Table II. Nmr Spectra of Methoxy- and Hydroxybenzoylnitropyrroles[a]

Compd	H(3)	H(4)	NH	OH	OMe	Solvent
1e	3.47	3.05	−0.53		6.22	$CDCl_3$
1g	3.35	2.9	Exchanged			DMSO-d_6
1d	3.75	3.08	−3.62		6.38	DMSO-d_6
1f	3.67	3.03	−3.62	0.37	6.38	DMSO-d_6
3	3.6	3.1	−0.5		6.25	$CDCl_3$

[a]In compounds **1**, $J_{1,3}$ = 2 Hz; in **3** H(1) is not split.

mass spectrum *m/e* 321 (90, M^+), 179 (100); nmr, see Table II.

5-Nitro-(2′-hydroxy-6′-methoxybenzoyl)pyrrole (1f). Demethylation of the dimethyl ether 1d by the method above gave 75% of monomethyl ether: mp 139–141° (crystallized from benzene–petroleum ether); mass spectrum *m/e* 262 (65, M^+), 150 (100). *Anal.* ($C_{12}H_{10}N_2O_5$) C, H, N. When boron tribromide was used for demethylation, a minor product was also obtained, shown by mass spectrometry to be a monobromo derivative of 1f with the bromine in the phenolic ring.

Preparation of 4-Chloro-2-(2′-methoxybenzoyl)-5-nitropyrrole (1j). Nitration of 0.2 g of 4-chloro-2-(2′-methoxybenzoyl)pyrrole in acetic acid as above gave 80 mg of nitro derivative: mp 141–142°; yellow cubes from benzene–petroleum ether; mass spectrum *m/e* 280 (50, M^+), 135 (100). *Anal.* ($C_{12}H_9ClN_2O_4$) C, H, Cl, N.

4-Chloro-2-(2′-hydroxybenzoyl)-5-nitropyrrole (1k). Demethylation of 1j as above gave the phenol 1k: 65%; mp 183–185° (crystallized from benzene–petroleum ether); mass spectrum *m/e* 266 (80, M^+), 120 (100). *Anal.* ($C_{11}H_7ClN_2O_4$) C, H, Cl, N.

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Hydroxylamine Derivatives as Potential Inhibitors of Nucleic Acid Synthesis†

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The antineoplastic activity of hydroxyurea **1a** has been the subject of numerous investigations.[1] Currently, its

1a, R = H
b, R = CH_3
c, R = C_2H_5

principal clinical utility lies in the treatment of chronic granulocytic leukemia.[2] Its *N*-methyl (**1b**) and *N*-ethyl (**1c**) derivatives have also been studied in considerable detail[3] but neither of these was shown to have a clear-cut chemotherapeutic advantage over the parent compound.

The analog of **1a**, hydroxyoxamide (oxamylhydroxamic acid) **2a**, as well as its acetylated derivative acetoxyoxamide **2b**, was shown to be a selective inhibitor of *in vitro* DNA synthesis having a similar level of potency to that of **1a**.[4,5]

2a, R = H
b, R = $COCH_3$

However, 2a displays considerably higher toxicity due at least in part to the fact that it readily oxidizes hemoglobin to methemoglobin.[4] Other close structural analogs of **1a** such as 1- and 3-hydroxybiuret (**3a** and **3b**) have also been investigated for antimitotic effects.[6]

3a

3b

In the present investigation, two modes of modification of **2a** were chosen in an effort to obtain reduced toxicity: (a) insertion of a substituent, preferably a bulky one, on the N′ nitrogen and (b) attaching a group at the hydroxyl which would be less susceptible to hydrolysis than **2b**. Compounds **5–13** (Table I) are representative of the former approach while **14** and **15** are examples of the latter.

Two synthetic routes were evaluated for the preparation of N′-substituted hydroxyoxamides. The more general route involving the reaction of ethyl *N*-hydroxyoxamate (**4**) with the appropriate amine was found to be unsatisfactory since complex mixtures were invariably formed even at low temperature. Therefore, these compounds were prepared from their corresponding esters, the properties of which are summarized in Table II.

Although admittedly equivocal, the structures of compounds **14** and **15** have been assigned the *O*-carbamyl configuration. This is based upon the fact that neither compound gave a positive reaction with aqueous iron(III) chloride. In addition, the infrared spectra of **14** and **15** have a band in common with **2a** and **2b** at 2.94–2.96 μ which is absent in the spectra of **7** and **11**.

Three new pyrimidine-5-carbohydroxamic acids **16–18** were prepared as potential antimetabolites by the reaction of their corresponding esters with hydroxylamine in the presence of excess base. The analogous reaction employing 5-carbethoxycytosine, however, resulted in hydrolysis to the corresponding carboxylic acid.

Each of the compounds presented in Table I was evaluated for inhibition of DNA, RNA, and protein synthesis in Ehrlich ascites tumor cells *in vitro.* Seven compounds showed inhibition of DNA synthesis at concentrations below $10^{-3}M$ and these are presented in Table III together with the corresponding data for **1a** and **2a**. It will be seen that none of the present compounds display the degree of selective inhibition of DNA synthesis which is shown by **1a** and **2a**, although the activity of **14** is considered significant.

With the exception of **8**, none of the derivatives of **2a** (**5–7** and **9–15**) were found to form methemoglobin (*cf.* Experimental Section). However, the structural modifications generally also resulted in loss of selective action. Only compounds **7** and **9** produced "unbalanced growth" of *Escherichia coli* as evidenced by the formation of long, filamentous cell forms which is often associated with selectivity of action against DNA synthesis. It will be noted

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Table I. Analogs of Hydroxyoxamide and Hydroxamates of Some Pyrimidine-5-carboxylic Acids

Name	No.	Mp, °C	Formula	Analyses[a]	Yield, %	Recrystn medium and no.	Molar reaction ratio (NH_2OH/ester)
Ethyl *N*-hydroxyoxamate	4	85–87[b]	$C_4H_7NO_4$	C, H, N	39[c]	Acetone (1)	2:3
N-Ethyl-*N*'-hydroxyoxamide	5	144–146 dec	$C_4H_8N_2O_3 \cdot NH_2OH$[d]	C, H, N	38	EtOH (2)	2:1
N-(2-Diethylaminoethyl)-*N*'-hydroxyoxamide	6	131–132 dec	$C_8H_{17}N_3O_3$	C, H, N	40	EtOH (1)	1:1
N-Hydroxy-*N*'-phenyloxamide	7	148–152 dec[e]	$C_8H_8N_2O_3$	C, H, N	47	H_2O (1)	2:1
N-Benzyl-*N*'-hydroxyoxamide	8	176–180 dec	$C_9H_{10}N_2O_3 \cdot NH_2OH$[d]	H, N[f]	44	EtOH (2)	2:1
N-Hydroxy-*N*'-(2-isopropylphenyl)oxamide	9	134–137	$C_{11}H_{14}N_2O_3$	C, H, N	42	Bz (2)	2:1
Ethylene-1,2-di(*N*'-hydroxyoxamide)	10	198 dec	$C_{10}H_{16}N_2O_6$	C, H, N	29	H_2O (1)	4:1
N-Diethyl-*N*'-hydroxyoxamide	11	138–139	$C_6H_{12}N_2O_3$	C, H, N	18	EtOH (1)	1:1
N-(2,6-Diisopropylphenyl)-*N*'-hydroxyoxamide	12	202–203	$C_{14}H_{20}N_2O_3$	C, H, N	39	Bz (1)	2:1
N-(1-Adamantyl)-*N*'-hydroxyoxamide	13	138–140 dec	$C_{12}H_{18}N_2O_3$	C, H, N	30	H_2O (1)	1:1
O-Phenylcarbamylhydroxyoxamide	14	167–169 dec	$C_9H_9N_3O_4$	C, H, N	16	EtOH (1)	
O-Diphenylcarbamylhydroxyoxamide	15	168–170	$C_{15}H_{13}N_3O_4$	C, H, N	30	$CHCl_3$ (1)	
Uracil-5-carbohydroxamic acid	16	>250 dec[h]	$C_5H_5N_3O_4$	C, H, N	76	DMSO–H_2O (1)[g]	2.7:1
2-Amino-4-hydroxypyrimidine-5-carbohydroxamic acid	17	234–236 dec	$C_5H_6N_4O_3$	C, H, N	77	DMAC (1)	2.7:1
2-Mercapto-4-hydroxypyrimidine-5-carbohydroxamic acid	18	>240 dec[h]	$C_5H_5N_3O_3S$	C, H, N	54	H_2O (1)	2.7:1

[a]Where analyses are indicated only by symbols of the elements, the results were within ±0.4% of the theoretical values. The analyses were performed by Galbraith Laboratories, Knoxville, Tenn. [b]G. Gilbert, T. Wagner-Jauregg, and G. M. Steinberg [*Arch. Biochem. Biophys.*, **93**, 469 (1961)] reported mp 85–87°. [c]Yield based upon NH_2OH. [d]Isolated as the NH_2OH salt. [e]H. Schiff and U. Monsacchi [*Justus Liebigs Ann. Chem.*, **288**, 313 (1895)] reported mp 195°. [f]C: calcd, 47.57; found, 48.27. [g]Reprecipitation. [h]Compound gradually darkened above this temperature.

Table II. Esters of N-Substituted Oxamic Acids

Name	No.	Mp or bp (mm), °C	Formula	Analyses[a]	Yield, %	Method of prepn	Recrystn medium
Ethyl *N*-phenyloxamate	19	63–65[b]	$C_{10}H_{11}NO_3$		38	A	H_2O–EtOH (75:25)
Ethyl *N*-benzyloxamate	**20**	46–47[c]	$C_{11}H_{13}NO_3$		36	B	Acetone–H_2O[d]
Ethyl *N*-(2-isopropylphenyl)-oxamate	21	120 (0.4)	$C_{13}H_{17}NO_3$	C, H, N	37	A	
Methyl *N*-(2,6-diisopropylphenyl)-oxamate	**22**	125–127	$C_{15}H_{21}NO_3$	C, H, N	47	C	Hexane
Ethyl *N*-ethyloxamate	**23**	80 (0.4)[e]	$C_6H_{11}NO_3$		49	B	
Ethyl *N*-(2-diethylaminoethyl)-oxamate	**24**	112–122 (1.0)[f]	$C_{10}H_{20}N_2O_3$		78	B	
Diethyl ethylene-1,2-dioxamate	**25**	128–129[g]	$C_{10}H_{16}N_2O_6$		36	B	EtOH
Methyl *N*-(1-adamantyl)oxamate	**26**	134–135	$C_{13}H_{19}NO_3$	C, H, N	78	C	H_2O–MeOH (50:50)

[a]*Cf.* footnote *a*, Table I. [b]A. G. Richardson, J. S. Pierce, and E. E. Reid [*J. Amer. Chem. Soc.*, 74, 4011 (1952)] reported mp 66°. [c]T. Curtis and W. Sandhaas [*J. Prakt. Chem.*, **125**, 90 (1930)] reported mp 48°. [d]Reprecipitation. [e]P. M. Kochargin and K. S. Bushueva, [*Zh. Prikl. Khim. (Leningrad)*, **35**, 2745 (1962)] reported bp 148–149° (23 mm). [f]F. K. Kirshner [U. S. Patent 3,096,373 (1963); *Chem. Abstr.*, **60**, 453 (1964)] reported bp 112–122° (1.0 mm). [g]J. van Alphen [*Recl. Trav. Chim. Pays-Bas*, **53**, 1159 (1934)] reported mp 129°.

that there is no correlation between this observation and the incorporation studies presented in Table III.

Experimental Section

Chemistry. Melting points were taken with a Mel-Temp apparatus and are uncorrected.

Esters of N-Substituted Oxamic Acids. Method A (19 and 21). The appropriate aniline in the presence of 2 equiv of diethyl oxalate was maintained at reflux for 4 and 48 hr, respectively. Cooling at 0° produced solids which were purified by recrystallization (*cf.* Table II).

Method B (20, 23, 24, and 25). The required amine was added dropwise with stirring to 2 equiv of diethyl oxalate (3 equiv for **25**) at 0°. The resulting mixtures were kept at *ca.* 25° for 24 hr (0° for 48 hr in the case of **23**). Concentration at reduced pressure and addition of hexane produced **20** as a solid, while the other esters were purified by vacuum distillation.

Method C (22 and 26). In a flask protected from moisture and maintained at 0° containing 9.96 g (0.066 mol) of 1-aminoadamantane in 25 ml of pyridine was added 9.1 g (0.066 mol) of methyloxalyl chloride over 1 hr. After standing at *ca.* 25° for 2 days, the mixture was neutralized with concentrated HCl, H_2O added, and the product separated by filtration.

***N*-Hydroxyoxamides (4–13).** Each of these compounds was prepared by reacting the appropriate ester with a neutral solution of NH_2OH in EtOH. The use of excess NH_2OH proved beneficial in the case of *N*-aryl compounds 7, 9, and 12 but led to NH_2OH complex formation in the case of **5** and **8** (*cf.* Table I). The following procedure for **13** is representative. A solution of 1.0 g (0.025 mol) of NaOH in 55 ml of EtOH was added with cooling and stirring to

Table III. Compounds Inhibiting Macromolecular Synthesis in Ehrlich Ascites Tumor Cells *in Vitro*

No.	IC_{50}, M^a DNA	RNA	Protein
1a	2×10^{-4}	$>1 \times 10^{-3}$	$>1 \times 10^{-3}$
2a	8×10^{-4}	$>1 \times 10^{-3}$	$>1 \times 10^{-3}$
4	3×10^{-5}	1×10^{-4}	3×10^{-6}
7	2×10^{-5}	5×10^{-5}	6×10^{-5}
9	1×10^{-5}	6×10^{-5}	4×10^{-5}
12	2×10^{-5}	4×10^{-5}	7×10^{-5}
13	2×10^{-5}	4×10^{-5}	4×10^{-5}
14	1×10^{-5}	$>1 \times 10^{-3}$	3×10^{-4}
15	5×10^{-4}	5×10^{-4}	5×10^{-4}

[a]Molar concentration which conferred 50% inhibition of incorporation of thymidine-*methyl*-^3H, uridine-*5*-^3H, and *l*-leucine-^{14}C into DNA, RNA, and protein, respectively.

1.74 g (0.025 mol) of $NH_2OH \cdot HCl$ in 60 ml of EtOH. The resulting solution was filtered and cooled to 0°, and 5.88 g (0.025 mol) of 26 was added. This mixture was left to stand at *ca.* 25° for 15 hr. The resulting solid was separated by filtration, washed with H_2O, and then recrystallized.

O-Phenylcarbamylhydroxyoxamide (14). A solution of 2.08 g (0.02 mol) of **2a** in 20 ml of pyridine was protected from moisture by an N_2 purge. To this was added dropwise 2.4 g (0.02 mol) of phenyl isocyanate over 0.5 hr at *ca.* 25°. After 3 hr the mixture was added to 25 ml of concentrated HCl and 25 g of ice. The solid was separated by filtration, washed with H_2O, and air-dried. Extraction with boiling EtOH (90 ml) yielded white crystals which upon vacuum drying without a desiccant amounted to 0.7 g (negative test with 1% $FeCl_3$ solution).

O-Diphenylcarbamylhydroxyoxamide (15). To a solution of 1.04 g (0.01 mol) of 2a in 10 ml of pyridine (N_2 purge, ice bath) was added dropwise 2.32 g (0.01 mol) of diphenylcarbamyl chloride over 1 hr. After stirring for 3 additional hr, the mixture was added to 12 ml of concentrated HCl and 12 g of ice. The resulting solid was separated by filtration, washed with H_2O, and vacuum dried without a desiccant. The recrystallized product gave a negative test with 1% $FeCl_3$ solution.

Pyrimidine-5-carbohydroxamic Acids (16–18). These compounds were prepared from the corresponding ethyl esters according to the procedure of Chang;[7] the basic reaction mixtures were kept at *ca.* 10°. In each case, tlc (silica gel) indicated that all the ester had been consumed (16, 5 days; 17, 2 days; 18, 7 days) before the reactions were neutralized to pH 3–3.5 with AcOH (glacial). The resulting solids were separated by filtration, washed with H_2O, and recrystallized. The analogous reaction with 5-carbethoxycytosine was unsuccessful, the principal product being 5-carboxycytosine as identified by mixture melting point, ir, and elemental analysis.

Biological. Methods of measuring the rates of DNA, RNA, and protein synthesis were substantially the same as those described earlier.[8] Of the seven active compounds, *cf.* Table III, the inhibitory action was not substantially reduced against any of the three parameters upon subsequent washing the cells with fresh medium devoid of the inhibitor. Each of the compounds in Table I was evaluated for its tendency to induce methemoglobin using the method of Leahy and Smith.[9] Of these, only **8** showed any measurable effect causing 26% methemoglobin after 1 hr of incubation (37°) at $1 \times 10^{-3} M$. Under the same condition, **2a** effected an 84% conversion.

Each of the compounds listed in Tables I and II was assayed against the growth of *Escherichia coli* strain B (ATCC No. 11303). This involves placing a filter paper disk impregnated with a 1% solution or suspension of the sample on a seeded agar plate.

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Examination of the Utility of the Topliss Schemes for Analog Synthesis

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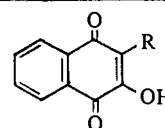
The recent formulations by Topliss of operational schemes for rational analog synthesis were suggested as an avenue "to maximize the chances of synthesizing the most potent compounds in the series as early as possible."[1] It is the purpose of this communication to present additional retrospective examples of the utility of the schemes. A scheme is considered useful if it either included the synthesis of the most active analog or if after the synthesis of the suggested analogs an examination of physical properties *vs.* potencies leads directly to the most potent compound. No example found in which the suggested compounds were tested has been omitted from this discussion.

The side-chain scheme can be examined by a study of the very complete series of 295 2-alkyl-3-hydroxy-1,4-naphthoquinones which were investigated as antimalarials.[2] In the total study, 26 molecules (9%) exhibited an ED_{95} of 7 mg/kg or less. If the Topliss schemes had been followed, the five molecules listed in Table I would have been synthesized. One of these (20%) exhibited an ED_{95} of 7 mg/kg. (Only one molecule of the 295 tested was significantly more active than the cyclohexyl: the 4'-cyclohexylcyclohexyl analog had an ED_{95} of approximately 0.6 mg/kg.) The relative potency of analogs in this series appears to depend not only on the partition coefficient of the molecule but also

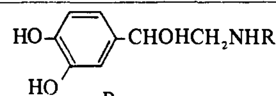
Table I. Antimalarial Activity of Naphthoquinones



Step no.[a]	R	ED_{95}, mg/kg[b]
1	CH_3	>400
2	*i*-C_3H_7	175
3	c-C_5H_9	26
4	c-C_6H_{11}	7
5	$CH_2C_6H_5$	>140

[a]Order of compound synthesis from Chart II, ref 1. [b]Reference 2.

Table II. Adrenergic Activity of Catechol Amines



Step no.	R	β_1 potency[a]
1	CH_3	61
2	*i*-C_3H_7	1000
3	c-C_5H_9	214
		β_2 potency[a]
1	CH_3	230
2	*i*-C_3H_7	1000
3	c-C_5H_9	350
		$1/\beta_2$ potency[a]
1	CH_3	0.0043
2	*i*-C_3H_7	0.0010
3	H	0.300

[a]Reference 3.