Antiviral Chemotherapy. I. The Activity of Pyridine and Quinoline Derivatives against Neurovaccinia in Mice

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Oral, in contrast to subcutaneous, administration of 4-formylpyridine thiosemicarbazone or 4-formylquinoline thiosemicarbazone affords marked protection to mice infected intracerebrally with the I.H.D. strain of neurovaccinia. It is suggested that this dependence on the route of administration may be due to the formation of an active metabolite in the gut. 3-Mercapto-5-(4-pyridyl)-1,2,4(H)-triazole, which could be formed by oxidative cyclization of 4-formylpyridine thiosemicarbazone, is also active orally but not subcutaneously, and the possibility of its being an active metabolite is discussed. No significant activity was found in derivatives of formylpyridine or formylquinoline thiosemicarbazones or in a series of triazoles.

The antiviral activity of a thiosemicarbazone *in vivo* was first reported by Hamre, *et al.*,[1] who found that *p*-aminobenzaldehyde thiosemicarbazone prolonged the survival time of mice infected intranasally with vaccinia virus. Thompson, *et al.*,[2] found that several heterocyclic aldehyde thiosemicarbazones had a similar action. Bauer[3] showed that isatin β-thiosemicarbazone afforded high protection when administered subcutaneously to mice infected intracerebrally with a neurotropic strain of vaccinia. Bauer and Sadler[4] examined structure–activity correlations in the isatin series and concluded that N-ethylisatin β-thiosemicarbazone was the most active compound against vaccinia. However, against *Variola major* in mice, N-methylisatin β-thiosemicarbazone appeared to be the most active[5] and, when administered orally, was reported to have prophylactic properties against human smallpox.[6] However, Ferguson[7] could not confirm this activity.

On the assumption that an orally administered drug would be preferable for clinical use, we reinvestigated the earlier literature work and found that 4-formylpyridine thiosemicarbazone and 4-formylquinoline thiosemicarbazone were the most active compounds against vaccinia in mice when given orally. In fact, the former was twenty times more active than N-ethylisatin β-thiosemicarbazone on a weight basis (Table I).

The high degree of protection afforded by these compounds prompted us to synthesize and test some nuclear-substituted derivatives (Table II). In general, it was found that nuclear substitution reduced or abolished antiviral activity. This parallels the observations on isatin thiosemicarbazones where substitution in the benzene ring is dystherapeutic.[4] Substitution of the H atom in the formyl group by methyl (**9**) reduces activity while larger groups abolish it completely (**10** and **11**) (Table II).

The relative positions of the thiosemicarbazone moiety and the ring nitrogen are critical as 3-formylpyridine thiosemicarbazone (**2**) is only marginally active and 2-formylpyridine thiosemicarbazone (**1**) is inactive. Furthermore, position isomers of 4-formylquinoline thiosemicarbazone (**12** and **14**–**16**) are inactive.

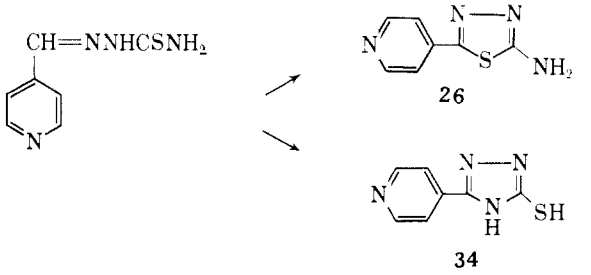
TABLE I

COMPARATIVE EFFECTS OF 4-FORMYLPYRIDINE THIOSEMICARBAZONE AND N-ETHYLISATIN β-THIOSEMICARBAZONE ON NEUROVACCINIA INFECTIONS IN MICE[a]

Compound	Daily dose, mg./kg.	Route	Mean survival time, days	Survivors (after 14 days)
N-Ethylisatin β-thiosemicarbazone	1.0	*p.o.*	10.2	5/9
	0.05	*p.o.*	4.0	0/10
	0.05	s.c.	13.1	9/10
4-Formylpyridine thiosemicarbazone	0.05	*p.o.*	10.3	6/10
	0.05	s.c.	4.0	0/9
Controls	0.0	...	3.8	0/10

[a] Virus dilution: 10^{-3}.

A significant feature of 4-formylpyridine thiosemicarbazone is that although possessing activity when administered orally, it is virtually inactive when administered subcutaneously. A possible explanation is that the compound is metabolized during absorption through the gut and that the metabolite is, in fact, the reactive entity. Two possible metabolites are the aminothiadiazole (**26**) and the mercaptotriazole (**34**) formed from the thiosemicarbazone by oxidative cyclization.



The aminothiadiazole (**26**) was reported active against vaccinia by Sadler,[8] who claimed to have prepared this compound by ferric chloride oxidation of 4-formylpyridine thiosemicarbazone. In our hands, this procedure afforded only the hydrochloride of the starting material, although the analogous 2-amino-5-

(1) D. Hamre, J. Bernstein, and R. Donovick, *Proc. Soc. Exptl. Biol. Med.*, **73**, 275 (1950).
(2) R. L. Thompson, S. A. Minton, J. E. Officer, and G. H. Hitchings, *J. Immunol.*, **70**, 229 (1953).
(3) D. J. Bauer, *Brit. J. Exptl. Pathol.*, **36**, 105 (1955).
(4) D. J. Bauer and P. W. Sadler, *Brit. J. Pharmacol.*, **15**, 101 (1960).
(5) D. J. Bauer, K. R. Dumbell, P. Fox-Hulme, and P. W. Sadler, *Bull. World Health Organ.*, **26**, 727 (1962).
(6) D. J. Bauer, A. W. Downie, C. H. Kempe, and L. St. Vincent, *Lancet*, ii, 494 (1963).
(7) D. L. Ferguson, *S. African Med. J.*, **38**, 868 (1964).
(8) P. W. Sadler, *J. Org. Chem.*, **26**, 1315 (1961).

TABLE II

THE ACTIVITY OF THIOSEMICARBAZONES AND RELATED COMPOUNDS AGAINST NEUROVACCINIA IN MICE

No.	Compound	Oral LD_{50}, mg./g.	Daily dose, (mg./g.) × 4	Mean survival time, days: Treated	Mean survival time, days: Controls	Activity[a]
1	2-Formylpyridine thiosemicarbazone[b]	0.07	0.02	3.8	3.8	0
2	3-Formylpyridine thiosemicarbazone[c]	0.20	0.05	5.8	3.8	+
3	4-Formylpyridine thiosemicarbazone[d]	0.20	0.05	10.3	3.8	++
4	2-Formyl-3-hydroxypyridine thiosemicarbazone[e]	0.93	0.23	2.4	7.4	0
5	4-Formyl-3-hydroxypyridine thiosemicarbazone[e]	1.00	0.25	4.9	8.0	0
6	4-Formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridine thiosemicarbazone[f]	>4.00	1.00	5.7	7.4	0
7	4-Formyl-3-methylpyridine thiosemicarbazone	0.60	0.15	10.0	7.4	+
8	2-Ethyl-4-formylpyridine thiosemicarbazone	0.32	0.08	4.2	3.8	0
9	Methyl 4-pyridyl ketone thiosemicarbazone[g]	0.027	0.006	8.4	5.3	+
10	*n*-Hexyl 4-pyridyl ketone thiosemicarbazone	0.20	0.05	5.8	5.1	0
11	Benzyl 4-pyridyl ketone thiosemicarbazone	0.45	0.11	5.0	5.1	0
12	2-Formylquinoline thiosemicarbazone[h]	>4.00	1.00	5.6	4.2	0
13	4-Formylquinoline thiosemicarbazone[h]	>4.00	1.00	11.3	4.1	++
14	6-Formylquinoline thiosemicarbazone[i]	0.16	0.04	4.1	4.2	0
15	7-Formylquinoline thiosemicarbazone[i]	0.04	0.01	5.7	5.3	0
16	8-Formylquinoline thiosemicarbazone[i]	3.80	0.90	3.3	5.4	0
17	4-Benzyloxy-2-formylquinoline thiosemicarbazone	>4.00	1.00	5.1	5.4	0
18	6-Chloro-4-formylquinoline thiosemicarbazone	1.00	0.25	3.9	4.2	0
19	7-Chloro-4-formylquinoline thiosemicarbazone	1.26	0.30	7.1	5.1	0
20	8-Chloro-4-formylquinoline thiosemicarbazone	1.71	0.42	6.7	5.1	0
21	4-Formyl-6-methoxyquinoline thiosemicarbazone	>4.00	1.00	5.1	4.9	0
22	4-Formyl-8-nitroquinoline thiosemicarbazone	>4.00	1.00	9.4	4.9	++
23	3-Formylisoquinoline thiosemicarbazone	>4.00	1.00	8.1	4.9	+
24	2-Amino-5-(2-pyridyl)-1,3,4-thiadiazole hydrochloride[k]	1.20	0.30	3.7	5.4	0
25	2-Amino-5-(3-pyridyl)-1,3,4-thiadiazole dihydrochloride[k]	1.26[l]	0.30[l]	5.0	4.2	0
26	2-Amino-5-(4-pyridyl)-1,3,4-thiadiazole dihydrochloride	0.50	0.10	4.2	4.8	0
		0.20[l]	0.05[l]	4.2	4.5	0
27	2-Acetamido-5-(4-pyridyl)-1,3,4-thiadiazole[k]	1.10	0.25	1.6	5.1	0
28	2-Amino-5-(4-quinolyl)-1,3,4-thiadiazole hydrochloride	0.045[l]	0.01[l]	5.1	5.5	0
29	5-(4-Pyridyl)-1,2,4(H)-triazole	0.79	0.20	5.2	5.7	0
30	3-Amino-5-(4-pyridyl)-1,2,4(H)-triazole[m]	0.68	0.17	4.9	4.6	0
31	3-Hydroxy-5-(4-pyridyl)-1,2,4(H)-triazole	0.58	0.15	8.0	5.7	+
32	3-Mercapto-5-(2-pyridyl)-1,2,4-(H)-triazole	0.44	0.11	4.5	4.8	0
33	3-Mercapto-5-(3-pyridyl)-1,2,4(H)-triazole	>4.00	1.00	6.2	8.0	0
34	3-Mercapto-5-(4-pyridyl)-1,2,4(H)-triazole[n]	>4.00	1.00	10.00	4.9	++
		>4.00[l]	0.5[l]	3.5	5.0	0
35	3-Mercapto-5-(4-quinolyl)-1,2,4(H)-triazole	>4.00	1.00	7.4	5.7	0
36	3-Mercapto-5-(2-ethyl-4-pyridyl)-1,2,4(H)-triazole	>4.00	1.00	5.1	4.8	0
37	3-Methylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole[o]	1.71	0.40	4.7	4.8	0
38	3-Ethylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole	2.00	0.50	4.7	4.8	0
39	3-*n*-Propylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole	1.7	0.40	6.8	4.8	0
40	3-*n*-Hexylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole	>4.00	1.00	4.7	6.0	0
41	3-Allylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole	2.33	0.60	7.8	6.0	0
42	3-Benzylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole	>4.00	1.00	5.4	4.8	0
43	3-(2-Pyridylmercapto)-5-(4-pyridyl)-1,2,4(H)-triazole	1.47	0.36	7.8	6.0	0
44	3-Acetonylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole	0.50	0.12	4.7	4.8	0
45	3-Carboxymethylmercapto-5-(4-pyridyl)-1,2,4(H)-triazole	>4.00	1.00	8.3	6.0	0
46	3-(2-Dimethylaminoethyl)-5-(4-pyridyl)-1,2,4(H)-triazole	0.36	0.09	5.6	6.0	0
47	3-Mercapto-4-methyl-5-(3-pyridyl)-1,2,4-triazole	0.85	0.21	7.0	5.1	0
48	3-Mercapto-4-methyl-5-(4-pyridyl)-1,2,4-triazole	1.10	0.25	5.1	5.7	0
49	4-Amino-3,5-dimethylmercapto-1,2,4-triazole[p]	3.80	0.90	4.4	6.0	0

[a] 0 = inactive, + = marginal activity, ++ = active (more than doubles the mean survival time). [b] R. E. Hagenbach and H. Gysin, *Experientia*, **8**, 184 (1952). [c] C. Levaditi, A. Girard, A. Vaisman, and A. Ray, *Compt. rend.*, **231**, 1174 (1950). [d] E. Grunberg and B. Leiwant, *Proc. Soc. Exptl. Biol. Med.*, **77**, 47 (1951). [e] D. Heinert and A. E. Martell, *Tetrahedron*, **3**, 49 (1958). [f] P. P. T. Sah and C. T. Peng, *Arch. Pharm.*, **293**, 501 (1960). [g] H. H. Fox, *J. Org. Chem.*, **17**, 555 (1952). [h] E. Hoggarth, A. R. Martin, N. L. Storey, and E. H. P. Young, *Brit. J. Pharmacol.*, **4**, 248 (1949). [i] J. Bernstein, H. L. Yale, K. Losee, M. Holsing, J. Martins, and W. A. Lott, *J. Am. Chem. Soc.*, **73**, 906 (1951). [j] F. Gialdi and R. Ponci, *Farm. sci. tec.* (Pavia), **6**, 332 (1951). [k] Ref. 9. [l] Subcutaneous administration. [m] R. Giuliano and G. Leonardi, *Farmaco* (Pavia), *Ed. sci.*, **9**, 529 (1954). [n] Ref. 11. [o] S. Yoshida and M. Asai, *J. Pharm. Soc. Japan*, **74**, 946 (1954). [p] J. Sandstrom, *Acta Chem. Scand.*, **15**, 1295 (1961).

(4-quinolyl)-**1**,3,4-thiadiazole was readily prepared by this method from 4-formylquinoline thiosemicarbazone. Authentic 2-amino-5-(4-pyridyl)-1,3,4-thiadiazole (**26**) prepared by peracid oxidation of 4-formylpyridine N,S-diacetylthiosemicarbazone[9] was completely inactive as was the quinoline analog (**28**). The mercaptotriazole (**34**) was active orally but inactive subcutaneously. It is therefore unlikely to be an active metabolite which, it is reasonable to suppose, would be active by both routes of administration. However, the possibilities remain that the mercaptotriazole (**34**) is either further metabolized in the gut to a more active

(9) P. Hemmerich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta*, **41**, 2058 (1958).

TABLE III

FORMYLQUINOLINE THIOSEMICARBAZONES

No.	Compound	M.p., °C.	Formula	Calcd., % N	Calcd., % S	Found, % N	Found, % S
17	4-Benzyloxy-2-formylquinoline thiosemicarbazone[a]	216–220	$C_{18}H_{16}N_4OS$	16.7	9.5	16.5	9.8
18	6-Chloro-4-formylquinoline thiosemicarbazone[a,b]	240–244	$C_{11}H_8N_4S \cdot HCl$	21.2	12.1	20.9	11.9
19	7-Chloro-4-formylquinoline thiosemicarbazone[b,c]	251–252	$C_{11}H_8N_4S \cdot HCl$	21.2	12.1	21.3	12.0
20	8-Chloro-4-formylquinoline thiosemicarbazone[a,b]	234–236	$C_{11}H_8N_4S \cdot HCl$	21.2	12.1	21.4	12.0
21	4-Formyl-6-methoxyquinoline thiosemicarbazone[c]	238–240	$C_{12}H_{12}N_4OS$	21.5	12.3	20.8	12.6
22	4-Formyl-8-nitroquinoline thiosemicarbazone[a]	240–245	$C_{11}H_9N_5O_2S$	25.5	11.6	25.2	11.9
23	3-Formylisoquinoline thiosemicarbazone[c]	238–240	$C_{12}H_{12}N_4OS$	21.5	12.3	20.8	12.6

[a] The aldehydes were prepared by SeO_2 oxidation of the appropriate methylquinoline by method I. [b] Hydrochloride. [c] The aldehydes were prepared by method II.

entity or merely reverts to the parent thiosemicarbazone **3**. The difference in biological activity of **3** and **34** by the two routes of administration is not considered to be a solubility effect because the very insoluble N-ethylisatin thiosemicarbazone displays greater activity subcutaneously than orally (Table I).

In the hope of developing any intrinsic antiviral activity in the mercaptotriazole series, many structural modifications were made (**29–49**). All were found to be inactive with the exception of 3-hydroxy-5-(4-pyridyl)-1,2,4(H)-triazole (**31**) which displayed marginal activity.

Experimental

Biological Methods.—All experiments were carried out with the I.H.D. strain of neurovaccinia; the mice were of an inbred albino strain bred in these laboratories. Under light ether anesthesia, 0.03 ml. of an appropriate dilution of virus was injected intracerebrally, and the daily mortalities were recorded. Animals dying within 24 hr. of infection were not included in the experiment.

The compounds examined were administered orally in a volume of 0.2 ml. once daily for 4 days, commencing at the time of infection.

For subcutaneous administration, insoluble compounds were finely ground in a mechanical Teflon grinder and then suspended in a suitable volume of 2.5% gum tragacanth mucilage.

Syntheses.[10]—All melting points were determined on an Electrothermal instrument and are corrected.

4-Formyl-3-methylpyridine Thiosemicarbazone (**7**).—A mixture of 3,4-dimethylpyridine (20.0 g., 0.2 mole), thiosemicarbazide (18.0 g., 0.2 mole), and sulfur (18.5 g., 0.6 mole) in xylene (100 ml.) was refluxed for 5 hr. The solvent was removed by steam distillation, and the cooled residue was filtered. The filter cake was stirred with 2 *N* NaOH (250 ml.) for 30 min. and filtered. The pH of the filtrate was adjusted to 6 with 2 *N* acetic acid to give 6.6 g. (17%) of product, m.p. 242–244°.

Anal. Calcd. for $C_8H_{10}N_4S$: N, 28.8; S, 16.5. Found: N, 29.4; S, 16.5.

2-Ethyl-4-formylpyridine Thiosemicarbazone (**8**).—Benzenesulfonyl chloride (68 g.) was added during 1 hr. to a solution of 2-ethylpyridine 4-carboxyhydrazide (57 g.) in pyridine (500 ml.) at 0°. After 2 hr. the mixture was poured onto ice to give 2-ethylisonicotinic acid 2-(phenylsulfonyl)hydrazide (100 g., 95%), m.p. 155° (from ethanol).

Anal. Calcd. for $C_{14}H_{15}N_3O_3S$: N, 13.8; S, 10.5. Found: N, 13.8; S, 10.2.

An intimately ground mixture of 2-ethylisonicotinic acid 2-(phenylsulfonyl)hydrazide (12.5 g., 0.041 mole), anhydrous Na_2CO_3 (12.0 g., 0.145 mole), and thiosemicarbazide (4.3 g., 0.041 mole) in glycerol (50 ml.) was stirred vigorously and heated to 150° for 2 min. The mixture was cooled and filtered. Dilution of the filtrate with an equal volume of water afforded 2.2 g. (26%) of the thiosemicarbazone, m.p. 218–222°.

Anal. Calcd. for $C_9H_{12}N_4S$: C, 51.9; H, 5.8; N, 26.9. Found: C, 51.7; H, 5.9; N, 27.3.

Benzyl 4-Pyridyl Ketone Thiosemicarbazone (**11**).—A solution of 4-cyanopyridine (20.8 g., 0.2 mole) in dry ether (100 ml.) was added dropwise during 30 min. to a stirred, refluxing solution of benzylmagnesium bromide [prepared from 76.8 g. (0.45 mole) of benzyl bromide and 11.4 g. (0.45 g.-atom) of magnesium]. The mixture was refluxed for 3 hr., cooled, and added cautiously to ice-cold 2 *N* H_2SO_4 (500 ml.). The ether layer was separated, and the pH of the aqueous layer was adjusted to 11 with 2 *N* NaOH solution. Ether extraction and fractional distillation afforded 11.5 g. (29%) of benzyl 4-pyridyl ketone, m.p. 90–91°, b.p. 194–196° (15 mm.). The ketone was refluxed with thiosemicarbazide (5.3 g.) in ethanol (200 ml.) for 3 hr. Cooling gave 13.1 g. (83%) of the thiosemicarbazone, m.p. 213–214°.

Anal. Calcd. for $C_{14}H_{14}N_4S$: C, 62.3; H, 5.5; S, 11.8. Found: C, 62.3; H, 5.2; S, 11.8.

***n*-Hexyl 4-Pyridyl Ketone Thiosemicarbazone** (**10**).—Similarly 4-cyanopyridine and *n*-hexylmagnesium bromide gave *n*-hexyl 4-pyridyl ketone (33%), b.p. 175–179° (24 mm.). The thiosemicarbazone crystallized in needles from ethanol, m.p. 143–144°.

Anal. Calcd. for $C_{13}H_{20}N_4S$: C, 59.1; H, 7.6; N, 21.2; S, 12.1. Found: C, 59.2; H, 7.8; N, 20.9; S, 12.1.

Oxidation of Methylquinolines to Formylquinolines. Method I.—A mixture of equimolecular quantities of the methylquinoline and freshly prepared SeO_2 in 5% aqueous dioxane was refluxed for 2 hr. The precipitated selenium was filtered off, and the filtrate was evaporated to dryness *in vacuo*.

Method II.—Equimolar quantities of the methylquinoline and SeO_2 were heated to 130–140° when a vigorous reaction occurred. Heating was continued for 10 min. and the mixture was cooled. The residues from each method were then stirred with 2 *N* HCl for 30 min. and filtered (charcoal). Treatment of the filtrates with 2 *N* Na_2CO_3 afforded the crude aldehydes.

Formylquinoline Thiosemicarbazones.—The crude aldehydes were converted into the thiosemicarbazones which were purified by crystallization from, or boiling with, ethanol (yields 70–90%). The products are listed in Table III.

Ferric Chloride Oxidation of Formylpyridine Thiosemicarbazones.—A mixture of 4-formylpyridine thiosemicarbazone (15.9 g.) and ferric chloride (30 g.) in water (300 ml.) was heated at 85° for 30 min. The reaction mixture was concentrated to *ca.* 100 ml. and cooled to give a solid (9.4 g.), m.p. 260–262° dec. (lit.[8] m.p. 260°), which did not depress the melting point of authentic 4-formylpyridine thiosemicarbazone hydrochloride. Treatment of the solid with 2 *N* NH_4OH gave a yellow product (6.5 g.), m.p. 236° undepressed by authentic 4-formylpyridine thiosemicarbazone (the product obtained by Sadler[8] had m.p. 226°). Authentic 2-amino-5-(4-pyridyl)-1,3,4-thiadiazole[9] had m.p. 249–251°.

Similar ferric chloride treatment of 3-formylpyridine thiosemicarbazone afforded 3-formylpyridine thiosemicarbazone hydrochloride while the 2-isomer immediately formed an intractable tar.

2-Amino-5-(4-pyridyl)-1,3,4-thiadiazole Dihydrochloride (**26**).—2-Amino-5-(4-pyridyl)-1,3,4-thiadiazole[9] was dissolved in the minimum volume of concentrated HCl and diluted with a large volume of acetone to give the product, m.p. 287–292° dec.

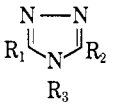
Anal. Calcd. for $C_7H_6N_4S \cdot 2HCl$: C, 33.3; H, 3.2; Cl, 28.2; S, 12.8. Found: C, 33.0; H, 3.3; Cl, 28.0; S, 12.9.

2-Amino-5-(4-quinolyl)-1,3,4-thiadiazole Hydrochloride (**28**). **Method I.**—4-Formylquinoline thiosemicarbazone (23 g.) was refluxed with acetic anhydride (50 ml.) for 30 min., cooled, and poured onto ice-cold saturated $NaHCO_3$ solution. The resulting 4-formylquinoline N,S-diacetylthiosemicarbazone was separated

(10) Microanalyses were performed by Mr. S. Bance and his staff.

TABLE IV

1,2,4-TRIAZOLES



No.	R_1	R_2	R_3	Yield, %	M.p., °C.	Formula	Calcd., % C	H	N	S	Found, % C	H	N	S
29	4-Pyridyl	H	H	36	226–227	$C_7H_6N_4$	57.5	4.1	38.4	...	57.4	3.8	38.6	...
31	4-Pyridyl	OH	H	41	262–263	$C_7H_6N_4O$	51.9	3.7	34.5	...	51.9	4.0	34.4	...
32	2-Pyridyl	SH	H	49	282–285	$C_7H_6N_4S$	47.2	3.4		18.0	47.2	3.6		17.6
33	3-Pyridyl	SH	H	42	299–304	$C_7H_6N_4S$	47.2	3.4	31.4	18.0	47.2	3.6	31.3	18.0
35	4-Quinolyl	SH	H	81	290–300	$C_{11}H_8N_4S$			24.6	14.1			24.4	13.8
36	2-Ethyl-4-pyridyl	SH	H	63	314–316	$C_9H_{10}N_4S$			27.2	15.6			27.0	15.3
38	4-Pyridyl	C_2H_5S	H	43	182–183	$C_9H_{10}N_4S$	52.4	4.9	27.2	15.6	52.7	5.5	27.6	15.8
39	4-Pyridyl	C_3H_7S	H	55	158–161	$C_{10}H_{12}N_4S$	54.5	5.5	25.4	14.6	54.1	5.3	24.9	14.7
40	4-Pyridyl	$CH_3(CH_2)_5S$	H	37	111–113	$C_{13}H_{18}N_4S$	59.5	6.9	21.4	12.2	59.6	6.4	21.4	12.1
41	4-Pyridyl	$CH_2{=}CHCH_2S$	H	60	149–151	$C_{10}H_{10}N_4S$	55.0	4.6	25.7	14.7	55.4	4.8	25.9	14.7
42	4-Pyridyl	$C_6H_5CH_2S$	H	34	188–190	$C_{14}H_{12}N_4S$	62.7	4.5	20.9	12.0	62.7	4.8	20.9	12.0
43	4-Pyridyl	2-PyridylS	H	62	188–190	$C_{12}H_9N_5S$			27.4	12.6			27.4	12.7
44	4-Pyridyl	CH_3COCH_2S	H	27	214–216	$C_{10}H_{10}N_4OS\cdot HCl$[a]	44.1	4.1	20.7	11.8	44.5	4.6	20.7	11.8
45	4-Pyridyl	$HO_2C\cdot CH_2S$	H	45	238	$C_9H_8N_4O_2S$	45.8	3.4	23.7	13.6	45.4	3.4	23.8	13.7
46	4-Pyridyl	$(CH_3)_2NCH_2CH_2S$	H	18	200–201	$C_{11}H_{15}N_5S\cdot HCl$[a]	46.2	5.9		10.8	46.2	5.6		11.2
47	3-Pyridyl	SH	CH_3	69	183–184	$C_8H_8N_4S$			29.1	16.7			28.7	16.5
48	4-Pyridyl	SH	CH_3	63	287	$C_8H_8N_4S$	50.0	4.2	29.1	16.7	49.8	4.5	29.0	16.6

[a] Hydrochloride.

and crystallized from dimethylformamide as colorless prisms (26.3 g., 83%), m.p. 228–231°.

Anal. Calcd. for $C_{15}H_{15}N_4O_2S$: N, 17.8; S, 10.2. Found: N, 17.6; S, 10.4.

4-Formylquinoline N,S-diacetylthiosemicarbazone (4.8 g.) was added portionwise to a mixture of glacial acetic acid (25 ml.) and H_2O_2 (100 vol., 8.0 g.) at 0°. The mixture was allowed to warm to room temperature and then heated to 65–70° for 10 min. Cooling gave 2-acetamido-5-(4-quinolyl)-1,3,4-thiadiazole (3.1 g., 78%) as colorless prisms, m.p. 296–299°.

Anal. Calcd. for $C_{13}H_{10}N_4OS$: N, 20.7; S, 11.9. Found: N, 20.7; S, 11.8.

2-Acetamido-5-(4-quinolyl)-1,3,4-thiadiazole (3.1 g.) and concentrated HCl (50 ml.) were refluxed for 1.5 hr. The reaction mixture was cooled and made alkaline to give 2-amino-5-(4-quinolyl)-1,3,4-thiadiazole (1.06 g., 37%), m.p. 271–273°.

Anal. Calcd. for $C_{11}H_8N_4S$: C, 57.9; H, 3.5; N, 24.6; S, 14.0. Found: C, 57.9; H, 3.8; N, 24.2; S, 14.0.

The thiadiazole was dissolved in the minimum volume of concentrated HCl and diluted with a large volume of acetone to give 2-amino-5-(4-quinolyl)-1,3,4-thiadiazole hydrochloride, m.p. 258–260°.

Anal. Calcd. for $C_{11}H_8N_4S\cdot HCl$: C, 49.8; H, 3.4; Cl, 13.4; S, 12.1. Found: C, 49.6; H, 3.4; Cl, 13.6; S, 12.3.

Method II.—A mixture of 4-formylquinoline thiosemicarbazone (10 g.) and ferric chloride hexahydrate (49.0 g.) in water (200 ml.) were heated at 80° for 2 hr. Cooling afforded 2-amino-5-(4-quinolyl)-1,3,4-thiadiazole hydrochloride (6.7 g.), m.p. 259–261° undepressed on admixture with the product obtained by A.

1,2,4-Triazoles (Table IV).—The methods are illustrated by the following examples.

5-(4-Pyridyl)-1,2,4(H)-triazole (29).—A solution of 3-mercapto-5-(4-pyridyl)-1,2,4(H)-triazole[11] (20 g.) in 2 *N* NH_4OH (100 ml.) was added to a slurry of Raney nickel[12] (10 g.) in water (10 ml.), and the mixture was refluxed for 3 hr. The reaction mixture was cooled and filtered, and the filtrate was concentrated to dryness under reduced pressure. Crystallization of the residue from ethanol gave colorless prisms of the product (5.9 g., 36%), m.p. 226–227°.

3-Hydroxy-5-(4-pyridyl)-1,2,4(H)-triazole (31).—Isonicotinoyl S-methylthiosemicarbazide[13] (21.0 g.) was heated at 195–200° for 5 min. The cooled mass was crystallized from water to give the product as colorless needles (12.3 g., 80%), m.p. 262–263°.

3-Mercapto-4-methyl-5-(4-pyridyl)-1,2,4-triazole (48).—A mixture of methyl isothiocyanate (7.3 g., 0.1 mole) and pyridine 4-carboxyhydrazide (13.7 g., 0.1 mole) in ethanol (75 ml.) was refluxed with stirring for 2 hr. The mixture was filtered hot and the filtrate was then cooled to give isonicotinoyl 4-methylthiosemicarbazide (12.3 g., 80%), as colorless needles from water, m.p. 221–223° dec.

Anal. Calcd. for $C_8H_{10}N_4OS$: N, 26.7; S, 15.3. Found: N, 26.3; S, 15.1.

Isonicotinoyl 4-methylthiosemicarbazide (10.0 g., 0.048 mole) and sodium methoxide (3.0 g., 0.056 mole) in dry methanol (100 ml.) was refluxed for 8 hr. and then evaporated to dryness under reduced pressure, and the residual solid was dissolved in water. Acidification with acetic acid to pH 6.0 afforded 3-mercapto-4-methyl-5-(4-pyridyl)-1,2,4-triazole (6.1 g., 63%) which crystallized from aqueous Ethyl Cellosolve in colorless prisms, m.p. 287°.

3-Mercapto-4-methyl-5-(3-pyridyl)-1,2,4-triazole (47).—Similarly pyridine 3-carboxyhydrazide and methyl isothiocyanate afforded nicotinoyl 4-methylthiosemicarbazide, m.p. 180–183°.

Anal. Calcd. for $C_8H_{10}N_4OS$: N, 26.7; S, 15.3. Found: N, 26.1; S, 15.2.

Sodium methoxide treatment afforded the triazole (69%), m.p. 183–184°.

3-Mercapto-5-(4-quinolyl)-1,2,4(H)-triazole (35).—Potassium thiocyanate (46.5 g., 0.48 mole), quinoline 4-carboxyhydrazide (45 g., 0.24 mole), and 6 *N* HCl (300 ml.) were heated at 100° for 12 hr. Cooling afforded quinol-4-oyl thiosemicarbazide (49 g., 83%), m.p. 235–238° dec.

Anal. Calcd. for $C_{11}H_{10}N_4OS$: N, 22.7. Found: N, 22.7.

Quinol-4-oyl thiosemicarbazide (49 g.) was heated at 150° at 15 mm. for 15 min. to give 3-mercapto-5-(4-quinolyl)-1,2,4(H)-triazole (37.5 g., 81%), m.p. 290–300°.

3-Mercapto-5-(3-pyridyl)-1,2,4(H)-triazole (33).—Pyridine 3-carboxyhydrazide (13.7 g., 0.1 mole) and thiourea (7.6 g., 0.1 mole) were heated in an oil bath to 190° when an exothermic reaction occurred. The mixture was heated at 195–200° for a further 30 min., cooled, and extracted with 2 *N* NH_4OH solution (100 ml.). The extract was acidified with glacial acetic acid to give the product (7.5 g., 42%), m.p. 299–304°.

Compounds **32** and **36** were prepared similarly.

General Procedure for S-Substitution.—3-Mercapto-5-(4-pyridyl)-1,2,4(H)-triazole (0.1 mole) and the halide (0.15 mole)

(11) H. C. Beyerman, J. S. Bontekoe, W. J. Van Der Burg, and W. L. C. Veer, *Rec. trav. chim.*, **73**, 109 (1954).

(12) A. A. Pavlic and A. Adkins, *J. Am. Chem. Soc.*, **68**, 1471 (1946).

(13) S. Yoshida and M. Asai, *J. Pharm. Soc. Japan*, **74**, 946 (1954).

were refluxed in dry ethanol (100 ml.) until a clear solution was obtained. Excess ethanol and halide were distilled under reduced pressure, and the residue was extracted with water and filtered. Treatment of the filtrate with saturated $NaHCO_3$ afforded the product which was filtered off and crystallized from ethanol or aqueous ethanol. In two cases (**44** and **46**), the hydrochlorides of the product separated from the reaction mixture on cooling.

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Antiviral Chemotherapy. II. Structure–Activity Relationships in a Series of Isothiazolealdehyde and Ketone Thiosemicarbazones[1a]

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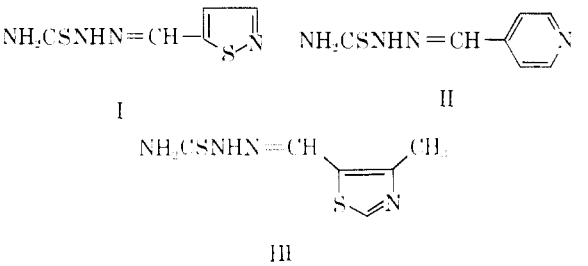
A series of isothiazolealdehyde and ketone thiosemicarbazones has been synthesized and tested for antiviral activity. Several of these compounds, in particular 4-bromo-5-formyl-3-methylisothiazole thiosemicarbazone,[1b] gave high protection to mice infected intracerebrally with the I.H.D. strain of neurovaccinia. Structure–activity relationships in the series are discussed.

We have reported previously[1a] that oral administration of 4-formylpyridine thiosemicarbazone affords marked protection to mice infected intracerebrally with neurovaccinia. It was further demonstrated that nuclear substitution abolished or reduced antiviral activity and that, of several possible metabolites, only 3-mercapto-5-(4-pyridyl)-1,2,4(H)-triazole had comparable activity. The present communication is concerned with another line of development, *viz.*, the synthesis and evaluation of thiosemicarbazones of heterocyclic carbonyl compounds chemically analogous to 4-formylpyridine thiosemicarbazone.

Our preliminary investigations showed that thiosemicarbazones derived from simple thiophene, furan, imidazole, and pyrazole carbonyl compounds were of little interest. Furthermore, 5-formyl-4-methylthiazole thiosemicarbazone, which was reported to be highly active against an intranasal infection of vaccinia in mice,[2] was only marginally active in our tests using the intracerebral route for infection. However, isothiazolecarbonyl thiosemicarbazones showed promising activity and accordingly a series was prepared and tested (Table I).

It is clear that antiviral activity is markedly dependent on the position of the thiosemicarbazone moiety in the isothiazole ring (compare **1**, **2**, and **3**). If isothiazole is regarded as electronically and geometrically related to pyridine, *e.g.*, the –S– of the former replacing –CH=CH– of the latter, activity in the two series follows a similar pattern. Thus, 5-formylisothiazole thiosemicarbazone (I) and the analogous 4-formylpyridine thiosemicarbazone (II) are both active, whereas thiosemicarbazones of 4-formylisothiazole and 3-formylpyridine are only weakly active, and 3-formylisothiazole and 2-formylpyridine thiosemicarbazones are totally inactive.[1a] The weak activity of the formylthiazole thiosemicarbazone (III) is explainable by its formal analogy to the weakly active 3-formylpyridine thiosemicarbazone.



I II

III

In the 5-formylisothiazole series, substitution by halogen in the 4-position increases activity (compare **2**, **11**, **12**, and **13**) with a decrease in toxicity, particularly the bromo derivative **12**. A 3-methyl substituent decreases toxicity slightly without decreasing activity (**2** and **4**), and a combination of a 3-methyl and a 4-chloro or -bromo substituent leads to highly active compounds with low toxicity (**15** and **16**). 4-Nitro and 4-methyl substituents have little effect on activity (compare **4** and **19**, and **2** and **5**) but a carboxy substituent (**18**) completely abolishes activity. Compounds substituted in the side chain are, in general, inactive (**7**, **8**, **21**, and **27–39**) with the exception of the highly active 5-acetyl-3-methyl derivative (**9**) and the marginally active 4-bromo-5-formyl-3-methylisothiazole 2-methylthiosemicarbazone (**26**). Cyclization of the side chain of an active thiosemicarbazone to a mercaptotriazole (compare **4** and **41**, and **16** and **42**) or an aminothiadiazole (compare **4** and **43**) considerably reduced or eliminated activity.

Six compounds (**9**, **11–13**, **15**, and **16**) satisfied our criterion for significant activity, namely, they more than doubled the mean survival time. Of these, three were of relatively low toxicity (**12**, **15**, and **16**) and one (**16**, M&B 7714) was eventually selected for more extended biological evaluation[3] and clinical trials against smallpox.[4]

No activity was shown by any of the thiosemicarbazones against murine infections of influenza virus,

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(2) E. Campaigne, R. L. Thompson, and J. E. Van Werth, *J. Med. Pharm. Chem.*, **1**, 577 (1959).

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