naphthyl)pentane], this compound was submitted to a more detailed pharmacological and toxicological study,[6-9] as well as to a preliminary clinical trial.[10]

(6) C. Bianchi, T. Bruzzese, S. Casadio, G. Coppi, G. Pala, G. P. Sanna, and C. Turba, *Experientia*, **23**, 243 (1967).
(7) C. Bianchi, G. P. Sanna, and C. Turba, *Arzneim. Forsch.*, **18**, 845 (1968).
(8) G. Coppi, G. Bonardi, and R. Perego, *ibid.*, **18**, 1343 (1968).
(9) G. Coppi, G. Bonardi, E. Marazzi-Uberti, and C. Bianchi, *ibid.*, **19**, 156 (1969).
(10) V. Casadio, E. Baldoni, and P. Serenthà, *Curr. Ther. Res. Clin. Exp.*, **9**, 429 (1967).

An investigation of other substances chemically related to the title compounds is also in progress, in order to shed more light on the structure–antiarrhythmic activity relationships.

Acknowledgments.—The authors wish to thank Dr. G. Sekules for performing the microanalyses, Mr. O. Boniardi for assistance in preparing the compounds, and Mrs. L. Pozzi and Miss L. Tomasi for carrying out the pharmacological tests.

Anticancer Agents. IV.[1a,b] The Antitumor Activity of Some 1,4- and 1,5-(Bisthiosemicarbazones) and of Related Heterocycles

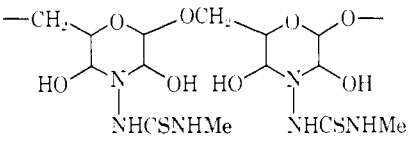
V. C. BARRY, M. L. CONALTY, JOAN E. MCCORMICK, R. S. MCELHINNEY,[1c] MARY R. MCINERNEY, AND J. F. O'SULLIVAN

Laboratories of the Medical Research Council of Ireland, Trinity College, Dublin 2, Ireland

Received September 10, 1968

4-Alkylthiosemicarbazide derivatives of 1,4-diketones (2:1 and to a lesser extent 1:1), of succindialdehyde, of 3-heteraglutaraldehydes (2:1 and 1:1), and of 2,5-dihydroxy-1,4-dithian are in general active against Sarcoma 180 in mice, while the corresponding unsubstituted thiosemicarbazones have no activity. This parallels the striking difference previously observed between thiosemicarbazide (TSC) and its 4-alkyl counterparts when condensed with periodate-oxidized polysaccharides and with other dicarbonyl compounds. Where the effect of varying the 4-alkyl substituent of the TSC has been investigated, it seems that Pr derivatives of 3-heteraglutaraldehydes are more effective than Me or β-hydroxyethyl derivatives, whereas increasing the chain length of the TSC substituent in the diketone series is detrimental (as in the oxypolysaccharides). The vitamin B_6 antagonism characteristic of the polymeric derivatives has also been observed in some of the compounds now described. Many of them display activity against HeLa cells *in vitro*.

The polyaldehydes resulting from periodate oxidation of polysaccharides condense with substituted TSC's to give products which show activity against Sarcoma 180 in mice.[2] The composition of these N-containing polymers approximates 1 molecule of TSC per pair of aldehyde groups. Investigation of the structure[2,3] has revealed that some of the TSC residues are linked to the polymeric backbone by single bonds (C–N–C), the others by normal thiosemicarbazone bonds (C=N). The former are often incorporated into morpholine rings while the latter help to constitute a polythiosemicarbazone. Two consecutive morpholine units from an oxidized xylan are shown in I, and two such thiosemicarbazone units from oxidized starch in II. In the present work, we have prepared TSC derivatives of simple dicarbonyl compounds and tested them for antitumor activity, in order to compare them with the polymers.



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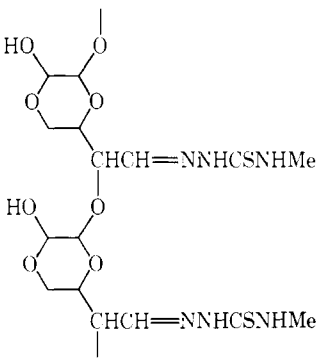


II

Chemistry.—In the first attempt to prepare ring compounds modeled on I, acetonylacetone was chosen as the most readily available comparable dicarbonyl compound. When this reacts with 1 mol of 4-methyl-TSC, the intermediate dihydroxy compound (corresponding to the morpholine unit in I) is not isolable. It spontaneously loses H_2O to yield the pyrrole IIIa, an example of the well-known Paal–Knorr synthesis. Other pyrroles IIIb–d were prepared similarly.[4]

Reaction of 2 mol of 4-Me-TSC with the diketone gives the bisthiosemicarbazone IVb (*cf.* ref 5). 4-Monosubstituted TSC's in general react in this way to give IVa–g, but we have so far been unable to prepare bis derivatives from TSC's with other types of substitution.

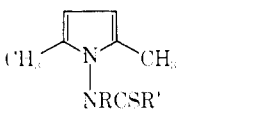
(1) (a) Paper III: V. C. Barry, M. L. Conalty, C. N. O'Callaghan, and D. Twomey, *Proc. Roy. Irish Acad. Sect. B*, **65**, 309 (1967). (b) Part of this work was presented before the Ninth International Cancer Congress, Tokyo, Japan, Oct 1966 (Abstracts, p 318). (c) To whom correspondence should be addressed.
(2) V. C. Barry, M. L. Conalty, J. E. McCormick, R. S. McElhinney, and J. F. O'Sullivan, *Proc. Roy. Irish Acad. Sect. B*, **64**, 335 (1966).
(3) J. E. McCormick, *J. Chem. Soc. C*, 2121 (1966).
(4) R. S. McElhinney, to be published.
(5) H. Beyer, T. Pyl, and C.-E. Völcker, *Justus Liebigs Ann. Chem.*, **638**, 150 (1960).

Table I

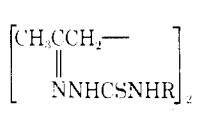
Activity[a] of Miscellaneous Bisthiosemicarbazones against Sarcoma 180

Compd	Bis derivative of	HeLa cell growth inhibition, 10^{-8} g ml	Dose, mg/kg		Effect in mice bearing S180		
			ip	po	Tumor, T/C, %	Survivors	Bodyweight change, T/C, g
3	$(CH_2CHO)_2$[b]	6–7		300	42	5/5	−2.8, +0.7
			200		32	5/5	+0.6, +2.8
			300		30	5/5	−2.3, +2.8
			450		13	4/5	−4.5, +2.8
4	$PhCO(CH_2)_2COMe$[c]	6–7		300[c]	76	4/5	−2.2, −0.6
			200		46	5/5	−3.6, +0.9
5[d]	$CH_2(COMe)_2$[b]	6–7		60	49	5/5	+0.8, +0.4
				200	11	5/5	−2.8, +0.4
			135		40	5/5	−0.2, +1.7
			450		12	5/5	−1.8, +1.7
6[d]	$CH_2(CH_2CHO)_2$[b]	5–6		27	45	5/5	+1.2, +0.4
				90	23	5/5	−1.6, +0.4
			18		56	5/5	−1.1, +1.4
			40		18	5/5	−1.8, +1.4
7	$CH_2(CH_2CHO)_2$[c]	6–7		200	40	5/5	−1.6, +2.6
				450	42	5/5	−2.9, +2.6
			90		45	4/5	−1.8, +2.7
			135		19	5/5	−3.4, +2.7
			200		11	5/5	−5.9, +2.7
8[d]	CH_3COCH_2CHO[c]	7–8		675	128	5/5	+0.8, +4.1
			300		34	5/5	−1.6, −0.8
			450		35	4/5	−3.5, +2.0

[a] Compounds 9[d] and 11 (x = 6–7 (for both), 12 and 13 (x <6 for both) and 10[d] were not significantly active vs. S180. [b] Bis(4-methylthiosemicarbazone). [c] Highest dose tested. [d] Prepared by Dr. D. Twomey. [e] Bis-(4-propylthiosemicarbazone).



IIIa, R = H; R' = NHMe
b, R = H; R' = NMe_2
c, R = H; R' = $NCH_2CH_2CH_2CH_2$
d, R = Me; R' = NH_2

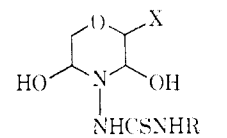


IVa, R = H
b, R = Me
c, R = C_3H_7
d, R = CH_2CH_2OH
e, R = $CH_2CH_2NMe_2$
f, R = CH_2Ph
g, R = Ph

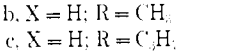
The bisthiosemicarbazones of other dicarbonyl compounds referred to in Table I were prepared by standard methods. Phenacylacetone reacted incompletely with 2 mol of 4-Me-TSC, giving a mixture of the bis and mono derivatives; the ir spectrum of the latter compound showed the presence of a free COPh group.

The synthesis of derivatives Va–f, VIa,b, of 3-oxaglutaraldehyde has been reported.[3,6] 3-Thiaglutaraldehyde, which exists as the cyclic hemialdal VII and gives thiazanes of the type VIIIa with amines in the Strecker reaction,[7] did not yield derivatives VIIIb using TSC's or isoniazid. Even under the conditions used to prepare the oxazanes V,[6] only bishydrazones IX could be isolated. Several such compounds have been described previously.[8,9]

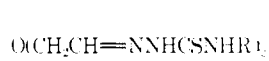
Although various derivatives of 3-azaglutaraldehyde have been prepared,[10] the N-phenyl compound was unknown before the present work. It was obtained by



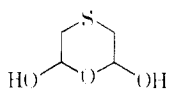
Va, X = H; R = H
b, X = H; R = CH_3
c, X = H; R = C_3H_7
d, X = H; R = CH_2CH_2OH
e, X = OCH_2Ph; R = CH_3
f, X = OCH_2Ph; R = C_3H_7



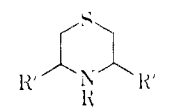
IXa, R = $CSNH_2$
b, R = $CSNHCH_3$
c, R = $CSNHC_3H_7$
d, R = $CSNHCH_2CH_2OH$
e, R = COC_5H_5N-4



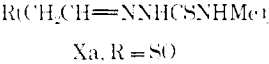
VIa, R = CH_3
b, R = C_3H_7



VII

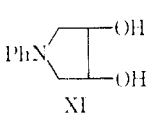


VIIIa, R' = CN
b, R' = OH

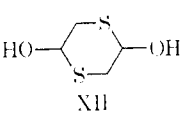


Xa, R = S0
b, R = SO_2
c, R = S,S
d, R = NPh

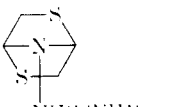
treating the pyrrolidine XI[11] with $NaIO_4$ and converted into its bis(4-methylthiosemicarbazone), isolated as the methanolate. When the experiment was



XI



XII



XIIIa, R = H
b, R = CH_3
c, R = C_3H_7
d, R = CH_2CH_2OH

repeated on a larger scale with only slight modifications, a mixture resulted. The composition of the derived

(6) V. C. Barry, J. E. McCormick, and R. S. McElhinney, *Carbohyd. Res.*, **7**, 299 (1968).

(7) R. D. Coghill, *J. Amer. Chem. Soc.*, **59**, 801 (1937); H. I. Miner, E. O. Hook, and R. D. Coghill, *ibid.*, **62**, 1613 (1940).

(8) (a) K.-D. Gundermann and C. Burba, *Chem. Ber.*, **94**, 2157 (1961); (b) C. L. Zirkle, F. R. Gerns, A. M. Pavloff, and A. Burger, *J. Org. Chem.*, **26**, 395 (1961).

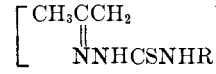
(9) O. Hromatka and R. Haberl, *Monatsh. Chem.*, **85**, 1088 (1954).

(10) L. Wolff and R. Marburg, *Justus Liebigs Ann. Chem.*, **363**, 109 (1908); N. Vinot, *C. R. Acad. Sci. Ser. C*, **248**, 3013 (1959).

(11) J. J. Roberts and W. C. J. Ross, *J. Chem. Soc.*, 4288 (1952).

TABLE II[a]

ACTIVITY[b] OF ACETONYLACETONE BISTHIOSEMICARBAZONES AND OF TWO RELATED COMPOUNDS AGAINST SARCOMA 180



Compd	R	HeLa cell growth inhibition, 10^{-x} g/ml	Effect in mice bearing S180				
			Dose, mg/kg		Tumor,		Bodyweight
		x	po	ip	T/C, %	Survivors	change, T/C, g
IVa[c]	H	9–10	135		51	5/5	−0.8/+3.2
				135	107	3/5	−1.1/+1.0
IVb	CH_3	7–8	70		18	5/5	−1.6/−0.2
			105		20	5/5	−3.0/−0.2
				40	66	5/5	+4.0/+3.2
				60	29	5/5	−1.2/+3.2
				90	27	5/5	−1.6/+3.2
IVc	C_3H_7	6–7	675		54	4/5	−2.5/−1.2
				90	53	5/5	−2.2/+1.0
				200	35	5/5	−3.4/+1.0
				450[d]	40	5/5	−5.6/+1.0
IVd	CH_2CH_2OH	7–8	450		37	5/5	+1.2/+1.7
			675[d]		17	5/5	−1.2/+1.7
				135	55	5/5	−2.4/+1.3
1	SMe for NHR	7–8	90		51	3/5	−3.8/+1.0
				27	44	4/5	−1.4/+1.5
IIIa		<5	60		34	4/5	−0.2/+0.6
			90		42	3/5	−3.6/+0.6
				60	61	3/5	−0.6/+0.5

[a] In the tables only the minimum effective doses and the maximum doses are usually recorded, but intermediate doses in every case yielded significant inhibition. [b] Compounds IVe (x = 8), IVf (x = 6–7), IVg[c] (x <5), and **2** (the analog of IIIa carrying carbethoxy groups at positions 3 and 4 in the pyrrole ring) were not significantly active *vs.* S180. [c] Prepared by Dr. D. Twomey. [d] Highest dose tested.

analytical sample suggested that it had 2 C atoms less than Xd. The experimental conditions for the oxidation of the diol XI and subsequent reaction of the product with 4-Me-TSC are accordingly quite critical. A more detailed study[6] has since enabled conditions for the preparation of Xd to be defined.

Finally, the mercaptoacetaldehyde dimer XII may be regarded as a potential 1,4-dialdehyde. This compound reacts with 1 mol of TSC to give the enediminodithian XIIIa.[12] We prepared the analogs XIIIb–d by an improved experimental modification of the published procedure.

Biological Activities.—Of the derivatives of the 1,4-diketone, acetonylacetone, the bisthiosemicarbazones were more easily prepared and were tested first. The results are summarized in Table II. The difference between IVb and its unsubstituted analog IVa[5,13] was very striking. Replacement of 4-Me by other 4-alkyl groups afforded at best no improvement in activity. The presence of basic centers, as in IVe, eliminates *in vivo* activity, although the compound is still very active against HeLa cells.

This structure–activity relationship follows the pattern established for the TSC derivatives of oxystarch. Moreover, the antitumor activity of IVb, like that of the polymer B.1190,[2] was antagonized by vitamin B_6 (Table III). These factors point to a similar mode of action for the two series of compounds. It is of interest that the high activity of the "mixed" bisthiosemicarbazones from pyruvaldehyde is not antagonized by vitamin B_6, and the structure–activity relationship is somewhat different from the present one.[14] Several diketones $MeCO(CH_2)_nCOMe$ with CO groups separated by various distances (n = 1,3 and, *e.g.*, 7,12) resemble acetonylacetone in that the bis derivatives from $NH_2NHCSNHR$ where R = Me are active, whereas those where R = H, C_6H_{11}, Ph have poor or no activity;[1a] those where R = C_3H_7 are referred to below.

Other dicarbonyl compounds were then examined (Table I). Succindialdehyde resembled the saturated diketones, while the inactivity of but-2-ene-1,4-dial derivatives is consistent with that of the vinylogous glyoxal and diacetyl bisthiosemicarbazones noted previously.[1a] Replacement of a diketone terminal Me group by Ph greatly reduces activity (*cf.* **4** and IVb); 1,4-dibenzoylbutane bis-(4-methylthiosemicarbazone)[1a] is completely inactive.

The type of antitumor activity shown by polymers which contain the polythiosemicarbazone structure such as II is thus shown in general by bisthiosemicarbazones of saturated aliphatic dicarbonyl compounds separated by at least one CH_2 group. The pyrrole IIIa which was designed to act as a model of structure I also proved active, but to a lesser degree (Table II). The analogous pyrrole (IIId) from 2-Me-TSC had no activity, nor had IIIb, IIIc and **2**.

The morpholines Va–f are closer analogs of structure I; they are derived from dialdehydes with β-hetero atoms as in the oxidized polysaccharides. Here, the 4-Pr-TSC products (Vc,f) are more active than those (Vb,e) from 4-Me-TSC (Table IV). This is also true for the bis-derivatives (*cf.* VIa,b, Table V). Again, in the case of the 3-thiaglutaraldehyde derivatives, IXc

(12) R. Haberl and O. Hromatka, *Monatsh. Chem.*, **88**, 996 (1957).

(13) B. A. Gingras, T. Suprunchuk, and C. H. Bayley, *Can. J. Chem.*, **40**, 1058 (1962).

(14) V. C. Barry, M. L. Conalty, and J. F. O'Sullivan, *Cancer Res.*, **26**, 2165 (1966).

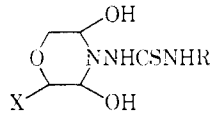
Table III

Effect of Added Pyridoxine on Antitumor Activity in Mice Bearing Sarcoma 180

Compd (route)	Dose, mg/kg Alone and + pyridoxine (po)	Tumor, T/C, %	Survivors	Bodyweight change, T/C, g
Polymer (B.1190) from oxidized starch and $NH_2NHCSNHMe$ (*po*)	500	33	10/10	−2.0/−0.9
	500 +50(p)	94	10/10	+0.3/−0.9
	50(p)	76	10/10	+0.4/−0.9
IVb (ip)	60	29	5/5	−1.2/+3.2
	60 +60(p)	96	5/5	+1.6/+3.2
$CH_3C(=NNHCSNH_2)_2$	200	10	5/5	+1.8/+2.8
$CH(=NNHCSNHMe)_2$ (*po*)	200 +200(p)	12	5/5	+4.2/+2.8
XIIIb (*po*)	575	22	5/5	−2.6/+2.6
	575 +115(p)	128	5/5	+2.6/+2.6

Table IV

Activity[a] of 3,5-Dihydroxy-4-(thioureylene-1′-)morpholines against Sarcoma 180



Compd	X, R	HeLa cell growth inhibition, 10^{-x} g/ml, x	Effect in mice bearing S180: Dose, mg/kg, *po*	Dose, mg/kg, ip	Tumor, T/C, %	Survivors	Bodyweight change, T/C, g
Vb	X = H	<6	200		68	3/5	+2.2/+2.9
	R = Me			135	53	3/5	+2.4/+0.8
Vc	X = H	<6	300		36	5/5	+0.8/+2.5
	R = C_3H_7		675[b]		27	5/5	−0.8/+2.5
				200	18	5/5	−3.6/+0.9
				300	37	5/5	−0.4/+0.9
Vc	X = OCH_2Ph	4–5	450		74	3/5	−2.3/+1.4
	R = Me			135	39	3/5	−1.2/+2.0
Vf	X = OCH_2Ph	4–5	450		43	4/5	−0.9/+2.2
	R = C_3H_7		675		25	5/5	−2.0/+2.2
			1013		38	5/5	−2.2/+2.2
				90	32	4/5	−1.6/+0.7
				135	24	3/5	−4.0/+0.7

[a] Compounds Va (x = 6–7) and Vd (x < 5) were not significantly active *vs.* S180. [b] Highest dose tested.

shows activity over a wider dose range ip than IXb and has some activity by the oral route. The 4-(β-hydroxyethyl) compounds Vd and IXd are virtually inactive; the "unsubstituted" compounds Va and IXa illustrate the characteristic difference between products from TSC and their 4-alkyl counterparts.

However, the pattern of activity just recounted for the 4-alkyl-TSC derivatives of 3-oxa- and 3-thiaglutaraldehyde is evidently somewhat different from the results obtained with those of acetonylacetone (Table II). Another diketone, pentane-2,4-dione, affords derivatives (Table I) which resemble those of the 1,4-diketone; in this case, the 4-propyl- (**9**) and 4-isobutylthiosemicarbazones (**10**) are inactive, in contrast to the 4-Me compound **5**.[1a] Glutaraldehyde and acetoacetaldehyde give bis-(4-propylthiosemicarbazones) (**7** and **8**) which have poor activity compared with, *e.g.*, **6**; in this they resemble the diketones rather than the 3-heteraglutaraldehydes.

Other hetero-substituted dialdehydes also yielded active bis-(4-methylthiosemicarbazones). The sulfone Xb and the disulfide Xc closely resemble the sulfide IXb (Table V). The sulfoxide Xa however is considerably more toxic and is active when administered *po*, albeit over a narrow dose range. The *N*-substituted aniline Xd is active only ip and then too over a narrow dose range.

The enediminodithian XIIIb showed high activity (Table VI) and was antagonized by Vitamin B_6 (Table III). The pattern of activity observed in other derivatives of the dithian XII recalls those of 3-heteraglutaraldehydes.

Most of the substances in Tables I, II, and V show good activity *in vitro* against HeLa cells which, however, does not parallel the *in vivo* activity. Derivatives of 3-oxaglutaraldehyde (Tables IV and V) and of 2,5-dihydroxy-1,4-dithian (Table VI) are, in general, ineffective against HeLa cells, while compounds such as IVa,e have useful activity (Table II). We have also given representative members of the agents in this study, without success, to mice bearing leukemias L 1210 and C 1498 and Ehrlich carcinoma (ascitic).

The activity against Sarcoma 180 of the bisthiosemicarbazones IVb,d, of the morpholine VI, and of

TABLE V

ACTIVITY OF 3-HETERAGLUTARALDEHYDE BISTHIOSEMICARBAZONES AGAINST SARCOMA 180

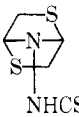
$X(CH_2CH{=}NNHCSNHR)_2$

Compd	X, R	HeLa cell growth inhibition, 10^{-x} g/ml	Effect in mice bearing S180				
			Dose, mg/kg		Tumor,		Bodyweight
		x	po	ip	T/C, %	Survivors	change, T/C, g
VIa[a]	X = O	5–6	[b]	200	27	4/5	+0.3/+4.7
	R = Me						
VIb	X = O	<5	200		43	4/5	+1.1/+1.8
	R = C_3H_7			135	41	4/5	−3.8/−1.8
				200	32	4/5	−1.0/−1.8
IXa	X = S	7–8	675		36	4/5	−4.8/+0.9
	R = H			135	64	5/5	+0.4/+1.1
IXb	X = S	7–8	[b]	450	27	5/5	+0.6/+3.1
	R = Me			675[c]	46	5/5	−1.6/+3.1
IXc	X = S	8–9	675[c]		43	3/5	+2.2/+3.9
	R = C_3H_7			135	50	5/5	−0.4/+2.4
				450	20	5/5	−2.8/+2.4
IXd	X = S		[b]	675	61	4/5	+2.5/+3.3
	R = CH_2CH_2OH			1013	17	4/5	−3.5/+3.3
Xa	X = SO	6–7	135		33	4/5	−0.4/+1.9
	R = Me			135	32	5/5	+1.0/+2.4
Xb	X = SO_2	6–7	[b]	300	51	5/5	−1.8/−1.3
	R = Me			450	24	4/5	−3.7/−1.3
Xc	X = S,S	5–6	[b]	450	42	4/5	−1.8/+2.4
	R = Me			675	22	4/5	+1.6/+2.4
Xd	X = NPh	5–6	[b]	675	20	4/5	−1.2/+3.1
	R = Me						

[a] 1 mol of H_2O associated.[6] [b] This compound was not significantly active by the oral route. [c] Highest dose tested.

TABLE VI

ACTIVITY[a] OF *N*-(THIOUREYLENE-1′-)2,5-ENDIMINO-1,4-DITHIANS AGAINST SARCOMA 180



Compd	R	HeLa cell growth inhibition, 10^{-x} g/ml	Effect in mice bearing S180				
			Dose, mg/kg		Tumor,		Bodyweight
		x	po	ip	T/C, %	Survivors	change, T/C, g
XIIIb	CH_3	5–6	110		47	5/5	−1.0/+1.3
			220		35	5/5	−1.0/+1.3
			440		33	5/5	−4.4/+1.3
				250	13	4/5	−2.2/+0.3
				360	29	4/5	−2.6/+0.3
				580	15	4/5	−0.6/+0.3
XIIIc	C_3H_7	<5	450		47	5/5	+0.6/+3.0
			675[b]		36	5/5	+0.6/+3.0
				300	46	4/5	+0.9/+3.2
				675	43	4/5	+0.6/+3.2
XIIId	CH_2CH_2OH	5–6	675[b]		74	5/5	−0.8/−0.4
				675	34	3/5	−2.2/−0.5

[a] Compounds XIIIa (x = 5–6) and XII were not significantly active *vs.* S180. [b] Highest dose tested.

the dithian XIIIb has been confirmed elsewhere, while the last-named compound had no effect on the Walker carcinosarcoma 256 in rats at the dose-levels employed.[15] In another center,[16] where 4 mouse tumor systems are routinely used, IVb showed slight inhibition of the growth of leukaemia C 1498 and of mammary gland adenocarcinoma DBRB, with slight increase in survival time in the former case. The compound was inactive against leukemia L 1210, but completely inhibited the growth of leukemia P 1534 when administered intraperitoneally at a level of 100 mg/kg daily for 10 days. The next dose level, 400 mg/kg, proved toxic. In the same laboratory, the bis-(4-methylthiosemicarbazones) of glutaraldehyde (**6**) and of heptane-2,6-dione[1a] were found inactive against all four tumors; the dithian XIIIb behaved similarly except for slight activity against adenocarcinoma DBRB.

(15) Results of Dr. E. Mihich, Roswell Park Memorial Institute, Buffalo, N. Y.

(16) Dr. C. L. Maddock, Children's Cancer Research Foundation, Boston, Mass.

Experimental Section[17]

Biological Activity.—The methods used for assessment of antitumor activity in mice have been described elsewhere.[2,14]

(17) We thank Mr. S. Bance of May and Baker Ltd., Dagenham, Essex, England, for microanalytical data. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ± 0.4% of the theoretical values. Melting points were determined using a Kofler block and are uncorrected.

Table VII

Dialdehyde Bisthiosemicarbazones

No.	Dialdehyde and 4-substituent of TSC	% Yield	Mp, °C	Formula	Anal.
11	A, H	78	187–188[a]	$C_6H_{12}N_6S_2$	C, H, S
3	A, Me	69	192–194[b]	$C_8H_{16}N_6S_2$	C, H, N, S
13	B, H	63	>300	$C_6H_{10}N_6S_2$	H, N, S[c]
12	B, Me	71	210–250[d]	$C_8H_{14}N_6S_2$	C, H, N, S
1Xa	C, H	95	174–176	$C_8H_{12}N_6S_3$	H, N, S[e]
1Xb	C, Me[f]	90	184–186	$C_9H_{16}N_6S_3$	C, H, N, S
1Xc	C, Pr[g]	57	119–120[h]	$C_{12}H_{24}N_6S_3$	C, H, N, S
1Xd	C, CH_2CH_2OH[i]	83	145–147[j]	$C_{10}H_{20}N_6O_2S_3$	H, N[k]

[a] From pyridine–H_2O. [b] From DMF–H_2O. [c] C: calcd, 31.3; found, 31.9. [d] Gradual decomposition. [e] C: calcd, 27.25; found, 27.8. [f] A similar experiment using 1 equiv of 4-methyl-TSC yielded an impure product of mp 154–171° from which no cyclic compound could be isolated. [g] The crude product, from the bisacetal (0.01 mol) and 4-propyl-TSC[2] (0.02 mol), was a yellow gum (3.25 g). In MeOH (7 ml) at 60° this gave a dark solution from which crystals separated in the yield indicated. In another experiment, the additional heating necessitated by charcoal treatment caused further darkening of the solution and gave a less pure product. [h] From 85% MeOH. [i] From 4-(β-hydroxyethyl)-TSC [R. S. McElhinney, *J. Chem. Soc. C*, 950 (1966)]. [j] From MeOH. [k] C: calcd, 34.05; found, 34.6.

The drugs were given in 0.5% CBMC once daily intraperitoneally or perorally (gavage by syringe fitted with a blunt needle) for 7 days commencing 24 hr after implantation of Sarcoma 180. Eight days after implantation the mice were killed and the tumors removed and weighed. A T/C% value (100 × mean weight of tumors from treated mice/mean weight of tumors from control mice) of 50 or less is regarded as significant. Each compound was given at doses (1.5-fold steps) ranging up to the maximum tolerated dose or 1013 mg/kg, whichever is the lesser.

Evaluation in HeLa cell cultures is by determining[2,14] the concentration of drug which inhibits cell growth in liquid medium (Earle's balanced salt solution to which is added lactalbumin hydrolysate, human serum, $NaHCO_3$, penicillin, and streptomycin) to 50% of that in the control flasks at 4 days. This concentration is recorded in the tables.

Materials.—The bisthiosemicarbazones IVa,[5,13] IVg,[14a] **5**,[14a,18] **9–10**,[18] **6–8**,[19] VIa-b,[6] and Xd[6] and the morpholines Va–d[6] and Ve–f[6] were prepared according to published methods. We thank our colleague Dr. D. Twomey for the compounds indicated in Tables I and II. He found that IVa had mp 196–197° (lit.[5,13] mp 195° and 272°, respectively). The preparation and properties of the acetonylacetone derivatives[4] IIIa-d, IVb-f, **1**, and **2**, and of the bisthiosemicarbazones[19] Xa-c, will be described elsewhere.

Bisthiosemicarbazones of (A) Succindialdehyde, (B) But-2-ene-1,4-dial, and (C) 3-Thiaglutaraldehyde.—Solutions of these dialdehydes were prepared by treatment of 2,5-diethoxytetrahydrofuran, 2,5-dimethoxy-2,5-dihydrofuran, and 1,1,5,5-tetramethoxy-3-thiapentane,[8b] respectively with warm 0.1 *N* HCl. Each was brought to pH 4 and treated with the TSC (2 equiv) dissolved in H_2O. The solid derivative was collected and recrystallized from DMF–MeOH unless otherwise specified (Table VII).

3-Thiaglutaraldehyde Bis(isonicotinoylhydrazone) (IXe).—To a neutralized dialdehyde solution obtained from the bisacetal (2.1 g) was added INH (1.37 g, 10 mmol) dissolved in H_2O (8 ml). After a few minutes, colorless crystals began to separate. Next day, 0.97 g (55%) was collected by filtration, mp 211–212°. After crystallization from MeOH, the melting point was 218–219°. *Anal.* ($C_{16}H_{16}N_6O_2S$) C, H, N, S.

Phenacylacetone 4-Methylthiosemicarbazones.—To a solution of phenacylacetone[20] (5.67 g, 0.032 mol) in warm MeOH (5 ml) was added 4-Me-TSC (6.83 g, 0.065 mol) dissolved in MeOH (15 ml) and H_2O (10 ml). The solution was warmed briefly, and when a few drops of AcOH were added, an oil separated. The mixture was allowed to cool during the next hour, and the oil gradually became semisolid. The mother liquor was replaced by MeOH, and the crystalline product filtered off: yield, 8.55 g; mp 90–135°. Recrystallization from MeOH gave material which still melted over a wide range, so it was treated briefly with a little boiling MeOH and the insoluble fraction, mp 178–179°, filtered off from the hot solution (A). In this way 3.42 g of phenacylacetone bis-(4-methylthiosemicarbazone) (4) was obtained. The melting point was not raised by crystallization from first MeOH and then DMF–MeOH. *Anal.* ($C_{15}H_{22}N_6S_2$) C, H, N, S.

The solution (A) on cooling yielded a mixture of crystals which were filtered off and discarded. Concentration of the mother liquor gave 0.63 g, mp 108–110°. After 3 further crystallizations from C_6H_6–petroleum ether (bp 40–60°), phenacylacetone mono-(4-methylthiosemicarbazone) had mp 109–111°. *Anal.* ($C_{13}H_{17}N_3OS$) C, H, N, S. A free benzoyl group was indicated by the very strong peak in the ir spectrum (KBr) at 1674 cm^{-1}. Moreover, this excluded the possibility that the product was the hydrate of an N-substituted 2-methyl-5-phenylpyrrole.

Periodate Oxidation of *cis*-3,4-Dihydroxy-1-phenylpyrrolidine.—When this diol (X1) or *N*,*N*-diethylaniline was oxidized at room temperature, deep blue colors quickly developed. These are characteristic of the nonspecific oxidation of arylamines by periodate.[21]

Accordingly, the diol[11] (0.36 g, 2 mmol) was dissolved in MeOH (10 ml) and added gradually to a solution of $NaIO_4$ (0.45 g, 2.1 mmol) in H_2O (20 ml), keeping the temperature below 10°. The resulting colorless solution was kept overnight in the refrigerator, filtered from some inorganic salt which had separated, and extracted with Et_2O (4 × 30 ml). The extract was washed with saturated brine, dried ($MgSO_4$), and evaporated, leaving a syrupy residue. This was taken up in MeOH (5 ml) and treated with a solution of 4-methyl-TSC (0.42 g, 4 mmol) in MeOH (12 ml). After being heated to boiling it was cooled and H_2O (40 ml) was added, causing separation of a gummy product. This soon crystallized and after a few hours was filtered off (0.54 g, 71%). It was recrystallized from MeOH and had mp 165–167°. *Anal.* Calcd for $C_{16}H_{26}N_7OS_2$: C, 47.0; H, 6.55; N, 25.55; S, 16.7. Found: C, 47.2; H, 6.5; N, 26.1; S, 16.8.

This corresponds to *N*-phenyliminodiacetaldehyde bis-(4-methylthiosemicarbazone) plus 1 MeOH. The MeOH is possibly linked in a methylglycoside type of ring structure, rather than solvent of crystallization.

In a similar experiment, the diol (8.06 g, 45 mmol) was oxidized and treated with 4-Me-TSC as before except that instead of being boiled the solution was left overnight at room temperature. Addition of H_2O caused gradual separation of a solid. This became much quicker when the pH was brought to 4 using AcOH. The product (13.0 g) was evidently a mixture. Several recrystallizations of a sample from DMF–MeOH gave crystals with mp 166–167°. *Anal.* Calcd for $C_{14}H_{21}N_7S_2$ [*N*-phenyliminodiacetaldehyde bis-(4-methylthiosemicarbazone)]: C, 47.85; H, 6.0; N, 27.9; S, 18.25. Calcd for $C_{12}H_{21}N_7S_2$: C, 44.0; H, 6.45; N, 29.95; S, 19.6. Found: C, 43.5; H, 5.8; N, 30.0; S, 20.2.

***N*-(Thioureylene-1′-)2,5-enedimino-1,4-dithians.**—The dithian XII is obtained as an almost colorless product of mp 106–110° in 80–85% yield by saturating 3 *N* NaOH (400 ml) with H_2S at 5° and adding chloroacetaldehyde (1 mol; as Eastman Kodak 40–45% aqueous solution which can be used after storage for several years at −20°) according to Hromatka and Haberl.[9] It

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Table VIII

No.	3′-Substituent	% Yield	Mp, °C	DMF, ml/g	Uv max[a] λ, mμ	Uv max[a] ε	Formula
XIIIa	H	86	189–191	18			[b]
XIIIb	Me	81	194–196	6	237.5	21,400	$C_6H_{11}N_3S_3$
XIIIc	Pr	88	169–170	[c]			$C_8H_{15}N_3S_3$
XIIId	CH_2CH_2OH	84	181–182	2[d]	245	15,000	$C_7H_{13}N_3OS_3$

[a] In MeOH. [b] Lit.[12] mp 200–201°. [c] Readily soluble in hot MeOH. [d] Analytical sample from MeOH.

dissolves fairly slowly in hot EtOH and some insoluble material is often formed, and for reaction with TSC's the following modification of the published method[12] is more convenient.

A mixture of XII (7.6 g, 0.05 mol; prepared as above) and the TSC (0.05 mol) was treated with EtOH (100 ml), H_2O (60 ml), and AcOH (3 ml). The suspension was heated under reflux on the water bath, and after 1–2 min a clear solution resulted. Heating was continued for 10 min in all, and on cooling, a mass of colorless crystals separated; in the case of TSC itself, the product crystallized from the clear solution during the heating. Next day the product was collected and washed with EtOH. For recrystallization, each of the derivatives XIIIa,b,d was dissolved in the indicated volume of DMF at 100° and the solution treated with 5–6 vol of boiling MeOH. The recovery was good and the melting point (dec) raised only a few degrees. *Anal.* C, H, N, S for the compounds with formulas recorded (Table VIII).

Acknowledgments.—We are grateful to the following organizations who supported this work financially: May & Baker Ltd., Dagenham, England; Arthur Guinness, Son and Co. (Dublin) Ltd.; Irish Cancer Society; Tenovus; and Irish Hospitals Trust Ltd.

2-Fluoropurine Ribonucleosides[1]

John A. Montgomery and Kathleen Hewson

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Received November 3, 1969

The preparation of the 2-fluoro derivative of the anticancer agent 6-methylthiopurine ribonucleoside from *S*-methylthioguanosine is described. A number of other 2-fluoropurine ribonucleosides were synthesized by the selective displacement of the 6-fluorine of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-difluoropurine followed by treatment with MeOH–NH_3 to remove the O-Ac groups. The cytotoxicity of these nucleosides is discussed.

Because of the high degree of broad-spectrum biologic activity of 2-fluoroadenosine,[2–13] a number of other nucleosides of 2-fluoroadenine were prepared and evaluated for cytotoxicity with interesting results.[14] This paper describes the synthesis and evaluation of another type of structural variants of 2-fluoroadenosine.

N-Methyladenosine and *N*,*N*-dimethyladenosine are readily phosphorylated by adenosine kinase to the 5′-monophosphates,[10] but the 5′-monophosphate of *N*-methyladenosine is not a substrate for adenylate kinase[15] and, therefore, is not converted in whole cells into the di- and triphosphates,[15] a conversion requisite for incorporation into RNA (presumably the same holds true for *N*,*N*-dimethyladenosine). It is reasonable to assume that 2-fluoro-*N*-methyladenosine (**11**) and 2-fluoro-*N*,*N*-dimethyladenosine (**12**) would probably be converted also into the 5′-monophosphates only and not incorporated into RNA, although *in vivo* demethylation to 2-fluoroadenosine might occur. These compounds and other 2-fluoropurine ribonucleosides have been prepared for biologic evaluation.

S-Methylthioguanosine (**2**) was converted into the 2-fluoro analog (**5**) of the anticancer agent, 6-(methylthio)purine ribonucleoside,[16] by the modified Schiemann reaction.[2] To prepare 2-fluoro-*N*-methyladenosine (**11**) by this method, 2-amino-6-chloro-9-β-D-ribofuranosylpurine (**1**) was treated with $MeNH_2$ to obtain the requisite intermediate, 2-amino-*N*-methyladenosine (**3**). In addition to **3**, 2-methylamino-*N*-methyladenosine (**4**), resulting from the displacement of the 2-NH_2 as well as the 6-Cl, was formed. When 2-amino-*N*-methyladenosine (**3**) was subjected to the modified Schiemann reaction a complex reaction mixture resulted from which an impure sample of the desired 2-fluoro-*N*-methyladenosine (**12**) was isolated by column chromatography. Fortunately the availability of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-difluoropurine (**6**)[17] provided an alternative route to this and other 2-

(1) This work was supported by funds from the Southern Research Institute, the C. F. Kettering Foundation, and Chemotherapy, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

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