5 mol of H_2/mol of **1** had been absorbed. The catalyst and solvent were removed. The residue was distilled *in vacuo* and a colorless liquid, bp 125–130° (0.5 mm), was collected, $n^{25}D$ 1.5302. *Anal.* ($C_{18}H_{27}N$) C, H. A picrate melted at 162–164°. *Anal.* ($C_{24}H_{30}N_4O_7$) C, H.

Nmr.—The nmr spectra of **1–4** were obtained on a Varian-A60 spectrometer in $CDCl_3$ (Me_4Si). Confirmation of the expected structures were provided through analysis of the changes in the spectra in going from **1** to **4**. The CH group connecting the indene and benzene rings couples with the protons of the aromatic ring of indene to produce a complicated multiplet. The reduction of the double bond between the two rings removes this coupling effect and a single peak for the four protons of the aromatic ring of indene is observed at δ 7.12 in the spectra of **3** and **4**. The absence of this peak in the spectrum of **2** is evidence for the retention of the double bond between the rings in this compound.

Uv-Visible Spectra.—These spectra were as expected for the structures given in Scheme I.

Acknowledgment.—We are grateful to Dr. Vito Morlino of Virginia Commonwealth University for nmr spectra and assistance in their interpretation. We are grateful to Professor Sir Alexander Haddow, Mr. J. E. Everett, and Mr. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor. We are also grateful to CCNSC for screening tests against the Walker 256 tumor.

Synthesis of Additional Arylhydroxamic Acids Which Inhibit Nucleic Acid Biosynthesis *In Vitro*[1]

GLEN R. GALE, JOHN B. HYNES, AND ALAYNE B. SMITH

Veterans Administration Hospital, Department of Pharmacology, and School of Pharmacy, Medical University of South Carolina, Charleston, South Carolina

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Prompted by the observation[2] that salicylhydroxamic acid selectively inhibits the synthesis of deoxyribonucleic acid (DNA) in Ehrlich ascites tumor cells *in vitro*, 11 arylhydroxamic acids were synthesized earlier and their activities were assessed.[3] Six were shown to possess varying degrees of selectivity in the test system. One of these, 4-hydroxybenzoylhydroxamic acid, has now been shown to possess significant antitumor activity *in vivo*.[4] In BDF_1 mice bearing the L-1210 leukemia, daily administration of 400 mg/kg per day intraperitoneally for 9 days increased the survival times of the animals 36% to 57% in four experiments, with no deaths due to toxicity of the compound. The present report is concerned with the synthesis and biological evaluation of additional arylhydroxamic acids (HAs) as regards their effects on biosynthesis of DNA, ribonucleic acid (RNA), and protein.

(1) This work was aided by Grant GM-13958 from the National Institutes of Health, U. S. Public Health Service; nine of the compounds were made available indirectly through support of U. S. Army Medical Research and Development Contract No. DADA17-67-C-7055.

(2) G. R. Gale, *Proc. Soc. Exp. Biol. Med.*, **122**, 1236 (1966).

(3) G. R. Gale and J. B. Hynes, *J. Med. Chem.*, **11**, 191 (1968).

(4) Personal communication, N. Greenberg, Cancer Chemotherapy National Service Center, Bethesda, Md.

Experimental Section[5]

Chemical.—Each of the compounds listed in Table I was prepared from the corresponding Me or Et ester by the well-known reaction with excess NH_2OH in basic solution.[6,7] In each case the base was NaOH and the solvent was MeOH or H_2O, or a combination of these depending upon the solubility of the individual ester. In the case of a phenolic compound, an extra equivalent of NaOH was used, while I, X, and XI, which are sensitive to oxidation in basic solution, were prepared in a N_2 atmosphere.

With the exception of I, IX, XIX, and XX, the required esters were obtained commercially. Methyl 3,5-diisopropylsalicylate, bp 142–143.5° (7 mm), was prepared in 35% yield by the HCl-catalyzed esterification of the corresponding acid in MeOH [lit.[8] bp 146° (7 mm), yield 15%]. Methyl 2-bromo-3,4,5-trimethoxybenzoate was synthesized by the bromination[9] of 3,4,5-trimethoxybenzoic acid followed by esterification using MeOH and anhydrous HCl. The compound exhibited bp 162–163.5° (2 mm), mp 31–32°, and gave an acceptable elemental analysis [lit.[10] bp 160–161° (2 mm), mp 34–36°]. Methyl 3,5-dichlorobenzoate was also prepared by the MeOH–HCl technique. The purified product melted at 58–60° (lit.[11] mp 58°). Methyl 3,4,5-trimethoxyphenylacetate was prepared by the H_2SO_4-catalyzed esterification of the corresponding acid, and was isolated and used as a viscous oil without further purification.

Biological.—These methods were as described in the corresponding section of the previous report.[3] The parameters investigated were (a) relative potency against DNA and RNA synthesis as measured by least-squares analysis of dose–response data; (b) slopes of the regression lines; (c) relative selectivity for DNA synthesis; (d) reversibility of the DNA and RNA inhibitory action upon removal of the inhibitor; and (e) effect upon preformed DNA and RNA, *i.e.*, depolymerization, to an acid-soluble form, of thymidine-methyl-^{3}H or uridine-5-^{3}H, respectively, which had been incorporated into nucleic acid of the cells prior to exposure to each inhibitor.

Results

The concentrations of each active compound which conferred 50% and 90% inhibition (IC_{50} and IC_{90}) of DNA synthesis in Ehrlich ascites tumor cells *in vitro* are shown in Table II. When the inhibitor and isotopic precursor were added simultaneously to the cell suspension, the slopes of the regression lines were numerically similar, with greater variations occurring after the 1 hr preincubation period. Compounds I and VI were of similar potency after the 1-hr preincubation as compared with no preincubation; compounds II, IV, V, VII, VIII, and IX were more active after 1 hr.

Four compounds suppressed RNA synthesis immediately upon contact with the cells, and the extent of inhibition was virtually the same as that obtained on DNA synthesis. Table III shows the IC_{50} and IC_{90} concentrations of these compounds when added to the cells simultaneously with the isotopic precursor. The relative potency and slopes of the regression lines of compounds V, IX, and XI were quite similar. Compound I was more active and the slope of the regression line was greater.

Figure 1 shows a comparison of inhibitory action of each of the 11 active compounds at a single (10^{-3} *M*)

(5) The melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. The elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(6) A. W. Scott and B. L. Wood, *J. Org. Chem.*, **7**, 515 (1942).

(7) H. L. Yale, *Chem. Rev.*, **33**, 209 (1943).

(8) G. Desseigne, *Bull. Soc. Chim. Fr.* 68 (1948).

(9) W. Mayer and R. Fikentscher, *Ber.*, **89**, 511 (1956).

(10) E. C. Horning and J. A. Parker, *J. Amer. Chem. Soc.*, **74**, 2107 (1952).

(11) E. Müller and E. Tietz, *Ber.*, **74B**, 807 (1941).

TABLE I

Hydroxamic acid	No.	Mp, °C	Formula	Analyses	% yield	Recrystallized from
3,5-Diisopropylsalicyl	I	129.5–131.5	$C_{13}H_{19}NO_3$	C, H, N	54	Hexane
4-Chlorobenzoyl	II	199–199.5[a]	$C_7H_6ClNO_2$	C, H, N, Cl	62	H_2O
4-Fluorobenzoyl	III	165–167[b]	$C_7H_6FNO_2$		68	H_2O
4-Bromobenzoyl	IV	208–210[c]	$C_7H_6BrNO_2$	C, H, N, Br	53	H_2O
3,4,5-Trimethoxybenzoyl	V	173–176	$C_{10}H_{13}NO_5$	C, H, N	34	H_2O
4-Nitrobenzoyl	VI	182–183.5[d]	$C_7H_6N_2O_4$		37	H_2O
4-Iodobenzoyl	VII	223–225 dec[e]	$C_7H_6INO_2$	C, H, N, I	68	MeOH–H_2O (4:1)
3-Methylsalicyl	VIII	147–149[f]	$C_8H_9NO_3$	C, H, N	36	H_2O
3,5-Dichlorobenzoyl	IX	188.5–189.5[g]	$C_7H_5Cl_2NO_2$	C, H, N, Cl	29	MeOH–H_2O (3:1)
2,5-Dihydroxybenzoyl	X	202 dec	$C_7H_7NO_4$	C, H, N	50	H_2O
2-Hydroxy-3,4,5-trimethoxybenzoyl	XI	120.5–122.5	$C_{10}H_{13}NO_6$	C, H, N	10	H_2O
5-Bromosalicyl	XII	239–244 dec[h]	$C_7H_6BrNO_3$	H, N[i]	68	MeOH–H_2O (1:1)
4-Ethoxysalicyl	XIII	156–158	$C_9H_{11}NO_4$	C, H, N	91	H_2O
3-Nitrosalicyl	XIV	159–160.5	$C_7H_6N_2O_5 \cdot 0.5H_2O$	C, H, N	54	H_2O
2-Aminobenzoyl	XV	146–148[j]	$C_7H_8N_2O_2$		55	H_2O
Isonicotinyl	XVI	160–163[k]	$C_6H_6N_2O_2$		48	H_2O
2-Hydroxy-1-naphthoyl	XVII	199–200.5	$C_{11}H_9NO_3$	C, H, N	72	MeOH–H_2O (1:1)
3-Hydroxy-2-naphthoyl	XVIII	206–207.5[l]	$C_{11}H_9NO_3$	C, H, N	66	MeOH–H_2O (1:1)
3,4,5-Trimethoxyphenylacetyl	XIX	136–136.5	$C_{11}H_{15}NO_5$	C, H, N	35[m]	EtOH–Pet ether (1:1)
2-Bromo-3,4,5-trimethoxybenzoyl	XX	193–194 dec	$C_{10}H_{12}BrNO_5$	C, H, N, Br	64	H_2O

[a] B. E. Hackley, R. Plapinger, M. Stolberg, and T. Wagner-Jauregg [*J. Amer. Chem. Soc.*, **77**, 3651 (1955)] reported mp 185°. [b] K. Buraczewski, E. Czerwinska, Z. Eckstein, E. Grochowski, R. Kowalik, and J. Plenkiewicz [*Przemysl Chem.*, **43**, 626 (1964)] reported mp 160–161.5°. [c] N. Kornblum and R. A. Brown [*J. Amer. Chem. Soc.*, **87**, 1742 (1965)] reported mp 189.5–190°. [d] N. Kornblum and R. A. Brown [*ibid.*, **87**, 1742 (1965)] reported mp 176.2–177 dec; B. E. Hackley, Jr., R. Plapinger, M. Stolberg, and T. Wagner-Jauregg [*ibid.*, **77**, 3651 (1955)] reported mp 186°. [e] K. Buraczewski, E. Czerwinska, Z. Eckstein, E. Grochowski, R. Kowalik, and J. Plenkiewicz [*Przemysl Chem.*, **43**, 626 (1964)] reported mp 195–196. [f] T. Urbanski, S. Hornung, S. Slopek, and J. Venulet [*Nature*, **170**, 753 (1952)] reported mp 148–150° dec. [g] K. Buraczewski, E. Czerwinska, Z. Eckstein, E. Grochowski, R. Kowalik, and J. Plenkiewicz [*Przemysl Chem.*, **43**, 626 (1964)] reported mp 150–151°. [h] T. Urbanski, S. Hornung, S. Slopek, and J. Venulet [*Nature*, **170**, 753 (1952)] reported mp 232°. [i] C: calcd 36.23; found 36.72. [j] A. W. Scott and B. L. Wood [*J. Org. Chem.*, **7**, 508 (1942)] reported mp 149°. [k] H. L. Yale, K. Losee, J. Martins, M. Holsing, F. M. Perry, and J. Bernstein [*J. Amer. Chem. Soc.*, **75**, 1933 (1953)] reported mp 163–164°. [l] T. Urbanski, S. Hornung, S. Slopek, and J. Venulet [*Nature*, **170**, 753 (1953)] reported mp 191–192° dec. [m] Overall yield based upon 3,4,5-trimethoxyphenylacetic acid.

TABLE II

EFFECTS OF CERTAIN ARYLHYDROXAMIC ACIDS ON DNA SYNTHESIS BY EHRLICH ASCITES TUMOR CELLS

	No preincubation[a]			1-hr preincubation		
Compd	IC_{50}, M	IC_{90}, M	Slope	IC_{50}, M	IC_{90}, M	Slope
I	2.9×10^{-5}	1.1×10^{-4}	−2.17	3.6×10^{-5}	1.5×10^{-4}	−1.91
II	8.7×10^{-4}	2.9×10^{-3}	−2.45	1.8×10^{-4}	3.6×10^{-4}	−4.27
III	7.8×10^{-4}	2.4×10^{-3}	−2.63	1.3×10^{-3}	4.0×10^{-3}	−2.62
IV	4.4×10^{-4}	5.2×10^{-3}	−1.18	1.6×10^{-4}	3.1×10^{-4}	−4.51
V	5.9×10^{-4}	4.6×10^{-3}	−1.44	2.8×10^{-4}	1.6×10^{-3}	−2.42
VI	6.6×10^{-4}	3.0×10^{-3}	−1.96	5.6×10^{-4}	1.4×10^{-3}	−1.83
VII	4.3×10^{-3}	3.5×10^{-2}	−1.40	9.6×10^{-5}	4.4×10^{-4}	−1.64
VIII	5.6×10^{-4}	2.0×10^{-3}	−2.31	1.6×10^{-4}	1.0×10^{-3}	−1.59
IX	6.7×10^{-4}	3.9×10^{-3}	−1.67	2.0×10^{-4}	1.3×10^{-3}	−1.78
X	6.0×10^{-4}	1.7×10^{-3}	−2.86	3.0×10^{-4}	8.7×10^{-4}	−2.76
XI	6.1×10^{-4}	2.8×10^{-3}	−1.93	3.4×10^{-4}	1.1×10^{-3}	−2.22

[a] IC_{50} and IC_{90} represent 50 and 90% inhibitory concentrations, respectively. The slope is the decrease in probit units for each tenfold increase in inhibitor concentration. Each reaction vessel contained 3 ml of a 1% tumor cell suspension in Eagle's minimum essential medium with Hank's balanced salt solution and 0.03 ml of DMSO with or without the test compound. After 20 min of pulse labeling with 1 μCi thymidine-^{3}H, 2-ml aliquots were added to 2 ml of cold 10% TCA. After 3 washings with 5% TCA, the samples were processed for liquid scintillation counting in PPO–POPOP phosphor solution.

concentration on DNA, RNA, and protein synthesis. Virtually complete inhibition of all 3 parameters occurred with compound I immediately upon exposure of the inhibitor to the cells. Compounds IX and XI were initially quite inhibitory to both DNA and RNA synthesis, with only slight effect on protein synthesis until after exposure of the cells to the inhibitor for 1 to 2 hr. The most singularly selective inhibitor was compound V, which depressed DNA and RNA synthesis over 50% throughout the 2-hr period with no effect whatsoever on protein synthesis. Degrees of selectivity between those of compounds I and V were obtained with the other 9 active agents.

Table IV shows the extent of reversibility of the action of each active compound upon washing the cells free of inhibitor. Inhibition conferred by compounds II, VIII, X, or XI at the higher concentration was readily abolished. The only partial reversibility of the action of compounds I, VII, and IX at the higher concentration indicates a binding of each compound to a critical cellular site or an otherwise irreversible alteration of the metabolic pathway(s) involved. Those compounds which also suppressed the rate of RNA synthesis yielded a similar reversibility pattern on this parameter.

Each of the active compounds was incubated at

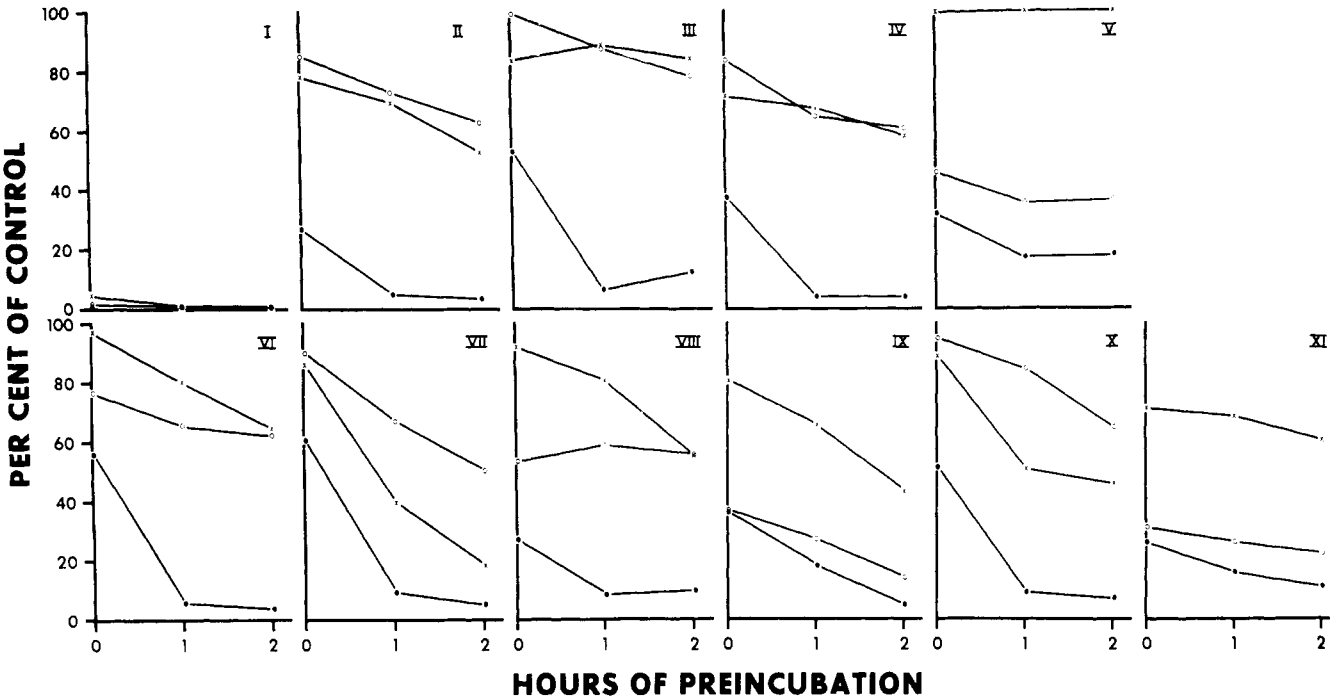

Figure 1.—Effects of certain arylhydroxamic acids on DNA, RNA, and protein synthesis by Ehrlich ascites tumor cells: (●) DNA; (○) RNA; (×) protein. A 1% cell suspension in Eagle's minimum essential medium with Hank's balanced salt solution was incubated with each compound for the interval designated on the abscissa. Isotopic precursors (1 μCi of thymidine-^{3}H, 1 μCi of uridine-^{3}H, and 0.2 μCi of L-leucine-^{14}C) were then added and samples were removed after 20 min and processed for liquid scintillation counting.

TABLE III
EFFECTS OF CERTAIN ARYLHYDROXAMIC ACIDS ON RNA SYNTHESIS BY EHRLICH ASCITES TUMOR CELLS

	No preincubation[a]		
Compd	IC_{50}, M	IC_{90}, M	Slope
I	8.9×10^{-5}	5.1×10^{-5}	−3.30
V	5.9×10^{-4}	1.4×10^{-3}	−1.56
IX	5.8×10^{-4}	1.5×10^{-3}	−1.43
XI	4.1×10^{-4}	1.4×10^{-3}	−1.43

[a] Conditions were the same as described for Table II, except that thymidine-^{3}H was replaced with uridine-^{3}H.

TABLE IV
REVERSIBILITY OF THE INHIBITORY ACTIONS OF ARYLHYDROXAMIC ACIDS ON DNA AND RNA SYNTHESIS IN ASCITES TUMOR CELLS

	DNA		RNA	
	Per cent of control at[a]		Per cent of control at	
Compd	IC_{50}, M	IC_{90}, M	IC_{50}, M	IC_{90}, M
I	112	0	97	0
II	111	111		
III	94	87		
IV	100	76		
V	100	87	85	70
VI	100	91		
VII	43	28		
VIII	111	110		
IX	96	55	83	66
X	110	112		
XI	106	97	98	79

[a] A 1% tumor cell suspension with and without inhibitor at indicated concentrations was incubated at 37° for 10 min. Cells not exposed to the inhibitor and an aliquot of cells incubated with inhibitor were washed three times with fresh medium. The remaining cell suspension containing inhibitor was washed three times with fresh medium containing the same concentration of inhibitor. After resuspension to 1% in the appropriate medium the cells were incubated for 20 min with 1 μCi/ml thymidine-^{3}H or uridine-^{3}H. Reactions were terminated with cold 10% TCA, and the samples were processed for liquid scintillation counting.

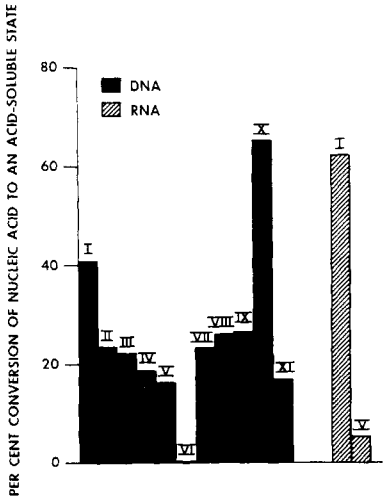

Figure 2.—Depolymerization of nucleic acids by certain arylhydroxamic acids. Cells were incubated at 37° for 20 min with thymidine-^{3}H or uridine-^{3}H (1 μCi/ml), washed three times with fresh medium, and resuspended to 1%. To each vessel containing 0.03 ml of DMSO with or without inhibitor, 3.0 ml of isotopic cell suspension was added, and after 3-hr continued incubation at 37°, 2-ml samples were added to 2 ml of cold 10% TCA. Following three washings in 5% TCA, cell pellets were dissolved in 0.5 ml of methanol and 2 ml of hydroxide of hyamine and combined with PPO–POPOP phosphor solution for liquid scintillation counting.

10^{-3} M for 3 hr at 37° with cells previously pulse-labeled with thymidine-^{3}H (DNA) or uridine-^{3}H (RNA) to detect any degradative action on preformed nucleic acid. All compounds except VI depolymerized preexisting DNA to varying extents (Figure 2). Compound I extensively degraded labeled RNA, V was only moderately active in this regard, and IX and XI were totally inactive.

The present report thus extends the work described earlier[3] and further demonstrates that arylhydroxamic acids are to varying degrees selectively inhibitory to nucleic acid synthesis. An interesting feature noted here is that the majority of the compounds which are active *in vitro* are substituted in the 4 position in relation to the hydroxamic acid group. The demonstrated inhibitory action of 4-hydroxybenzoylhydroxamic acid on growth of experimental tumors[4] suggests that this class of compounds should be subjected to screening in various tumor systems *in vivo*.

Glycylureas and Quaternary Salts[1]

PRICE TRUITT AND J. T. WITKOWSKI

Department of Chemistry, North Texas State University, Denton, Texas 76203

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Although several 1-(*N*,*N*-dialkylglycyl)ureas have been prepared and tested for analgetic properties,[2-6] it seemed worthwhile to prepare a number of such compounds and to convert them into quaternary salts for further physiological testing.

The reaction of chloroacetyl chloride with urea and substituted ureas according to the procedure of Piggott and Rose[2] was utilized in this work to prepare 1-chloroacetylurea and 1-chloroacetyl-3-alkylureas. The reaction of these compounds with secondary amines gave the desired glycylurea derivatives plus some hydantoin. The quaternary salts were readily prepared by reaction of the dialkylaminoacetylureas with various halides. Attempts to prepare *N*-nitroso derivatives of these urea compounds proved futile.

Physiological Activity.—Representative compounds were tested for antibacterial, antiinflammatory, diuretic, shistosomiasis, and trichomonicidal effects.[7] Compounds **12** and **16** were not active against *Trypanosoma cruzi* in chick embryo tissue culture.[8,9] Compound **10**, 1-butyl-3-(chloroacetyl)urea, was cidal when tested *in vitro* against *Trichomonas vaginalis*. Compound **16** was inactive against *T. cruzi* in mice at 0.25% in diet.

Compounds **15** and **16** failed to show activity against measles virus, polio virus, and herpes virus when tested at 100 μg/ml.[10]

TABLE I

SUBSTITUTED UREAS, $RNHCONHCOCH_2R'$

	R	R′	Mp, °C	Yield, %	Formula	Anal
1	H	Pyrrolidino	150–151	85	$C_7H_{13}N_3O_2$	CHN
2	H	Morpholino	137–138	68	$C_7H_{13}N_3O_3$	N
3	H	Me_2N	148–150	55	$C_5H_{11}N_3O_2$	N
4	H	n-Bu_2N	123–124	88	$C_{11}H_{23}N_3O_2$	N
5	n-Bu	Pyrrolidino	69–70	65	$C_{11}H_{21}N_3O_2$	CHN
6	n-Bu	Piperidino	78–79	93	$C_{12}H_{23}N_3O_2$	CHN
7	Et	Pyrrolidino	84–85	41	$C_9H_{17}N_3O_2$	N
8	Et	Piperidino	85–86	67	$C_{10}H_{19}N_3O_2$	CHN
9	Et	Morpholino	86–88	50	$C_9H_{17}N_3O_3$	N
10	n-Bu	Cl	115–116	80	$C_7H_{13}ClN_2O_2$	N

TABLE II

QUATERNARY SALTS, $RR_2'N^+CH_2CONHCONHR''$

R	R′	R″	X	Mp, °C	Yield, %	Formula	Anal
CH_2	n-Bu	H	I	195–196	73	$C_{12}H_{26}IN_3O_2$	N
CH_2	$(CH_2)_4$	H	I	160–161	95	$C_8H_{18}IN_3O_2$	CHN
$C_6H_5CH_2$	$(CH_2)_4$	H	Cl	185–186	41	$C_{14}H_{20}ClN_3O_2$	N
p-$NO_2C_6H_4CH_2$	$(CH_2)_4$	H	Br	171–172	88	$C_{14}H_{19}BrN_4O_4$	CHN
p-$NO_2C_6H_4CH_2$	$(CH_2)_4$	n-Bu	Br	179–180	95	$C_{18}H_{27}BrN_4O_4$	CHN
p-$NO_2C_6H_4CH_2$	$(CH_2)_5$	n-Bu	Br	150–155	81	$C_{19}H_{29}BrN_4O_4$	CHN
p-$NO_2C_6H_4CH_2$	$(CH_2)_4$	Et	Br	191–192	74	$C_{16}H_{23}BrN_4O_4$	N
p-$NO_2C_6H_4CH_2$	$(CH_2)_5$	Et	Br	150–151	82	$C_{17}H_{25}BrN_4O_4$	N
p-$NO_2C_6H_4CH_2$	Et	H	Br	174–175	94	$C_{14}H_{21}BrN_4O_4$	N

Experimental Section[11]

1-Alkyl-3-(dialkylglycyl)ureas were prepared by refluxing 1 mol of 1-alkyl-3-chloroacetylurea with 2 mol of dialkylamine or cyclic secondary amine in C_6H_6. The products were recrystallized from MeOH or C_6H_6 (see Table I).

These compounds were converted into quaternary salts by heating with the desired halide in MeCN. The salt precipitated and rarely needed to be recrystallized (see Table II).

(11) Melting points were determined in a Thomas–Hoover melting point apparatus with a calibrated thermometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Antitumor Activity of Some Azine and Hydrazone Derivatives of 1,4-Dimethoxy-2-butanone[1,2]

WILLIAM J. HAGGERTY, JR., AND C. C. CHENG

Midwest Research Institute, Kansas City, Missouri 64110

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During our investigation of the preparation of certain pyridazine derivatives, three intermediates, 1,4-dimethoxy-2-butanone azine (I), ethyl pyruvate azine with 1,4-dimethoxy-2-butanone (II), and 1,4-dimethoxy-2-butanone hydrazone (III), were prepared and found to possess confirmed activity against Walker 256 (intramuscular, 5WM) tumor system in rats[3] (see Table I).

This interesting activity led us to search the literature for compounds of this type with oncolytic activity. It was found that little information has been published relative to hydrazones as anticancer agents and studies of azines as potential antitumor

(1) Supported by a Grant from Parke, Davis & Company and a Faculty Grant from North Texas State University.

(2) H. A. Piggott and J. D. Rose, U. S. Patent 2,203,506, *Chem. Abstr.*, **34**, 6735 (1940).

(3) P. F. Wiley, *J. Amer. Chem. Soc.*, **71**, 1310 (1949).

(4) T. Takahashi, H. Fujimura, and K. Okamura, *Yakugaku Zasshi*, **82**, 1597 (1962); *Chem. Abstr.*, **59**, 611c (1963).

(5) T. Takahashi and K. Ogiu; Japan Patent 6173 (1961); *Chem. Abstr.*, **58**, P10247*a* (1963).

(6) Y. Matsuo; *Yakugaku Zasshi*, **83** (5) 480 (1963); *Chem. Abstr.*, **59**, 7401*e* (1964).

(7) These tests were arranged through Dr. Ed Elslager of Parke, Davis and Co., Ann Arbor, Mich.

(8) F. A. Neva, M. F. Malone, and B. R. Meyers, *J. Trop. Med. Hyg.*, **10**, 140 (1961).

(9) F. Hawking, *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 345 (1946).

(10) Antiviral screening was carried out by Dr. Frank Schabel, Southern Research Institute, Birmingham, Ala.

(1) This investigation was supported by contract PH 43-65-94 with Chemotherapy, National Cancer Institute, National Institutes of Health.

(2) Presented in part before the Division of Medicinal Chemistry, 155th National Meeting of the American Chemical Society, San Francisco, Calif., March 1968 (N-055).

(3) Test results were provided by contract screeners of CCNSC.