Antibacterial Nitrofuran Derivatives. I. 5-Nitro-2-furaldehyde Semicarbazones and Thiosemicarbazones

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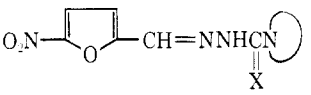
Received December 8, 1966

A series of 5-nitro-2-furaldehyde semicarbazones and thiosemicarbazones has been synthesized. All compounds exhibited an antibacterial activity *in vitro* comparable to that of 5-nitro-2-furaldehyde semicarbazone (**12**) and thiosemicarbazone (**13**). Several thiosemicarbazones had antifungal *in vitro* activity against *Trichophyton mentagrophytes* and *Candida albicans* while **13** had no activity. Compounds **2** and **9** showed significant *in vivo* activity in the mouse against *Micrococcus pyogenes*, whereas **12** was not active. Some other compounds were active against *Trypanosoma congolense in vivo*.

The antibacterial activity of 5-nitro-2-furaldehyde semicarbazone and the importance of the thiosemicarbazone group in chemotherapic agents have been known for some time.[1] Recently O'Sullivan, *et al.*, have studied modifications of antiviral activities in a series of compounds disubstituted on the terminal nitrogen atom of the side chain of isatin thiosemicarbazone.[2] The purpose of this paper was to synthesize a series of compounds with the following structure in order



X = O, S

to study the influence of substituents at the terminal nitrogen atom on antibacterial activity.

Chemistry.—5-Nitro-2-furaldehyde semicarbazones and thiosemicarbazones were prepared as usual. The preparation of aliphatic thiosemicarbazides had been described by O'Sullivan, *et al.*,[2] and N-heterocyclic thiocarbonylhydrazines had been prepared by Kazakov, *et al.*[3]

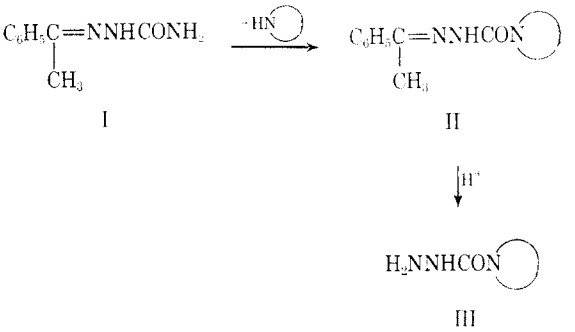
We have prepared all thiosemicarbazides in analogy to O'Sullivan's procedure, and the N'-methyl-N-piperazinothiocarbonylhydrazine by both procedures. The thiocarbamoylthioglycolic acids of diisobutylamine and diisopropylamine and acetophenone N-diisobutylaminothiocarbonylhydrazone have also been prepared, but we have not been able to obtain the corresponding thiosemicarbazides. All semicarbazides were prepared according to Scheme I, as described for the piperidine derivative.[4]

In synthesizing II from N-methylpiperazine, we have isolated a side product IV. Its structure was

IV

suggested by the observation[4] that an analogous product was formed when I was treated with piperidine in moist toluene.

SCHEME I



(1) H. H. Fox and E. G. Klarmann in "Medicinal Chemistry," A. Burger, Ed., Interscience Publishers Inc., New York, N. Y., 1960, pp 977, 980, 1135.

(2) D. G. O'Sullivan, P. W. Sadler, and C. Webley, *Chemotherapia*, **7**, 17 (1963).

(3) V. Ya Kazakov and I. Ya Postovskii, *Dokl. Akad. Nauk SSSR*, **134**, 824 (1960); *Chem. Abstr.*, **55**, 6483*b* (1961).

(4) J. M. Stratton and F. J. Wilson, *J. Chem. Soc.*, 1154 (1931).

Biological Results.—The acute toxicity was determined intraperitoneally in mice for all compounds and orally in rats for some of them (see Table II).

All compounds were tested for bacteriostatic activity *in vitro* on the following microorganisms: *Escherichia coli* 100, *Salmonella typhimurium* 1090, *Pseudomonas aeruginosa* H2, *Proteus vulgaris* OX, *Micrococcus pyogenes* SG511, *Streptococcus pyogenes* A88, *Bacillus subtilis* ATCC 9466, *Clostridium novyi*, *Mycobacterium tuberculosis* $H_{37}Ra$, *Trichophyton mentagrophytes* 1236, and *Candida albicans* 28. The results are summarized in Table I.

Some products were tested in mice infected septically with *S. pyogenes* C203, on peritonitis with *E. coli* 100, of subacute intramuscular staphylococcus infection on the leg, and on *Trypanosoma brucei* and *congolense*. The urinary elimination of some drugs was determined in rats (see Table II).

Some compounds exhibited antiinflammatory activity against formalin edema, but were ineffective as analgesics in Randall and Selitto's test.[5] No activity was observed when the compounds were screened for smooth muscle relaxing activity, for coronary vasodilatation, and for anticonvulsant activity.[6]

All compounds exhibited an antibacterial activity *in vitro* comparable to that of 5-nitro-2-furaldehyde semicarbazone (**12**) and 5-nitro-2-furaldehyde thiosemicarbazone (**13**). All compounds were compared for their activity *in vivo* with **12**.

The thiosemicarbazones **2**, **3**, **5**–**7** with the disubstituted terminal nitrogen atom exhibited antifungal activity against *T. mentagrophytes* and *C. albicans*.

(5) L. O. Randall and J. J. Selitto, *Arch. Intern. Pharmacodyn.*, **111**, 409 (1957).

(6) E. Massarani, D. Nardi, L. Degen, and M. J. Magistretti, *J. Med. Chem.*, **9**, 617 (1966).

TABLE I

MINIMAL INHIBITORY CONCENTRATION (μg/ml) OF 5-NITRO-2-FURALDEHYDE THIOSEMICARBAZONES AND SEMICARBAZONES

No.	*E. coli*	*S. typhimurium*	*Ps. aeruginosa*	*P. vulgaris*	*M. pyogenes*	*S. pyogenes*	*B. subtilis*	*C. novyi*	*M. tuberculosis*	*T. mentagrophytes*	*C. albicans*
1	10	20	80	40	10	40	5	80	40	80	100
2	5	100	100	100	10	20	10	80	100	40	40
3	40	100	100	100	20	40	20	100	100	40	40
4	10	100	100	100	20	80	10	80	100	100	100
5	5	80	80	80	5	20	5	80	100	40	40
6	2.5	20	100	20	2.5	40	2.5	100	100	80	80
7	5	20	100	20	10	40	5	100	100	10	80
8	20	100	100	100	20	100	10	40	10	100	100
9	20	100	100	100	20	80	10	10	100	100	100
10	20	40	100	100	20	20	10	80	100	100	100
11	10	40	100	100	40	5	40	100	100	100	100
12[a]	10	40	100	80	5	5	5	80	20	80	100
13[b]	5	10	100	40	2.5	20	1.25	80	10	100	100

[a] 5-Nitro-2-furaldehyde semicarbazone. [b] 5-Nitro-2-furaldehyde thiosemicarbazone.

TABLE II

ACTIVITY *in Vivo* OF 5-NITRO-2-FURALDEHYDE THIOSEMICARBAZONES AND SEMICARBAZONES

											Trypanosoma			
											brucei		*congolense*	
No.	LD_{50}, mg/kg ip (mice)	LD_{50}, mg/kg *po* (rats)	Antiinflam. act.,[a] mg/kg	*S. pyogenes* sepsis mM	Act.	*E. coli* peritonitis mM	Act.	*M. pyogenes* abscess mM	Act.[b]	Urinary elim., %	Suppressed	Cured	Suppressed	Cured
1	130	750	0	0.25	−	0.5	−	0.50	−	6.2[c]	1	0	*e*	5
2	450	750	50	0.5	−	0.75	−	0.75	+	0[d]	0	0	0	0
4	420	750	100			0.25	−	0.75	−	0[c,d]	*e*	2	2	0
5	42	750	0	0.14	−	0.25	−	0.14	±	0[d]	0	0	0	0
6	50		25					0.03	−	0[d]	0	0	0	0
7	200		50			0.25	−	0.12	−	0[d]	5	2	3	0
8	2400		25			0.5	−			0[d]	0	0	1	0
9	250		15			0.25	−	0.75	+	0[d]	0	0	0	0
10	150		20	0.28	−	0.25	−	0.28	−	0[d]	*e*	2	5	0
11	150	750	0	0.27	−	0.25	−			8.5[c]	0	0	5	0
12[f]	96	>2500		0.75	−	0.75	−	0.75	−	4.75[c]	5	3	5	4

[a] Dose which provoked a statistically significant diminution of edema over 3 hr. [b] + statistically significant, ± statistically insignificant. [c] Rat. [d] Mouse. [e] Not determined. [f] 5-Nitro-2-furaldehyde semicarbazone.

No activity was exhibited by the corresponding thiosemicarbazone **13**.

Compounds **2** and **9** were significally active on subacute intramuscular *M. pyogenes* infection of the mouse leg, whereas **12** was not active.

For *T. brucei*, **4**, **7**, and **10** possessed curative activity comparable with **12**; for *T. congolense*, **1** exhibited curative activity comparable with **12**, whereas **10** and **11** exhibited suppressive activity only.

Experimental Section[7]

Thiocarbamylthioglycolic Acids. Method A.—Carbon disulfide (7.6 g, 0.1 mole) was added dropwise to a solution of amine (0.1 mole) and KOH (5.6 g, 0.1 mole) in a water–ethanol (5:15 ml) mixture, keeping the temperature at 0°. Sodium chloroacetate (11.7 g, 0.1 mole) was then added and the mixture was left overnight at 25°. Addition of concentrated HCl precipitated the thiocarbamylthioglycolic acid, which was washed with water, dried, and crystallized.

In the case of N′-methyl-N-piperazine derivative, the reaction mixture was neutralized with HCl to pH 6.7, filtered with charcoal, and evaporated to dryness. The residue was extracted with hot 2-propanol and the solution was acidified with anhydrous HCl. On cooling, the N′-methyl-N-piperazinothiocarbamylthioglycolic acid hydrochloride crystallized (see Table III).

Acetophenone N′-Methyl-N-piperazinothiocarbonylhydrazone. Method B.—A mixture of 3-α-methylbenzylidenedithiocarbazate[3] (2.24 g, 0.01 mole), N-methylpiperazine (1 g, 0.01 mole), and methanol (25 ml) was refluxed for 15 hr. After cooling, the solution was filtered with charcoal and evaporated *in vacuo*, and the residue was crystallized (see Table IV).

Acetophenone N′-Methyl-N-piperazinocarbonylhydrazone. Method C.—A mixture of acetophenone semicarbazone (56.70 g, 0.32 mole), N-methylpiperazine (32 g, 0.32 mole), and xylene (160 ml) was refluxed for 20 hr. After cooling, the precipitate was filtered and crystallized (see Table IV). The acetophenone ε,N′-methyl-N-piperazinocarbonyl carbohydrazone (IV) was removed by filtration from hot benzene. It crystallized from ethanol, mp 226°.

Anal. Calcd for $C_{16}H_{22}N_6O_2$: C, 56.59; H, 6.97; N, 26.40. Found: C, 56.60; H, 7.15; N, 26.83.

N-Pyrrolidinothiocarbonylhydrazine. Method D.—A solution of N-pyrrolidinothiocarbamoylthioglycolic acid (20.5 g, 0.1 mole) in water (70 ml) containing NaOH (4 g, .1mole and hydrazine hydrate (20.02 g, 0.4 mole) was refluxed for 30 min. After cooling, the crystals were filtered, yield 9.8 g (68%), mp 177–178° (lit.[3] 163–165°). The **N-piperidinothiocarbonylhydrazine**[3] (yield 60%), and the **N-morpholinothiocarbonylhydrazine**[3] (yield 50%) were obtained by this procedure.

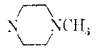
N′-Methyl-N-piperazinothiocarbonylhydrazine. Method E.—A mixture of acetophenone N′-methyl-N-piperazinothiocarbonylhydrazone (2.76 g, 0.01 mole) and 1% aqueous HCl (200 ml) was refluxed for 30 min. The solution was filtered with charcoal, cooled, made basic to pH 8 with dilute NaOH, and evaporated to dryness *in vacuo*. The residue was crystallized from 2-propanol by removing the inorganic salt by filtration (see Table V).

N-Pyrrolidinocarbonylhydrazine Hydrochloride. Method F.—Acetophenone N-pyrrolidinocarbonylhydrazone (1.15 g, 0.005 mole), when added to a hot 1% aqueous HCl (100 ml), dissolved immediately. The solution was filtered with charcoal and evaporated to dryness *in vacuo*. The residue was crystallized (see Table V).

-Nitro-2-furaldehyd Thi semicarbazone. Met'od G.—A solution of dimethylaminothiocarbonylhydrazine[2] (1.19 g, 0.01

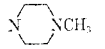
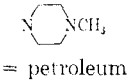
(7) All melting points are corrected and were determined on a Kofler Heiztischmikroskop melting point apparatus.

TABLE III
THIOCARBAMYLTHIOGLYCOLIC ACIDS
RCS_2CH_2COOH

R	Method	Yield, %	Solvent of crystn[a]	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
$N[CH(CH_3)_2]_2$	A	42	B	123	$C_9H_{17}NO_2S_2$	45.95	7.28	5.96	27.20	45.97	7.00	6.12	27.26
$N[CH_2CH(CH_3)_2]_2$	A	75	H	99	$C_{11}H_{21}NO_2S_2$	50.18	8.04	5.32	24.31	50.29	8.17	5.56	24.09
	A	60	I	203	$C_8H_{14}N_2O_2S_2 \cdot HCl$[b]	35.48	5.58	10.35	23.69	35.74	5.72	10.59	23.54

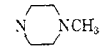
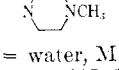
[a] B = benzene, H = hexane, I = 2-propanol. [b] *Anal.* Calcd: Cl, 13.10. Found: Cl, 13.58.

TABLE IV
ACETOPHENONE THIOSEMICARBAZONES AND SEMICARBAZONES
$C_6H_5C(CH_3)=NNHCXR$

X	R	Method	Yield, %	Solvent of crystn[a]	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S
S	$N[CH_2CH(CH_3)_2]_2$	B	78	P	61	$C_{17}H_{27}N_3S$	66.85	8.91	13.76	10.48	66.80	9.11	13.99	10.47
S		B	50	H	90	$C_{14}H_{20}N_4S$	60.85	7.30	20.28	11.58	60.87	7.32	20.67	11.59
O		C[b]	85	E	140	$C_{13}H_{17}N_3O$	67.50	7.41	18.17		67.26	7.62	18.47	
O		C[b]	65	E–W–B	150–151	$C_{13}H_{17}N_3O_2$	63.14	6.93	16.99		63.42	7.10	16.87	
O		C	50	B	129	$C_{14}H_{20}N_4O$	64.59	7.74	21.52		64.34	7.70	21.78	

[a] P = petroleum ether (bp 40–70°), H = hexane, E = ethanol, W = water, B = benzene. [b] The reaction was carried out for 40 hr.

TABLE V
THIOCARBAMYL- AND CARBAMYLHYDRAZINE
$R_2NNHCXR$

X	R	Method	Yield, %	Solvent of crystn[a]	Mp, °C	Formula	Calcd, % C	H	N	Cl	Found, % C	H	N	Cl
S		D[b]	89	B	155–156	$C_6H_{14}N_4S$	41.37	8.10	32.17		41.26	8.49	32.35	
		E	60	I										
O		F	85	E	192	$C_5H_{11}N_3O \cdot HCl$	36.25	7.30	25.37	21.41	36.35	7.42	25.56	21.30
O		F	65	E	200	$C_5H_{11}N_3O_2 \cdot HCl$	33.06	6.66	23.14	19.52	33.17	6.81	23.17	19.72
O		F	80	M–Et	207–208	$C_6H_{14}N_4O \cdot 2HCl$	31.18	6.97	24.74	30.68	31.07	7.20	24.82	30.57

[a] W = water, M = methanol, B = benzene, I = 2-propanol, E = ethanol, Et = ethyl ether. [b] The reaction was carried out at 60° with 2 moles of NaOH.

mole), in water (10 ml), was added to a solution of 5-nitro-2-furaldehyde (1.41 g, 0.01 mole) in ethanol (10 ml) and the mixture was stirred for 3 hr at 25°. After cooling the crystals were filtered and recrystallized (see Table VI).

5-Nitro-2-furaldehyde N-Pyrrolidinocarbonylhydrazone. Method H.—A solution of 5-nitro-2-furaldehyde (1.41 g, 0.01 mole) in ethanol (30 ml) was added to a solution of N-pyrrolidinocarbonylhydrazine hydrochloride (1.65 g, 0.01 mole) and sodium acetate hydrate (1.36 g, 0.01 mole) in water (5 ml). The mixture was refluxed for 1 hr. After cooling the crystals were collected and recrystallized (see Table VI).

Pharmacological Methods.—For all tests NMRI albino mice (18–20 g) and Wistar albino rats (200–250 g) were used. For trypanosomal infection, mice of 22–24 g were used. Acute toxicity, antinflammatory activity, and antimicrobial and antifungal activity *in vitro* (Table I) were determined as previously described.[6]

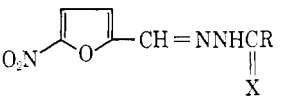
For antimicrobial methods *in vivo* (Table II) groups of ten mice or five rats were used. For trypanosomal infection groups of five mice were used.

(a) **Sepsis with *Streptococcus pyogenes* C 203.**—The infection was produced in mice by intraperitoneal injection of 0.5 ml of diluted 6-hr broth (Difco brain heart infusion broth + 10% rabbit blood) culture of *Streptococcus pyogenes*, β-hemolytic strain C203, containing 100 ID_{95} (infectious dose 95) with a mortality rate of 100% and an average survival time of 24 hr for nontreated control animals. Mice were treated by single oral intubation immediately after infection with the doses indicated in the table.

(b) **Peritonitis with *E. coli* 100.**—Mice were infected with 0.5 ml of diluted 5-hr broth culture, as in (a), of *E. coli* 100 containing about 10 ID_{95} with a mortality rate of 100% within 24 hr for the untreated control mice. Therapy was effected by oral intubation 3 hr before infection, immediately after infection, and 3 hr after infection with the total dose indicated in the table.

(c) **Subacute Intramuscular *M. pyogenes* SG511 Infection of the Mouse Leg.**—The test was performed by inoculating mice with 0.2 ml of the 1:2 dilution of the broth culture (18 hr at 37° in Difco brain heart infusion broth) of *M. pyogenes* 742 into the midthigh of one hind leg (muscular adductor magnus). Immediately thereafter 0.2 ml of the diluent was injected into the

TABLE VI
5-NITRO-2-FURALDEHYDE THIOSEMICARBAZONES AND SEMICARBAZONES



No.	X	R	Method	Yield, %	Solvent of crystn[a]	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Calcd, % S	Found, % C	Found, % H	Found, % N	Found, % S
1	S	$N(CH_3)_2$	G	87	A	165	$C_8H_{10}N_4O_3S$	39.67	4.16	23.14	13.24	39.72	4.47	23.09	13.00
2	S	$N(C_2H_5)_2$	G	85	E	145	$C_{10}H_{14}N_4O_3S$	44.44	5.22	20.73		44.39	4.98	20.50	
3	S	$N(C_3H_7)_2$	G	90	E	138	$C_{12}H_{18}N_4O_3S$	48.31	6.08	18.78	10.75	48.05	6.17	19.07	10.79
4	S		G	76	E	198–199	$C_{10}H_{12}N_4O_3S$	44.78	4.51	20.89	11.93	44.51	4.80	21.08	11.56
5	S		G	65	E	150	$C_{11}H_{14}N_4O_3S$	46.81	5.00	19.85	11.33	46.57	5.22	20.00	10.90
6	S		G	91	E	152	$C_{10}H_{12}N_4O_4S$	42.25	4.26	19.71	11.26	42.39	4.40	19.88	11.23
7	S		G[b]	76	I	162–163	$C_{11}H_{16}N_6O_3S$	44.44	5.09	23.56	10.76	44.47	5.29	23.47	10.78
8	O		H	95	E	226–227	$C_{10}H_{12}N_4O_4$	47.24	5.55	22.04		47.33	5.37	21.98	
9	O		H	100	E	177–178	$C_{11}H_{14}N_4O_4$	49.62	5.30	21.04		49.92	5.37	21.07	
10	O		H	80	A	205–206	$C_{10}H_{12}N_4O_5$	44.71	4.51	20.89		44.68	4.71	20.62	
11	O		H[c]	60	A	188	$C_{11}H_{16}N_6O_4$	46.97	5.38	24.90		47.13	5.48	25.05	

[a] A = ethyl acetate, E = ethanol, I = 2-propanol. [b] The reaction was carried out in anhydrous ethanol. [c] The reaction mixture was evaporated to dryness *in vacuo* and the residue was crystallized from ethyl acetate by removing the inorganic salt.

muscle of the opposite leg. The legs were measured at the point of maximal swelling by means of a caliper. The mean of the differences of the leg diameters was plotted daily. Mice were treated by daily oral intubation with the doses indicated in the table.

(d) **Urinary Elimination of the Drug.**—Urinary levels were determined in rats or in mice as follows. The urine of each mouse was collected with a 5-mm filter paper disk at 30, 60, 90, 120, 180, 240, and 300 min after single oral dose of 0.5 mmole of the drugs. The relative drug concentration was estimated evaluating the inhibition zones of the paper disks on agar plates inoculated with *B. subtilis*. For quantitative determinations, urine samples were assayed for drug concentrations by the method of the U. S. Pharmacopeia, Vol. XVII for antibiotics (cylinder cup method). Each drug was used as its own standard.

(e) **Antitrypanosomal Activity.**—The tests were performed with 22–24 g mice, infected with *T. brucei* or *congolense* by the method of Hawking.[8] Groups of five mice were treated by a single subcutaneous dose of $0.5DL_{50}$. When the trypanosomes disappeared from the blood permanently (more than 30 days), the animals were classified as "cured"; when the trypanosomes disappeared and then reappeared within the period of observation, the animals were classified as "suppressed."

(8) F. Hawking, *Exptl. Chemotherapy*, **1**, 137 (1963).

Insect Sex Attractants. VI. 7-Dodecen-1-ol Acetates and Congeners[1]

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Received December 14, 1966
Revised Manuscript Received March 6, 1967

cis- and *trans*-7-dodecen-1-ol acetates and several congeners were synthesized. Prior work demonstrating the *cis* isomer to be very attractive to male cabbage looper moths, *Trichoplusia ni* (Hübner), was confirmed. The other compounds were inactive.

The activity of certain insect attractants has been shown to depend on their stereochemical configuration. For example, the attractancy of *sec*-butyl *trans*-6-methyl-3-cyclohexene-1-carboxylate for the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is greatly superior to that of the *cis* isomer,[2,3] and the four *trans* isomers of trimedlure run the gamut from inactive to highly potent.[4]

When the sex attractant of the cabbage looper, *Trichoplusia ni* (Hübner), was revealed by Berger to be *cis*-7-dodecen-1-ol acetate,[5] we decided to prepare this compound to evaluate its attractancy. The synthesis of several analogs was undertaken in order to explore their attractancy–structure relationships.[6]

To prepare *cis*-7-dodecen-1-ol acetate, Berger first coupled 1-hexyne with 1-chloro-5-iodopentane in liquid

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