procedures for the Preparation of the Oxadiazolethiones 8−10. Method 1 for 8 and 9. Acetic anhydride (7.3 mmol) was added dropwise to a mixture of 7.3 mmol of the respective amidoxime and 7.3 mmol of Et_3N in 30 mL of CH_2Cl_2. The mixture was stirred at room temperature for 4 h. The solids were filtered, washed with CH_2Cl_2 and H_2O, and air-dried. The filtrate was washed with 30 mL of H_2O and evaporated. The solids were combined with the ones obtained from the mother liquor and dissolved in 40 mL of DMF. The solution was cooled to 0 °C in an ice−water bath, and 25.5 mmol of CS_2 and 19.2 mmol of a 50% dispersion of NaH in mineral oil were added. The mixture was stirred for 1 h. A 1 N HCl solution (80 mL) was added very carefully, and the mixture was stirred for another hour and refrigerated overnight. The crystals were collected by filtration, washed with H_2O, and recrystallized twice from EtOH to give **8** or **9**.

Method 2 for 8−10. To a stirred suspension of 7.3 mmol of the respective amidoxime in 60 mL of MeCN was added 10.9 mmol ofTCDI (90%) in one portion. A clear solution formed for a moment, and then a yellow precipitation followed. To this mixture, 29.2 mmol of DBU was added dropwise. A clear solution was obtained again, which was stirred at room temperature overnight. The mixture was poured into 120 mL of H_2O and neutralized using 6 N HCl. The precipitate was filtered and washed with H_2O. The mother liquor was refrigerated overnight, and the solids were filtered, combined with the first crop, and recrystallized from ethanol to give **8**−**10**. **8**: yield method 1: 35%, method 2: 80%. ^{1}H NMR (DMSO-d_6): δ 8.91 (dd, 2H), 8.06 (dd, 2H), 6.19 (br s, 1H). **9**: yield method 1: 28%, method 2: 79%. ^{1}H NMR (DMSO-d_6): δ 9.08 (d, 1H), 8.84 (dd, 1H), 8.34 (ddd, 1H), 7.70 (dd, 1H). **10**: yield 89%. ^{1}H NMR (DMSO-d_6): δ 9.25 (d, 1H), 8.90 (m, 2H).

General Procedure for the Alkylation of Oxadiazolethiones 8−10 (11−13). Chloromethyl pivalate (7.1 mmol) was added dropwise to a stirred solution of 5.9 mmol of the respective oxadiazolethione and 7.1 mmol of Et_3N in 10 mL of DMF. The solution was stirred at room temperature for 7 h, and another equivalent of chloromethyl pivalate and Et_3N was added. The solution was stirred at room temperature for a total of 24 h. For the last 1.5 h, it was heated at 50 °C. The mixture was cooled to room temperature and poured into crushed ice with vigorous stirring. In the case of **11** and **13**, the solids were collected by filtration, washed with water, and recrystallized twice from petroleum ether to give **11** and **13**. Compound **12** separated as an oil, which was extracted with 3 × 50 mL of EtOAc. The solution was dried over Na_2SO_4 and filtered, and the solvent was evaporated. The residue was dissolved heating slightly in hexanes and filtered. Hexanes were removed under vacuum. A tan oil was obtained, which was chromatographed on silica gel using hexanes/EtOAc (9:1) as eluent. The product solidified in the freezer and was recrystallized from petroleum ether in the freezer to give **12**. **11**: yield 83%. ^{1}H NMR ($CDCl_3$): δ 8.80 (dd, 2H), 7.94 (dd, 2H), 5.83 (s, 2H), 1.22 (s, 9H). **12**: yield 78%. ^{1}H NMR ($CDCl_3$): δ 9.31 (dd, 1H), 8.77 (dd, 1H), 8.34 (m, 1H), 7.44 (m, 1H), 5.83 (s, 2H), 1.22 (s, 9H). **13**: yield 84%. ^{1}H NMR ($CDCl_3$): δ 9.37 (d, 1H), 8.77 (m, 2H), 5.86 (s, 2H), 1.22 (s, 9H).

General Procedure for the Preparation of Oxathiazolinones 14−16.[18] A mixture of 30 mmol of the respective carboxamide and 10 mmol of chlorocarbonylsulfenyl chloride in 100 mL of toluene was refluxed for 3 h. The precipitate was filtered and washed with 50 mL of EtOAc. The filtrate and the washings were combined and evaporated. The residue was purified by column chromatography using hexanes/EtOAc (1:1) as eluent and recrystallized from a mixture of hexanes and EtOAc to give **14**−**16**. **14**: yield 43%. ^{1}H NMR ($CDCl_3$): δ 8.83 (dd, 2H), 7.82 (dd, 2H). **15**: yield 43%. ^{1}H NMR ($CDCl_3$): δ 9.15 (d, 1H), 8.75 (dd, 1H), 8.20 (ddd, 1H), 7.42 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 172.93, 155.33, 153.04, 148.48, 134.46, 123.64, 122.04. **16**: yield 33%. ^{1}H NMR ($CDCl_3$): δ 9.29 (d, 1H), 8.77 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 172.11, 154.42, 147.11, 144.62, 144.14, 140.07.

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.[19] Percent inhibition was calculated as 1 − (growth index of test sample/growth index of control) × 100. The minimum inhibitory concentration is defined as the minimum concentration that inhibited 99% of the inoculum.

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(14) 3-Cyanopyridine (6.24 g, 0.06 mol) and 8.34 g (0.12 mol) of NH_2-OH·HCl were dissolved in 60 mL of H_2O. A solution of 12.72 g (0.12 mol) of Na_2CO_3 in 45 mL of H_2O was cautiously added, and the resulting solution was stirred and heated at 70 °C overnight. The solution was cooled to room temperature, saturated with NaCl, and extracted with 4 × 50 mL of EtOAc. The solution was dried over anhydrous Na_2SO_4, and the solvent was evaporated, giving 5.12 g of colorless crystals (62%). Reported yield was 38%.

(15) 4-Cyanopyridine (6.24 g, 0.06 mol) was dissolved in 9 mL of EtOH (slightly heated). NH_2OH·HCl (8.34 g, 0.12 mol) was dissolved in 9 mL of H_2O and added to the 4-cyanopyridine solution. Na_2CO_3 (12.72 g, 0.12 mol) was dissolved in 75 mL of H_2O and added cautiously to the above solution. The suspension was heated at 70 °C overnight. The mixture was cooled to room temperature and kept at 4 °C for a few hours. The crystals were separated by filtration and washed with H_2O, giving 7.40 g of colorless crystals (90%). Reported yield was 57%.

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(17) Cyanopyrazine (6.3 g, 0.06 mol) was diluted with 9 mL of EtOH. Hydroxylamine hydrochloride (8.34 g, 0.12 mol) was dissolved in 9 mL of H_2O and added to the cyanopyrazine solution. Two phases separated. Na_2CO_3 (12.72 g, 0.12 mol) was dissolved in 75 mL of H_2O and added cautiously to the above mixture. After the addition of the first few milliliters of the Na_2CO_3 solution, a large amount of white substance precipitated, which did not dissolve upon the addition of the rest of the solution. The whole mixture was heated at 70 °C for 5 h and left standing at room temperature overnight. The crystals were filtered and washed with H_2O. The filtrate was saturated with NaCl and extracted with 3 × 50 mL of EtOAc. Solution was dried over anhydrous Na_2SO_4, and the solvent was evaporated. The crystals were combined with the initial precipitate giving 7.98 g of colorless needles (97%).

(18) The synthesis of **15** was recently described as a microbicide with no physical or analytical data: Holzl, W.; Schnyder, M. Microbicidal Active Substances. Int. Pat. Appl., WO 00/07446, 2000.

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