was 5.8 g (45% based on 1-pyridiniumethylisoquinoline-5-sulfonate), mp 314–316° dec.

General Procedure for Oxidation Reaction.—SeO_2 (1 mole-equiv) was added portionwise to a solution of the Me derivative (1 mole-equiv) in dioxane and heated slowly to reflux temp and maintained at reflux for 2 hr. The Se ppt was removed by filtration and the filtrate evapd under vacuum. The residue was extracted with dilute HCl and filtered and the filtrate made alkaline with $NaHCO_3$ to ppt the carboxaldehyde. The aldehyde was filtered off, washed with H_2O, dried over P_2O_5 in a desiccator, and crystallized from petroleum ether (bp 60–110°).

Thiosemicarbazones.—Except where noted the thiosemicarbazones (Tables III and IV) were prepared by the method of French and Blanz[32] from the corresponding aldehyde and thiosemicarbazide.

Acknowledgments. We thank Dr. Harry B. Wood, Jr., Professor John Broomhead, Dr. R. Wallace Brockman, Professor Alan C. Sartorelli, and Dr. T. W. Brooks for helpful consultations and encouragement. We express our appreciation to Arvia Hosking and June French for the antitumor evaluations and to Dre J. Glasby and David Chu for excellent technical assistance.

Carcinostatic Activity of Thiosemicarbazones of Formyl Heteroaromatic Compounds. VII. 2-Formylpyridine Derivatives Bearing Additional Ring Substituents[1]

Erwin J. Blanz, Jr., Frederic A. French,*
Jefferson R. DoAmaral, and Douglas A. French

Mount Zion Hospital and Medical Center, Chemotherapy Research Laboratory, Palo Alto, California 94305

Received March 20, 1970

Sixteen thiosemicarbazones of 2-formylpyridines bearing additional ring substituents were synthesized and tested for antitumor activity against 5 mouse tumor systems *in vivo* and compared with the parent unsubstituted derivative. The major tumors used were L-1210 leukemia, sarcoma 180 (ascites), L-5178Y lymphoma, C-1498 myelogenous leukemia, and the Lewis lung carcinoma. Occasional studies were also performed on sarcoma 180 (solid), B-16 melanoma, and the Ehrlich ascites carcinoma. The substituents studied were: 3-carboxy, 3-fluoro, 3-methyl, 4-methyl, 5-fluoro, 5-chloro, 5-bromo, 5-iodo, 5-methyl, 5-ethyl, 5-trifluoromethoxy, 5-trifluoromethyl, 5-dimethylamino, 5-methylsulfonyl, 5-hydroxy, and 5-acetoxy. The effect of additional substituents on activity against a particular tumor system follows no simple parametric rules. Furthermore, the order of substituent effects changes markedly from one tumor system to another. A number of the compounds studied have been found, in other laboratories, to be extremely potent inhibitors of tumor-derived ribonucleotide diphosphate reductase and hence the synthesis of DNA. The 5-hydroxy derivative is, in general, the most interesting compound studied. On certain dose–time regimens it yields a significant cure rate in L-1210 leukemia.

The original observation of the antitumor activity of 2-formylpyridine thiosemicarbazone[2] stood in isolation for several years. However, preliminary theorizations on possible modes of action were formulated.[3] In 1963 a concerted attack on the overall problem was initiated. This led, in the pyridine series, to the discovery that 3-hydroxy-2-formylpyridine thiosemicarbazone and especially 5-hydroxy-2-formylpyridine thiosemicarbazone displayed markedly superior activity.[4–7] This was not the result of an increase in gravimetric potency *per se* but was essentially due to a large improvement in therapeutic index and hence the practical attainability of much higher and protracted dose levels.

In the companion paper on related isoquinoline derivatives the question of mechanism of action is dealt with in detail.[8] In this paper attention is focused on the pyridine derivatives.

It gradually became apparent, during the course of this investigation, that the pyridine derivatives possessed a strong advantage over the isoquinoline compounds due to the simple fact that, in general, they are more water soluble and readily absorbed *in vivo*. In the pyridine group only 2 out of 17 compounds studied yielded drug deposits in mice. In contrast, 17 out of 23 compounds in the isoquinoline series gave rise to this problem.

Chemistry.—The substituted 2-formylpyridine thiosemicarbazones were prepared from the appropriate substituted 2-picolines (Scheme I,[9] Tables I–VI). Most of the 3- and 5-halogenated 2-picolines (Ia–e) utilized in this study are known compounds and were prepared according to or with slight modification of published procedures.[10,11] 2-Methyl-5-trifluoromethylpyridine (If) was prepared by heating 6-methylnicotinic acid with SF_4 and HF at 120°. 2-Methyl-5-trifluoromethoxypyridine (Ig) was prepared essentially by the method of Sheppard.[12] 3-Hydroxy-6-methylpyridine and COF_2 were allowed to react at 100–150° for 4 hr followed by reaction with SF_4 and anhydrous HF to form the desired product.

* To whom correspondence should be addressed.

(1) (a) This investigation was supported by Grant CA-03287 from the National Cancer Institute; (b) presented in part at the 169th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract MEDI-76.

(2) R. W. Brockman, J. R. Thompson, M. J. Bell, and H. E. Skipper, *Cancer Res.*, **16**, 167 (1956).

(3) F. A. French and B. L. Freedlander, *ibid.*, **18**, 1290 (1958).

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(9) 1h was oxidized to 5-dimethylamino-2-picoline *N,N'*-dioxide which was converted into IIh with SO_2; Ii was prepared from oxidation of 5-methylthio-2-picoline; when R = OH it becomes CH_3COO in formula III.

(10) R. Graf, *J. Prakt. Chem.*, **133**, 19 (1932).

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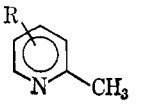
Table I

5-Substituted 2-Picolines

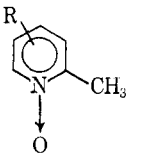


Compd[a]	R	Mp or bp (mm), °C	Yield, %	Crystn solvent	Formula	Analyses
Ib	5-F	120–121 (760)	35		C_6H_6FN	C, H, F, N
If	5-CF_3	24–25.5, 129–130 (760)	74		$C_7H_6F_3N$	C, H, F, N
Ig	5-OCF_3	129–131 (760)	32		$C_7H_6F_3NO$	C, H
Ih	5-$N(CH_3)_2$[b]	198–200 (HCl salt)	c	*i*-PrOH	$C_8H_{12}N_2 \cdot HCl$	C, H, N; Cl[d]
	5-$OCN(C_2H_5)_2$ (S)	61–62	48	Petr ether	$C_{11}H_{16}N_2OS$	C, H, N, S
	5-$SCN(C_2H_5)_2$ (O)	40–41	87	Petr ether	$C_{11}H_{16}N_2OS$	C, H, N, S
	5-SH	121.5–123	80	Benzene	C_6H_7NS	C, H, N, S
	5-SCH_3	94–95 (8.7)	81		C_7H_9NS	C, H; N, S[e]

[a] Compounds were synthesized utilizing standard procedures as described in the Chemistry and Experimental Sections. [b] Bp 68–69 (1 mm), 49% yield. [c] Analytical sample. [d] Cl: calcd, 20.53; found, 19.8. [e] N: calcd, 10.06; found, 9.58; S: calcd, 23.03; found, 22.12.

Table II

3- and 5-Substituted 2-Picoline *N*-Oxides



Compd[a]	R	Mp or bp (mm), °C	Yield, %	Crystn solvent	Formula	Analyses
III	3-COOEt	50–52	84	Petr ether	$C_9H_{11}NO_3$	C, H, N
IIb	5-F	146–148	80	*i*-PrOH	$C_6H_6FNO \cdot HCl$	C, H, N[b]
IIc	5-Cl	155–157	66	*i*-PrOH	$C_6H_6ClNO \cdot HCl$	C, H, Cl, N
IId	5-Br	117–118	91	Petr ether	C_6H_6BrNO	C, H, Br, N
IIe	5-I	154–155	79	Benzene	C_6H_6INO	C, H, I, N
IIf	5-CF_3	66–68 (1.5)	89		$C_7H_6F_3NO$	C, H, F, N
IIg	5-OCF_3	74–78 (1.2)	82		$C_7H_6F_3NO_2$	C, H, F, N
	5-$N(CH_3)_2$ (O)	184–185 dec	89	*i*-PrOH–acetone	$C_8H_{12}N_2O_2$	C, H, N[c]
IIh	5-$N(CH_3)_2$	96–97	80	Petr ether	$C_8H_{12}N_2O$	C, H, N[d]
IIi	5-SO_2CH_3	172.5–173.5	88	EtOH	$C_7H_9NO_3S$	C, H, N, S
IIj	5-OH	188.5–189.5	83	EtOH	$C_6H_7NO_2$	C, H, N

[a] Compounds were synthesized utilizing standard procedures as described in the Chemistry and Experimental Sections. [b] Analyzed as the picrate, mp 98.5–99°. [c] This compound yielded inaccurate analyses because it is very hygroscopic. Calcd for $C_8H_{12}N_2O_2 \cdot 0.75H_2O$: C, 52.9; H, 7.5; N, 15.4. Found: C, 53.3; H, 6.9; N, 16.1. [d] Analyzed as the picrate, mp 162–163°.

5-Dimethylamino-2-picoline (Ih) was prepared from 5-amino-2-picoline by the method of Binz and v. Schickh who converted 3-aminopyridine into 3-dimethylaminopyridine.[13]

5-Methylsulfonyl-2-picoline (Ii) was not synthesized but instead 5-methylthio-2-picoline was utilized to prepare IIi–VIi. 5-Methylthio-2-picoline was prepared from the alkylation of the Na salt of 5-mercapto-2-picoline with MeI. The synthesis of 5-mercapto-2-picoline was accomplished from 3-hydroxy-6-methylpyridine by the method of Newman and Karnes who converted 3-pyridinol into 3-mercaptopyridine.[14] The 3- and 5-halogenated 2-picolines (Ia–g) were converted into the *N*-oxide derivatives (IIa–g) by using commercially available *m*-chloroperbenzoic acid or 40% AcO_2H. 5-Dimethylamino-2-picoline *N*-oxide (IIh) cannot be directly prepared from Ih because 5-dimethylamino-2-picoline *N*,*N*′-dioxide is formed. When an aromatic tertiary amino group is present on an N-heteroaromatic ring, the formation of an aromatic and ring heteronitrogen *N*,*N*′-dioxide is generally observed. In such a case, if the *N*,*N*′-dioxide is allowed to stand in aq H_2SO_3 or SO_2–EtOH–H_2O at room temperature, only the aromatic *N*-oxide group is reduced and the ring heteronitrogen *N*-oxide group remains, in general, intact, resisting such a reduction.[15–17] In this manner 5-dimethylamino-2-picoline *N*,*N*′-dioxide was con-

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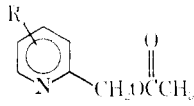
TABLE III

SUBSTITUTED 2-PYRIDYLMETHANOL ACETATES

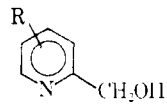


Compd[a]	R	Mp or bp (mm), °C	Yield, %	Crystn solvent	Formula	Analyses
IIIl	3-COOEt	122–127 (1.0)	58		$C_{11}H_{13}NO_4$	C, H, N
IIIa	3-F	60–65 (1.2)	53		$C_8H_8FNO_2$	C, H, N; F[b]
IIIb	5-F	62–65 (1.3)	51		$C_8H_8FNO_2$	C, H, F, N
IIIc	5-Cl	70–76 (1.2)	57		$C_8H_8ClNO_2$	C, H, Cl, N
IIId	5-Br	42.5–43.5	54	Petr ether	$C_8H_8BrNO_2$	C, H, Br, N
IIIe	5-I	45–46	46	Petr ether	$C_8H_8INO_2$	C, H, I, N
IIIf	5-CF_3	68–72 (1.8)	49		$C_9H_8F_3NO_2$	C, H, N; F
IIIg	5-OCF_3	67–68 (1.7)	62		$C_9H_8F_3NO_3$	C, H, N; F[d]
IIIh	5-$N(CH_3)_2$	116–120 (1.0)	86		$C_{10}H_{14}N_2O_2$	C, H; N[e]
IIIi	5-O_2SCH_3	95–96	31	EtOH	$C_9H_{11}NO_4S$	C, H, N, S
IIIk	5-AcO	122–128 (0.8)	74		$C_{10}H_{11}NO_4$	C, H, N

[a] Compounds were synthesized utilizing standard procedures as described in the Chemistry and Experimental Sections. [b] F: calcd, 11.23; found, 10.72. [c] F: calcd, 26.01; found, 25.22. [d] F: calcd, 24.24; found, 25.60. [e] Analyzed as the picrate, mp 159–159.5. N: calcd, 16.54; found, 15.76.

TABLE IV

SUBSTITUTED 2-PYRIDYLMETHANOLS

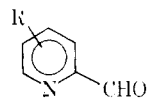


Compd[a]	R	Method	Mp or bp (mm), °C	Yield, %	Crystn solvent	Formula	Analyses
IVa	3-F	A	50–52 (1.3)	42		C_6H_6FNO	C, H, F, N
IVb	5-F	A	141.5–142.5	82	*i*-PrOH	$C_6H_6FNO \cdot HCl$	C, H, F, Cl, N
IVc	5-Cl	A	179–181	70	*i*-PrOH	$C_6H_6ClNO \cdot HCl$	C, H, Cl, N
IVd	5-Br	A	211–212	71	*i*-PrOH	$C_6H_6BrNO \cdot HCl$	C, H, N
IVe	5-I	A	68–69	87	Petr ether	C_6H_6INO	C, H, I, N
IVf	5-CF_3	A	63–68 (1.9)	79		$C_7H_6F_3NO$	C, H, F, N
IVg	5-OCF_3	B	68–70 (2)	85		$C_7H_6F_3NO_2$	C, H, F, N
IVh	5-$N(CH_3)_2$	B	121.5–122.5	73	Benzene	$C_8H_{12}N_2O$	H, N; C[b]
IVi	5-SO_2CH_3	A	190–191 dec	94	EtOH	$C_7H_9NO_3S \cdot HCl$	C, H, Cl, N, S

[a] Compounds were synthesized utilizing standard procedures as described in the Chemistry and Experimental Sections. [b] C: calcd 63.13; found, 63.72.

TABLE V

SUBSTITUTED 2-FORMYLPYRIDINES



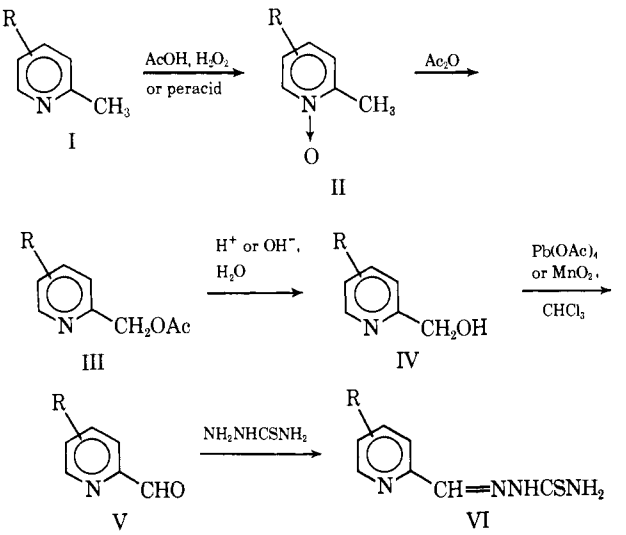
Compd[a]	R	Method	Mp or bp (mm), °C	Yield, %	Crystn solvent	Formula	Analyses
Va	3-F	C	95–98 (18)	41		C_6H_4FNO	C, H, F, N
Vb	5-F	C	72–74 (20.8)[b]	39		C_6H_4FNO	C, H, N
Vc	5-Cl	C	60–62	67	Petr ether	C_6H_4ClNO	C, H, Cl, N
Vd	5-Br	C	85–87	76	Petr ether	C_6H_4BrNO	N; C, H, Br
Ve	5-I	C	102–103	61	Petr ether	C_6H_4INO	C, H, I, N
Vf	5-CF_3	D	65–68 (21)	52		$C_7H_4F_3NO$	H, N; C, F[c]
Vg	5-OCF_3	D	73–76 (20)	59		$C_7H_4F_3NO_2$	C, H, N; F
Vh	5-$N(CH_3)_2$	C	86–88	81	Petr ether	$C_8H_{10}N_2O$	C, H, N
Vi	5-SO_2CH_3	C	167–169	82	EtOAc	$C_7H_7NO_3S$	C, H, N, S
Vj	5-OH	C	183–184	52	EtOAc	$C_6H_5NO_2$	C, H, N

[a] Compounds were synthesized utilizing standard procedures as described in the Chemistry and Experimental Sections. [b] Mp, 31–33. [c] C: calcd, 38.07; found, 38.65. H: calcd, 2.19; found, 2.79. Br: calcd, 43.43; found, 42.88. [d] C: calcd, 48.01; found, 47.05. F: calcd, 32.55; found, 31.57. This aldehyde is very sensitive to air oxidation. The analysis was in excellent agreement if one assumed the sample analyzed contained 25% of the carboxylic acid derivative. [e] F: calcd, 29.83; found, 30.94.

verted into IIh with SO_2 in 95% EtOH at room temperature. 5-Methylsulfonyl-2-picoline *N*-oxide (IIi) was prepared from the oxidation of 5-methylthio-2-picoline with AcOH–H_2O_2.

All the *N*-oxides (IIa–j) were rearranged to the substituted 2-pyridinemethanol acetates (IIIa–j) with Ac_2O at reflux temperature, followed by hydrolysis of the esters to the substituted 2-pyridinemethanols (IVa–

Scheme I



R: a = 3-F, b = 5-F, c = 5-Cl, d = 5-Br, e = 5-I, f = 5-CF_3, g = 5-OCF_3, h = 5-$N(CH_3)_2$, i = 5-SO_2CH_3, j = 5-OH[9]

j). The esters IIIg and IIIh were hydrolyzed to the corresponding substituted 2-pyridinemethanols IVg and IVh with aq NaOH and the remainder of the acetates (III) were hydrolyzed with boiling HCl. The substituted 2-formylpyridines Vf and Vg were prepared from the $Pb(OAc)_4$ oxidation of the corresponding 2-pyridinemethanols IVf and IVg in $CHCl_3$ at room temperature. The remainder of the substituted 2-formylpyridines were prepared by oxidation of their pyridinemethanols with active MnO_2. The thiosemicarbazones VIa–j were prepared from the substituted 2-formylpyridines and thiosemicarbazide by the usual procedure.[5]

It has been shown that substituted 2-picoline *N*-oxides rearrange on refluxing with Ac_2O to give substituted 2-acetoxymethylpyridines (Scheme I). Repetition of this reaction with substituted 2-acetoxymethylpyridine *N*-oxides gave substituted picolinaldehyde diacetates in moderate yields (Table VII).[18] The 5-acetoxypicolinaldehyde thiosemicarbazone (VIk) and 2-formylnicotinic acid thiosemicarbazone (VIl) were prepared from 3-hydroxy-6-methylpyridine *N*-oxide and ethyl-2-methylnicotinate *N*-oxide, respectively. 5-Acetoxypicolinaldehyde diacetate was hydrolyzed cautiously with cold aq HCl in order to form 5-acetoxypicolinaldehyde *in situ;* with more drastic conditions 5-hydroxypicolinaldehyde (Vj) was formed.

Structure–Activity Relations.—Typical screening data are presented in Table VIII. These data, in the case of positive compounds, are not chosen as the best values but are representative of a large amount of data. Using L-1210 leukemia as an index of antitumor activity, the ranking in activity of the different 5-substituted-2-formylpyridine thiosemicarbazones is: 5-hydroxy (**16**) >> 5-dimethylamino (**14**) > 5-trifluoromethyl (**13**) > 5-acetoxy (**17**) = 5-methyl (**10**) = 5-ethyl (**11**) > 5-fluoro (**6**) = 5-chloro (**7**) > the unsubstituted derivative (**1**). The 5-bromo (**8**), 5-iodo (**9**), 5-trifluoromethoxy (**12**), and 5-methylsulfonyl (**15**) derivatives were not significantly active. For the L-5178Y lymphoma the order is: 5-acetoxy (**17**) > 5-hydroxy (**16**) > 5-dimethylamino (**14**) >> 5-ethyl (**11**) > 5-methyl (**10**) = 5-fluoro (**6**) = 5-chloro (**7**) = 5-bromo (**8**) = 5-methylsulfonyl (**15**). The other (5-) derivatives tested were inactive. On the C-1498 myelogenous leukemia the order of activity is: 5-chloro (**7**) = 5-methyl (**10**) = 5-ethyl (**11**) > 5-dimethylamino (**14**) = 5-hydroxy (**16**) = 5-acetoxy (**17**). The other (5-) derivatives tested were not significantly active. With the Lewis lung carcinoma the order is: 5-acetoxy (**17**) ≧ 5-trifluoromethyl (**13**) > 5-fluoro (**6**) > 5-dimethylamino (**14**) = 5-chloro (**7**) = 5-ethyl (**11**) = 5-hydroxy (**16**). No compounds tested were significantly active on the B-16 melanoma or sarcoma 180 (solid phase). In contrast, with sarcoma 180 (ascites) a number of compounds were active and several yielded a significant 60-day cure rate. The order of activity for noncured mice is: 5-hydroxy (**16**) ≫ 5-bromo (**8**) > 5-fluoro (**6**) > 5-dimethylamino (**14**). The order for curative activity is: 5-hydroxy (**16**) > 5-dimethylamino (**14**) = 5-trifluoromethyl (**13**) > 5-fluoro (**6**). Only 4 compounds were tested on the Ehrlich ascites tumor. The unsubstituted parent compound **1** was toxic. The 5-methylsulfonyl derivative **15** yielded 2/10 60-day cures, the 5-acetoxy (**17**) yielded 1/10 survivor, but the 5-hydroxy derivative (**16**) yielded 90% 60-day survivors and was active over a dose range of 9 to 100 mg/kg per day. The order of toxicity is, roughly: 5-hydroxy (**16**) < 5-acetoxy (**17**) < 5-methylsulfonyl (**15**) < 5-dimethylamino (**14**) < 5-trifluoromethyl (**13**) < 5-methyl (**10**) < 5-iodo (**9**) < 5-ethyl (**11**) < 5-fluoro (**6**) < 5-trifluoromethoxy (**12**) = 5-bromo (**8**) = 5-chloro (**7**) < the unsubstituted derivative (**1**).

Of the compounds substituted in other than the 5 position, the 3-fluoro derivative **3** was modestly but significantly active on L-1210, L-5178Y, and the Lewis lung carcinoma. The 3-methyl derivative **4** showed interesting activity on C-1498 and barely significant activity on L-1210 and L-5178Y. The 4-methyl derivative **5** was similarly uninteresting and yielded barely significant activity on L-1210, L-5178Y, and C-1498.

It may be seen from the foregoing that even within the confines of a single tumor test system the substituent effects do not fall into any simple order relating to electronic parameters, steric effects, or lipophile–hydrophile character. Compounding this difficulty is the fact that the order of substituent effects changes quite drastically from one test system to another.

Since the active compounds in this series, that have been studied in the cell-free enzyme system, are potent inhibitors of some mammalian tumor derived ribonucleoside diphosphate reductases[19–22] and hence of DNA synthesis it would be useful to have knowledge of the fine structure of the active Fe-containing site of this enzyme. It might then be possible to relate structural parameters of the enzyme to that of the inhibitor.

Of all the compounds studied the 5-hydroxy-2-formylpyridine derivative **16** is by far the most interesting found in either the pyridine or isoquinoline series. It has exceptionally good solubility at pH 7, low toxic-

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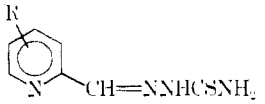
(20) A. C. Sartorelli, M. S. Zedeck, K. C. Agrawal, and E. C. Moore, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 650 (1968).

(21) R. W. Brockman, R. W. Sidwell, G. Arnett, and S. Shaddix, *Proc. Soc. Exp. Biol. Med.*, **133**, 609 (1970).

(22) R. W. Brockman, personal communication, 1970.

TABLE VI

SUBSTITUTED 2-FORMYLPYRIDINE THIOSEMICARBAZONES

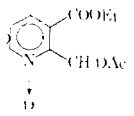
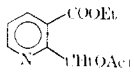
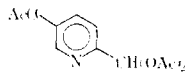


Compd[a]	R	Mp, °C, dec	Formula	Analyses
VII	3-COOH	221–222	$C_8H_8N_4O_2S \cdot H_2O$	H, N, S; C[b]
VIa	3-F	222.5–223.5	$C_7H_7FN_4S$	C, H, N
VIb	5-F	236–237	$C_7H_7FN_4S$	C, H, N
VIc	5-Cl	235–236	$C_7H_7ClN_4S$	C, H, Cl, S; N[c]
VId	5-Br	241–242	$C_7H_7BrN_4S$	C, H, Br, N, S
VIe	5-I	247–247.5	$C_7H_7IN_4S$	C, H, I, N, S
VIf	5-CF_3	208–209	$C_8H_7F_3N_4S \cdot C_2H_5OH$	C, H, N
VIg	5-OCF_3	206–207	$C_8H_7F_3N_4OS$	C, H, N
VIh	5-$N(CH_3)_2$	230–231	$C_9H_{13}N_5S$	C, H, N, S
VIi	5-SO_2CH_3	255–255.5	$C_8H_{10}N_4O_2S_2$	H, N, S; C[d]
VIj	5-OH	236–237	$C_7H_8N_4OS$	C, H, N, S
VIk	5-AcO	200–201	$C_9H_{10}N_4O_2S$	C, H, N, S

[a] Compounds were synthesized utilizing standard procedures as described in the Chemistry Section. [b] C: calcd, 39.66; found, 39.16. [c] N: calcd, 26.10; found, 25.42. [d] C: calcd, 37.19; found, 36.69.

TABLE VII

MISCELLANEOUS SUBSTITUTED PYRIDINES

Compd[a]	Mp or bp (mm), °C	Yield, %	Crystn solvent	Formula	Analyses
	180–200 (<1)	97		$C_{11}H_{13}NO_5$	C, H, N
	100–103	32	Petr ether	$C_{13}H_{15}NO_6$	C, H, N
	86–87.5	c	Benzene	$C_{12}H_{13}NO_6$	C, H, N

[a] Compounds were synthesized utilizing standard procedures as described in the Chemistry Section. [b] Prepared from 5-acetoxy-2-pyridinemethanol acetate *N*-oxide (crude). [c] An overall yield of 39% was obtained based on 5-acetoxy-2-pyridinemethanol acetate.

ity, and a broad spectrum of activity. It produces synergistic effects with some standard antitumor agents.[23] In common with other OH derivatives in both the pyridine and isoquinoline series the biological effect is highly dependent on dose schedule.[24,25] Thus, for example, with 5-hydroxy-2-formylpyridine thiosemicarbazone we have found that a dosage of 40 or 50 mg/kg twice daily yielded a 10–50% cure rate in L-1210 leukemia. This independently substantiates extensive test results at the CCNSC. No other compound studied so far in either the pyridine or isoquinoline series has yielded this level of effect. 5-Hydroxy-2-formylpyridine thiosemicarbazone (5-HP) has been selected for clinical trial at the National Cancer Institute.

Experimental Section

Antitumor Tests.—The methods used in this laboratory for the evaluation of antitumor activity in mice have been described elsewhere.[5]

Chemical Procedures.—Melting points are corrected and were measured on a Thomas-Hoover capillary melting point apparatus. Microanalyses were performed by Berkeley Analytical Laboratory, Berkeley, Calif. and by Micro-Analysis, Inc., Wilmington, Del. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.4% of the theoretical values.

5-Fluoro-2-picoline (Ib).—5-Amino-2-picoline (31 g, 0.29 mole) was dissolved in 110 ml of 50% HBF_4 diluted with 216 ml of 95% EtOH. The stirred solution was cooled to 0° and EtONO bubbled in slowly for 3 hr. The diazonium fluoroborate salt pptd after a short time. Et_2O (110 ml) was added and the ppt was filtered, washed with ice-cooled petroleum ether, and kept moist with petroleum ether at −20°. The diazonium fluoroborate was divided into 5 equal portions. Each portion was decomposed as follows: the salt, covered with cold high-boiling petroleum ether (60–110°), was allowed to warm slowly until decomposition started. The temp was kept below 55°. Decomposition was uncontrollable above this temp. After decomposition was complete the dark red oil was sepd from the petroleum ether, made alkaline with NaOH solution, and steam distd. The distillate was satd with solid NaOH and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$) and carefully distd through a 7.5-cm Vigreux column. After removal of the CH_2Cl_2 the product distd at 120–121°. The yield was 11.6 g (35% from the amine); $n^{24}D$ 1.4696.

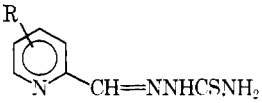
2-Methyl-5-trifluoromethylpyridine (If).[26]—A 1.4-l., 316 stainless steel autoclave was charged with 75 g (0.55 mole) of 6-methylnicotinic acid and sealed. After leak testing by pressuring with N_2, the autoclave was cooled in Dry Ice–acetone and 110 g (5.5 moles) of anhyd HF was introduced by siphoning. SF_4 (178 g, 1.65 moles) was then condensed into the mixture, the autoclave degassed and finally heated at 120° for 8 hr. After cooling to room temp and venting excess gases, the contents of the autoclave was poured into ice-chilled 10% NaOH. The basic mixture was extracted several times with Et_2O and the extract dried (K_2CO_3).

(23) Dr. J. H. Burchenal, personal communication, 1969.
(24) K. C. Agrawal and A. C. Sartorelli, *J. Pharm. Sci.*, **57**, 1948 (1968).
(25) Dr. H. B. Wood, Jr., personal communication, 1969.

(26) This intermediate was purchased from Peninsular Chemresearch, Calgon Corp., Gainesville, Fla. We thank Dr. T. W. Brooks for providing us with the synthetic details for publication.

TABLE VIII

ANTITUMOR ACTIVITY OF SUBSTITUTED 2-FORMYLPYRIDINE THIOSEMICARBAZONES[a]



No.	R	L-1210 Dose, mg/kg	L-1210 % T/C[b]	S-180 ascites Dose, mg/kg	S-180 ascites % T/C	L-5178Y Dose, mg/kg	L-5178Y % T/C	C-1498 Dose, mg/kg	C-1498 % T/C	LL-Ca Dose, mg/kg	LL-Ca % T/C
1	H	10	130	10	124	10	121	10[c]	112	10[c]	47
2	3-COOH	200	104	100	93					100	63
3	3-F	25	137	20	140	25	160	25	121	25	24
4	3-CH_3[d]	12	125	8	119	12	136	8	150	8	58
5	4-CH_3[e]	20	125	20	100	15	139	20	129	15	63
6	5-F	10	136	10	170 (10)[f]	10	132	10	123	10	17
7	5-Cl	15	136	10	91	15	125	15	147	10	24
8	5-Br	20	113	20	191	15	125			20	52
9	5-I	60	114	50	115	60	100			30	83
10	5-CH_3[d]	40	144	25	128	40	134	30	145	35	64
11	5-C_2H_5[g]	20	144	5	134	15	149	20	145	10	25
12	5-OCF_3	30	118	20	137					15	71
13	5-CF_3	30	158	20	128 (20)	25	112	25	110	25	9
14	5-$N(CH_3)_2$	50	164	30	162 (20)	50	179	50	132	35	23
15	5-SO_2CH_3	71	108	71	134	71	127	71	94	71	97
16	5-OH	141	215	100	309 (50)	141	203	71	131	100	26
17	5-AcO	100	147	100	146	100	235	100	133	100	6

[a] See ref 8. Detailed data too numerous to be reported here will be published in Cancer Chemotherapy Reports. The drugs were given ip daily, at approximately maximum tolerated doses, starting 24 hr after tumor inoculation; 6–10 mice were used in each experiment. [b] % T/C = treated/control × 100. Criteria for activity: L-1210, L-5178Y, and C-1498, % $T/C \geq 125$ S-180 ascites, ≥ 150; LL-Ca, ≤ 30. [c] Drug given every other day. [d] W. Mathes and W. Sauermilch, *Ber.*, **90**, 758 (1957). [e] S. Furukawa and Y. Kuroiwa, *Chem. Pharm. Bull.*, **3**, 232 (1955). [f] No. in parentheses are per cent 60-day survivors. [g] W. Mathes and W. Sauermilch, *Chem. Z.*, **80**, 475 (1956).

Et_2O was removed by distillation through a 1200-mm packed column until the pot temp reached 105°. The pot residue (83 g) was then distd carefully through a small Vigreux column to yield 65.3 g (73.7%) of product, bp 129–130°, mp 24–25.5°.

2-Methyl-5-trifluoromethoxypyridine (Ig).[26]—A 1.4-l. stainless steel autoclave was charged with 65 g (0.60 mole) of 3-hydroxy-6-methylpyridine and 60 g (0.9 mole) of COF_2. The autoclave was heated for 1 hr at 100° and then 3 hr at 150°. After cooling to 0° and venting excess COF_2, the autoclave was charged with 194 g (1.8 moles) of SF_4 and 120 g (6.0 moles) of anhyd HF. The mixture was then heated at 165° for 6 hr. The autoclave was cooled to room temp and vented to release excess gases. The contents was poured into ice-chilled 10% $NaHCO_3$ and the aq mixture extracted thoroughly with Et_2O. After drying the extract (K_2CO_3), the Et_2O was removed by stripping through a 900-mm packed distilling column to a head temp of 45°. The pot residue (50 g) was distd carefully through a small Vigreux column to yield 33.4 g (31.5%), bp 129–131°.

5-Dimethylamino-2-picoline (Ih).—5-Amino-2-picoline (40 g, 0.37 mole) was added portionwise with cooling to 740 ml of concd H_2SO_4. The H_2SO_4 solution was diluted carefully with 230 ml of cold H_2O. While the temp was maintained at 40° 81 g (1.13 moles) of 37.4% CH_2O solution was added dropwise with stirring to the H_2SO_4 solution. Zn (148 g, 2.26 g-atoms) was added over a period of 1.5 hr; the temp rose from 40 to 50°. The reaction mixture was stirred and heated at 100–105° for an additional hour and allowed to stand overnight at room temp. It was then filtered through a sintered glass funnel and neutralized with concd NaOH solution. The organic layer was extracted with C_6H_6, dried ($MgSO_4$), and distd to yield 38.2 g of product, bp 75–93° (1.5 mm). It was found by tlc that there were 3 components in the distillate. These turned out to be the starting material, the monomethylamino derivative, and the 5-dimethylamino derivative. The mixture was subjected to the Hinsberg reaction for separation of the amines. Pure 5-dimethylamino-2-picoline (14.3 g) had a bp 68–69° (1 mm). The residue of *N*-methyl-*N*-5-(2-methylpyridyl)benzenesulfonamide was hydrolyzed with 80% H_2SO_4 and recycled as above to obtain an additional 10.4 g, bp 68–69° (1 mm). The combined total of 24.7 g was 49% of theoretical yield.

Substituted 2-Picoline *N*-Oxides (IIa–g).—A 25–30% excess of peracid was added cautiously to the unsubstituted 2-picoline (1 equiv wt) dissolved in $CHCl_3$. Picolines (IIa–e) were oxidized with *m*-chloroperbenzoic acid and picolines (IIf–g) were oxidized with 40% AcO_2H. The reaction was usually exothermic and the $CHCl_3$ solution was cooled with an ice bath. The contents was allowed to react overnight at ambient temp and then neutralized with excess satd Na_2CO_3 solution. The $CHCl_3$ layer was sepd and the aq layer extracted with $CHCl_3$ several times. All the extracts were combined, dried ($MgSO_4$), and the $CHCl_3$ evapd. If the crude *N*-oxide crystallized, it was recrystd with the appropriate solvent. If the *N*-oxide was volatile enough, it was distd *in vacuo*.

5-Dimethylamino-2-picoline *N*,*N'*-Dioxide (Table II).—5-Dimethylamino-2-picoline (Ih) (10 g, 0.074 mole), dissolved in 20 ml of $CHCl_3$, was treated dropwise with 40% AcO_2H (36.8 g, 0.19 mole). The reaction was very exothermic and the solution was cooled with an ice bath until all the oxidant was added. The resulting solution was kept at 45–50° for 24 hr and then treated with excess satd Na_2CO_3 solution. It was found that the dioxide was not in the $CHCl_3$ layer but had dissolved in the alkaline aq phase. The aq phase was evapd to dryness and the remaining solid was extracted with two 150-ml portions of *i*-PrOH. The *i*-PrOH extracts were evapd to 100 ml and treated with 1 l. of Me_2CO. The solution was refrigerated overnight yielding 7.0 g of the dioxide, mp 184–185° dec. The mother liquor was evapd to 100 ml and a second crop was obtained, 4.1 g, mp 179–181° dec. When the combustion analysis was performed on the compd it was found to be hygroscopic.

5-Dimethylamino-2-picoline *N*-Oxide (IIh).—The foregoing dioxide (11.2 g, 0.067 mole) was dissolved in 100 ml of EtOH and the resulting solution was satd with SO_2. The dark red solution was allowed to stand 18 hr at room temp and then made alkaline with 20% NaOH solution. The reaction mixture was evapd to dryness and extracted with $CHCl_3$. The extract was evapd to a solid which was triturated with boiling petroleum ether (60–110°). The petroleum ether extracts were carefully cooled and IIh crystallized to yield 8.1 g (80%), mp 96–97°.

5-Methylsulfonyl-2-picoline *N*-Oxide (IIi).—5-Methylthio-2-picoline (8 g, 0.058 mole) was added to 100 ml of glacial AcOH containing 25 ml of 31% H_2O_2. The solution was heated at 90° for 2 hr and then flash evapd to a white solid, which was crystallized from 90 ml of abs EtOH to yield 9.5 g (88%), mp 172.5–173.5°.

General Method for 2-Pyridylmethanol Acetates (IIIa–j).—A substituted picoline *N*-oxide (0.2 mole) was added slowly with

stirring to 100 ml of Ac_2O at 100–120°. After the exothermic reaction subsided, the dark reaction mixture was stirred and refluxed for 0.5–1.0 hr. EtOH was cautiously added until the excess Ac_2O was converted to EtOAc and AcOH. The resultant solutions were coned by flash evaporation to dark oils except in those cases where they were suspected of being highly volatile. The reaction mixture was then cooled and neutralized with $KHCO_3$ solution. The organic layer was extracted with $CHCl_3$, dried ($MgSO_4$), and distd *in vacuo*. Some of the acetates (IIId, IIIe, IIIi) crystallized on standing and were recrystd from petroleum ether or EtOH.

Substituted 2-Pyridylmethanols (IVa–i). A.—Coned HCl (50 ml) was added to 0.1 mole of a substituted 2-pyridylmethanol acetate and refluxed for 1 hr. The solution was evapd to dryness *in vacuo* to give the HCl salt of the substituted 2-pyridylmethanol. Some of the HCl salts were neutralized with $KHCO_3$ solution and the organic material was extracted with $CHCl_3$, dried ($MgSO_4$), and distilled. In some cases the free base crystallized on standing (IVd and IVe). Some of the HCl salts were neutralized without further purification and utilized in the oxidn reaction (C).

B.—NaOH (1.2 equiv wt) and a substituted 2-pyridylmethanol acetate (1.0 equiv wt) were added to H_2O and refluxed for 1 hr. The substituted methanol solidified on cooling and was filtered and dried or (if it did not solidify) extracted with $CHCl_3$, dried ($MgSO_4$), and distd.

Substituted 2-Formylpyridines (Va–i). C.—To a substituted 2-pyridylmethanol dissolved in $CHCl_3$ was added 2 to 3 times its wt of active MnO_2. The reaction mixture was allowed to stir and reflux for 2 hr and then filtered, and the MnO_2 cake washed well with boiling $CHCl_3$. The $CHCl_3$ extracts were combined, dried ($MgSO_4$), and distd to furnish the aldehydes. In the cases where the aldehydes were solids after evaporation of the $CHCl_3$, they were crystallized from petroleum ether. IVj was oxidized with MnO_2 in *i*-PrOH because of its poor solubility in $CHCl_3$.

D.—$Pb(OAc)_4$ (1.1 equiv wt) was added portionwise to the 2-pyridylmethanol (1.0 equiv wt) dissolved in dry $CHCl_3$. The yellow solution was allowed to stand 3 days at room temp, then treated with excess $KHCO_3$ solution, and filtered. The organic layer was sepd from the aq layer, dried ($MgSO_4$), and distd to give the pure aldehyde.

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Irreversible Enzyme Inhibitors. CLXXIII.[1,2] Cure of Walker 256 Ascites by Reversible and Irreversible Inhibitors of Dihydrofolic Reductase[3] Derived from 1-(Substituted-phenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine

B. R. BAKER,[4a] NICOLAAS M. J. VERMEULEN,[4b] WALLACE T. ASHTON, AND ADRIAN J. RYAN[5]

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

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Nine active-site directed irreversible inhibitors and seven potent reversible inhibitors of dihydrofolic reductase were assayed against Walker 256 ascites, Dunning leukemia ascites, and intramuscular Walker 256 in the rat. Some of the irreversible and reversible inhibitors were remarkably effective in promoting cures of the 2 ascitic tumors; however, there was no correlation between tissue specificity of irreversible inhibition and *in vivo* activity, indicating that other unknown factors were playing important roles in these cures. The best compounds against Walker 256 ascites were the 1-phenyl-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazines substituted on the Ph group with *p*-$(CH_2)_2CONHC_6H_3$-3-Me-4-SO_2F (**1**), *m*-$(CH_2)_4C_6H_4SO_2F$-*p*, (**7**), *p*-$(CH_2)_2CONHC_6H_4$-3-Me (**15**), 3-Cl-4-$O(CH_2)_3OC_6H_5$ (**16**), 3-Cl-4-$(CH_2)_2C_6H_5$ (**18**), or *p*-$(CH_2)_4C_6H_5$ (**19**) moieties. The best compounds against the Dunning leukemia ascites were **18**, **19**, and the phenyltriazine substituted by the 3-Cl-4-$(CH_2)_2C_6H_4$-SO_2F-*p* (**9**) and 3-Cl-4-$OCH_2CONHC_6H_5$ (**17**) moieties.

The design of enzyme inhibitors as possible chemotherapeutic agents has the advantage that direct answers on inhibition of the target can be obtained by assay with the isolated enzyme. With this approach complications such as transport through membranes and metabolism are avoided in order to gain insight on selectivity of attack of the target enzyme.[6] Concepts emerged over a period of 10 years[7] that allowed design of enzyme inhibitors so highly specific that they could differentiate between isozymes[8] or even the same enzyme (such as dihydrofolic reductase) from two or more different tissues in the same animal.[9]

Once this specificity at the isolated enzyme level had been achieved,[10] it was time to return to assay of these inhibitors in whole animals bearing a tumor; if these highly specific enzyme inhibitors failed to work *in vivo*, there was some assurance that the difficulty was not in failure to attack the target enzyme, but was due to the other *in vivo* factors such as transport and metabolism that had been deliberately avoided to this point. The first studies on these dihydrofolic reductase inhibitors were done with L1210 mouse leukemia;[11] although significant life extensions were

(1) This work was supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper in this series see R. Cardinaud and B. R. Baker, *J. Med. Chem.*, **13**, 467 (1970).

(3) For the previous paper on this enzyme see B. R. Baker, N. M. J. Vermeulen, and A. J. Ryan, *ibid.*, **13**, 281 (1970).

(4) (a) To whom correspondence should be addressed. (b) N. M. J. V. wishes to thank, the Council of Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

(5) On sabbatical leave from the University of Sydney, Australia.

(6) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.

(7) B. R. Baker, *Cancer Chemother. Rep.*, **4**, 1 (1959).

(8) B. R. Baker and R. P. Patel, *J. Pharm. Sci.*, **53**, 714 (1964).

(9) (a) For a summary of this approach to highly selective inhibitors of dihydrofolic reductase see B. R. Baker, *Accounts Chem. Res.*, **2**, 129 (1969). (b) The tissue selectivity with these crude enzyme preparations could be due to selective irreversible inhibition of isozymes or to the rapid destruction of the irreversible inhibitor in normal tissue or both.

(10) (a) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968), paper CXIX; (b) B. R. Baker and P. C. Huang, *ibid.*, **11**, 495 (1968), paper CXX.

(11) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXIII.