Monocyclic Pteridine Analogues. Inhibition of *Escherichia coli* Dihydropteroate Synthase by 6-Amino-5-nitrosoisocytosines

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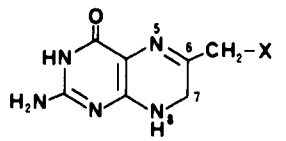
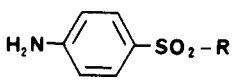
A variety of 5,6-disubstituted isocytosine derivatives were evaluated in vitro as inhibitors of dihydropteroate synthase from *Escherichia coli*. A number of 6-(alkylamino)-5-nitrosoisocytosines have in vitro potency equivalent with or superior to that of therapeutically effective sulfonamide inhibitors of the synthase. The sulfonamide drugs are known to compete for the *p*-aminobenzoic acid binding site of the synthase, and kinetic analysis of inhibition of the synthase by 6-(methylamino)-5-nitrosoisocytosine (**16**; I_{50} = 1.6 μM) and by the 6-(3-phenoxypropyl)amino analogue (**33**; I_{50} = 3.7 μM) indicated that the nitrosoisocytosine inhibitors compete with the pteridine substrate for the enzyme. Structure–activity studies demonstrated that the enzyme surface has a low tolerance for steric bulk in the region surrounding the isocytosine 6-amino function. However, this steric intolerance may be counterbalanced to a significant degree by positive allosteric interactions achieved by certain analogues that have a 6-(ω-phenylalkyl)amino substituent. For example, 6-[(7-phenylheptyl)amino]-5-nitrosoisocytosine (**28**) is as effective an inhibitor (I_{50} = 1.4 μM) as the 6-methylamino compound **16**. Although several members of the 5-nitroso series were potent synthase inhibitors, none of the nitrosoisocytosines exhibited significant antibacterial activity. This observation may reflect poor transport of these compounds through the bacterial cell wall or, alternatively, may result from a rapid metabolic inactivation process.

Folic acid is a vitamin in man but must be synthesized de novo by bacteria.[1] On the biosynthetic pathway to tetrahydrofolic acid, dihydropteroate synthase (EC 2.5.1.15) is the enzyme responsible for condensation of *p*-aminobenzoic acid (PABA) with 7,8-dihydro-6-(hydroxymethyl)pterin pyrophosphate (**1**) to provide 7,8-dihydropteroic acid (**2**).[1] Inhibition of dihydropteroate synthase is a viable target for antibacterial chemotherapy, as evidenced by the clinical success of the sulfonamide inhibitors.[1b] The sulfonamide drugs, e.g. sulfamethoxazole (**3**) and sulfathiazole (**4**), compete with PABA for the synthase and are also effective alternate substrates for the enzyme.[2] Despite the clinical effectiveness of the sulfonamide drugs, the only reported studies on inhibition of the synthase by analogues of the pteridine substrate have involved evaluation of certain pterin–sulfa adducts[2] and homopteroate analogues[3] as inhibitors. Our interest in monocyclic analogues of biologically active pteridines[4] led us to consider the design of isocytosine derivatives that might compete with pteridine **1** for binding to the synthase. Of particular interest were isocytosine derivatives (cf. **5**) bearing an alkylamino function at the pyrimidine C-6 position as a potential mimic of the pteridine C-7/N-8 dihydro region and an unsaturated substituent at C-5 of the pyrimidine as a potential mimic of the pteridine N-5/C-6 imine linkage. In the study described herein, we evaluated a series of isocytosine analogues related to general structure **5** as dihydropteroate synthase inhibitors and report that certain 6-alkylamino derivatives of 5-nitrosoisocytosine are potent inhibitors of the synthase from *Escherichia coli*.



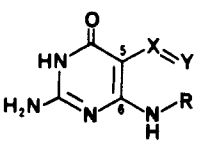
1 X = -OPP

2 X = -NH-⟨⟩-CO_2H

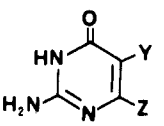


3 R = -NH-⟨isoxazole, Me⟩

4 R = -NH-⟨thiazole⟩



5



	Y	Z
6	NO	SMe
7	H	Cl
8	NO_2	Cl
9	CHO	Cl

Chemistry. The 5-nitrosoisocytosines listed in Table I were typically prepared by nitrosation of an appropriate 6-substituted isocytosine (method A)[5–9] or through condensation of the appropriate amine with 6-(methylthio)-5-nitrosoisocytosine (**6**; method B), which was prepared by methylation of 6-mercaptoisocytosine followed by nitrosation. Condensation of 4-phenylbutylamine with 6-chloro-5-nitroisocytosine (**8**)[6,10] provided the 5-nitro compound **29** (method C), and the 5-formyl and 5-cyano compounds **30** and **31** were obtained as previously described.[11] Amines required for condensation with **6** generally were available from the appropriate alkyl halide by

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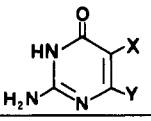
Table I. Dihydropteroate Synthase Inhibition and Chemical Properties of the Isocytosines



no.	X	Y	I_{50},[a] μM	mp, °C	method[b,c]	yield, %	formula[d]
10	H	H	(500)		e		$C_4H_5N_3O$
11	H	NHMe	(110)	262–263 dec[f]	g		$C_5H_8N_4O$[g]
12	CHO	NHMe	(300)	ca. 350 dec	h	23	$C_6H_8N_4O_2$·HCl
13	NO_2	NHMe	2.8	>300[i]	C[j]		$C_5H_7N_5O_3$
14	NO	OH	(200)	>300	k	32	$C_4H_2N_4O_3Na_2·H_2O$[k]
6	NO	SMe	17% @ 20	>225 dec	h	95	$C_5H_6N_4O_4S·0.3H_2O$[l]
15	NO	NH_2	5.8	>360	m		$C_4H_5N_5O_2$
16	NO	NHMe	1.6	>360[n]	A	65	$C_5H_7N_5O_2$
17	NO	NHEt	11.0	269 dec[o]	A	13	$C_6H_9N_5O_2$
18	NO	NEt_2	197	199–200	A[p]	24	$C_8H_{13}N_5O_2$
19	NO	NH-*i*-Pr	208	>360	A	68	$C_7H_{11}N_5O_2$[q]
20	NO	$NH(CH_2)_3OH$	35	246–247	A	61	$C_7H_{11}N_5O_3$
21	NO	NHPh	(100)	>293 dec[r]	B	38	$C_{10}H_9N_5O_2$
22	NO	$NHCH_2Ph$	12.9	>290 dec[s]	A	28	$C_{11}H_{11}N_5O_2$
23	NO	$NH(CH_2)_2Ph$	4.2	274–278 dec	A	35	$C_{12}H_{13}N_5O_2$
24	NO	$NH(CH_2)_3Ph$	7.8	252–253	A	66	$C_{13}H_{15}N_5O_2$
25	NO	$NH(CH_2)_4Ph$	2.5	235–240 dec	B	49	$C_{14}H_{17}N_5O_2$[t]
26	NO	$NH(CH_2)_5Ph$	5.9	219–220.5	B	61	$C_{15}H_{19}N_5O_2$
27	NO	$NH(CH_2)_6Ph$	9	224–225.5	B	47	$C_{16}H_{21}N_5O_2$
28	NO	$NH(CH_2)_7Ph$	1.4	211–214.5	A	18	$C_{17}H_{23}N_5O_2$
29	NO_2	$NH(CH_2)_4Ph$	21	255.5–257.5	C	37	$C_{14}H_{17}N_5O_3$
30	CHO	$NH(CH_2)_4Ph$	(100)	274–278 dec	u	u	$C_{15}H_{18}N_4O_2$·HCl
31	CN	$NH(CH_2)_4Ph$	290	213–215.5	u	u	$C_{15}H_{17}N_5O$
32	NO	$NH(CH_2)_3SO_2Ph$	18	240–243.5	B	26	$C_{13}H_{15}N_5O_4S$
33	NO	$NH(CH_2)_3OPh$	3.7	229–234.5 dec	B	50	$C_{13}H_{15}N_5O_3$

[a] Micromolar concentration to inhibit the synthase by 50%. Numbers in parentheses indicate no activity (≤10% *I*) at the designated concentration. [b] Methods A, B, and C refer to the general methods outlined in the Chemistry Section; exceptions are noted. [c] For detail see the Experimental Section. [d] Unless otherwise indicated, all compounds gave satifactory (±0.4% of theory) elemental analyses for C, H, and N. [e] Sigma Chemical Co. [f] Literature mp 265–266 °C (ref 8), 255–257 °C (ref. 9). [g] Obtained from methylamine and chloro compound 7 (cf. ref 8 and 9). N: calcd, 39.98; found, 39.49. We thank Dr. R. Morrison for providing this sample. [h] See the Experimental Section for details. [i] As reported in ref 12. Reference 6 reports mp 360 °C dec, and ref 10b reports mp >350 °C. [j] We thank Prof. H. C. S. Wood (Strathclyde) for this sample (cf. ref 12). [k] Obtained and assayed as the disodium salt monohydrate from basic hydrolysis of **15**. Satisfactory Na analysis also obtained. See also ref 13. [l] Satisfactory S analysis. [m] See ref 14. We thank Dr. B. Roth for this sample. [n] Literature[9] mp >300 °C. [o] Literature[15] mp 265–266 °C dec. [p] Isolated from nitrosation of 2-amino-6-(diethylamino)-4-methoxypyrimidine[16] which was provided by Dr. H. Openshaw of the Wellcome Research Laboratories, U.K. [q] Obtained as the hemihydrate. [r] Literature[5] mp 294 °C. [s] Literature[5] mp 289 °C. [t] N: calcd, 24.38; found, 23.90. [u] Prepared as described in ref 11.

displacement with potassium phthalimide and subsequent liberation of the primary amino group by hydrazinolysis. Catalytic reduction of the corresponding nitrile provided 3-(phenylsulfonyl)propylamine, which afforded **32** upon condensation with **6**. Aldehyde **12** was prepared from **9**[11] and methylamine.

Results and Discussion

The isocytosines prepared in this study were evaluated in vitro as inhibitors of dihydropteroate synthase from *E. coli*.[3] The inhibition data for the series are presented in Table I along with the properties of the new compounds. Isocytosine (**10**) is inactive as a synthase inhibitor, and the additional presence of the 6-methylamino group in **11** is insufficient to elicit binding to the enzyme. The effect of an additional substituent in the 5-position was examined, and although the 5-formyl derivative **12** of 6-(methylamino)isocytosine is inactive, the 5-nitro analogue **13** (I_{50} = 2.8 μM) and the 5-nitroso analogue **16** (I_{50} = 1.6 μM) are potent synthase inhibitors. These compounds exhibit inhibitory effects at least equipotent with those observed, under equivalent in vitro conditions, for sulfonamide drugs such as sulfamethoxazole (**3**; I_{50} = 4.7 μM) and sulfathiazole (**4**; I_{50} = 2.5 μM). The role of the heteroatom linkage at C-6 was also evaluated. In the 5-nitroso series, the 6-hydroxy (**14**) and 6-methylthio (**6**) derivatives suffer dramatic losses in activity relative to the 6-methylamino compound **16**. Furthermore, the dialkylamino compound **18** is significantly less active than its 6-monoalkylamino counterpart **17**, which indicates the superiority of 6-monoalkylamino substitution at C-6. We selected the 5-nitroso series for exploration of the effect of structural variations of the 6-alkylamino function.

The inhibition data in Table I clearly demonstrate that the 6-methylamino analogue **16** is outstanding among the compounds with relatively small aliphatic residues attached to the pyrimidine 6-amino group. This inhibitor is more potent than either the 6-amino compound **15** or the 6-ethylamino derivative **17**, and a dramatic loss in activity was observed for the 6-isopropylamino analogue **19**. These data are suggestive of a rather restrictive bulk intolerance on the enzyme surface near the binding site for the pyrimidine 6-amino function. The slightly enhanced binding of the 6-methylamino compound **16** compared with the analogue **15**, which bears the smaller 6-amino group, may reflect an electronic (pK_a) effect, or may arise from a strict steric requirement for one-carbon substitution at the amino group. In either case, as a mimic of the 7,8-dihydro region of pteridine **1**, the methylamino group of **16** is more appropriate than an unsubstituted amino group and thus **16** is more tightly bound than **15**.

Although the activity of the 6-amino-5-nitrosoisocytosines decreases as steric effects predominate with

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nitrogen substituents larger than methyl, it is interesting to note that this steric effect may be largely overcome by a positive allosteric binding contribution from an aromatic ring appended at the terminus of an alkyl group of appropriate length. This is apparent in the series of 6-(ω-arylalkyl)amino analogues **22–28** (Table I). Although the 6-anilino compound **21** is inactive, the *N*-benzyl derivative **22** exhibits considerable activity, and compounds with longer aliphatic chains linking the aromatic ring to the pyrimidine have substantial affinity for the enzyme. This is especially evident for analogues with four (cf. **25**) and seven (cf. **28**) methylene units separating the 6-amino function and the phenyl ring. The nature of the bridging chain also influences activity. For the tetramethylene compound **25**, replacement of the benzylic methylene with a sulfonyl moiety, as in compound **32**, results in a considerable loss of activity. By contrast, isosteric replacement of the benzylic methylene of **25** with an ether linkage, as in the phenoxypropyl analogue **33**, is an acceptable alteration in the bridging chain.

The significant role of the terminal phenyl ring is dramatically illustrated by the 10-fold difference in activity of the hydroxypropyl compound **20** compared with the phenoxypropyl derivative **33**. We considered the possibility that an altered mode of pyrimidine binding, relative to that of the less structurally complex analogues such as **16**, might be induced by the ancillary binding of the phenyl ring and might contribute positively to the binding of certain of the ω-phenylalkyl compounds. If this were the case, the substituent effects on the pyrimidine ring might also be altered. This possibility was evaluated by variation of the functionality at C-5 in a small series of 6-[(4-phenylbutyl)amino]isocytosines for comparison with the active 5-nitroso compound **25**. The 5-formyl (**30**) and 5-cyano (**31**) derivatives are inactive, and the 5-nitroso and 5-nitro (**29**) substituents again emerge as the preferred functionality. This suggests that the pyrimidine binding orientation is similar in both the 6-alkylamino and 6-(ω-phenylalkyl)amino series. In any event, the binding contribution of the terminal phenyl ring indeed negates the adverse steric effects of the bridging chain in the 5-nitroso series such that these steric effects are completely overcome in certain members of this series (e.g., **25**, **28**, **33**).

We then sought to validate our initial premise that the nitrosoisocytosines should function as monocyclic pteridine analogues and compete with the pterin substrate **1** for the synthase. Methylamino compound **16** and phenoxypropyl analogue **33** were selected as representatives of the alkyl and ω-aryl series, respectively, for more detailed kinetic study. Kinetic analysis of synthase inhibition by **16** confirmed that this compound competitively inhibits binding of pterin **1** (Figure 1a) and is noncompetitive with PABA (Figure 1b). Similarly, **33** was also shown to be competitive with **1** (Figure 1c). These results are the reverse of those obtained previously[2] for sulfamethoxazole, which competes with PABA, and demonstrate that the nitrosoisocytosines function as monocyclic analogues of **1**. The K_{is} value for phenoxypropyl compound **33** is 0.69 μM, and the K_{is} value for methylamino compound **16** is 0.085 μM.

Despite the potent synthase inhibition shown by several members of the series, no significant antibacterial activity was observed at 100 μg/mL for these compounds and there was no evidence of potentiation of trimethoprim or sulfamethoxazole (see the Experimental Section). It is possible that this reflects either poor transport of these compounds through the bacterial cell wall or a rapid metabolic inactivation once the compounds reach the interior of the cell.

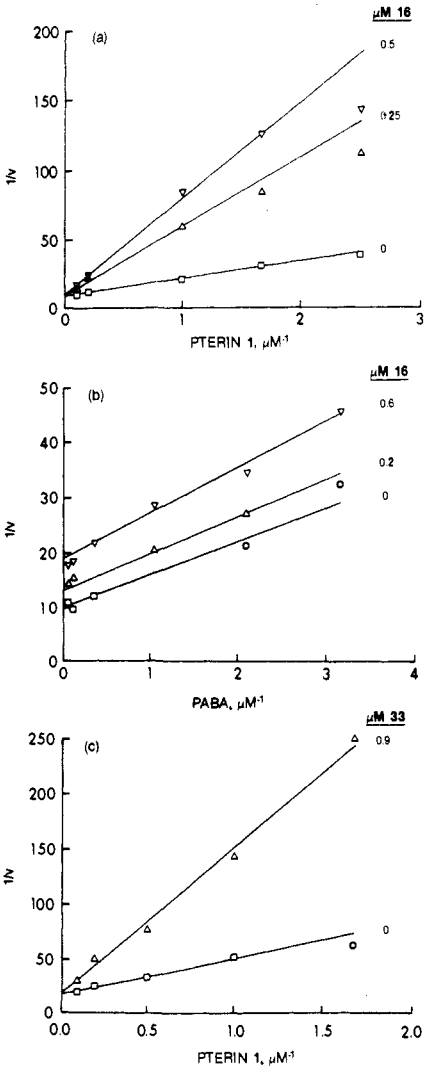

Figure 1. Double-reciprocal plots of dihydropteroate synthase inhibition of (a) compound **16** and (c) compound **33** with varied concentrations of pterin substrate **1** and (b) compound **16** with varied concentrations of PABA. Velocity (v) was measured as nmoles of dihydropteroate produced in a 12-min incubation at 37 °C.

In summary, we have shown that certain 6-(alkylamino)-5-nitrosoisocytosines effectively inhibit dihydropteroate synthase by competing with pteridine **1** for the synthase. Although the enzyme appears to be intolerant of bulk around the 6-amino function of the isocytosine inhibitors, a phenyl ring appropriately spaced from the 6-amino group by an aliphatic linker appears to interact with an allosteric binding site. This positive ancillary interaction counterbalances, and in certain cases totally overrides, the adverse steric effect of the aliphatic chain.

Experimental Section

Melting points were determined with a Buchi 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. Chloropyrimidine **7** was purchased from Aldrich Chemical Co.

2-Amino-5-formyl-6-(methylamino)pyrimidin-4(3*H*)-one (12). A mixture of chloropyrimidine **9**[11] (4.85 g, 28 mmol), 40% aqueous methylamine (4.3 mL, 56 mmol), triethylamine (2.83 g, 28 mmol), and MeOH (75 mL) was heated in a bomb at 100 °C for 2 h and then allowed to cool. The solid present was collected, triturated with boiling 2-methoxyethanol (1.2 L), and then treated

with hot 1 N HCl (350 mL). After cooling in ice water, the mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was treated with hot 1 N HCl (135 mL) and the mixture was filtered while hot. Upon cooling, the filtrate deposited the monohydrochloride of **12** (1.32 g (23%)) as orange crystals.

2-Amino-6-(diethylamino)-5-nitrosopyrimidin-4(3*H*)-one (18). To a warm (40 °C) stirred slurry of 2-amino-6-(diethylamino)-4-methoxypyrimidine[16] (0.50 g, 2.6 mmol) and a solution of $NaNO_2$ (0.18 g, 2.6 mmol) in water (2 mL) was added slowly 2 N aqueous acetic acid (1.8 mL). After 1 h at room temperature, the mixture was cooled in ice water and filtered. The solid was treated with refluxing EtOH (20 mL), and the mixture was filtered while hot. The filtrate provided a red solid in two crops (0.24-g total). This solid was then refluxed in EtOH (70 mL) to obtain complete solution. Upon cooling, the solution provided pure **18** as a red solid, 0.127 g (24%).

Method A. 2-Amino-5-nitroso-6-(3-phenylpropyl)aminopyrimidin-4(3*H*)-one (24). A. A mixture of 6-chloroisocytosine hemihydrate (**7**; 3.86 g, 25 mmol) and 3-phenylpropylamine (6.75 g, 50 mmol) was refluxed in 2-methoxyethanol (50 mL) for 3.5 h, and then the solvent was removed under reduced pressure. The residual oil was dissolved in a hot mixture of EtOH (30 mL) and water (40 mL), and upon cooling a solid was obtained. The solid was collected and was treated with hot EtOAc (800 mL). The mixture was filtered while hot, and upon cooling the filtrate deposited white crystals, 2.09 g. A portion (1.50 g) of this material was recrystallized from EtOAc–EtOH (ca. 400 mL, 9:1) to provide 2-amino-6-(3-phenylpropyl)aminopyrimidin-4(3*H*)-one (0.81 g) as white crystals, mp 192.5–194.5 °C. Anal. ($C_{13}H_{16}N_4O$) C, H, N.

B. To a warm (40 °C) stirred mixture of the above 6-(3-phenylpropyl)amino derivative (0.50 g, 2.1 mmol) and a solution of $NaNO_2$ (0.152 g, 2.1 mmol) in water (2 mL) was added aqueous HOAc (2N, 1.5 mL) dropwise, followed by additional water (2 mL). After 1 h at 25 °C, the mixture was cooled in ice water and filtered. The solid (0.562 g) was recrystallized from 2-methoxyethanol (45 mL) to provide **24** (0.379 g, 67%) as a dark pink solid.

Method B. 2-Amino-5-nitroso-6-(3-phenoxypropyl)-aminopyrimidin-4-(3*H*)-one (33). A. A mixture of potassium phthalimide (17.41 g, 94 mmol) and 3-phenoxypropyl bromide (20.15 g, 94 mmol) was refluxed in DMF (250 mL) for 1.5 h. The mixture was cooled and then filtered. The filtrate was evaporated under reduced pressure, and the residue was recrystallized from 95% EtOH to afford *N*-(3-phenoxypropyl)phthalimide (19.75 g, 75%) as a white solid, mp 88–90 °C. Anal. ($C_{17}H_{15}NO_3$) C, H, N.

B. The phthalimide obtained above (9.80 g, 35 mmol) was refluxed with 85% hydrazine (4.1 mL) in 95% EtOH (100 mL) for 3 h. The mixture was cooled, and water (335 mL) and 1 N NaOH (70 mL) were then added. The solution was extracted three times with ether (100-mL portions), and the ether layers were washed with water (100 mL) and then dried (Na_2SO_4). The ethereal solution was treated with HCl and then evaporated to dryness under reduced pressure. The residue was recrystallized from ether–ethanol to give 3-phenoxypropylamine hydrochloride (4.49 g, 68%) as a white solid, mp 173–175 °C. Anal. ($C_9H_{14}ClNO$) C, H, N.

C. The free base of 3-phenoxypropylamine (2.75 g, 18 mmol) and (thiomethyl)pyrimidine **6** (3.36 g, 18 mmol) were heated with stirring in 50% aqueous 2-methoxyethanol (160 mL) for 2.5 h. The reaction mixture was filtered, and the solid was washed with a small amount of 95% EtOH to provide **33** as a dark red solid, 2.61 g (50%).

Method C. 2-Amino-5-nitro-6-[(4-phenylbutyl)amino]pyrimidin-4(3*H*)-one (29). A mixture of nitropyrimidine **8**[6,10] (hydrate; 1.00 g, 4.8 mmol), 4-phenylbutylamine (0.716 g, 4.8 mmol), and triethylamine (2.43 g, 24 mmol) was stirred in 50% aqueous EtOH (20 mL) for 3 h, then cooled to 0 °C, and filtered to provide a yellow solid. The solid was treated with boiling 95% EtOH (1.8 L), and the mixture was filtered while hot. Upon cooling, the filtrate provided **29** (0.542 g, 37%) as pale yellow crystals.

2-Amino-5-nitroso-6-[[3-(phenylsulfonyl)propyl]amino]-pyrimidin-4(3*H*)-one (32). 3-(Phenylsulfonyl)propylamine hydrochloride [mp 219–223.5 °C. Anal. ($C_9H_{14}ClNO_2S$) C, H, N] was prepared by catalytic reduction of 3-(phenylsulfonyl)-propionitrile. The amine salt (2.02 g, 8.6 mmol) was then heated at ca. 110 °C with (thiomethyl)pyrimidine **6** (1.59 g, 8.6 mmol) and 1 N NaOH (8.6 mL, 8.6 mmol) in 50% aqueous 2-methoxyethanol (40 mL). After 1 h, the reaction mixture was cooled in ice and filtered. The solid was treated with boiling 95% EtOH (700 mL), and the mixture was filtered while hot. The filtrate was concentrated under reduced pressure, and the residue was triturated with aqueous HOAc and then recrystallized from 95% EtOH to provide **32** in two crops: 0.38 g, mp 240–243.5 °C; 0.37 g, mp 244–246 °C; total yield 26%.

2-Amino-6-(methylthio)-5-nitrosopyrimidin-4(3*H*)-one (6). A. 2-Amino-6-mercaptopyrimidin-4(3*H*)-one[17] (36.9 g, 0.258 mol) was dissolved in 1 N NaOH (270 mL), and the mixture was filtered and then diluted with 50% aqueous EtOH (900 mL). Methyl iodide (38.3 g, 0.270 mol) was added, and after stirring for 1 h the reaction mixture was cooled and the precipitate collected, washed with water, and dried in vacuo. This provided 2-amino-6-(methylthio)pyrimidine-4(3*H*)-one: 31.8 g (78%); mp 271–273 °C. Anal. ($C_5H_7N_3OS$) C, H, N, S.

B. The methylthio compound (24 g, 0.153 mol) obtained above was suspended in a solution of $NaNO_2$ (10.8 g, 0.156 mol) in water (180 mL), and the stirred mixture was warmed to 40 °C. Aqueous HOAc (2 N, 108 mL, 0.216 mol) was added in portions during a 0.5-h period, and stirring was continued at ambient temperature for an additional 0.5 h. The mixture was cooled in an ice bath and then filtered. The product was washed with cold water and then dried in vacuo. This provided the 0.3 hydrate of **6** (27 g, 95%) as a deep greenish blue powder.

2-Amino-4,6-dihydroxy-5-nitrosopyrimidine Disodium Salt (14). Pyrimidine **15**[14] (1.00 g, 6.45 mmol) was dissolved in boiling 1 N aqueous NaOH. The purple solution was diluted with EtOH (25 ml) and then cooled in ice. The resulting purple solid was collected and dried in vacuo at 80 °C to provide the disodium salt of **14** as the monohydrate, 0.449 g (32%).

Biological Methods. Enzyme Assays. Dihydropteroate synthase was prepared and assayed as described previously.[3] Briefly, the enzyme was partially purified from *E. coli* B and was assayed by paper chromatographic separation of unreacted [^{14}C]carboxy-*p*-aminobenzoic acid from enzymatically formed radioactive dihydropteroate. In the early stages of this study, the pterin substrate **1** was formed in situ with *E. coli* (hydroxymethyl)dihydropterin phosphokinase, ATP, and 7,8-dihydro-6-(hydroxymethyl)pterin in a combined assay.[18] The majority of the compounds, however, were tested with chemically prepared **1** as substrate, as previously described.[3] Data from kinetic analysis of enzyme inhibition were fitted by computer to models for competitive or noncompetitive inhibition and were statistically tested for conformity to the models as described by Spector and Hajian.[19]

In Vitro Antibacterial Assays. The in vitro antibacterial activity of the compounds at 100 μg/mL against more than 20 test organisms was determined as described previously.[20] The compounds were also tested for ability to potentiate the growth inhibitory activities of trimethoprim (TMP) and sulfamethoxazole (SMX). The concentration of TMP and SMX needed to produce 50% inhibition (ED_{50}) of the growth of *Staphylococcus aureus* (CN491) and *Pseudomonas aeruginosa* (CN200) were each determined in the presence and absence of 50 μg/mL of the test compound. Growth was measured turbidometrically after overnight incubation at 37 °C in oxoid sensitivity test broth. For this procedure, a decrease of 2-fold or greater in the ED_{50} values of TMP or SMX was considered evidence of potentiation by the test compound.

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Registry No. **6**, 98525-47-4; **7**, 1194-21-4; **8**, 1007-99-4; **9**, 85840-20-6; **10**, 108-53-2; **11**, 54004-20-5; **12**, 98510-35-1; **12**·HCl, 98510-49-7; **13**, 879-44-7; **14**, 55482-22-9; **15**, 2387-48-6; **16**, 86296-75-5; **17**, 78523-14-5; **18**, 98510-36-2; **19**, 98510-37-3; **20**, 98510-38-4; **21**, 33344-20-6; **22**, 33344-24-0; **23**, 98510-39-5; **24**, 98510-40-8; **25**, 98510-41-9; **26**, 98510-42-0; **27**, 98510-43-1; **28**, 98510-44-2; **29**, 98510-45-3; **30**, 98510-46-4; **31**, 85840-24-0; **32**, 98510-47-5; **33**, 98510-48-6; NH_2Ph, 62-53-3; $NH_2(CH_2)_4Ph$, 13214-66-9; $NH_2(CH_2)_5Ph$, 17734-21-3; $NH_2(CH_2)_6Ph$, 17734-20-2; $NH_2(CH_2)_3SO_2Ph$, 98510-52-2; $NH_2(CH_2)_3OPh$, 7617-76-7; methylamine, 74-89-5; 2-amino-6-(diethylamino)-4-methoxypyrimidine, 98525-48-5; 3-phenylpropylamine, 2038-57-5; 2-amino-6-(3-phenylpropyl)aminopyrimidin-4(3*H*)-one, 98510-50-0; potassium phthalimide, 1074-82-4; 3-phenoxypropyl bromide, 588-63-6; *N*-(3-phenoxypropyl)phthalimide, 83708-38-7; 3-phenoxypropylamine hydrochloride, 83708-39-8; 3-(phenylsulfonyl)-propylamino hydrochloride, 98510-51-1; 3-(phenylsulfonyl)-propionitrile, 10154-75-3; 2-amino-6-mercaptopyrimidin-4(3*H*)-one, 6973-81-5; 2-amino-6-(methylthio)pyrimidine-4(3*H*)-one, 6307-40-0; dihydropteroate synthase, 9055-61-2.

Synthesis and Biological Activities of Some Pseudo-Peptide Analogues of Tetragastrin: The Importance of the Peptide Backbone

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Pseudo-peptide analogues of the C-terminal tetrapeptide of gastrin, in which a peptide bond has been replaced by a CH_2–NH bond, i.e. (*tert*-butyloxycarbonyl)-L-tryptophyl-$\psi(CH_2$–NH)-L-leucyl-L-aspartyl-L-phenylalanine amide (**8**), (*tert*-butyloxycarbonyl)-L-tryptophyl-L-leucyl-$\psi(CH_2$–NH)-L-aspartyl-L-phenylalanine amide (**13**), (*tert*-butyloxycarbonyl)-L-tryptophyl-L-leucyl-L-aspartyl-$\psi(CH_2NH)$-L-phenylalanine amide (**20**), were synthesized. The pseudo-peptides **8** and **13** were shown to have the same affinity as (*tert*-butyloxycarbonyl)-L-tryptophyl-L-leucyl-L-aspartyl-L-phenylalanine amide (**21**) for the gastrin receptor on isolated mucosal cells. The pseudo-peptide **20** exhibited lower affinity ($IC_{50} \simeq 10^{-5}$ M). The biological activity of these pseudo-peptides was studied on acid secretion in the anesthetized rat. Compound **8** stimulated acid secretion, identically with that of **21**. Compound **13** did not exhibit any agonist activity but was able to antagonize the action of gastrin (ED_{50} = 0.3 mg/kg). Compound **20** did not show any agonist activity but was able to inhibit gastrin-induced acid secretion, with lower potency (ED_{50} = 15 mg/kg). The importance of the peptide bonds in the mode of action of gastrin is discussed, and a hypothetical approach of the mechanism of action is presented.

Gastrin, a 17 amino acid hormone isolated from hog antral mucosa,[1] plays a major role in the stimulation of gastric acid secretion. It was early recognized that, of the 17 amino acid residues of the molecule, only the C-terminal tetrapeptide sequence, Trp-Met-Asp-Phe-NH_2 (which is also found in cholecystokinin and caerulein) is required for the remarkable range of physiological effects displayed by the natural hormone.[2] The finding of the whole activity in such a small molecule offered scope for an investigation of structure–function relationships on an unprecedented scale.[3] Changes can be made in the Trp, Met, and Phe positions, giving active analogues providing evidence that these positions were concerned only with binding at the site of action. On the other hand, even small changes at the Asp position resulted in loss of activity, pointing toward a functional rather than a binding role for the aspartyl residue. However, despite the numerous studies in the gastrin area, the mechanism of action of the hormone remains unclear. We recently showed that elimination of the C-terminal phenylalanyl residue resulted in potent gastrin antagonists,[4,18,19] and we concluded that, although the phenylalanyl residue was essential for the biological activity, it was not crucial for binding to gastrin receptors. Morley reported that the side chains of the binding amino acid residues rather than the peptide backbone are important in the binding interactions and that the peptide backbone serves mainly in providing correct spacing of the side chains.[3] We recently pointed out the significance of the peptide bonds in the mode of action of gastrin.[5] In the present report, we present the synthesis and the biological activities of three pseudo-peptide analogues of the C-terminal tetrapeptide of gastrin in which an amide bond has been replaced by a CH_2–NH bond, an isosteric modification that has already been used in the series of renin inhibitors.[6] For avoiding side reactions related to methionine and because it is known that leucine can replace methionine in the gastrin family without any loss of activity, we chose to prepare analogues containing leucine instead of methionine. Binding of these pseudo-peptides to isolated gastric mucosal cells and their biological activity on acid secretion in anaesthetized rats are reported.

Chemistry. The pseudo-peptide Boc-Trp-$\psi(CH_2$-NH)-Leu-Asp(Bzl)-Phe-NH_2 (**8**) was synthesized according to Scheme I. The tripeptide Boc-Leu-Asp(Bzl)-Phe-NH_2 (**3**) was prepared stepwise starting from the C-terminal residue, with BOP as coupling reagent.[7] It was partially deblocked by trifluoroacetic acid to produce the tri-

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