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THE SYNTHESIS OF COMPOUNDS FOR THE CHEMOTHERAPY OF TUBERCULOSIS. IV. THE AMIDE FUNCTION[1]

THOMAS S. GARDNER, E. WENIS, AND JOHN LEE

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The discovery by Chorine (1) of the *in vivo* activity of nicotinamide against tuberculosis in the mouse and the rediscovery of this activity by Kushner, *et al.* (2, 3) has encouraged further development of this line of approach to the problem. A large number of nicotinamide derivatives were prepared by the latter group and were found to be less active than nicotinamide itself.

Concurrent with our investigations of thiosemicarbazones (4) and hydroxamic acid compounds (5), we prepared a number of amide and thioamide type compounds. Our work emphasizes the difficulty of predicting activity from structural changes in the molecule. Table I illustrates this point. The relative activities of the pyrazinyl and pyridinyl amides, thioamides, and functional amide derivatives (hydroxamic acids, hydrazides) are given. These have been, in part, previously reported by us (4, 5), by our colleagues in these laboratories, (9, 14, 15), or by others (1, 3, 16, 17). For comparison the activity of the corresponding related thiosemicarbazones is given.

The relatively high activity of thioisonicotinamide in tuberculosis in the mouse introduces a new class of synthetic antitubercular compounds. The previously known, but not hitherto tested, amides: 2-pyridineacetamide, 3-pyridineacetamide, 4-pyridineacetamide, 5-methyl-3-isoxazolecarboxamide, and 4-pyridinethioacetomorpholide, were found to be inactive.

The following new isonicotinyl compounds were found to be inactive: N, N′-ethylene-bis-(isonicotinamide), N-(4-pyridyl)isonicotinamide, N-(carbethoxy)-isonicotinamide hydrochloride, 4-pyridineacetomorpholide hydrochloride, 4-isonicotinamidine, N-isonicotinylglycine, N-isonicotinylisoleucine, and N-(isonicotinyl)phenylalanine.

Details on the thioamides prepared and on the above compounds are given in Table II and in the Experimental section.

Table III gives toxicity data for related pyridine thioamides.

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EXPERIMENTAL

General procedure for the preparation of thioamides. The procedure of Karrer (8) for thionicotinamide and thiopicolinamide was used. One part by weight of the pure nitrile was added to five parts by weight of 30% ammonical methanol. The resulting solution was then saturated with dry hydrogen sulfide at 25°, and permitted to stand 1–2 days. The thioamide was separated by filtration. In the majority of cases, the recovered thioamide was recrystal-

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TABLE I[a, b]

COMPARISON OF ACTIVITIES IN TUBERCULOSIS IN THE MOUSE

CARBOXYL FUNCTION	HETEROCYCLIC NUCLEUS			
	Pyrazinyl	2-Pyridyl	3-Pyridyl	4-Pyridyl
Amide (1, 3, 6, 9)	+	0	+	0
Thioamide (7, 8, 16)	0[c]	0	0	++
Hydrazide (15, 16)	0	+	0	++++
Hydroxamic acid (5, 16, 17)	0	0	0	0
Aldehydethiosemicarbazone (4, 14, 16)	0	0	+++	+++

[a] Where the compound was first described elsewhere, the activity was verified in our laboratories with the single exception of pyrazinecarboxaldehyde thiosemicarbazone (16) which Kushner, *et al.* reported as having questionable activity and herein is rated inactive. [b] Compounds are rated in order of activity. Under this classification, nicotinamide and pyrazinecarboxamide are rated in the same class. Zero is used to indicate lack of activity in the screening tests performed. Slight traces of activity might be found by more sensitive methods. [c] Independently prepared by us with the same characteristics and lack of activity reported by Kushner, *et al.* (16).

lized from an appropriate solvent, usually water, and in most cases was obtained as a yellow crystalline material.

N,N'-Ethylene bis-(isonicotinamide). Isonicotinic acid (25 g.) in the form of the chloride hydrochloride, prepared by refluxing in thionyl chloride, was added to 150 ml. of pyridine containing 5 g. of ethylenediamine. The mixture was allowed to stand at 25° for five days and the pyridine hydrochloride was then removed and the filtrate was evaporated to dryness *in vacuo*. The residue was added to water and washed on a filter with water. The colorless, water-insoluble product was purified by exhaustive extraction with hot water. It was insoluble in hot ethanol, hot acetone, boiling water, hot ethyl acetate, and 10% sodium carbonate solution. It was soluble in acetic acid and in hydrochloric acid solution. Yield, 22 g., m.p. > 250°.

Anal. Calc'd for $C_{14}H_{14}N_4O_2$: N, 20.7. Found: N, 20.7.

N-(Carbethoxy)isonicotinamide hydrochloride. Isonicotinic acid (25 g.) in the form of the chloride hydrochloride was added to 100 ml. of pyridine containing 36 g. of urethan and the mixture was allowed to stand at 25° for five days. The pyridine hydrochloride was separated from the pyridine solution and the solution was concentrated to dryness. Toluene was added twice and traces of pyridine were removed by distillation. The product was dissolved in ethanol, decolorized by activated charcoal, and then was one-half saturated with hydrochloric acid gas by weight. A small quantity of pyridine hydrochloride separated and was removed. On complete saturation with hydrochloric acid gas and addition of ether the product crystallized as a colorless material. It was recrystallized by solution in ethanol and addition of ether. Yield, 18 g., m.p. 193–195°.

Anal. Calc'd for $C_9H_{10}N_2O_3 \cdot HCl$: C, 46.8; H, 4.8.
Found: C, 46.6; H, 4.9.

N-(4-Pyridyl)isonicotinamide. Isonicotinic acid (18 g.) in the form of the chloride hydrochloride was reacted with 9.4 g. of 4-aminopyridine in 200 ml. of pyridine at 25°C. for five days. The product separated with the pyridine hydrochloride. Addition of water and sodium bicarbonate to the separated solid gave a colorless material. The product was dissolved in ethanol (100 ml.) and 5% sodium bicarbonate solution (100 ml.), and on addition of more water, gave a relatively pure product which was recrystallized from boiling water. Yield, 14 g., m.p. 193–194°.

Anal. Calc'd for $C_{11}H_9N_3O$: N, 21.1. Found: N, 20.8.

4-(N-Methylformamido)benzoic acid. N-Methyl-*p*-aminobenzoic acid (11) was dissolved

TABLE II

AMIDES AND THIOAMIDES

NAME	FORMULA	CRYST. FROM	M.P., °C.	YIELD, %	COLOR	SULFUR	
						Calc'd	Found
Thioisonicotinamide	$C_6H_6N_2S$	Water	209–210	86	Yellow	23.2	22.8
Thio-3-pyridineacetamide	$C_7H_8N_2S$	Water	133–134	71	Yellow	21.0	20.5
5-Methyl-thio-3-isoxazolecarboxamide	$C_5H_6N_2OS$	Water	170–171	76	Yellow	22.5	22.6
4-Acetamidothiobenzamide[a]	$C_9H_{10}N_2OS$	Ethanol	230–231	79	Yellow	[b]	[b]
3-Acetamidothiobenzamide	$C_9H_{10}N_2OS$	Water	182–183	82	Lt. yellow	16.5	16.7
4-Amino-2-methyl-thio-5-pyrimidinecarboxamide	$C_6H_8N_4S$	Water	262–263	98	Lt. yellow	19.0	18.7
Thio-4-pyridineacetamide	$C_7H_8N_2S$	Water	178–179	66	Brown	21.0	21.0
2-Mercapto-4-imidazolecarboxamide	$C_4H_5N_3OS$	Water	>250	72	Cream	[c]	[c]
Thio-2-pyridineacetamide HCl	$C_7H_8N_2S \cdot HCl$	Methanol	193–195	70	Yellow	14.8	14.6
Thio-4-pyridinepropionamide[d]	$C_8H_{10}N_2S$	Water	174–175	26	Lt. brown	19.3	19.4

[a] *p*-Aminobenzonitrile (Eastman P-5803) used for this experiment contained a small quantity of impurity as x-ethyl-y-aminobenzonitrile. The thioamides were separated by crystallization from water, the x-ethyl-y-acetamidothiobenzamide being less soluble than the 4-acetamido. The x-ethyl-y-acetamidothiobenzamide melted at 233–234°.

Anal. Calc'd for $C_{11}H_{14}N_2OS$: C, 59.5; H, 6.3; S, 14.4; N, 12.6.
Found: C, 59.8; H, 6.3; S, 14.2; N, 12.4.

[b] *Anal.* Calc'd: C, 55.7; H, 5.2.
Found: C, 55.8; H, 5.2.

[c] *Anal.* Calc'd: C, 33.6; H, 3.5.
Found: C, 33.3; H, 3.8.

[d] Attempts to prepare 4-pyridineacrylonitrile were unsuccessful. Acetic anhydride, and also phosphorus pentoxide dehydration of the amide gave only tarry products.

in 250 ml. of anhydrous formic acid and the solution was evaporated to dryness. The residue was obtained from ethanol as colorless crystals. Yield, 5 g., m.p. 214–215°.

Anal. Calc'd for $C_9H_9NO_3$: C, 60.3; H, 5.1; N, 7.8.
Found: C, 60.3; H, 5.0; N, 7.6.

4-Pyridineacetomorpholide hydrochloride. This was prepared by the incomplete hydrolysis of 4-pyridinethioacetomorpholide (250 g.) in 1200 ml. of 95% ethanol containing 68 g. of potassium hydroxide. The reaction solution was refluxed for 72 hours, poured into two volumes of water, and then concentrated to one-third volume. The separated amide (207 g.) was crystallized from ethanol. The colorless product melted at 204–205°.

Anal. Calc'd for $C_{11}H_{14}N_2O_2 \cdot HCl$: C, 54.4; H, 6.2; N, 11.5.
Found: C, 54.8; H, 6.5; N, 11.6.

Hydrolysis of 4-pyridineacetomorpholide in 15% hydrochloric acid solution gave 4-

TABLE III

TOXICITY DATA FOR RELATED PYRIDINE THIOAMIDES

COMPOUND	LD_{50} (MICE) *per os* mg./kg.
Thiopicolinamide	1700
Thionicotinamide	1750
Thioisonicotinamide	1250
Thio-2-pyridineacetamide•HCl	750[a]
Thio-3-pyridineacetamide	[b]
Thio-4-pyridineacetamide	1750
Thio-4-pyridinepropionamide	1750

[a] S.C. 750 mg./kg. [b] S.C. 1000 mg./kg.

pyridylacetic acid and heating with ethanol containing an equal weight of conc'd sulfuric acid gave ethyl 4-pyridylacetate.

N-Isonicotinylglycine (12). Isonicotinylhydrazide (13.7 g.) was treated in 40 ml. of conc'd hydrochloric acid and 20 ml. of water at 0° with 30 g. of sodium nitrite in 40 ml. of cold water. The reaction was vigorous and at completion the solution was brought to *p*H 7.5 and extracted with ether. At *p*H 4 only isonicotinic acid was obtained. The ether solution was concentrated to a viscous syrup and dissolved in 200 ml. of N/100 sodium hydroxide solution (12) to which 10 g. of glycine was added. After standing 48 hours at 25° the solution was evaporated to dryness at 80°, and the residue was dissolved in 100 ml. of water, neutralized with sodium carbonate, and 20 g. of copper sulfate pentahydrate in 100 ml. of water was added. The precipitated copper salt was recovered by filtration, suspended in 500 ml. of hot water, and decomposed with hydrogen sulfide. The clear, hot filtrate was concentrated and the product crystallized out on cooling. It was recrystallized from aqueous ethanol. Yield, 5 g., m.p. 230–232°.

Anal. Calc'd for $C_8H_8N_2O_3$: N, 15.6. Found: N, 15.8.

N-Isonicotinylisoleucine. This compound was prepared in a manner similar to the synthesis of N-isonicotinylglycine. From 10 g. of isoleucine, 6 g. of N-isonicotinylisoleucine were obtained. m.p., froths at 180°, resolidifies and melts at 200–210°.

Anal. Calc'd for $C_{12}H_{16}N_2O_3$: N, 11.86. Found: N, 11.6.

N-(Isonicotinyl)phenylalanine. Phenylalanine (14 g.) gave 10 g. of N-(isonicotinyl)-phenylalanine by the same procedure as described above, m.p. > 250°.

Anal. Calc'd for $C_{15}H_{14}N_2O_3$: N, 10.4. Found: N, 10.6.

All the isonicotinyl amino acid amides were colorless compounds.

Isonicotinamidine hydrochloride. Crude isonicotinimidic acid ethyl ester (imino ether) (85 g.) was prepared from 4-cyanopyridine (60 g.) in a standard procedure (13) and was refluxed for 4 hours in 150 ml. of ethanol containing 50 ml. of water and 12 g. of ammonium chloride. On cooling, the separated crude amidine hydrochloride was recovered and recrystallized four times from ethanol to obtain a colorless compound. Yield, 13 g., m.p. 242–244°.

Anal. Calc'd for $C_6H_7N_3$•HCl: C, 45.7; H, 5.1.
Found: C, 45.8; H, 5.4.

This compound has recently been reported, m.p. 230–232° (10).

SUMMARY

A number of amides and thioamides were prepared for testing in tuberculosis in the mouse. Only thioisonicotinamide was found to be active.

NUTLEY 10, N. J.

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