Inhibitors of Dihydropteroate Synthase: Substituent Effects in the Side-Chain Aromatic Ring of 6-[[3-(Aryloxy)propyl]amino]-5-nitrosoisocytosines and Synthesis and Inhibitory Potency of Bridged 5-Nitrosoisocytosine–*p*-Aminobenzoic Acid Analogues

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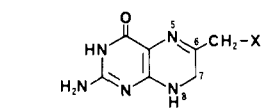
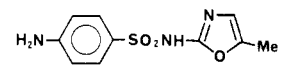
We previously reported that 6-(methylamino)-5-nitrosoisocytosine (**5**) is a potent inhibitor (I_{50} = 1.6 μM) of *Escherichia coli* dihydropteroate synthase. It was noted that 6-amino substituents larger than methyl were detrimental to binding, although the adverse steric effect could be overcome by a positive ancillary binding contribution of a phenyl ring attached at the terminus of certain 6-alkylamino substituents. We selected the 6-[[3-(aryloxy)propyl]amino]-5-nitrosoisocytosine structure as a parent system and explored the effects of aromatic substituents on synthase inhibition. The nature of the aryl substitution influences binding, as shown by a 30-fold range of inhibitory potencies observed for the 15 aryl analogues (I_{50} values = 0.6–18 μM), although there is no apparent correlation between synthase inhibition and the electronic or hydrophobic characteristics of the aryl substituents. To explore the possibility that the aryl ring of these inhibitors might interact with the synthase binding site for the substrate *p*-aminobenzoic acid (PABA), three compounds were synthesized in which a PABA analogue is bridged to the nitrosoisocytosine moiety by linkage to an amino group at C-6 of the isocytosine. The bridged analogues significantly inhibited the synthase (I_{50} values = 2.5–8.9 μM) but were of unexceptional potency compared with other members of the (aryloxy)propyl series. Structure–activity considerations and inhibition kinetics did not support the PABA binding site as the synthase region that interacts with the aryl ring of these inhibitors. Despite the potent synthase inhibition exhibited by many of the nitrosoisocytosines studied, none of the 18 new analogues showed significant antibacterial activity.

A crucial stage in the biosynthesis of tetrahydrofolic acid (THF) is the formation of 7,8-dihydropteroic acid (**2**) through condensation of *p*-aminobenzoic acid (PABA) with the pyrophosphate (**1**) of 6-(hydroxymethyl)-7,8-dihydropterin. The enzyme dihydropteroate synthase carries out this step, and the sulfonamide drugs, e.g., sulfamethoxazole (**4**), exert their therapeutic antibacterial effects by competing with PABA for the synthase.[2] Although the sulfonamide drugs have been employed successfully as therapeutic agents for decades, surprisingly little attention has been directed toward potential synthase inhibitors that might interfere with the binding of the pteridine substrate **1**.[1-3] Pterin–sulfa adducts such as **3** are competitive inhibitors,[2] and we recently reported that certain derivatives of 6-amino-5-nitrosoisocytosine also inhibit dihydropteroate synthase from *Escherichia coli* by effectively competing with **1** for the enzyme.[1] For example, the 6-methylamino compound **5** inhibited the partially purified synthase with an in vitro potency (I_{50} = 1.6 μM) equivalent to that shown by sulfonamides (e.g., **4**; I_{50} = 4.7 μM). Structure–activity considerations indicated a lack of steric tolerance around the isocytosine 6-amino function, such that the 6-ethylamino analogue of **5** was sevenfold less active than **5**, and the 6-(3-hydroxypropyl)amino compound **6** was more than 20-fold less effective than **5** as a synthase inhibitor. However, significant affinity for the synthase was restored in the 3-phenoxypropyl derivative **7** (I_{50} = 3.7 μM), which differs from **6** in the presence of a phenyl group at the terminus of the side chain.

This observation, along with related results in a series of 6-(ω-phenylalkyl)amino analogues,[1] suggested that a suitably disposed phenyl ring provides a positive auxiliary binding interaction that can overcome the adverse steric effects of the bridging aliphatic chain. To assess the influence of aromatic substituents on this ancillary binding, **7** was selected as the parent structure, and in this study we report the synthesis and dihydropteroate synthase inhibition properties of a series of 15 analogues of **7** that are variously functionalized in the aromatic ring. Although the observed variations in inhibitory potency do not correlate with either the electronic or lipophilic character of the substituent, the nature of the aryl substitution does affect affinity for the synthase and a number of the substituted aryl compounds are more active inhibitors than the aryl parent **7**. We also considered the possibility that the aryl ring of inhibitors such as **7** might at least partially



1 X = OPP
2 X = NH–⟨⟩–CO_2H
3 X = NH–⟨⟩–SO_2NH–⟨isoxazole⟩–Me



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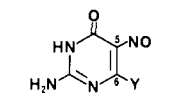
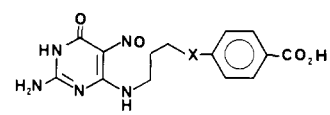


5 Y = NHMe
6 Y = $NH(CH_2)_3OH$
7 Y = $NH(CH_2)_3OPh$
8 Y = SMe



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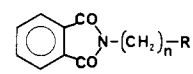

10 n = 3, R = Br
11 n = 4, R = Br
12 n = 2, R = CHO

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(1) Lever, O. W., Jr.; Bell, L. N.; McGuire, H. M.; Ferone, R. *J. Med. Chem.* **1985**, *28*, 1870.
(2) Roland, S.; Ferone, R.; Harvey, R. J.; Styles, V. L.; Morrison, R. W. *J. Biol. Chem.* **1979**, *254*, 10 337.
(3) Ferone, R.; Webb, S. R. In "Chemistry and Biology of Pteridines"; Pfleiderer, W., Ed.; Walter de Gruyter: New York, 1975; pp 61–71.

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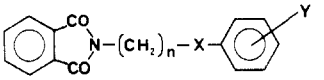
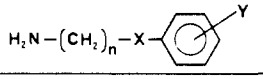
Table I. Chemical Properties of the Phthalimide Intermediates



no.	n	X	Y	mp, °C	yield, %	method[a,b]	formula[c]
13	3	O	4-OCH_2Ph	139–140	56	A	$C_{24}H_{21}NO_4$
14	3	O	4-OMe	98–99[d]	46	A	$C_{18}H_{17}NO_4$
15	3	O	4-*O*-*n*-Bu	67–69	45	A	$C_{21}H_{23}NO_4$
16	3	O	4-SO_2Me	165–166.5	76	A	$C_{18}H_{17}NO_5S$
17	3	O	4-CF_3	118–122	80	A	$C_{18}H_{14}F_3NO_3$[e]
18	3	O	4-NO_2	182–184[f]	83	A	$C_{17}H_{14}N_2O_5$
19	3	O	4-NH_2	93–94[g]	83	*h*	$C_{17}H_{16}N_2O_3$
20	3	O	3,5-Cl_2	129–133.5	89	A	$C_{17}H_{13}Cl_2NO_3$
21	3	O	3,5-$(OMe)_2$	102–105	40	A	$C_{19}H_{19}NO_5$
22	3	O	3,4,5-$(OMe)_3$	151–153	56	A	$C_{20}H_{21}NO_6$
23	3	O	4-(4-NHtosyl)	130–132	85	*i*	$C_{24}H_{22}N_2O_5S$[j]
24	3	O	4-CO_2Et	116–117	70	A	$C_{20}H_{19}NO_5$
25	3	NH	4-CO_2Et	142–144	60	*k*	$C_{20}H_{20}N_2O_4$
26	4	Ntosyl	4-CO_2Et	133–135	91	*l*	$C_{28}H_{28}N_2O_6S$[j]

[a] Method A as in Scheme I; exceptions are noted. [b] For details, see the Experimental Section. [c] Elemental analyses within ±0.4% of theory for C, H, and N were obtained for all compounds. [d] Lit.[6] mp 105–106 °C. [e] Obtained as the hemihydrate. [f] Lit.[7] mp 183 °C. [g] Lit.[7,8] mp 92–94, 92–93 °C. [h] Prepared by catalytic reduction (H_2, Pd/C) of 18. [i] Prepared by tosylation (*p*-toluenesulfonyl chloride, pyridine, 90 °C) of 19. [j] Satisfactory S analysis obtained. [k] Obtained by reductive amination of 12 with ethyl 4-aminobenzoate. [l] Prepared by alkylation of the *N*-tosyl derivative of ethyl 4-aminobenzoate with 11.

Table II. Chemical Properties of Salts of the Amine Intermediates



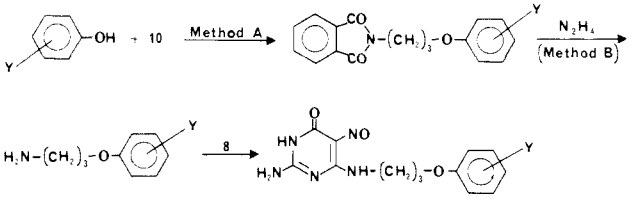
no.	n	X	Y	mp, °C	yield, %	method[a,b]	formula[c]
27	3	O	4-OCH_2Ph	231–232	69	B	$C_{16}H_{19}NO_2$·HCl
28	3	O	4-OH	191–192	84	d	$C_9H_{13}NO_2$·HCl
29	3	O	4-OMe	208–210	41	B	$C_{10}H_{15}NO_2$·HCl
30	3	O	4-O-*n*-Bu	>190 dec	88	B	$C_{13}H_{21}NO_2$·HCl
31	3	O	4-SO_2Me	196.5–200	48	B	$C_{10}H_{15}NO_3S$·HCl
32	3	O	4-CF_3	166–167	71	B	$C_{10}H_{12}F_3NO$·HCl
33	3	O	4-NO_2	159–161[e]	54	B	$C_9H_{12}N_2O_3$·HCl
34	3	O	3,5-Cl_2	160–162	73	B	$C_9H_{11}Cl_2NO$·HCl
35	3	O	3,5-$(OMe)_2$	145–147.5	58	B	$C_{11}H_{17}NO_3$·HCl
36	3	O	3,4,5-$(OMe)_3$	162–164	75	B	$C_{12}H_{19}NO_4$·HCl
37	3	O	2-OCH_2Ph	123–126	20	*f*	$C_{16}H_{19}NO_2$·HCl
38	3	O	4-(NHtosyl)	146–150	53	B	$C_{16}H_{20}N_2O_3S$·HCl
39	3	O	4-CO_2Et	186–188	32	B	$C_{12}H_{17}NO_3$·HCl
40	3	NH	4-CO_2Et	186–190	26	B	$C_{12}H_{18}N_2O_2$·2HCl
41	3	NH	4-CO_2Na		74	*g*	
42	4	Ntosyl	4-CO_2Et	66–76	95[h]	B	$C_{20}H_{26}N_2O_4S$·HCl
43	4	NH	4-CO_2Et	221–222 dec	74	*i*	$C_{13}H_{20}N_2O_2$·2HBr

[a] Method B refers to hydrazinolysis of the appropriate phthalimide precursor in Table I; exceptions are noted. [b] For detail, see the Experimental Section. [c] Elemental analyses within ±0.4% of theory for C, H, N, and Cl (Br in the case of 43) were obtained for all compounds except 35, for which a Cl analysis was not obtained, and sodium salt 41, which was not analyzed. A satisfactory analysis for S was also obtained for 38 and 42. [d] Prepared by reductive debenzylation (H_2, Pd/C) of 27. [e] Lit.[9] mp 155 °C. [f] Obtained from 1-[2-(benzyloxy)-phenoxy]-3-bromopropane[4] and methanolic ammonia. [g] Isolated as the sodium salt from hydrolysis of 40. [h] Yield refers to free base; only a portion of this compound was converted to the hydrochloride. [i] Prepared by detosylation of 42.

occupy the binding site of the sulfonamide drugs, and therefore of the substrate PABA. If this were the case, analogues in which the aryl moiety more closely resembles PABA might bridge the synthase binding sites for 1 and for PABA and might be multisubstrate inhibitors.[4] Accordingly, we also synthesized potential multisubstrate inhibitors, represented by **9**, in which a 5-nitrosoisocytosine is bridged through a 6-amino substituent to a PABA analogue.

Chemistry. The synthesis of the 6-[[3-(aryloxy)-propyl]amino]-5-nitrosoisocytosines (Table III) was accomplished (Scheme I) by condensation of the appropriate primary amine with 6-(methylthio)-5-nitrosoisocytosine (**8**; method C), prepared as previously described.[1] The required (aryloxy)propylamines (Table II) generally were

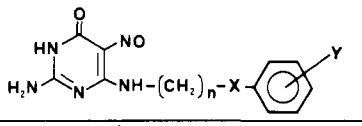
Scheme I



obtained by hydrazinolysis (method B) of the corresponding phthalimides (Table I), which in turn typically were generated by alkylation of a phenoxide anion with *N*-(3-bromopropyl)phthalimide (method A). Phthalimide **23** was prepared by tosylation of the aniline derivative **19**, which was available from reduction of the nitro compound **18**. Phenolic amine **28** was prepared by reductive debenzylation of **27**, and amine **37** was obtained from the reaction of 1-[2-(benzyloxy)phenoxy]-3-bromopropane[4] with methanolic ammonia.

(4) Lever, O. W., Jr.; Hyman, C.; White, H. L. *J. Pharm. Sci.* 1984, *73*, 1241 and references therein.

Table III. Dihydropteroate Synthase Inhibition and Chemical Properties of the 6-[[3-(Aryloxy)propyl]amino]-5-nitrosoisocytosines



no.	*n*	X	Y	I_{50},[a,b] μM	mp, °C	yield,[c] %	formula[d]
7	3	O	H	3.7[e]			
44	3	O	4-OH	2.2	242–244	76	$C_{13}H_{15}N_5O_4$[f]
45	3	O	4-OMe	7.3	259–260 dec	84	$C_{14}H_{17}N_5O_4$
46	3	O	4-O-*n*-Bu	2.1	246–251 dec[g]	23	$C_{17}H_{23}N_5O_4$
47	3	O	4-OCH_2Ph	3[h]	257–258	76	$C_{20}H_{21}N_5O_4$[f]
48	3	O	4-SO_2Me	2.5	265–268 dec	58	$C_{14}H_{17}N_5O_5S$
49	3	O	4-CF_3	1.4	244.5–246 dec	70	$C_{14}H_{14}F_3N_5O_3$
50	3	O	4-NO_2	1.0	259–261 dec	77	$C_{13}H_{14}N_6O_5$
51	3	O	4-Cl	1.0	255–259 dec[g]	53[i]	$C_{13}H_{14}ClN_5O_3$[j]
52	3	O	3,5-Cl_2	1.4	256–257.5 dec[g]	23	$C_{13}H_{13}Cl_2N_5O_3$[j]
53	3	O	3,5-$(OMe)_2$	0.7	236–237.5 dec[g]	48	$C_{15}H_{19}N_5O_5$
54	3	O	3,4,5-$(OMe)_3$	2.0	219.5–223.5 dec	28	$C_{16}H_{21}N_5O_6$
55	3	O	2-OCH_2Ph	18	233–234	79	$C_{20}H_{21}N_5O_4$[k]
56	3	O	4-(NHtosyl)	0.6	152–156 dec	73	$C_{20}H_{22}N_6O_5S$[l]
57	3	O	4-CO_2Et	2.9	256 dec	69	$C_{16}H_{19}N_5O_5$
58	3	O	4-CO_2H	6.6	265–267 dec	80[m]	$C_{14}H_{15}N_5O_5$[n]
59	3	NH	4-CO_2H	8.9	234 dec	13	$C_{14}H_{16}N_6O_4$[n]
60	4	NH	4-CO_2Et	3.0	239 dec	43	$C_{17}H_{22}N_6O_4$[k]
61	4	NH	4-CO_2H	2.5	206 dec	91[o]	$C_{15}H_{18}N_6O_4$[n]

[a] Micromolar concentration to inhibit synthase activity by 50%. [b] Assay method as described in ref 3. [c] Yields are for preparation by condensation of the appropriate amine with **8** (method C) unless otherwise noted. [d] Elemental analyses were within ±0.4% of the theoretical values for C, H, and N for all compounds. [e] Inhibition data from ref 1. [f] Obtained as the hemihydrate. [g] Sample slowly darkened on heating prior to melting with decomposition at the indicated temperature. [h] This compound was poorly soluble in the assay medium and the I_{50} value could not be determined with high precision. [i] This compound was prepared through condensation of **8** with 3-(4-chlorophenoxy)-propylamine, which was provided by Dr. H. Hodson of the Wellcome Research Laboratories, U.K. [j] Satisfactory Cl analysis also obtained. [k] Elemental analysis indicated the additional presence of H_2O (0.3 mol for **55**; 0.25 mol for **60**) and 0.1 EtOH for both; the ethanol was not removed by prolonged drying under vacuum and its presence was confirmed by NMR. [l] Satisfactory analysis for S also obtained. [m] From basic hydrolysis of **57**. [n] Obtained as the monohydrate. [o] From basic hydrolysis of **60**.

In the preparation of the bridged compounds, the pyrimidine moiety was linked to the PABA analogues by condensation of the appropriate amino ester (**39**, **43**) or amino acid (**41**) with **8** (method C). Alkylation (K_2CO_3, DMF) of ethyl *p*-hydroxybenzoate with bromide **10** provided phthalimide **24**, which was converted to amino ester **39** by hydrazinolysis in refluxing ethanol. Condensation of **39** with **8** gave isocytosine **57**, and subsequent hydrolysis of the ester function furnished the phenoxy carboxylic acid **58**.

Reductive amination ($Na(CN)BH_3$, EtOH, HOAc) of aldehyde **12** with ethyl *p*-aminobenzoate gave the phthalimide **25**, which after hydrazinolysis provided amino ester **40**. Basic hydrolysis of the ester group of **40** afforded amino acid **41**, which was isolated as the sodium salt and was directly condensed with **8** to provide the anilino analogue **59**.

Phthalimide **26** was obtained by alkylation of the *N*-tosyl derivative of ethyl *p*-aminobenzoate with the phthalimido bromide **11**. Hydrazinolysis of **26** afforded **42**, and detosylation (HBr, phenol, HOAc) of **42** provided diamino ester **43**. Condensation of **43** with **8** gave isocytosine **60**, which was converted to **61** by basic hydrolysis.

Results and Discussion

The functionalized aryl compounds were evaluated in vitro as inhibitors of the partially purified dihydropteroate synthase of *Escherichia coli*.[1-3] As shown in Table III, all of the aryl analogues were inhibitors of the synthase. Relative to the parent **7**, substitution enhanced activity for some analogues and led to reduced activity for others. The range of observed potencies is 30-fold, and the bulky ortho-functionalized compound **55** is the least effective inhibitor.

A variety of substituents were explored to probe for a possible relationship between synthase affinity and the electronic (σ parameter) and/or hydrophobic (π parameter) characteristics of the substituents.[5] Statistical analysis of the data does not support a correlation between potency and either the σ or the π parameter, or combinations of σ and π. The absence of an apparent correlation between biological activity and substituent parameters may reflect a lack of structural definition, i.e., considerable conformational mobility, for the aryl binding region of the synthase, or may reflect interaction of differently functionalized aryl rings in the series with topologically distinct, or partially overlapping, regions of the enzyme.

Interestingly, the potent inhibitor **56** has an aryloxy moiety that bears a remote resemblance to the sulfonamides (cf. **4**) and to the side chain of the pterin–sulfa synthase inhibitors (cf. **3**). Although the sulfonamide linkage per se is transposed in **56** relative to the orientation in **3** and **4**, we considered the possibility that the ancillary aryl binding region of the synthase may at least partially be composed of the binding site of the sulfonamide inhibitors and, consequently, of the substrate PABA. To explore this possibility, analogues (cf. **58**, **59**, **61**) in which the aromatic ring has a strong structural similarity to PABA were synthesized as potential multisubstrate inhibitors[4] that might bridge the synthase binding sites for **1** and for PABA.

The inhibition results in Table III demonstrate that all of the bridged analogues are effective inhibitors of the synthase, with the carboxylic acid derivatives and the corresponding ethyl esters essentially equivalent in potency. Although the anilino compound **59** has a stronger structural resemblance to PABA than does the phenoxy

(5) Substituent parameters were obtained from: (a) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. *J. Med. Chem.* **1973**, *16*, 1207. (b) Leo, A. Pomona College Data Base, Pomona College, Claremont, CA 91711.

analogue **58**, these two compounds are equivalent as synthase inhibitors. The tetramethylene anilino analogue **61**, which might provide a greater degree of flexibility in bridging the binding sites of **1** and PABA, is the most potent inhibitor of the bridged compounds but is only slightly more active than the unsubstituted phenoxypropyl compound **7**.

The range of potencies exhibited by the bridged analogues is narrow (less than fourfold), and although the levels of activity are significant, they are unexceptional when compared with the activities of other compounds in the (aryloxy)propyl series. Neither the carboxylic acid moiety nor an anilino nitrogen provided the enhanced synthase affinity that might be expected for multisubstrate analogues, and therefore it appears that the aryl groups of the (aryloxy)propyl inhibitors and of the bridged analogues interact at a site on the enzyme that is distinct from, or which involves only a portion of, the PABA binding site. An alternative explanation for these observations could be that the multisubstrate approach is inappropriate because the synthase reaction involves a ping-pong mechanism rather than a sequential ordered binding. In this case, an adequate conformation for PABA binding at the active site may not be induced by the binding of the nitrosoisocytosine ring but may only be achieved after formation of the pteridine–enzyme adduct. Kinetic studies of the synthase have not permitted absolute discrimination between an ordered sequential binding mechanism and a ping-pong mechanism, i.e., enzyme alkylation by activated pteridine **1** followed by PABA displacement of enzyme from the pteridine–enzyme adduct.[3]

To explore the mode of binding of the bridged compounds, **59** was selected for further study. The inhibition data in Figure 1 demonstrate that, despite the PABA-like nature of the side-chain aryl ring, **59** does not compete with PABA for the synthase but does competitively inhibit the binding of pterin **1**, as noted previously[1] for nitrosoisocytosines **5** and **7**.

Whatever the origin of the differences in synthase affinity for the substituted aryl analogues, the 30-fold range of observed inhibitory effects indicates that aryl functionalization does indeed have an influence on binding. These results further demonstrate that, for the 6-amino-5-nitrosoisocytosine synthase inhibitors, the significantly detrimental effect on binding observed for 6-substituents larger than methylamino may be completely overcome in appropriately functionalized 6-[3-(aryloxy)propyl]amino analogues. This clearly illustrates the powerful role of such ancillary binding effects in enzyme–inhibitor interactions.

The synthase inhibitors described in this study were evaluated in vitro for antibacterial activity. Disappointingly, although the level of synthase inhibition shown by most of these compounds is equivalent with or superior to that of the clinically effective sulfonamide synthase inhibitors such as sulfamethoxazole (I_{50} = 4.7 μM) and sulfathiazole (I_{50} = 2.5 μM), none of the nitrosoisocytosines exhibited significant antibacterial activity at 100 μg/mL and there was no evidence of potentiation of trimethoprim or sulfamethoxazole. As we suggested in our previous study,[1] the lack of antibacterial activity in this series may reflect an intrinsic inability of these compounds to penetrate the bacterial cell wall and a corresponding failure to reach the intracellular target enzyme. Alternatively, the lack of antibacterial activity may be due to metabolic inactivation once intracellular access is achieved.

Experimental Section

Melting points were determined with a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. ^{1}H NMR Spectra (Varian T-60, XL-100, and CFT-20 and Hitachi Perkin-Elmer R24B spectrometers) for intermediates and target compounds were consistent with the assigned structures. Methods A–C are illustrated by the experimental procedures described for the indicated compounds: method A (**13**, **24**, **26**); method B (**27**, **38**, **39**, **40**, **42**); method C (**44**, **47**, **56**, **57**, **59**, **60**). Amines prepared by method B typically were characterized as hydrochloride salts. The corresponding free bases, either formed in situ as described for the synthesis of **57** or generated in a separate step, were used in the condensation step of method C.

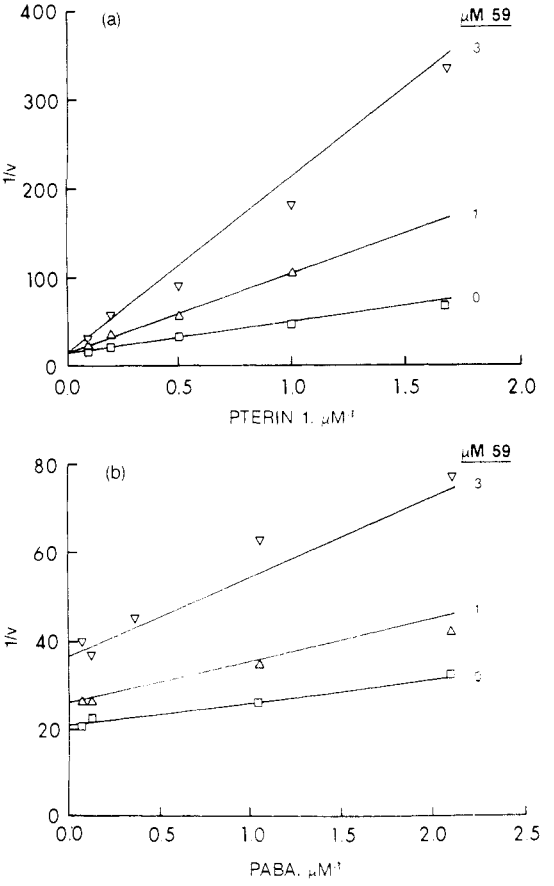


Figure 1. Double-reciprocal plots of inhibition of dihydropteroate synthase by compound **59** with varied concentrations of pterin **1** (panel a) and PABA (panel b). Velocity (v) was measured as nanomoles of dihydropteroate produced in a 12-min incubation at 37 °C.

Biological Methods. Dihydropteroate synthase was prepared and assayed as described previously.[3] The plots in Figure 1 were generated by computer fit of inhibition data to models for competitive or noncompetitive inhibition. Statistical conformity to the models was tested as described by Spector and Hajian.[11]

The in vitro antibacterial activity of the compounds at 100 μg/mL against more than 20 test organisms and the ability of the compounds to potentiate the growth inhibitory activities of trimethoprim and sulfamethoxazole were determined as previously described.[1,12]

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***N*-[3-[4-(Benzyloxy)phenoxy]propyl]phthalimide (13) (Method A).** To a stirred mixture of sodium hydride (0.72 g, 30 mmol; obtained from 1.44 g of a 50% NaH dispersion in oil by washing with pentane under nitrogen) in dry Me_2SO (40 mL) under nitrogen was added dropwise a solution of 4-(benzyloxy)phenol (6.0 g, 30 mmol) in Me_2SO (30 mL). After the mixture had stirred at 25 °C for 3 h, a solution of bromide **10** (8.0 g, 30 mmol) in Me_2SO (25 mL) was added, and the mixture was stirred at 25 °C for 72 h. The mixture was carefully poured into ice–water slush (700 mL), and the resulting solid was collected and dissolved in chloroform. The solution was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was recrystallized from EtOAC to give **13** (4.5 g, 56%).

3-[4-(Benzyloxy)phenoxy]propylamine (27) Hydrochloride (Method B). A mixture of phthalimide **13** (38.7 g, 0.10 mol) and hydrazine hydrate (85%, 11.7 g, 0.20 mol) was heated under reflux for 4 h in 95% EtOH (400 mL). The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc (500 mL) and 5% aqueous NaOH (200 mL). The organic layer was dried (Na_2SO_4) and then treated with ethanolic HCl (13.8%, 50 mL). The precipitate (30.7 g) was collected and recrystallized from aqueous ethanol to give the hydrochloride of **27** (20.3 g, 69%) as off-white plates.

2-Amino-6-[[3-[4-(benzyloxy)phenoxy]propyl]amino]-5-nitroso-4(3*H*)-pyrimidinone (47) (Method C). A mixture of methylthio compound **8** (0.37 g, 2 mmol) and amine **27** (free base, 0.514 g, 2 mmol) was heated under reflux in EtOH (35 mL) for 21 h and then cooled and filtered. The solid was heated with EtOH (100 mL) and then collected by filtration and washed with ether to yield pyrimidine **47** as a light brown powder (0.60 g, 76%).

3-(4-Hydroxyphenoxy)propylamine (28) Hydrochloride. The hydrochloride of amine **27** (5.88 g, 20 mmol) was hydrogenated in 20% aqueous MeOH (250 mL) at ca. 40 psi over 20% Pd-$(OH)_2$/C (0.20 g) for 1 h. The catalyst was removed by filtration, and the solvent was removed under reduced pressure. The residual solid was washed with ether and then with EtOAc to provide the hydrochloride of phenol **28** (3.42 g, 84%) as a light tan powder.

2-Amino-6-[[3-(4-hydroxyphenoxy)propyl]amino]-5-nitroso-4(3*H*)-pyrimidinone (44) (Method C). The free base of amine **28** (0.84 g, 5 mmol) and pyrimidine **8** (0.93 g, 5 mmol) were heated under reflux in EtOH (20 mL) for 22.5 h. The reaction mixture was cooled and filtered, and the solid was washed with EtOH to provide compound **44** (1.17 g, 76%) as a tan powder.

Ethyl 4-(3-Phthalimidopropoxy)benzoate (24) (Method A). To a stirred mixture of ethyl 4-hydroxybenzoate (16.6 g, 0.10 mol) and K_2CO_3 (13.8 g, 0.10 mol) in dry DMF (100 mL) was added bromide **10** (26.8 g, 0.10 mol). The mixture was heated at 65 °C for 4 h and then stirred at 25 °C for 18 h. The solvent was removed under reduced pressure, and the residue was extracted with $CHCl_3$. The extract was washed with aqueous NaOH (4%) followed by water and was then dried (Na_2SO_4) and concentrated under reduced pressure. The residue was recrystallized from EtOAc to provide compound **24** (24.9 g, 70%) as a white solid.

Ethyl 4-(3-Aminopropoxy)benzoate (39) Hydrochloride (Method B). A mixture of phthalimide **24** (10.5 g, 0.03 mol) and hydrazine hydrate (85%, 3.53 g, 0.06 mol) was heated under reflux for 45 min and then the solvent was removed under reduced pressure. Water (50 mL) was added followed by 6 N HCl (20 mL) and the mixture was filtered. The filtrate was evaporated to dryness under reduced pressure, and the residue was extracted with absolute EtOH. The EtOH solution was concentrated to dryness in vacuo, and the residue was partitioned between $CHCl_3$ and aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated to give a viscous oil. The oil was dissolved in ether and the hydrochloride salt of **39** (2.52 g, 32%) precipitated as a white powder upon addition of ethereal HCl.

2-Amino-6-[[3-[4-(ethoxycarbonyl)phenoxy]propyl]amino]-5-nitroso-4(3*H*)-pyrimidinone (57) (Method C). A mixture of pyrimidine **8**[1] (0.744 g, 4 mmol), hydrochloride **39** (1.30 g, 5 mmol), and triethylamine (0.51 g, 5 mmol) was heated under reflux in absolute EtOH (10 mL) under nitrogen for 8 h. The mixture was allowed to cool and was then filtered. The solid was sequentially washed with EtOH, ether, and then $CHCl_3$ to provide nitrosopyrimidine **57** (1.00 g, 69%) as a light rust powder.

2-Amino-6-[[3-(4-carboxyphenoxy)propyl]amino]-5-nitroso-4(3*H*)-pyrimidinone (58). Ester **57** (0.723 g, 2 mmol) was heated at 50 °C with 1 N NaOH (6 mL, 6 mmol) and MeOH (5 mL) for 1.5 h. The mixture was allowed to cool to room temperature, and acetic acid (0.48 g, 8 mmol) was added. The resulting precipitate was collected and sequentially washed with H_2O, EtOH, and ether. The solid (0.68 g) was dissolved in aqueous Na_2CO_3, and the aqueous solution was filtered. Addition of HOAc to the filtrate precipitated the acid **58** (0.53 g, 80%) as a tan powder.

Ethyl 4-[[(4-Methylphenyl)sulfonyl](4-phthalimidobutyl)amino]benzoate (26) (Method A). A mixture of K_2CO_3 (11.06 g, 80 mmol) and ethyl 4-(4-methylbenzenesulfonamido)-benzoate[10] (12.77 g, 40 mmol) in acetonitrile (100 mL) was heated to reflux, and bromide **11** (11.29 g, 40 mmol) was added in portions during a 4-h period. After an additional 23-h reflux, the solvent was removed under reduced pressure and the residue was extracted with EtOAc. The EtOAc was removed under reduced pressure, and the solid residue was triturated with Et_2O and then chromatographed on silica gel with CH_2Cl_2 to provide **26** as an off-white powder (8.46 g, 41%).

Ethyl 4-[(4-Aminobutyl)tosylamino]benzoate (42) Hydrochloride (Method B). Phthalimide **26** (6.86 g, 13.2 mmol) and hydrazine hydrate (85%, 1.55 g, 26.4 mmol) were heated under reflux in 95% EtOH (100 mL) for 3 h. After removal of the solvent under reduced pressure, the residue was treated with aqueous HCl (1 N, 50 mL), and the mixture was filtered. The filtrate was made slightly basic with $KHCO_3$ and a gum separated. The aqueous layer was decanted and the gum was dissolved in EtOAc. This solution was dried (Na_2SO_4) and concentrated under reduced pressure to provide **42** (4.90 g, 95%) as a viscous colorless syrup. A portion (0.4 g) of the syrup was converted to the hydrochloride of **42** by treatment with ethereal HCl.

Ethyl 4-[(4-Aminobutyl)amino]benzoate (43) Dihydrobromide. Tosyl compound **42** (free base, 4.5 g, 11.5 mmol) and phenol (3.9 g) were stirred in 32% HBr in AcOH (20 mL) for 5 h. The reaciton mixture was diluted with ether whereupon the dihydrobromide of **43** (3.40 g, 74%) precipitated as a white powder.

Ethyl 4-[[4-[(2-Amino-1,6-dihydro-5-nitroso-6-oxo-4-pyrimidinyl)amino]butyl]amino]benzoate (60) (Method C). The free base of amine **43** (0.47 g, 2.0 mmol) was heated under reflux in EtOH (20 mL) with pyrimidine **8** (0.335 g, 2 mmol) for 24 h. The reaction mixture was cooled to room temperature and filtered. The solid was continuously extracted with hexane and was then mixed with water and lyophilized to provide compound **60** as a brown powder (0.29 g, 43%).

4-[[4-[(2-Amino-1,6-dihydro-5-nitroso-6-oxo-4-pyrimidinyl)amino]butyl]amino]benzoic Acid (61). Ester **60** (0.748 g, 2 mmol) was heated under reflux in a mixture of water (6 mL), MeOH (10 mL), and aqueous NaOH (1 N, 4 mL, 4 mmol) for 2.5 h. After the mixture had cooled, HOAc (0.24 g) dissolved in water (2 mL) was added and the precipitate was collected and washed with EtOH to provide **60** (0.630 g, 91%) as a tan powder.

3-[2-(Benzyloxy)phenoxy]propylamine (37) Hydrochloride. A mixture of 1-[2-(benzyloxy)phenoxy]-3-bromopropane[4] (16 g, 0.05 mol) and MeOH (220 mL) containing anhydrous ammonia (34 g, 2.0 mol) was sealed in a stainless steel bomb and allowed to stand at 25 °C for 5 days. The solvent was then removed under reduced pressure, and the residue was treated with ethereal HCl. The solid was collected and was then partitioned between ether and aqueous K_2CO_3. The ether layer was concentrated, and the residue was distilled to provide 4.0 g of a clear oil [bp 153–160 °C (0.024 mm)]. The oil was triturated with ether, and the ether layer was evaporated to provide the free base of **37** (2.56 g, 20%) as a colorless oil which readily absorbed CO_2 from the air. The hydrochloride of **37** was obtained by treatment of the free base with ethereal HCl.

Ethyl 4-[(3-Phthalimidopropyl)amino]benzoate (25). To a stirred mixture of ethyl 4-aminobenzoate (8.26 g, 50 mmol), aldehyde **12** (10.15 g, 50 mmol), and HOAc (10 mL) in EtOH (120 mL) under nitrogen was added $Na(CN)BH_3$ (3.77 g, 60 mmol). The mixture was stirred for 21 h at 45 °C and was then quenched with ice and extracted with EtOAc. The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuo. Trituration of the residue with ether provided phthalimide **25** (10.6 g, 60%) as a pale yellow powder.

Ethyl 4-[(3-Aminopropyl)amino]benzoate (40) Dihydrochloride (Method B). A mixture of phthalimide **25** (24.67 g,

0.07 mol) and hydrazine hydrate (85%, 8.25 g, 0.14 mol) was heated under reflux in 95% EtOH (200 mL) for 1.5 h. The solvent was removed in vacuo, and water (200 mL) and concentrated HCl (35 mL) were added. The phthalhydrazide was removed by filtration, and the filtrate was evaporated under reduced pressure. The solid residue was extracted with several portions of hot EtOH, and the EtOH was then removed in vacuo. The residue was recrystallized from EtOH to provide the dihydrochloride of **40** (5.35 g, 26%) as an off-white solid.

2-Amino-6-[[3-(4-carboxyanilino)propyl]amino]-5-nitroso-4(3*H*)-pyrimidinone (59) (Method C). The dihydrochloride of diamino ester **40** (5.9 g, 20 mmol) was placed in water (50 mL) and EtOH (20 mL) containing NaOH (10 g, 250 mmol), and the mixture was heated under reflux briefly to obtain a clear solution. Additional water (ca. 50 mL) was added, and the reaction mixture was concentrated in vacuo to a final volume of 30 mL. The mixture was refrigerated overnight and then filtered, and the solid was washed with 2-propanol to afford sodium salt **41** (3.1 g, 74%) as a white powder.

Sodium salt **41** (1.95 g, 9 mmol) was heated under reflux with pyrimidine **8** (1.12 g, 6 mmol) in EtOH (10 mL) under nitrogen for 22 h. The solvent was removed in vacuo and the residue was dissolved in water (50 mL). Addition of HOAc (9 mmol) provided a precipitate, which was chromatographed on silica gel with 5% aqueous ammonia in MeOH and then rechromatographed on silica gel with 10% aqueous MeOH. Appropriate fractions were pooled to provide **59** (0.256 g, 13%) as a tan powder.

***N*-[3-(4-Aminophenoxy)propyl]phthalimide (19).** Nitro compound **18**[7] (32.6 g, 0.10 mol) was hydrogenated at 50–60 °C in dry DMF (200 mL) at 40 psi over 20% $Pd(OH)_2/C$ (0.80 g). The catalyst was filtered off and the DMF was removed in vacuo. The residue was dissolved in CH_2Cl_2 (200 mL) and the solution was filtered through Celite. The solvent was removed under reduced pressure, and the residue was recrystallized from EtOH to afford an 83% yield (24.7 g) of **19**.

***N*-[3-[4-(4-Methylbenzenesulfonamido)phenoxy]propyl]phthalimide (23).** A solution of aniline **19** (14.82 g, 50 mmol) and tosyl chloride (10.49 g, 55 mmol) in dry pyridine (75 mL) was heated at 90 °C for 3 h, and then the solvent was removed in vacuo. The residue was triturated with two 100-mL portions of water and then dissolved in EtOAc (150 mL). This solution was washed with water and with aqueous HCl and was then dried (Na_2SO_4) and evaporated to dryness in vacuo. Extraction of the solid residue with ether followed by evaporation of the ether provided 4.5 g of pure **23**. The portion that had not dissolved in ether was recrystallized from absolute EtOH to provide an additional 14.7 g of **23**, for a total yield of 85%.

3-[4-(4-Methylbenzenesulfonamido)phenoxy]propylamine (38) Hydrochloride (Method B). A mixture of compound **23** (2.25 g, 5 mmol) and 97% hydrazine (0.33 g, 10 mmol) in 95% EtOH (50 mL) was heated under reflux for 3 h. The solvent was removed in vacuo, and the residue was dissolved in 0.03 N NaOH (35 mL). Treatment of this solution with 1 N HCl (5 mL) led to the precipitation of the free base of **38** as a gum which solidified. The solid was collected by filtration and was then dissolved in 1 N HCl (30 mL). The solvent was removed in vacuo and the dry residue was triturated with ether to yield **38** (0.95 g, 54%) as the hydrochloride.

2-Amino-6-[[3-[4-(4-methylbenzenesulfonamido)phenoxy]propyl]amino]-5-nitroso-4(3*H*)-pyrimidinone (56) (Method C). The free base of **38** (2.10 g, 6.5 mmol) was heated under reflux with pyrimidine **8** (1.12 g, 6.0 mmol) in EtOH (50 mL) for 5 days. After cooling, the insoluble material was separated, triturated with EtOH, and then dissolved in 1 N NaOH (9.6 mL). Following the addition of aqueous HOAc (9.6 mL, 1 N), the mixture was filtered and the precipitate was washed successively with EtOH, EtOAc, and ether. This gave 2.0 g (73%) of nitrosopyrimidine **56**.

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