Synthesis and Antimicrobial Activity of Azasteroid-Type Compounds and Related Systems. Effect of Hydrophilic and Lipophilic Groups on Activity

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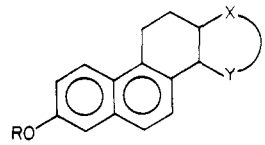
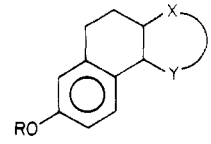
Pyrazole-, pyrazolone- and isoxazole-containing systems were prepared from 3,4-dihydro-6-(hexyloxy)-1(2*H*)-naphthalenone, 3,4-dihydro-6-(hexadecyloxy)-1(2*H*)-naphthalenone, 3,4-dihydro-6-(2-dimethylaminoethyloxy)-1-(2*H*)-naphthalenone, 3,4-dihydro-7-hexyloxy-1(2*H*)-phenanthrone, and 3,4-dihydro-7-(2-dimethylaminoethyloxy)-1(2*H*)-phenanthrone. A number of compounds derived from 7,8-dihydro-5-(6*H*)-quinolinone were also synthesized and characterized. Both hydrophilic and lipophilic groups were incorporated into certain systems as well as cidal groups. The compounds were screened for their in vitro inhibitory activity against *Bacillus subtilis* and *Pseudomonas fluorescens*. Structure–activity relationships among the molecular systems are discussed.

It has been reported from this laboratory that certain pyrazolo steroids and isoxazolo steroids and related model compounds possessed a wide range of biological activity.[1] In a continuing effort to evaluate the bacteriocidal and bacteriostatic ability of azasteroids and model systems and on the basis of previous screening data, we have synthesized specific members with certain fused heterocyclic rings as illustrated in **1** and **2**. On the supposition that improving the lipophilicity of the molecule could improve



1, R = $CH_3(CH_2)_5$-, $(CH_3)_2NCH_2CH_2$-



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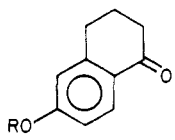
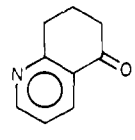
cell membrane penetration,[2] R was increased from methyl to a C_2, C_4, C_6, and C_{16}. The microorganisms used for growth studies were *Bacillus subtilis* and *Pseudomonas fluorescens*. One objective of this work was to determine the activity dependence upon the lipophilic nature of the substituent in ring A. Lipid–water balance has been suggested to be an important factor in the membrane transport process.[2a,b]

Chemistry. Key starting materials for syntheses described herein are ketones **3–5**. 6-Hydroxytetralone[3] (**3b**), prepared by cleavage of 6-methoxytetralone (**3a**, R = CH_3)





3a, R = CH_3
b, R = H
c, R = $(CH_2)_5CH_3$
d, R = CH_2CH_2OH
e, R = $CH_2CH_2N(CH_3)_2$
f, R = $C_{16}H_{33}$

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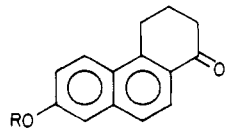


5a, R = CH_3
b, R = H
c, R = $CH_2CH_2N(CH_3)_2$
d, R = C_6H_{13}

with boiling 48% HBr, was required for **3c–f**, **6b–e,g**, **7b–d**, **8b,c** and **9b**. Similarly, hydroxy ketone **5b** was obtained from the methoxy-substituted precursor **5a**.

Alkylation of 6-hydroxytetralone (**3b**) or ketone **5b** with halogen-substituted compounds in the presence of K_2CO_3 proceeded smoothly in acetone to give **3c–f** and **5c,d**, respectively. Azasteroid-type systems **10b,c** and **11** were also prepared through condensation of hydrazine or hydroxylamine with the appropriately substituted hydroxymethylene ketone **12** by techniques previously described.[1b,c,4] In similar fashion 7,8-dihydro-5(6*H*)-quinolinone (**4**) was converted to **13**. Although **13** formed the methyl iodide salt smoothly at the pyridine nitrogen atom, attempts to introduce a long alkyl group (for improved lipophilicity), such as with octyl or cetyl bromide, gave only the hydrobromide salt.[5] Removal of HBr from the bromoalkane in an elimination may be competitive with N-alkylation and has been observed in related systems.[6]

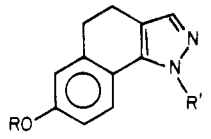
In order to incorporate a hydrophilic group and lipophilic group at opposite ends of the molecule, pyrazole **6d** was converted to the N-substituted glucose derivative **6h** by general techniques described elsewhere.[1c] Assignment of N(1) alkylated product was based on ^1H NMR arguments, summarized previously[1a,c] for **6i**.

Another approach to improve the activity members of **6** was to incorporate a cidal group such as a nitrogen mustard and/or sulfur mustard functions. Such groups are well known as conveying anticancer activity to melphalan (NSC-8806), nitrogen mustard (NSC-762), and chlorambucil (NSC-3088) to name only a few. Thus, pyrazole **6a** was treated as shown in Scheme I to give **18**.

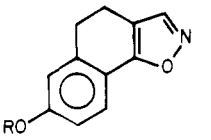
Conversion of **6d** to **6j** was effected in a manner like that shown in Scheme I. Treatment of **15** with mercaptoethanol in the presence of $NaOCH_3$ in absolute methanol gave the mercaptoethanol derivative **6k**. Formation of the sulfur mustard **6l** from **6k** was successfully achieved by heating **6k** with thionyl chloride in chloroform.

The general approach to thiazoles has been reported.[1a] Consequently, the synthesis of **9b** involved a combination of the reported technique and that O-alkylation process as discussed to give **3c–f** and **5c,d**. Tables I–IV contain properties of model compounds prepared.

Biological Activity. To evaluate the lipophilic properties of these compounds, a comparison was made of the biological activities of the compounds with respect to *O*-alkyl chains of varying length and with the nature of the heterocycle. *Bacillus subtilis* and *Pseudomonas fluorescens* were chosen as microbial screens. These data along with growth inhibition of KB cells are tabulated on Table V. All of the basic techniques for the biological

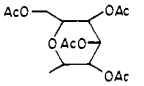


6a, R = CH_3; R' = H
b, R = CH_2CH_2OH; R' = H
c, R = $CH_2CH_2N(CH_3)_2$; R' = H
d, R = $(CH_2)_5CH_3$; R' = H
e, R = $(CH_2)_{15}CH_3$; R' = H
f, R = CH_3; R' = $SO_2N(CH_3)_2$
g, R = $(CH_2)_5CH_3$; R' = $SO_2N(CH_3)_2$

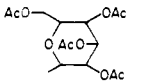


7a, R = CH_3
b, R = CH_2CH_2OAc
c, R = $CH_2CH_2N(CH_3)_2$·HCl
d, R = $(CH_2)_5CH_3$

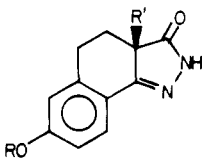
h, R = $(CH_2)_5CH_3$; R' =



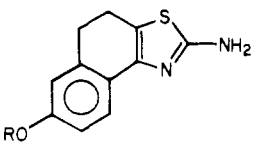
i, R = CH_3; R' =



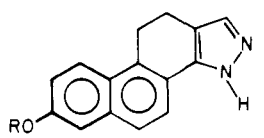
j, R = $(CH_2)_5CH_3$; R' = $CH_2CH_2N(CH_2CH_2Cl)_2$·2HCl
k, R = CH_3; R' = $CH_2CH_2SCH_2CH_2OH$
l, R = CH_3; R' = $CH_2CH_2SCH_2CH_2Cl$·HCl



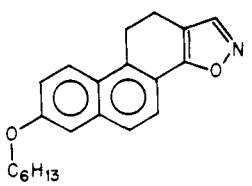
8a, R = CH_3; R' = CH_3
b, R = $(CH_2)_5CH_3$; R' = CH_3
c, R = $(CH_2)_5CH_3$; R' = H



9a, R = CH_3
b, R = $(CH_2)_5CH_3$



10a, R = CH_3
b, R = $CH_2CH_2N(CH_3)_2$
c, R = $(CH_2)_5CH_3$



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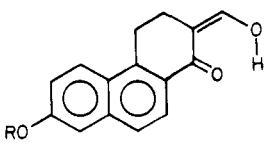

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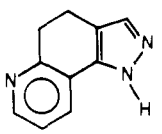

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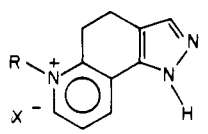


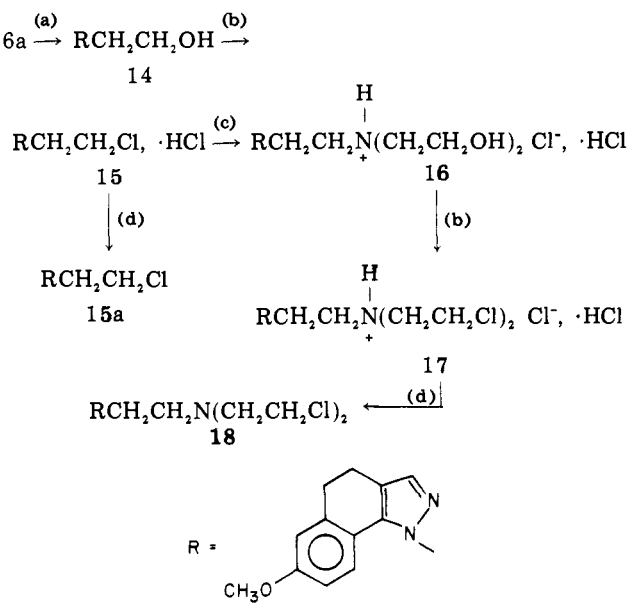
13a, R = CH_3; X = I
b, R = H; X = Br

assays have been previously reported from our laboratory.[1]

It is apparent from Table V that growth inhibition of *B. subtilis* with heterocyclic systems **6**, **7**, **8**, and **9** is found when R is an alkyl of six carbons (**6d**, **7d**, **8b**, and **9b**). The HCl salts of **9a** and **9b** are also slightly active against *P. fluorescens*. *N*-Glucosides **6h** and **6i** likewise effected growth inhibition of *B. subtilis* and **6i** was active against *P. fluorescens*. Thus, the sugar moiety appears to be as effective as the C_6 chain in providing bacteriostatic action.

Steroid-type systems **10b,c** and **11** were effective against *B. subtilis*. Curiously, **10a** was not very inhibitory with this organism but had a modest influence on the growth of *P. fluorescens*.

Scheme I



(a) $NaOCH_3$; $ClCH_2CH_2OH$-DMF. (b) $SOCl_2$-$HCCl_3$. (c) $HN(CH_2CH_2OH)_2$-diglyme; HCl. (d) CH_3CO_2Na-C_2H_5OH.

Quinoline derivatives **13** and **13a** were only moderate in ability to inhibit growth of *B. subtilis*. Thus, the nitrogen in the A ring has little influence on activity in this system.

Cidal-substituted analogues **17** and **6j** differed in that the latter was more active by a factor of 4 against growth of *B. subtilis*. Again the only structural difference is the chain length of the alkyl group at C-7. A viable cell count with *B. subtilis* in the presence of **6j** revealed a reduction from 3.3×10^7 cells/mL to 3.1×10^3 cells/mL suggesting **6j** had a cidal effect. After 24 h the cell count had risen to only 5% of the original value and the cells were still vulnerable to inhibition of growth by a fresh solution of **6j** at the same concentration.

In summary, there appears to be a correlation between the length of the alkyl chain on oxygen and the ability of these compounds to possess bacteriostatic action. A bacteriocidal effect was noted when a bis(chloroethyl)-aminoethyl group was attached to the system as in **6j**.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary apparatus and were uncorrected. NMR spectra were recorded in parts per million downfield from tetramethylsilane on a Varian XL-100 (15) high-resolution spectrometer in $DCCl_3$ and are unavailable upon request. Peak multiplicity is depicted as s = singlet, d = doublet, t = triplet, and m = multiplet. Where analyses have been indicated only by symbols of elements, analytical results obtained for these elements were within ±0.4% of the theoretical values and were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

6-Hydroxy-1-tetralone (3b). 6-Methoxy-1-tetralone (17.62 g, 0.1 mol) was boiled with aqueous 48% hydrobromic acid (250 mL) for 5 h. The reaction mixture was cooled, filtered, and washed (H_2O) several times and finally recrystallized (H_2O) to yield 12.5 g (77%) of a white solid: mp 156–157 °C (lit.[3] mp 154 °C).

3,4-Dihydro-7-hydroxy-1(2*H*)-phenanthrone (5b). 3,4-Dihydro-7-methoxy-1(2*H*)-phenanthrone[4] (**5a**) (3.5 g, 0.0154 mol) was boiled with aqueous 48% hydrobromic acid (60 mL) under N_2. A yellow precipitate formed after 20 min. Heating was continued for another 3 h. Remaining work-up was similar to that used for **3b** above and gave 3.0 g (92%) of 3,4-dihydro-7-hydroxy-1(2*H*)-phenanthrone (**5b**) (crystallized from aqueous

Table I. Substituted Tetralones and Phenanthrones

Compd	Method	Yield, %	Recrystn solvent	Mp or bp (mm), °C	Formula	Analyses
3c[a]	A	80		174 (0.75)	$C_{16}H_{22}O_2$	C, H
3d	A	73	Benzene	91-92[c]		
3e[b]	A_1	72	Petr ether (bp 30-60 °C)	51-52	$C_{14}H_{19}NO_2$	C, H, N
3f	A	74	Methanol	48-50	$C_{26}H_{42}O_2$	C, H
5c	A_1	85	Hexane	69-70	$C_{18}H_{21}NO_2$	C, H
5d	A	80	Aq ethanol	66-67	$C_{20}H_{24}O_2$	C, H

[a] Semicarbazone from ethanol: mp 203-204 °C. Anal. ($C_{17}H_{25}N_3O_2$) N. Thiosemicarbazone from ethanol: mp 147-148 °C. Anal. ($C_{17}H_{25}N_3OS$) N. [b] Methiodide crystallized from absolute methanol-ether: mp 197-198.5 °C. Anal. ($C_{15}H_{22}NO_2I$) C, H. [c] Lit.[7] mp 92 °C.

Table II. Physical Constants, Yields, and Spectral Data of Model Compounds

Compd	Method	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses
6b	C	79	Aq ethanol	162-163	$C_{13}H_{14}N_2O_2$	C, H, N
6c[a,b]	C	70	Aq ethanol	62-63	$C_{15}H_{17}N_3O \cdot H_2O$	N
6d	C	86	*n*-Heptane	67-68	$C_{17}N_{22}NO$	C, H, N
6e	C	68	Ethanol-water	88-90	$C_{27}H_{42}N_2O$	C, H, N
6f	E	85	Aq ethanol	133-134	$C_{14}H_{17}N_3O_3S$	N, S
6g	E	91	Aq ethanol	89-90	$C_{19}H_{27}N_3OS$	N, S
6k		88	*n*-Hexane	86-87	$C_{16}H_{20}N_2O_2S$	N, S
6l		56	$HCCl_3$-ether	158-159	$C_{16}H_{20}N_2OSCl_2$	N, S, Cl
7b	D	64	Aq ethanol	98-99	$C_{15}H_{15}NO_4$	C, H, N
7c	D_1	70	Abs methanol	216-217	$C_{15}H_{19}N_2O_2Cl$	N, Cl
7d	D	65	*n*-Heptane	50-51	$C_{17}H_{21}NO_2$	C, H, N
14		63	Benzene	140-141	$C_{14}H_{16}N_2O_2$	C, H, N
15		80	$HCCl_3$-ether	188-189	$C_{14}H_{16}N_2OCl_2$	C, H, N, Cl
16		56	Abs ethanol	166-167.5	$C_{18}H_{27}N_3O_3Cl_2$	C, H, N, Cl
18		84	Skelly B	47-47.5	$C_{18}H_{23}N_3OCl_2$	C, H, N, Cl
17		73	Abs ethanol	183-184 dec	$C_{18}H_{25}N_3OCl_4$	C, H, N, Cl

[a] Isolated as the hydrochloride. [b] The dihydrochloride was crystallized from absolute ethanol-ether: mp 243-244 °C. Anal. ($C_{15}H_{19}N_3OCl_2 \cdot H_2O$) N, Cl.

Table III. Pyrazolo Steroid and Isoxazolo Steroid Systems

Compd	Method	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses
10b	D_1	86	Benzene	154-155	$C_{19}H_{21}N_3O$	C, H, N
10c	C	77	Aq ethanol	127-128	$C_{21}H_{24}N_2O$	C, H, N
11	D	67.9	Aq ethanol	94-95	$C_{21}H_{23}NO_2$	C, H, N

Table IV. 4,5-Dihydro-1*H*-pyrazolo[3,4-*f*]quinoline and Its Derivatives

Compd	Yield, %	Recrystn solvent	Mp, °C	Formula	Analyses
13	61	2-Propanol-benzene	209-210.5	$C_{10}H_9N_3$	N
13a	52	Water-2-propanol	222.5-224	$C_{11}H_{12}N_3I$	C, H, N
13b	42	Methanol-ether	263-266	$C_{10}H_{10}N_3Br$	C, H, N

ethanol, mp 238–239 °C). Anal. ($C_{14}H_{12}O_2$) C, H.

Substituted 6-Alkoxy-1-tetralones and 7-Alkoxy-1-phenanthrones. Method A. A mixture of 6-hydroxy-1-tetralone (**3b**) (0.05 mol), 1-bromohexane (0.05 mol), 100 mL of acetone, and 8.5 g of K_2CO_3 was boiled (1 h). The mixture was diluted with 1 L of H_2O and extracted with two 100-mL portions of ether. The combined extracts were washed with (2 × 100 mL) 5% NaOH and water and then dried ($MgSO_4$). Removal of the solvent in vacuo gave the desired ketone **3c**. Relevant details are given in Table I.

Method A_1. A mixture of 6-hydroxy-1-tetralone (**3b**) (16.2 g, 0.1 mol), K_2CO_3 (41.5 g, 0.3 mol), 2-dimethylaminoethyl chloride hydrochloride (43.2 g, 0.3 mol), and acetone (100 mL) was boiled (12 h) under N_2. After removal of the solvent, the residue was poured into H_2O (200 mL). The mixture was extracted with ether (2 × 50 mL), and the ether layer was washed with 5% NaOH (2 × 100 mL) and water and then dried ($MgSO_4$). Evaporation of solvent and recrystallization of the residue gave pure **3e**.

Hydroxymethylene Intermediates. The procedure used for the synthesis of intermediate hydroxymethylene precursors to pyrazoles and isoxazoles was similar to that published[1a,b] but with crucial modifications especially in method B_1.

Method B. Hydroxymethylene Derivatives. Tetralone **3c** (8.5 g, 0.03 mol) was dissolved in anhydrous benzene (50 mL) and slowly added to a cooled (5–10 °C) mixture of $NaOCH_3$ (5.4 g, 0.1 mol) and ethyl formate (7.4 g, 0.1 mol) in anhydrous benzene (75 mL). After stirring under N_2 at 5–10 °C for 1 h, the mixture was stirred for 6 h at room temperature. The rest of the procedure was very similar to that published[1b] for related hydroxymethylene compounds. The product was used directly without further purification.

Method B_1. A solution (T = 10–15 °C) of ketone **3e** (4.6 g, 0.02 mol) in dry benzene (50 mL) was added to a cooled mixture of $NaOCH_3$ (2.10 g, 0.04 mol) and ethyl formate (2.56 g, 0.04 mol) in anhydrous benzene (75 mL). When the addition was completed, the reaction mixture was allowed to warm to room temperature overnight, whereupon it became a yellow semisolid, and stirring ceased. Hydrolysis was effected with distilled water (200 mL), and the resulting organic layer was washed (5% NaOH). The combined aqueous extracts were washed (ether) and acidified [dilute CH_3CO_2H (20%)]. The acid solution was neutralized [$NaHCO_3$ solution (20%)]. A yellowish hydroxymethylene ketone obtained upon neutralization was filtered, dried, and used directly.

Method C. Hydroxymethylene ketone (0.01 mol) was dissolved in absolute CH_3OH (50 mL) and 0.025 mol of 95% hydrazine was added. The solution was boiled (4 h) under N_2, and the resulting mixture was poured into ice water (250 mL). (See Table II.)

Method D. A solution of $HONH_2 \cdot HCl$ (1.38 g, 0.02 mol) and $CH_3CO_2Na \cdot 3H_2O$ (2.72 g, 0.02 mol) in 5 mL of water was added to a solution of the required hydroxymethylene ketone (0.01 mol)

Table V

(a) Effect of Compounds on Growth of Microorganisms

Compd	Concn, μg/mL	% inhibition B.s.[a]	% inhibition P.f.[a]	Time of inhibn, h
5c	91	100		12
6a	91	0		24
6b	91	0		24
6c	91	0		24
6d	45	100	0	24
6e	91	0		24
6h	91	100	0	24
	50	100	0	24
6i	91	100	100	24
	5	100		24
6j	5	100		24
6l	45	100		24
7a	91	100	0	24
7c	91	100	0	24
7d	91	100		11
	45	0	0	24
8a	91	0		24
8b	91	0	0	24
9a	5	40		8
9b	5	100		24
9a·HCl	90	100	25	24
9a·HCl	5	47	0	8
9b·HCl	90	60	25	24
	5	12	11	24
10a	91	7.8		24
	90		48	24
10b	91	100	0	24
10c	91	100		24
13	10	100		12
	90		0	12
13a	91	50		20
17	20	100		12

(b) Effect of Compounds on Plating Efficiencies with KB Cells

Compd	Concn, μg/mL	% plating eff (% inhibn)
6i	50	0 (100)
7a	150	0 (100)
	25	67 (33)
	12.5	100 (0)
9b	25	0 (100)

[a] *B.s.* = *Bacillus subtilis*; *P.f.* = *Pseudomonas fluorescens*.

in CH_3CO_2H (30 mL). The reaction mixture was heated on a water bath (2 h). The resulting solution was cooled and the solid was filtered and recrystallized.

Method D_1. The procedure was similar to method **D** except that acetic acid was removed under vacuum. The mixture was poured into water (50 mL) and basified (saturated aqueous $NaHCO_3$). The precipitated solid was filtered and recrystallized (Table II).

Method E. To an ice-cooled solution of pyrazole **6d** (1.35 g, 0.005 mol) in anhydrous pyridine (15 mL) was added 1.43 g (0.01 mol) of dimethylsulfamoyl chloride, and the solution was kept overnight at room temperature. The solution was poured onto ice water and precipitated **6g** was collected.

2-Amino-4,5-dihydro-7-*n*-hexyloxynaphtho[1,2-*d*]thiazole (9b). A solution of 6.3 g (0.025 mol) of 6-*n*-hexyloxy-1-tetralone (**3c**) in 100 mL of 1:1 $HCCl_3$–ether was placed in a flask cooled by an ice bath. Bromine (4 g, 0.025 mol) in $HCCl_3$ (10 mL) was added dropwise (0.5 h), and the mixture was stirred for 3 h. The remaining procedure was similar to that published for related systems.[1c] Evaporation of the solvent resulted in an oil (9.55 g, 90%) which was used without purification.

To a solution of the bromo ketone (4.06 g, 0.0125 mol) in 75 mL of 1:1 $HCCl_3$–alcohol was added thiourea (0.78 g, 0.0125 mol); the mixture was boiled with stirring under N_2 for 3 h. A procedure similar to that reported[1c] for related compounds was then followed. Recrystallization of crude **9b** (C_2H_5OH) gave 3 g (80%) of **9b**: mp 115–116 °C; NMR δ 0.80–1.04 (m, 3 H, CH_3–), 1.22–1.88 [m, 8 H, $-(CH_2)_4-$], 3.95 (t, 2 H, $-OCH_2$), 2.70–3.08 [m, 4 H, C(4)-H, C(5)-H], 4.94–5.28 [br s, 2 H, C(2)-NH_2], 7.52–7.62 [m, 1 H, C(9)-H], and 7.68–7.80 [m, 2 H, C(6)-H, C(8)-H]. Anal. ($C_{17}H_{22}N_2OS$) N, S.

The HCl salt of **9b** was obtained by treating a solution of **9b** in dry ether with a saturated ether solution of dry hydrogen chloride. Recrystallization (absolute alcohol–ether, 1:1) of the solid gave white crystals of the salt: yield, quantitative; mp 179–180 °C. Anal. ($C_{17}H_{23}H_2OSCl$) N, Cl.

By identical procedures, **9a** and its HCl salt were obtained from 6-methoxytetralone and **9a**, respectively. The yield of **9a** was 73%: mp 184–185 °C. Anal. ($C_{12}H_{12}N_2OS$) C, H, S. The salt was obtained in quantitative yield: mp 290–292 °C. Anal. ($C_{12}H_{13}N_2OSCl$) C, H, N.

2,3a,4,5-Tetrahydro-7-*n*-hexyloxy-3*H*-benz[*g*]indazol-3-one (8c). Freshly distilled (over NaH) dimethyl carbonate (30 mL) was placed in a flask to which was added 1.62 g (0.03 mol) of $NaOCH_3$ and 7.38 g (0.03 mol) of 6-*n*-hexyloxy-1-tetralone (**3c**) under N_2; the mixture was boiled for 4 h with stirring. During this period, a pink solid was separated. The remaining procedure was similar to that reported[1c] for related systems. Recrystallization (C_2H_5OH) of crude product gave pure methyl 1,2,3,4-tetrahydro-6-*n*-hexyloxy-1-oxo-2-naphthoate: 7.75 g (85%); mp 41–42 °C; NMR δ 0.80–1.02 (m, 3 H, CH_3–), 1.22–1.94 [m, 8 H, $-(CH_2)_4-$], 3.90–4.08 (m, 2 H, $-OCH_2$), 3.74–3.86 (m, 3 H, $-O_2CCH_3$), 6.64–6.88 [m, 2 H, C(5)-H, C(7)-H], 7.95–8.03 [m, 1 H, C(8)-H], and 3.44–3.63 [m, 1 H, C(2)-H]. Anal. ($C_{18}H_{24}O_4$) C, H.

To a solution of the above β-keto ester (3.04 g, 0.01 mol) in absolute CH_3OH (25 mL) was added 0.96 g (0.03 mol) of hydrazine (95%), and the mixture was boiled (3 h) under N_2. When the reaction was complete, the resulting solution was poured into water (250 mL). The product formed was recrystallized (C_2H_5OH–H_2O) and gave 2.9 g (92.3%) of **8c**: mp 177–178 °C; NMR δ 0.88–1.04 (m, 3 H, CH_3–), 1.22–1.92 [m, 8 H, $-(CH_2)_4-$], 3.87 (t, 2 H, OCH_2), 2.25–2.82 [br s, 4 H, C(4)-H, C(5)-H], 6.58–6.72 [m, 2 H, C(6)-H, C(8)-H], 7.44–7.64 [m, 2 H, C(9)-H], and 11.44–12.10 (br s, 2 H, NH, –OH). Anal. ($C_{17}H_{22}N_2O_2$) N.

2,3a,4,5-Tetrahydro-7-*n*-hexyloxy-3a-methyl-3*H*-benz[*g*]indazol-3-one (8b). 6-*n*-Hexyloxy-1-tetralone (**3c**) (4.92 g, 0.02 mol) and 2.16 g (0.04 mol) of $NaOCH_3$ were dissolved in 30 mL of anhydrous $(CH_3O)_2C{=}O$ (distilled over NaH), and the mixture was boiled (3 h). A pink solid formed and was dissolved in absolute methanol (100 mL). Iodomethane (14.5 g, 0.1 mol) was added, and the reaction mixture was boiled (3 h). The solvent was evaporated, and the resulting solid was dissolved in 100 mL of water. The remaining procedure was similar to that used for **8c**, with **8b** being obtained as a crude but clear oil when the ether was evaporated.

To 0.795 g (0.025 mol) of the above crude β-keto ester was added 0.96 g (0.03 mol) of 95% hydrazine and absolute methanol (10 mL). The mixture was heated to reflux (3 h), then cooled to room temperature, and poured into ice water (150 mL). A white precipitate formed and was filtered, washed (water), and dried. Recrystallization (aqueous ethanol) gave pure **8b**: 0.58 g (71%); mp 126–127 °C; NMR δ 0.82–0.98 (m, 3 H, CH_3–), 1.35 (s, 3 H, CH_3), 1.56–2.32 [m, 8 H, $-(CH_2)_4-$], 3.97 (t, 2 H, OCH_2), 2.72–3.22 [m, 2 H, C(5)-H], 6.68–6.90 [m, 2 H, C(6)-H, C(8)-H], 7.68–7.72 (m, 3 H, Ar-H), and 10.2 (s, 1 H, NH). Anal. ($C_{18}H_{24}N_2O_2$) C, H, N.

1-D-Glucopyranosyl-7-(hexyloxy)-4,5-dihydro-1*H*-benz[*g*]indazole 2,3,4,6-Tetraacetate (6h). 7-(Hexyloxy)-4,5-dihydro-1*H*-benz[*g*]indazole (**6d**) (4.59 g, 0.0016 mol) was dissolved in ethanol (100 mL) containing 0.0016 mol of sodium hydroxide. Mercuric chloride (4.59 g, 0.0016 mol) in ethanol (50 mL) was added to the stirred solution from which a gray solid precipitated. Upon trituration and washing with cold water, cold ethanol, and ether, 8.1 g (96%) of the chloromercurio derivative was obtained.

The chloromercurio derivative (8.1 g, 0.0158 mol) was added to dry xylene (300 mL). The vigorously stirred suspension was dried by distillation to approximately three-fourth volume. Tetra-*O*-acetyl-α-glucopyranosyl bromide (6.53 g, 0.00158 mol) was added to the hot, stirred mixture. Traces of water were removed azeotropically by distilling out xylene, and the reaction mixture was boiled (24 h). Filtration (suction) removed the light brown, inorganic by-products from the xylene solution. Evaporation of the xylene solution gave a brown mass which was

dissolved in $HCCl_3$; the resulting solution was washed with aqueous NaI (30%) and then evaporated. Purification was achieved by soxhleting the product with benzene over alumina (neutral). Removal of the benzene produced a black, gummy solid. Recrystallization (hexane) yielded the glucose derivative **6h** (5.4 g, 61.6%): mp 115–116 °C; NMR δ 0.84–0.98 (m, 3 H, CH_3–), 1.26–1.54 [m, 8 H, $-(CH_2)_4-$], 1.36 [s, 2 H, $-C(O)CH_2$], 2.05 (s, 12 H, $-COCH_3$), 2.80 [m, 4 H, C(4)-H, C(5)-H], 3.96 (t, 2 H, $-OCH_2$), 4.25 (m), 5.20–5.70 (m), 6.57 (s), 6.85 (d), 7.35 (s), and 7.80 (d) [9 H, ArH, =NCH, C(O)OCH]. Anal. ($C_{31}H_{40}N_2O_{10}$) C, H, N.

7-Methoxy-4,5-dihydro-1*H*-benz[*g*]indazole-1-ethanol (14). 7-Methoxy-4,5-dihydro-1*H*-benz[*g*]indazole (**6a**) (10.00 g, 0.05 mol) was dissolved in a solution of 3.24 g (0.06 mol) of $NaOCH_3$ in dimethylformamide (100 mL). To this solution was added 4.93 g (0.06 mol) of 2-chloroethanol in one portion. The resulting mixture was boiled and stirred (8 h) and allowed to cool. The mixture was poured into ice water (500 mL) to give crude product which crystallized from benzene to yield 7.6 g (65%) of pure **14**, mp 140–141 °C. Anal. ($C_{14}H_{16}N_2O_2$) C, H, N.

7-Methoxy-4,5-dihydro-1-(2-chloroethyl)benz[*g*]indazole (15a). Freshly distilled thionyl chloride (7 mL, 0.1 mol) was added to an ice-cold solution of 4.84 g (0.02 mol) of **14** in chloroform (50 mL). After the addition, the solution was allowed to stand at room temperature (15 min). A white solid formed and the mixture was boiled (2 h). Solvent and excess thionyl chloride were removed by evaporation to give a light yellow solid. Recrystallization ($HCCl_3$–ether) yielded the monohydrochloride [4.8 g (80%)] **15**, mp 188–190 °C. Anal. ($C_{14}H_{16}N_2OCl_2$) C, H, N, Cl.

A solution of the hydrochloride (2 g) in ethanol (5 mL) was poured into a cold saturated sodium acetate solution, and the solution was extracted (ether). After washing (H_2O), drying ($MgSO_4$), and evaporation of solvent, an oil formed and solidified. Recrystallization (pentane) gave 1.6 g (92.5%) of **15a**, mp 51–52 °C. Anal. ($C_{14}H_{15}N_2OCl$) N, Cl.

1-[2-[Bis(2′-chloroethyl)amino]ethyl]-7-methoxy-4,5-dihydro-1*H*-benz[*g*]indazole (18). To a solution of 2.0 g (0.0068 mol) of **15** in 200 mL of freshly distilled diglyme 7.36 g (0.070 mol) of diethanolamine was added, and the mixture was boiled (8 h). After cooling, dry HCl was passed into the diglyme layer. A viscous oil separated and was triturated (absolute ethanol) to yield a solid which was recrystallized (absolute alcohol) to give pure **16**, 2.3 g (56%). Anal. ($C_{18}H_{27}N_3O_3Cl_2$) C, H, N.

Thionyl chloride (5 mL, 0.068 mol) was added to a solution of **16** (1 g, 0.0025 mol) in 20 mL of $HCCl_3$ (cooled, 0 °C). After the addition, the mixture was allowed to stand at room temperature (15 min). After boiling for 2 h, solvent and excess thionyl chloride were removed, and the residue was crystallized (absolute ethanol) to give 0.8 (73%) of **17**, mp 183–184 °C. Anal. ($C_{18}H_{25}N_3OCl_4$) C, H, N, Cl.

To a cold saturated solution of CH_3CO_2Na in water was added a solution of **17** [0.5 g in ethanol (5 mL)]. The resulting solution was extracted twice with ether (2 × 25 mL), washed (H_2O), dried (Na_2SO_4), and evaporated to give an oil, which crystallized readily from Skelly B to yield 0.35 g (84.1%) of **18**, mp 47–47.5 °C. Anal. ($C_{18}H_{23}N_3OCl_2$) C, H, N, Cl.

1-[2-[Bis(2′-chloroethyl)amino]ethyl]-7-(hexyloxy)-4,5-dihydro-1*H*-benz[*g*]indazole Dihydrochloride (6j). A stirred suspension of $NaOCH_3$ (2.97 g, 0.055 mol), KI (500 mg), and 12.2 g (0.045 mol) of 4,5-dihydro-7-*n*-hexyloxy-1*H*-benz[*g*]indazole (**6d**) was boiled (1 h). The mixture was cooled to about 50 °C and 4.83 g (0.06 mol) of 2-chloroethanol was added dropwise. The resulting mixture was boiled (12 h); the resulting dark brown mixture was poured into ice. A light brown solid precipitated and was crystallized from Skelly B to yield 7.4 g (52%) of 7-(hexyloxy)-4,5-dihydro-1*H*-benz[*g*]indazole-1-ethanol: mp 95–96 °C; NMR ($DCCl_3$) δ 0.82–0.94 (m, 3 H, CH_3), 1.20–1.86 [m, 8 H, $(CH_2)_4-$], 2.62–2.94 [m, 4 H, C(4)-H, C(5)-H], 3.18 (br s, 1 H, –OH), 3.95 (t, 2 H, OCH_2), 4.04 (t, 2 H, NCH_2), 4.20 (t, 2 H, CH_2OH), and 6.72–7.78 [m, 4 H, C(3)-H, ArH]. Anal. ($C_{19}H_{26}N_2O_2$) C, H, N.

To a solution of this alcohol [7.0 g (0.022 mol) in 100 mL of anhydrous chloroform] was added thionyl chloride (8 mL). After boiling (2 h), the solvent and excess thionyl chloride were evaporated. The light yellow residue was dissolved in $HCCl_3$ (25 mL) and filtered. Anhydrous ether (100 mL) was added to the filtrate and the salt obtained was recrystallized (ether–absolute ethanol) to yield 6.9 g (83.9%) of 7-(hexyloxy)-4,5-dihydro-1-(2-chloroethyl)benz[*g*]indazole monohydrochloride: mp 150–151 °C; NMR ($DCCl_3$) δ 0.82–0.96 (m, 3 H, CH_3), 1.22–1.86 [m, 8 H, $(CH_2)_4-$], 2.72–3.10 [m, 4 H, C(4)-H, C(5)-H], 4.0 (t, 2 H, $-OCH_2$), 4.18 [t, 2 H, $=^+N(H)CH_2$], 4.86 (t, 2 H, $-CH_2Cl$), 6.82–8.24 [m, 4 H, C(3)-H, ArH], 12.4–13.0 [br s, 1 H, $-^+N(=)-H$]. Anal. ($C_{19}H_{26}N_2OCl_2$) C, H, N, Cl.

To a solution of the HCl salt of 7-(hexyloxy)-4,5-dihydro-1-(2-chloroethyl)benz[*g*]indazole (4.0 g, 0.0108 mol) in diglyme (200 mL), diethanolamine (15 mL) was added, and the mixture was boiled (8 h). After cooling, the diglyme layer was separated and saturated with dry hydrogen chloride. A white crystalline solid formed quickly (ca. 5 min). Three recrystallizations (absolute ethanol) yielded 3.4 g (72.3%) of 1-[2-[bis(2′-hydroxyethyl)amino]ethyl]-7-(hexyloxy)-4,5-dihydro-1*H*-benz[*g*]indazole monohydrochloride: mp 145–146 °C; NMR (Me_2SO-d_6) δ 0.80–0.92 (m, 3 H, CH_3), 1.20–1.76 [m, 8 H, $(CH_2)_4$], 2.60–2.84 [m, 4 H, C(4)-H, C(5)-H], 3.02 (br s, 2 H, –OH), 3.4 [br s, 4 H, $-^+N(H)-(CH_2-)_2$], 3.62–4.02 (m, 6 H, $-OCH_2$, $-CH_2OH$), 6.82–8.24 [m, 4 H, C(3)-H, Ar-H], and 11.2–11.6 [br s, 1 H, $-^+N(=)-H$]. Anal. ($C_{23}H_{36}N_3O_3Cl$) C, H, N, Cl.

To a solution of 3 g (0.0068 mol) of 1-[2-[bis(2′-hydroxyethyl)amino]ethyl]-7-hexyloxy-4,5-dihydro-1-*H*-benz[*g*]indazole monohydrochloride in anhydrous chloroform (75 mL) was added thionyl chloride (7 mL). After boiling (2 h), the solvent and excess thionyl chloride were stripped off. The yellow residue was recrystallized ($HCCl_3$–ether, 25:75) to yield 2.92 g (83.4%) of **6j**: mp 149–150 °C; NMR (Me_2SO-d_6) δ 0.82–0.96 (m, 3 H, $-CH_3$), 1.18–1.90 [m, 8 H, $-(CH_2)_4$], 2.84 [br s, 8 H, C(4)-H, C(5)-H, $-^+N(H)(CH_2-)_2$], 3.98 (t, 2 H, $-OCH_2$), 4.18 (br s, 8 H, $-CH_2Cl$, $>N^+CH_2CH_2{}^+N<$), 6.68–7.20 [m, 4 H, C(3)H, ArH], and 14.2–14.4 (br s, 2 H, ^+NH, $-^+N(H)(CH_2-)_2$]. Anal. ($C_{23}H_{35}N_3OCl_4$) C, H, N, Cl.

1-[(2-Chloroethyl)mercapto]ethyl-7-methoxy-4,5-dihydro-1*H*-benz[*g*]indazole Hydrochloride (6l). To an ice-cooled solution of $NaOCH_3$ (1.08 g, 0.02 mol) in 15 mL of methanol was added mercaptoethanol (0.86 g, 0.011 mol) at one time. While this mixture was stirred, a solution of **15** (2.09 g, 0.0068 mol) in absolute methanol (15 mL) was added. The mixture was boiled (2 h) and then heating was stopped and stirring was continued overnight. Evaporation of the solvent left a residue that was taken up in ether (200 mL), and the ether layer was dried ($MgSO_4$) and filtered; the solvent was evaporated to an oil which crystallized on standing. Recrystallization (hexane) gave 1.7 g (88%) of **6k**, mp 86–87 °C. Anal. ($C_{16}H_{20}N_2O_2S$) N, S.

To an ice-cold solution of **6k** (1.0 g, 0.0034 mol) in chloroform (30 mL) was added 5 mL (0.0689 mol) of thionyl chloride, and the reaction mixture was allowed to warm to room temperature (15 min). The remaining work-up was as described for **6j**. An analytical sample was obtained by recrystallization ($HCCl_3$–ether, 1:2), mp 158–159 °C, to yield 0.75 g (56%) of **6l**. Anal. ($C_{16}H_{20}N_2OSCl_2$) N, S, Cl.

4,5-Dihydro-1*H*-pyrazolo[3,4-*f*]quinoline (13). Sodium methoxide (2.42 g, 0.9448 mol) was suspended in anhydrous benzene (30 mL) and ethyl formate (3.40 g, 0.0448 mol) in 10 mL of anhydrous benzene was then added dropwise to the suspension cooled to 0 °C (ice bath) under N_2. Distilled 7,8-dihydro-5(6*H*)-quinoline [3.352 g, 0.0228 mol, bp 131–132 °C (12 mm)] (Aldrich Chemical Co.) was dissolved in anhydrous benzene (50 mL); the solution was added dropwise to the reaction mixture. The color of the mixture turned from white to yellow (5 min). By the end of the addition (about 30 min), the whole mixture was a bright yellow slurry, and the mixture was allowed to react at room temperature (24 h) with constant stirring. Stirring was stopped and two layers resulted, a top clear layer and a bottom layer of grayish yellow solid. To this mixture was added absolute methanol (40 mL) which gave a homogeneous, dark brown solution. To this dark brown solution, 95% hydrazine (3.65 g, 0.114 mol) was added and the resulting solution was boiled (3 h). The solvents were evaporated (rotary evaporator) completely. A yellow solid obtained was recrystallized from 2-propanol–benzene (1:1) to yield 2.39 g (61%) of **13**, mp 209–210.6 °C. Anal. ($C_{10}H_9N_3$) N.

6-Methyl-4,5-dihydro-1*H*-pyrazolo[3,4-*f*]quinolinium Iodide (13a). A yellowish suspension of 0.513 g (0.003 mol) of pyrazole

13 in absolute 2-propanol (30 mL) (distilled over CaO) was placed in standard apparatus under N_2. While the suspension was rapidly stirred, 1.9 mL (4.26 g, 0.03 mol) of methyl iodide was added slowly. After all the methyl iodide was added, the suspension cleared and a transparent yellowish solution resulted which was then boiled at about 85 °C by means of oil bath (3 h). After the first 30 min, the solution became cloudy and a white solid appeared and accumulated up to 3 h. When the mixture cooled, the solid was filtered (0.59 g, 63%, mp 220–222 °C) and recrystallized (water–2-propanol). Yellow needles of salt **13a** were obtained [0.486 g (52%), mp 222.5–224 °C]. Anal. ($C_{11}H_{12}N_3I$) C, H, N.

Attempted Preparation of 6-Hexadecyl-4,5-dihydro-1*H*-pyrazolo[3,4-*f*]quinolinium Bromide. A solution of 0.513 g (0.003 mol) of pyrazole **13** dissolved in absolute ethanol (25 mL) was boiled with 3.05 g (0.01 mol) of cetyl bromide with constant stirring under N_2. The solution was then poured into an excess (ca. 150 mL) of absolute ether, and a cloudy mixture formed. Filtration gave a white precipitate, 1.1 g (70%), mp 200 °C; this was recrystallized three times (absolute methanol–ether) and gave white crystals, 0.5 g (32%), mp 262–265 °C. NMR spectral data and the elemental analysis support the structure of the hydrobromide **13b**. Anal. ($C_{10}H_{10}N_3Br$) C, H, N. A variety of other conditions produced the same result.

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Thymidylate Synthetase Inhibitors. Synthesis of N-Substituted 5-Aminomethyl-2'-deoxyuridine 5'-Phosphates

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A series of substituted 5-aminomethyl-2'-deoxyuridines was synthesized as analogues of 5-thymidylyltetrahydrofolic acid, a proposed intermediate in the thymidylate synthetase catalyzed reaction. 1-(3,5-Di-*O*-*p*-toluoyl-2-deoxy-β-D-ribofuranosyl)-5-chloromethyluracil (**3**) was treated with the appropriate amine to give the ester protected 5-aminomethyl nucleoside. Removal of the ester groups was accomplished with anhydrous potassium carbonate in methanol to afford the free β-nucleoside. In this way 5-(2-dimethylaminoethylaminomethyl)-2'-deoxyuridine (**5a**), 5-dimethylaminomethyl-2'-deoxyuridine (**5b**), 5-*N*-methylpiperazinylmethyl-2'-deoxyuridine (**5c**), and 5-pyrrolidinylmethyl-2'-deoxyuridine (**5d**) were prepared. Compounds **5a**,**b**,d were converted to the respective 5'-phosphates **6a**,b,d. All three compounds were substrate competitive inhibitors of thymidylate synthetase purified from *Escherichia coli*, calf thymus, and Ehrlich ascites tumor cells. The most active compound was **6a** with K_I's of 6, 3.1, and 14 μM observed for the respective enzymes.

As part of a program to design selective cancer cell thymidylate synthetase inhibitors, a series of substituted 5-aminomethyl-2'-deoxyuridines with variations in the amine substituent was synthesized in order to elucidate structural criteria that provided strong binding of a 5-substituted 2'-deoxyuridine to the enzyme. Further modifications on the derived structure could then be made to probe for differences between cancer cell and normal cell thymidylate synthetase.[1] Ultimately, it is hoped that a study of this kind will lead to the synthesis of an agent that will selectively and irreversibly inhibit tumor cell thymidylate synthetase. Only one example of isozyme selectivity has been demonstrated for thymidylate synthetase.[2]

The rationale for the synthesis of the 5-aminomethyl-2'-deoxyuridine series of compounds is based on the postulated formation of 5'-thymidylyltetrahydrofolic acid (**1**) as an intermediate in the conversion of deoxyuridine 5'-phosphate to thymidine 5'-phosphate.[3] According to Friedkin's proposed mechanism, **1** undergoes a rearrangement via a 1,3-hydride shift to give the observed products.[3,4]

Compounds **6a** and **6c** are analogues of 5-thymidylyltetrahydrofolic acid (**1**). As such, they include binding sites of both the normal substrate, dUMP, and N_5 and N_8 of the cofactor, tetrahydrofolate, and should, therefore, have a strong binding affinity for the enzyme and act as potent inhibitors of it. Compounds **6b** and **6d**, containing only