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

Yvonne C. Martin

Experimental Therapy Division, Abbott Laboratories, North Chicago, Illinois 60064

and William J. Dunn, III

Department of Medicinal Chemistry, University of Illinois at the Medical Center, Chicago, Illinois 60680.

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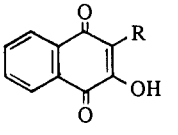
The recent formulations by Topliss of operational schemes for rational analog synthesis were suggested as an avenue "to maximize the chances of synthesizing the most potent compounds in the series as early as possible."[1] It is the purpose of this communication to present additional retrospective examples 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

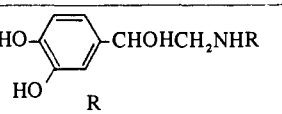
Table I. Antimalarial Activity of Naphthoquinones



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.

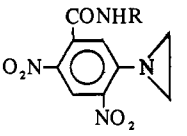
Table II. Adrenergic Activity of Catechol Amines



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.

Table III. Antitumor Activity of 5-Aziridino-2,4-dinitrobenzamides



Step no.	R	ED_{90}, mg/kg[a]
1	CH_3	<2.5
2	*i*-C_3H_7	1.6
3	c-C_5H_9	40.0
4	c-C_3H_5	1.0
5	*tert*-C_4H_9	20.0

[a]Reference 4.

on the structure of the side chain.[2] Thus, the scheme leads one to the correct molecule even when specific hydrophobic bonding is a critical feature in potency.

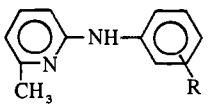
The β-adrenergic agonist activities of nine analogs of norepinephrine have been reported.[3] Table II summarizes the results which would have been obtained if the scheme had been used for this series. For β_1 (lipolytic) activity the most potent of the nine was found in the three-step scheme, a threefold enhancement efficiency. In a similar fashion the most potent β_2 (bronchodilator) agonist was found in the three-step scheme. For a cardiovascular agent it would be preferable to reduce β_2 activity; thus, the bottom of the table traces the synthetic scheme which would be used when the optimization procedures are used to *decrease* potency. Again, the most favorable of the nine compounds was found when the proposed schemes are used to direct synthesis of three molecules.

A final example involving the variation in the aliphatic substituents is of the variation of antitumor activity with variation of the amide nitrogen of the parent compound, 5-aziridino-2,4-dinitrobenzamide. The series of molecules to be synthesized is presented in Table III.[4] The study reported data for 11 compounds, yet using the proposed scheme led to the most active in the series in five steps. An examination of the scheme reveals, as does that for the antimalarials presented earlier, that the structure, and not just the hydrophobic nature, of the side chain is important for enhanced activity. Further work with this series[5] led to synthesis of the parent structure (R = H) with an ED_{90} = 0.4 mg/kg.

A study of the relative rate of oxidation of benzylamines by monoamine oxidase[4] provides a test for the scheme for aromatic substitution (Table IV). A total of 13 compounds were tested; the four-step scheme results in the synthesis of the second most potent analog, *m*-chlorobenzylamine. The most potent is the *m*-iodo derivative, which seems to be a logical follow-up to the chloro compound. Thus, five steps led to the most potent of 13 compounds.

In a set of seven antibacterial detergents[5] the most active was found in the three-step scheme (Table V).

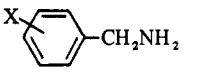
Bailey, *et al.*,[6] synthesized a series of 19 2-anilinopyridines of general structure I for their activity against *Escherichia*



I

coli and *Staphylococcus aureus*. By following the Topliss scheme optimum activity against *E. coli* is obtained after synthesis of five analogs. These steps are shown in Table VI. Also shown in Table VI is the scheme leading to the most active compound in the series against *S. aureus*. In this case

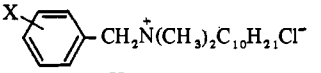
Table IV. Substrates of Monoamine Oxidase



Step no.	X	Log rate of oxidation[a]
1	H	2.3
2	4-Cl	1.8
3	4-OMe	1.3
4	3-Cl	2.5
	3-I	2.9

[a]C. Hansch, E. J. Lien, and F. Helmer, *Arch. Biochem. Biophys.*, 128, 319 (1968).

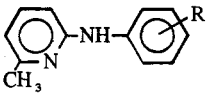
Table V. Detergent Inhibitors of *Staphylococcus aureus*



Step no.	X	Log 1/MIC[a]
1	H	2.8
2	4-Cl	3.6
3	3,4-Cl_2	3.8

[a]C. Hansch and W. R. Glave, *Mol. Pharmacol.*, 7, 337 (1971).

Table VI



Step no.	R	MIC, μg/ml[a]
	Minimum Inhibitory Concentration against *E. coli*	
1	H	250
2	4-Cl	31.3
3	3,4-Cl_2	62.5
4	4-Br	25.0
5	4-NO_2	>125
	Minimum Inhibitory Concentration against *S. aureus*	
1	H	250
2	4-Cl	62.5
3	3,4-Cl_2	3.9
4	3-CF_3, 4-Cl	0.5

[a]Reference 6.

the most active compound is obtained after four steps. "Most active" must, in this case, be qualified since the scheme suggests synthesis of the 3-CF_3, 4-NO_2 derivative after the 3-CF_3,4-Cl analog. This compound was not prepared so this set of analogs is still incomplete.

These examples suggest that if one followed the schemes proposed by Topliss he would synthesize the most potent compound of his series in approximately one-third the number of drugs as if he followed the traditional approach. The success of the schemes is perhaps due as much to their formulation by a person experienced in the intuitive aspects of structure-activity analysis as by their roots in the more formalized Hansch technique.

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