Potential Antitumor Agents: Derivatives of 2-Hydrazino-5-nitropyridine

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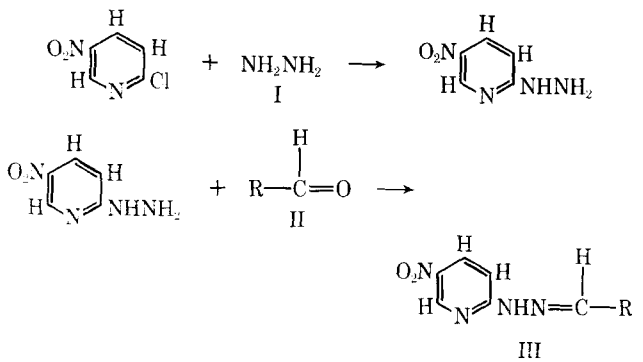
Abstract □ Fifty-one hydrazones of 2-hydrazino-5-nitropyridine have been synthesized for tolerance in DBA mice and for study as potential antitumor agents against the three mouse tumor systems (sarcoma 180, adenocarcinoma 755, and leukemia 1210). Of four compounds with activity against the sarcoma 180 tumor, two derivatives—*p*-acetaminobenzaldehyde and *p*-nitrobenzaldehyde—showed good inhibition and confirmed activity. The salicylaldehyde derivative showed slight activity against the adenocarcinoma 755 tumor. None of the compounds were active in the leukemia 1210 mouse tumor system. The highest tolerated dose of the active compounds in mice by intraperitoneal injection was 2 g./kg. The compounds were thus of low toxicity.

Keyphrases □ 2-Hydrazino-5-nitropyridines—synthesis □ Acute toxicity, mice—2-hydrazino-5-nitropyridines □ Biological activity —2-hydrazino-5-nitropyridines

The synthesis of thiosemicarbazones and hydrazones has received considerable attention since Domagk's (1) report on the antituberculous activity of tibione (*p*-acetylaminobenzaldehyde thiosemicarbazone). Numerous reports followed which indicated that a number of different hydrazones possessed potential antimicrobial, antifungal, and antitumor activity. Freedlander and Furst (2) studied the effects of substituted hydrazines and related compounds on myeloid mouse leukemia C-1498. Petersen and Domagk (3) synthesized the guanylhydrazone of *p*-benzoquinone-monothiosemicarbazone and found the product to possess strong bacteriostatic action on *Mycobacterium tuberculosis* and various other organisms. In addition Chang (4) reported that the isonicotinylhydrazones of 2-carboxymethoxy-3-methoxybenzaldehyde and 2-carboxymethoxybenzaldehyde were effective against mouse leprosy. Following these reports Jucker (5) published a review of hydrazone derivatives used as medicinals.

A few hydrazone derivatives of 2-hydrazino-5-nitropyridine were prepared by Mangini and Frenguelli (6). However, no biological properties were reported concerning these compounds. In extensive investigations from this laboratory into the bacteriostatic, fungistatic, and carcinostatic effects of hydrazones (7, 8) particularly with a view to their possible application as potential chemotherapeutic agents, results indicated that certain hydrazones may possess significant biological activity.

The present report also describes the synthesis of 51 5-nitro-2-pyridylhydrazones with analyses, tests for acute toxicity in mice, and for their effectiveness as potential antimicrobial and antitumor agents. Studies with this series of hydrazones reported herein have shown that certain derivatives have some effect as potential anticancer agents.



Scheme I—5-Nitro-2-pyridylhydrazone; I, *preparation of 2-hydrazino-5-nitropyridine;* II, *aldehyde where R = aliphatic, aromatic, or heterocyclic group;* III, *hydrazone derivative.*

MATERIALS AND METHODS

The desired 2-hydrazino-5-nitropyridine was synthesized from 2-chloro-5-nitropyridine by a modification of the method of Mangini and Frenguelli (6). Seventy-nine grams (0.5 mole) of 2-chloro-5-nitropyridine was warmed to solution in 2 l. of 95% EtOH, in a 3-l. three-necked flask equipped with a stirrer, thermometer, and dropping funnel. Twenty-five grams (0.5 mole) of 95% hydrazine hydrate were added dropwise to the stirred solution and the mixture cooled in an ice bath. The alcoholic solution turned orange colored immediately after the hydrazine was added and a voluminous yellowish-green precipitate began to separate with the evolution of a gas. The mixture was stirred for an additional 2 hr. at room temperature after all the hydrazine had been added and held at room temperature overnight. The heavy precipitate that formed was filtered, washed with petroleum ether, and air-dried. The crude greenish insoluble condensation product was crystallized from a large volume of 70% EtOH. The yellow fine crystals melted at 203–205° with decomposition.

Anal.—Calcd for $C_5H_6N_4O_2$: C, 38.96%; H, 3.92%; N, 36.25%. Found: C, 38.87%; H, 3.87%; N, 36.5%. The 2-hydrazino-5-nitropyridine readily reacted with aldehydes to form crystalline hydrazones which exhibited intense colors ranging from bright yellow, orange, through shades of red. All the aldehydes employed were commercial preparations. The chemical structures of the hydrazones are shown in Scheme I.

The general procedure for the synthesis of the hydrazones was as follows: a solution of the aldehyde (0.1 mole) in 95% ethanol (50 ml.) was added to a warm solution of the 2-hydrazino-5-nitropyridine (0.1 mole) in 95% ethanol (200 ml.) and refluxed gently for 30 min. on a hot plate. In numerous instances immediate reaction took place as seen by the formation of a solid. The solutions were cooled to room temperature and the insoluble products filtered, washed with cold water and petroleum ether, and air-dried. The products were purified by recrystallization from 70% ethanol in presence of decolorizing carbon. The compounds appeared as colorful, shiny crystals with yields from 90–95%. Table I gives a summary of the aldehydes used and the analysis of the compounds.

Biological Studies—Acute toxicity studies were performed in the DBA strain of mice as maintained at the National Institutes of Health, Bethesda, Md. The chemicals were suspended in 0.25% methocel (Dow methylcellulose) so that the dose per 20-g. mouse was contained in 0.25-ml. volume for intraperitoneal injection and the results judged by 72-hr. survival. The tolerated dose of each of the fifty-one compounds is 2 g./kg. The compounds are of low toxicity and may be administered in larger doses than are commonly used in the administration of various anticancer drugs.

Antitumor Studies—The compounds were tested for antitumor activity in the three tumor (sarcoma 180, adenocarcinoma 755, and leukemia 1210) mouse screening program by screeners under contract to the Cancer Chemotherapy National Service Center. The

Table I—5-Nitro-2-pyridylhydrazones—Chemical and Physical Properties

Derivative of Aldehyde	M.p. °C.[a]	Empirical Formula	Anal., % Calcd.	Found
Formaldehyde	220–225	$C_{11}H_{12}N_8O_4$	C, 41.25 H, 3.78 N, 34.99	C, 41.42 H, 4.10 N, 34.19
2-Ethylbutyraldehyde	152–155	$C_{11}H_{16}N_4O_2$	C, 55.91 H, 6.82 N, 23.71	C, 55.49 H, 6.79 N, 23.69
Heptaldehyde	191–194	$C_{12}H_{18}N_4O_2$	C, 57.58 H, 7.25 N, 22.38	C, 57.71 H, 7.30 N, 22.53
2-Ethylhexanal	136–138	$C_{13}H_{20}N_4O_2$	C, 59.07 H, 7.63 N, 21.19	C, 59.14 H, 7.81 N, 21.38
Nonal	108–111	$C_{14}H_{22}N_4O_2$	C, 60.41 H, 7.96 N, 20.13	C, 60.68 H, 7.24 N, 20.34
Decanal	88	$C_{15}H_{24}N_4O_2$	C, 61.62 H, 8.28 N, 19.16	C, 61.38 H, 8.77 N, 19.47
Lauraldehyde	78–80	$C_{17}H_{28}N_4O_2$	C, 63.72 H, 8.81 N, 17.49	C, 63.49 H, 8.36 N, 17.34
Crotonaldehyde	174–177	$C_9H_{10}N_4O_2$	C, 52.42 H, 4.88 N, 27.17	C, 52.69 H, 4.84 N, 27.13
Tiglaldehyde	216	$C_{10}H_{12}N_4O_2$	C, 54.53 H, 5.49 N, 25.44	C, 54.39 H, 5.22 N, 25.43
Methyl glyoxal	>300	$C_{13}H_{12}N_8O_4$	C, 45.35 H, 3.51 N, 32.55	C, 45.32 H, 3.86 N, 32.41
Benzaldehyde[b]	227–229	$C_{12}H_{10}N_4O_2$	C, 59.47 H, 4.20 N, 23.12	C, 59.72 H, 4.76 N, 23.17
4-Fluorobenzaldehyde	228	$C_{12}H_9FN_4O_2$	C, 55.38 H, 3.49 N, 21.53	C, 55.48 H, 3.70 N, 21.27
2-Chlorobenzaldehyde	210	$C_{12}H_9ClN_4O_2$	C, 52.09 H, 3.28 N, 20.25	C, 52.41 H, 3.50 N, 29.05
4-Chlorobenzaldehyde	218–219	$C_{12}H_9ClN_4O_2$	C, 52.09 H, 3.28 N, 20.25	C, 52.37 H, 3.50 N, 20.29
2,4-Dichlorobenzaldehyde	211	$C_{12}H_8Cl_2N_4O_2$	C, 46.32 H, 2.59 N, 18.00	C, 46.10 H, 2.52 N, 18.50
3,4-Dichlorobenzaldehyde	168–170	$C_{12}H_8Cl_2N_4O_2$	C, 46.32 H, 2.59 N, 18.00	C, 46.01 H, 2.41 N, 18.21
2-Nitrobenzaldehyde	217–222	$C_{12}H_9N_5O_4$	C, 50.18 H, 3.16 N, 24.39	C, 50.83 H, 3.40 N, 24.42
3-Nitrobenzaldehyde	248–250	$C_{12}H_9N_5O_4$	C, 50.18 H, 3.16 N, 24.39	C, 50.42 H, 3.62 N, 24.76
4-Nitrobenzaldehyde	278	$C_{12}H_9N_5O_4$	C, 50.18 H, 3.16 N, 24.39	C, 50.38 H, 3.85 N, 24.72
Phenylacetaldehyde	137–138	$C_{13}H_{12}N_4O_2$	C, 60.93 H, 4.72 N, 21.87	C, 60.07 H, 4.50 N, 21.59
4-Tolylaldehyde	212–216	$C_{13}H_{12}N_4O_2$	C, 60.93 H, 4.72 N, 21.87	C, 60.72 H, 4.45 N, 21.39
Hydrocinnamaldehyde	126–128	$C_{14}H_{14}N_4O_2$	C, 62.21 H, 5.22 N, 20.73	C, 62.32 H, 5.40 N, 20.68
4-Isopropylbenzaldehyde	160	$C_{15}H_{16}N_4O_2$	C, 63.36 H, 5.67 N, 19.71	C, 64.05 H, 5.51 N, 19.53

(*continued on next page*)

Table I—(*continued*)

Derivative of Aldehyde	M.p. °C.[a]	Empirical Formula	Anal., % Calcd.	Found
Cinnamaldehyde	237	$C_{14}H_{12}N_4O_2$	C, 62.68 H, 4.51 N, 20.88	C, 62.31 H, 4.64 N, 20.92
α-Methylcinnamaldehyde	230–232	$C_{15}H_{16}N_4O_2$	C, 63.36 H, 5.67 N, 19.71	C, 63.70 H, 5.90 N, 19.64
1-Naphthaldehyde	195–198	$C_{16}H_{12}N_4O_2$	C, 65.74 H, 4.14 N, 19.17	C, 65.06 H, 3.78 N, 19.70
Salicylaldehyde	217	$C_{12}H_{10}N_4O_3$	C, 55.81 H, 3.90 N, 21.69	C, 55.16 H, 4.03 N, 21.38
2-Methoxybenzaldehyde	275–276	$C_{13}H_{12}N_4O_3$	C, 57.35 H, 4.44 N, 20.58	C, 57.22 H, 4.74 N, 20.35
2-Ethoxybenzaldehyde	228	$C_{14}H_{14}N_4O_3$	C, 58.73 H, 4.93 N, 19.57	C, 58.32 H, 4.44 N, 19.25
5-Chlorosalicylaldehyde	290–292	$C_{12}H_9ClN_4O_3$	C, 49.24 H, 3.10 N, 19.14	C, 49.43 H, 3.27 N, 19.64
5-Bromo-2-hydroxybenzaldehyde	298	$C_{12}H_9BrN_4O_3$	C, 42.75 H, 2.69 N, 16.62	C, 42.86 H, 3.01 N, 16.90
3-Hydroxybenzaldehyde	248–250	$C_{12}H_{10}N_4O_3$	C, 55.82 H, 3.90 N, 21.69	C, 55.69 H, 4.21 N, 21.66
4-Hydroxybenzaldehyde	>300	$C_{12}H_{10}N_4O_3$	C, 55.82 H, 3.90 N, 21.69	C, 56.46 H, 3.68 N, 21.33
4-Methoxybenzaldehyde	195–197	$C_{13}H_{12}N_4O_3$	C, 57.35 H, 4.44 N, 20.58	C, 57.68 H, 4.33 N, 20.82
2-Methoxy-5-*tert*-butylbenzaldehyde	199–203	$C_{17}H_{20}N_4O_3$	C, 62.17 H, 6.14 N, 17.07	C, 62.08 H, 6.00 N, 17.21
2-Hydroxy-1-naphthaldehyde	277–279	$C_{16}H_{12}N_4O_3$	C, 62.33 H, 3.92 N, 18.18	C, 62.48 H, 4.14 N, 18.09
2-Hydroxy-3-methoxybenzaldehyde	190–195	$C_{13}H_{12}N_4O_4$	C, 54.12 H, 4.20 N, 19.44	C, 54.33 H, 4.31 N, 19.11
2,3-Dimethoxybenzaldehyde	244	$C_{14}H_{14}N_4O_4$	C, 55.62 H, 4.67 N, 18.54	C, 55.85 H, 4.66 N, 18.54
3,4-Dimethoxybenzaldehyde	238–240	$C_{14}H_{14}N_4O_4$	C, 55.62 H, 4.67 N, 18.54	C, 55.69 H, 4.45 N, 18.07
2,4-Dihydroxybenzaldehyde	>300	$C_{12}H_{10}N_4O_4$	C, 52.55 H, 3.68 N, 20.43	C, 52.66 H, 3.85 N, 20.46
3-Ethoxy-4-hydroxybenzaldehyde	197–198	$C_{14}H_{14}N_4O_4$	C, 55.62 H, 4.67 N, 18.54	C, 55.32 H, 4.77 N, 18.86
3,4-Diethoxybenzaldehyde	190–191	$C_{16}H_{18}N_4O_4$	C, 58.17 H, 5.49 N, 16.96	C, 58.02 H, 5.53 N, 16.67
Phthalaldehydic acid	273–275	$C_{13}H_{10}N_4O_4$	C, 54.54 H, 3.52 N, 19.57	C, 54.18 H, 3.64 N, 19.40
2-Benzaldehyde sulfonic acid (Na)	>300	$C_{12}H_9N_4NaO_5S$	C, 41.85 H, 2.64 N, 16.25	C, 41.18 H, 2.88 N, 15.97
4-Dimethylaminobenzaldehyde	263–264	$C_{14}H_{15}N_5O_2$	C, 58.93 H, 5.30 N, 24.55	C, 58.53 H, 5.22 N, 24.53

(*Continued on next page*)

Table I—*(continued)*

Derivative of Aldehyde	M.p. °C.[a]	Empirical Formula	Anal., % Calcd.	Anal., % Found
4-Diethylaminobenzaldehyde	150–151	$C_{16}H_{19}N_5O_2$	C, 61.32 H, 6.11 N, 22.35	C, 61.56 H, 5.96 N, 22.47
4-Acetaminobenzaldehyde	273–275	$C_{14}H_{13}N_5O_3$	C, 56.25 H, 4.38 N, 23.41	C, 56.42 H, 4.58 N, 23.51
Furfural	213–215	$C_{10}H_8N_4O_3$	C, 51.72 H, 3.47 N, 24.13	C, 51.94 H, 3.43 N, 23.83
Piperonal	195–200	$C_{13}H_{10}N_4O_4$	C, 54.54 H, 3.52 N, 19.57	C, 54.20 H, 3.47 N, 19.20
Ribose	155 dec.	$C_{10}H_{14}N_4O_6$	C, 41.96 H, 4.93 N, 19.58	C, 41.66 H, 4.71 N, 19.09
Glucose	185–188	$C_{11}H_{16}N_4O_7$	C, 41.77 H, 5.10 N, 17.72	C, 41.70 H, 4.92 H, 17.35

[a] All melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus. [b] Degussa reported m.p. 226–228° (Beilstein, 4th ed., 2nd Suppl. vol. 22, p. 488).

testing procedures employed have been described previously (9). Among the fifty-one compounds, four derivatives, salicyaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, and 4-acetaminobenzaldehyde were found to exhibit significant inhibition against the S-180 mouse tumor system with the latter two compounds showing confirmed activity. The salicylaldehyde derivative showed slight activity against the adenocarcinoma 755 tumor in a dose of 450 mg./kg. None of the compounds in the series showed any significant or reproducible antitumor effects in the L-1210 mouse tumor system. The results of the biological tests supplied by the Cancer Chemotherapy National Service Center are summarized in Table II.

Table II—Antitumor Activity of Certain 5-Nitro-2-pyridylhydrazones against Sarcoma 180

Derivative	Dose, mg./kg.	Survivors, Mice	Animal wt. Difference, T/C	Tumor wt., mg., Test/Control	T/C, %
4-Nitrobenzaldehyde	250	4/6	−0.1	488/1730	28
	185	6/6	−1.3	317/1014	31
	82.2	5/6	−1.6	371/784	47
	41.1	5/6	−0.8	508/784	64
	20.5	6/6	−0.4	481/784	61
	10.2	6/6	0	696/784	88
4-Acetaminobenzaldehyde	562	4/6	−3.8	335/710	47
	500	6/6	−2.8	333/1371	24
	375	6/6	−4.4	342/710	48
	250	6/6	−3.4	159/710	22
	166	6/6	−3.1	547/710	77
4-Chlorobenzaldehyde	500	6/6	−2.2	442/1371	32
	500	6/6	−2.7	450/1064	42
Salicylaldehyde	350	6/6	−6.2	675/1852	36
	350	6/6	−5.7	362/1063	34

Antibacterial Activity—*In vitro* bacteriostatic studies were performed on the fifty-one compounds with *Staphylococcus aureus* FD 209 and with *Mycobacterium tuberculosis* B 103. None of the compounds exhibited any appreciable antibacterial activity. The highly active *p*-acetaminobenzaldehyde derivative was also studied by Y. T. Chang of the Laboratory of Pharmacology and Toxicology, National Institutes of Health, for its effect on rat leprosy in mice and found to be inactive. In the light of the results with the *p*-acetaminobenzaldehyde and *p*-nitrobenzaldehyde derivatives as antitumor agents a test of their effectiveness in clinical tests is indicated.

REFERENCES

(1) G. Domagk, R. Behnisch, F. Mietzsch, and H. Schmidt, *Naturwissenschaften*, **10,** 315(1946).

(2) B. S. Freedlander and A. Furst, *Proc. Soc. Exptl. Biol. Med.*, **81,** 638(1952).

(3) S. Petersen and G. Domagk, *Naturwissenschaften*, **41,** 10 (1954).

(4) Y. T. Chang, *Leprosy*, **25,** 130(1957).

(5) E. Jucker, *Angew. Chem.*, **71,** 317(1959).

(6) A. Mangini and B. Frenguelli, *Gazz. Chim. Ital.*, **69,** 97 (1939).

(7) B. Prescott, G. Caldes, W. R. Piggott, and W. B. Hill, *Chimica Therap.*, **2,** 228(1967).

(8) B. Prescott, G. Lones, and G. Caldes, *Intern. J. Clin. Pharmacol.*, **1,** 529(1968).

(9) J. Leiter, A. R. Bourke, S. A. Schepartz, and I. Wodinsky, *Cancer Res.*, **20,** 734(1960).

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