Synthesis and Biological Activity of 5,11-Methylenetetrahydro-5-deazahomofolic Acid[1a,b]

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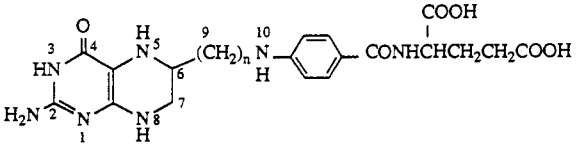
The synthesis of 5,11-methylene-5-deazatetrahydrohomofolate (**5**), a stable, semirigid mimic of 5,10-methylenetetrahydrofolate (**4**) is reported as a potential inhibitor of thymidylate synthases (TS). The key intermediate 3-amino-1-oxo-tetrahydropyrimido[4,5-*c*][2,6]naphthyridine (**6**) was obtained by the regiospecific cyclocondensation of 2,4,6-triaminopyrimidine with ethyl 1-benzyl-3-oxo-4-piperidinecarboxylate followed by halogenation (of the resulting lactam **9**) and catalytic hydrogenolysis. Selective reduction of **6** followed by arylation with *tert*-butyl *p*-fluorobenzoate, saponification, and coupling with diethyl L-glutamate followed by saponification afforded the target compound **5**. The title compound was tested as an inhibitor of the growth of Manca human lymphoma cells and also as an inhibitor of TS from Manca cells and *Lactobacillus casei* and was found to be inactive. In addition, compound **5** also failed to inhibit glycinamide ribonucleotide formyltransferase from L. casei and from Manca cells.

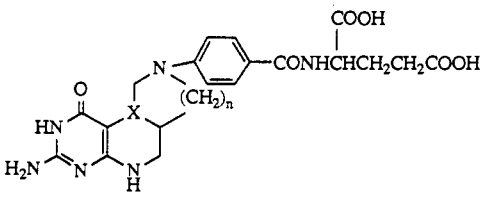
Thymidylate synthase (TS) is involved in the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). Since this is the sole de novo pathway for the synthesis of dTMP, TS is a pivotal enzyme in the biosynthesis of an essential precursor for DNA synthesis and is an important chemotherapeutic target.[2]

(6*R*,*S*)-Tetrahydrohomofolate (**1**) a homologue of (6*R*,*S*)-tetrahydrofolic acid (**2**) functions as an inhibitor of certain thymidylate synthases (TS)[3] and as a substrate for TS from other sources.[4a,b] The ability of the 6*S* form of **1** to transfer a one-carbon unit suggests that it forms a tricyclic intermediate 5,11-methylenetetrahydrohomofolate (**3**) which mimics the natural cofactor 5,10-methylenetetrahydrofolate (5,10-CH_2THF) (**4**).



1 n = 2

2 n = 1



3 n = 2, X = N

4 n = 1, X = N

5 n = 2, X = CH

Tricyclic **4** has been postulated as a bound cosubstrate in the mechanism of the TS-catalyzed synthesis of dTMP from dUMP.[5] Since **4** has been proposed to bind to TS[5] in order to transfer the one-carbon unit, it is logical to suggest that **3** in its function as a cosubstrate must also bind to TS in a conformation similar to **4**.[5] This concept was initially proposed by Mosher and co-workers[6] in an attempt to explain the inhibitory activity of **1** with respect to TS. Tricyclic systems such as **3** and **4** which contain a carbon flanked by two nitrogens are inherently unstable under catalytic conditions.[5] We reasoned that replacement of the N-5 of **3** with a carbon would afford a stable, semirigid analogue 5,11-methylenetetrahydro-5-deazahomofolic acid (**5**) with the potential to bind to TS without the ability to function as a cosubstrate. Thus compound **5** was anticipated to exhibit antitumor activity through the inhibition of TS and consequently the inhibition of DNA synthesis.[2] This report concerns the synthesis of a novel tricyclic 5-deazahomofolate **5** and its biological evaluation.

Chemistry

The synthesis of the target compound **5** commenced with the key intermediate 3-amino-1-oxo-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,6]naphthyridine (**6**) (Scheme I). A one-step facile entry into this novel heterocyclic ring system was accomplished by the cyclocondensation of 2,4,6-triaminopyrimidine (**7**) with ethyl 1-benzyl-3-oxo-4-piperidinecarboxylate (**8**) in diphenyl ether. This cyclocondensation can occur with the 5-position of the pyrimidine **7** being attached to the ketone carbonyl of **8** and the 6-amino (or 4-amino) moiety being attached to the ester carbonyl of **8**. Such a mode of condensation affords the desired angular isomer **9**. A reversal of the direction of ring closure would, however, afford the linear isomer **10**. We[7] and others[8,9] have established that similar cyclo-

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(1) (a) Taken in part from the Thesis submitted by I.O.D. to the Graduate School of Duquesne University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, March 1988. (b) Presented in part at the American Chemical Society 195th National Meeting, Toronto, Canada, June 1988.
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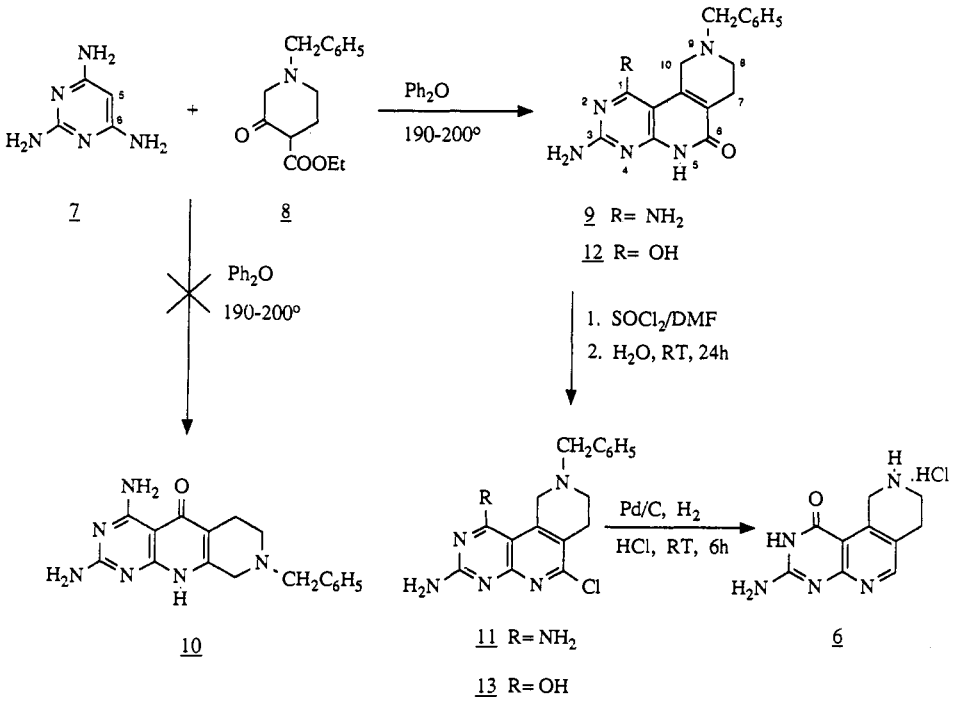
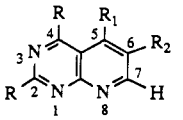
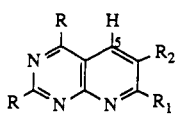
Scheme I



condensation reactions of aminopyrimidines and β-ketoesters in diphenyl ether afford regiospecifically the 5,6-disubstituted products for bicyclic systems and the angular, rather than linear, isomer for tricyclic systems. The product of the cyclocondensation of **7** and **8** was homogenous on TLC in three different solvent systems indicating a regiospecific reaction. The angular structure of the product was established by comparison of the chemical shift position of the lactam carbonyl in the ^{13}C NMR spectrum with literature values of isomeric fused α-pyridones (162–165 ppm) and γ-pyridones (177–179 ppm).[10,11] Compound **9** had the largest chemical shift at 162.92 ppm assigned to the lactam carbonyl. This shift position confirms that the compound is an α-pyridone and hence must be the angular isomer 1,3-diamino-9-benzyl-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,6]naphthyridin-6-(5*H*,9*H*)-one (**9**). The 6-oxo moiety of **9** was chlorinated following a modification of the procedure of Grivsky et al.[8] with use of DMF/$SOCl_2$ to afford **11** in 40% yield. Compound **11** was homogenous on TLC and its IR and ^1H NMR spectra and elemental analysis were in accord with structure **11**. Selective hydrolysis of the 1-amino moiety of **9** and of **11** in 20% HCl for 1.5 h gave the 3-amino-1-oxo derivatives **12** and **13**, respectively. The structures of **12** and **13** were consistent with their spectral data and elemental analysis. Hydrogenolysis of **11** using 10% palladium on carbon at room temperature and atmospheric pressure for 6 h in HCl/H_2O/*i*-PrOH (4:1:1 v/v) as solvent afforded the key intermediate **6**. This procedure of hydrogenolysis of **11** was accompanied by the concomitant debenzylation and hydrolysis of the 1-amino to a 1-oxo moiety. Further support for the regiospecific nature of the cyclization of **7** and **8** was obtained by establishing the angular structure of **6** by comparison with literature reports.[11-16] This structural assignment is based on the position of the aromatic proton H-6 in the ^1H NMR, the C-6 carbon position, and the one bond coupling constant $^1J_{C-H}$ in the ^{13}C NMR. In general for bicyclic 5,6-disubstituted isomers such as **14** the aromatic proton (at C-7) resonates at about 8.50 ppm in acidic solvents as does the aromatic proton (at C-6) for tricyclic angular isomers such as **16**. While the 6,7-disubstituted isomers such as **15** have



14



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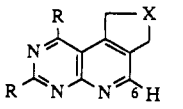

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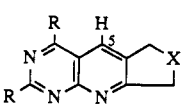
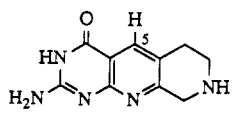


17



18

their aromatic proton (at C-5) resonate close to 9.00 ppm similar to tricyclic linear isomers **17**. The aromatic signal for **6** occurred at 8.57 ppm, strongly supporting the angular structure **6**. Further comparison of the chemical shift position of the C-6 carbon of **6** at 153.17 and the one bond coupling constant $^1J_{C-H}$ of 186 Hz with those of pyridines,

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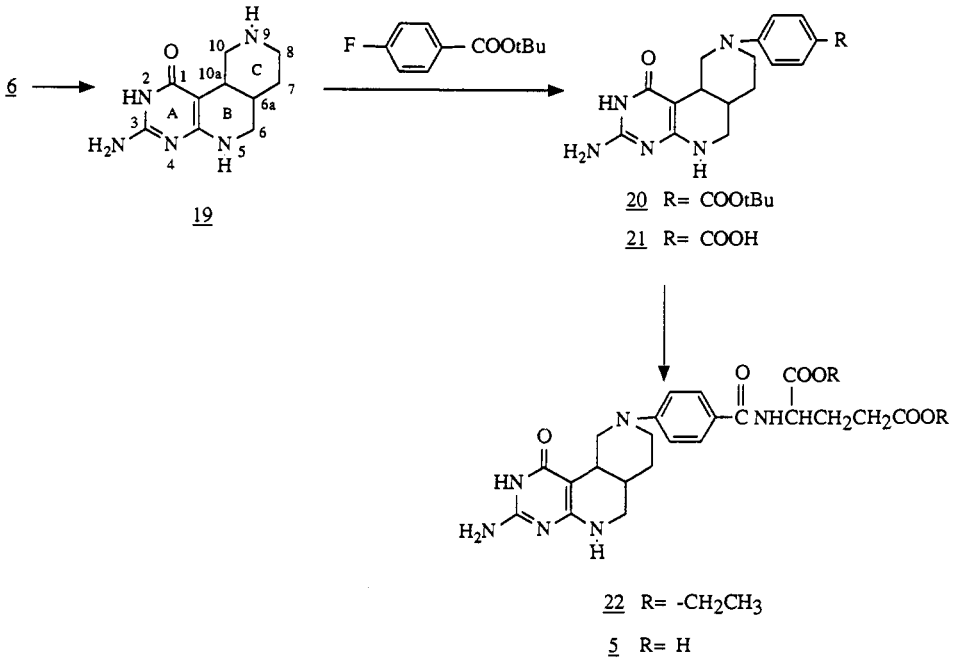
Scheme II



quinolines,[17] and pyrido[2,3-*d*]pyrimidines[12] support an aromatic carbon α to the nitrogen of the pyridine ring and hence establishes the angular structure of **6** as designated.

To unequivocally establish the angular structure of **6**, the linear isomer **18** was also synthesized.[18] This was accomplished by a regiospecific cyclocondensation of the sodium salt of 1-benzyl-4-(hydroxymethylene)-3-piperidone with 2,6-diamino-4-hydroxypyrimidine followed by hydrogenolysis. The aromatic carbon γ to the pyridine ring nitrogen in **18** had a chemical shift position of 140.21 ppm with a one bond coupling constant $^1J_{C-H}$ of 169 Hz. Both the chemical shift position and the magnitude of the coupling constant established the linear nature of **18** as distinct from that of **6**.

Having synthesized and established the angular structure of **6** we turned our attention to coupling the *p*-aminobenzoyl glutamate moiety onto the N-9 of **6**. First the B ring of **6** was hydrogenated (Scheme II) with PtO_2 in 50% TFA to afford a product **19** which was homogenous on TLC in three different solvent systems. In addition, compound **19** had a sharp melting point of 230 °C and the ^{13}C NMR showed the presence of 10 carbons. This hydrogenation results in the generation of two chiral centers at C-6a and C-10a thus producing a possible mixture of cis and trans isomers. However, hydrogenation using platinum catalysts usually affords predominantly if not exclusively cis products.[19,20] On the basis of the TLC, melting point, and ^{13}C NMR data for **19** coupled with the preference of platinum catalysts for cis products, we believe that since only one product was obtained in the hydrogenation it must be the cis isomer. Thus **19** was a racemic mixture in which one enantiomer has its C ring oriented above the plane of the A ring (i.e. 6a*S*,10a*S*) and the other enantiomer has its C ring below the plane of the A ring (i.e. 6a*R*,10a*R*) as shown in Figure 1 part a and b, respectively. On the basis of the proposed conformation of 5,10-CH_2THF,[21] the desired enantiomer of **19** was that in which the C ring was above the plane of the A ring. However, no separation of enantiomers was attempted.

Nucleophilic aromatic displacement of *tert*-butyl *p*-fluorobenzoate[22] with **19** afforded 3-amino-9-[4-(*tert*-butoxycarbonyl)phenyl]-1-oxo-5,6,6a,7,8,9,10,10a-octahydro-2*H*-pyrimido[4,5-*c*][2,6]naphthyridine (**20**). Hydrolysis of the ester with TFA at room temperature afforded **21**. This product **21** was a single spot on TLC and had a sharp melting point at 253 °C. The fact that monosubstitution had occurred was supported by elemental analysis and ^1H NMR. The presence of an aromatic NH proton at 6.44 ppm, corresponding to the N_5-H confirmed that arylation had occurred at the desired alicyclic N_9-position. The 300-MHz ^1H NMR spectrum of **21** indicated that the C-10 axial proton was shielded by the anisotropy of the C-1 carbonyl, as has been reported for 5,10-CH_2THF,[23] and occurs at 2.84 ppm about 1 ppm upfield from the C-10 equatorial proton. The bridgehead methine proton α to an aromatic ring at C-10a resonated as a multiplet at 2.84 ppm and forms part of the multiplet with the C-10 axial proton. The other bridgehead proton at C-6a resonated as a multiplet at 1.96 ppm along with one C-7 proton. The proton assignments were supported by spin–spin decoupling experiments and also by homonuclear shift correlated two-dimensional NMR spectroscopy.

Carboxyl activation of **21** via the mixed anhydride method with isobutyl chloroformate followed by coupling with diethyl L-glutamate afforded the glutamate diester **22** as a diastereomeric pair. Saponification of **22** with 1 N NaOH at room temperature gave the target compound *cis*-5,11-methylenetetrahydro-5-deazahomofolate (**5**) in 36% yield as a diastereomeric pair. The ^1H NMR of **5** indicated a single proton doublet at 8.26 ppm corresponding to the NH of the amide bond thus confirming the formation of the coupled product. TLC analysis on cellulose with 0.1 M sodium phosphate buffer of pH 6.8 indicated, as antici-

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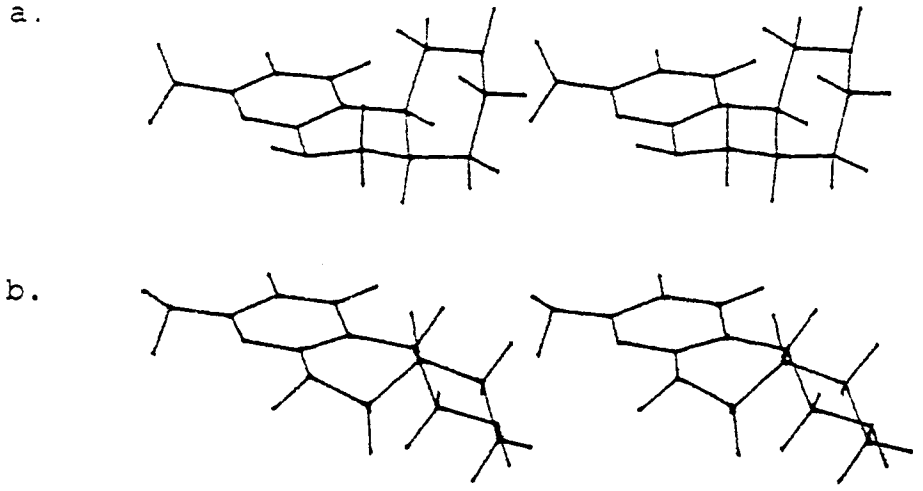

Figure 1. Stereoview of the low-energy conformation of the cis enantiomers of **19**, determined by Maximin2 on Tripos Associates SYBYL 5.3: (a) C ring above the plane of the A ring (6a*S*,10a*S*)-**19**, (b) C ring below the plane of the A ring (6a*R*,10a*R*)-**19**.

Table I. Cell Growth and Enzyme Inhibition Data (IC_{50}, μM) for Compound **5**[a]

	L. casei	Manca cells[b]
growth	–	>14
thymidylate synthase	>100	>200
Gar T′ fase	>20	>20

[a] Compound was dissolved in DMSO. Controls indicated that DMSO did not interfere with the assays at the concentrations used. [b] Manca human lymphoma cells[24] were obtained from Dr. F. M. Sirotrak, Memorial Sloan-Kettering Institute for Cancer Research, NY and grown in RPMI medium with 10% fetal calf serum. Growth was estimated by tetrazolium reduction. Thymidylate synthase and glycinamide ribonucleotide formyltransferase were assayed spectrophometrically as referenced in the text.

pated, two spots of R_f 0.20 and 0.41, corresponding to the diastereomeric mixture of the (±)-*cis*-L-glutamate **5**.

Biological Results and Discussions

The biological evaluation results for compound **5** are listed in Table I. Compound **5** was evaluated as an inhibitor of the growth of Manca human lymphoma cells[24,25] and was found to be inactive. In addition compound **5** also failed to inhibit thymidylate synthase[26] or glycinamide ribonucleotide formyltransferase[27] from *Lactobacillus casei* and from Manca human lymphoma cells. Diastereomers of **5** were not separated for biological evaluation. The inactivity of the target compound **5** was surprising. One possible explanation is that the orientation of the benzoyl glutamate portion of the molecule cannot be accommodated by TS. In the tricyclic folate cofactor **4** and also in the proposed tricyclic tetrahydrohomofolate **3** the C ring is perhaps more flexible than in **5** and thus can more readily adopt the required conformation of the benzoyl glutamate moiety such as to function as substrates. Thus, perhaps, the limited flexibility of the tricyclic nucleus of **5** is partially responsible for its inactivity. A second possibility is that the N-5 is required for binding to TS and that in its absence compound **5** does not bind appreciably. A third possibility is that 5,10-CH_2THF **4** is bound only transiently to the enzyme and that TS facilitates ring opening of **4** to the bicyclic imminium ion which then forms the ternary complex with TS and dUMP. Since compound **5** was designed specifically not to open to a bicyclic system, this property would preclude its action as an inhibitor.

We are currently synthesizing analogues which are designed to address these possibilities and which will be the topic of future publications.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Perkin Elmer Model 1430, in Nujol mulls. Nuclear magnetic resonance spectra for proton (^{1}H NMR) were recorded on a Varian EM-360 (60 MHz) or Brucker WH-300 (300 MHz) and for carbon-13 (^{13}C NMR) on a Brucker WH-300 at 75.46 MHz, 90° pulse, and 14 s. The data was accumulated by 16K size with 0.5 second delay time and 70° tip angle, with internal standard TMS; s = singlet, d = doublet, t = triplet, m = multiplet. Thin-layer chromatography (TLC) was performed on silica gel plates and cellulose plates with fluorescent indicator or as otherwise indicated and were visualized with light at 254 and 366 nm. Elemental analysis was performed by Atlantic Microlabs Inc., Norcross, GA.

1,3-Diamino-9-benzyl-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,6]naphthyridin-6(5*H*,9*H*)-one Hydrochloride (9). 2,4,6-Triaminopyrimidine (**7**) (2.4 g, 19 mmol), ethyl 1-benzyl-3-oxo-4-piperidinecarboxylate (**8**) (5.0 g, 19 mmol), and diphenyl ether (30 mL) were placed in a flask fitted with a Dean–Stark trap. The mixture was heated rapidly with vigorous stirring to 180 °C and then maintained at 190–210 °C until distillation of ethanol/water mixture ceased (5 h). The reaction mixture was allowed to cool to room temperature after which methanol (120 mL) was added, and the crude pale yellow solid was collected by filtration. The solid was suspended in boiling water (600 mL) filtered hot, washed with hot water (600 mL) followed by methanol (120 mL) and anhydrous ether (20 mL), and then air dried. The solid was recrystallized from 20% aqueous HCl at 60 °C and set aside at room temperature to deposit 4.0 g (65%) of **9** as yellow needles: TLC (a) *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.82, (b) MeOH/ethyl acetate (1:1)/silica gel, R_f = 0.53, (c) MeOH/ethyl acetate/pyridine (4:4:1)/silica gel, R_f = 0.50; mp >300 °C; IR (KBr) 3390 (NH_2), 3300 (NH_2), 3150 (NH), 1630 (C=O) cm^{-1}; ^{1}H NMR (300 MHz) (TFA-d) δ 2.80 (m, 2 H, 7-CH_2), 3.10 (m, 1 H, one of 8-CH_2), 3.72 (m, 1 H, one of 8-CH_2), 4.25 (d, J = 13.0 Hz, 1 H, 10-CH_2), 4.38 (d, J = 13.0 Hz, 1 H, 10-CH_2), 4.53 (d, J = 15.4 Hz, 1 H, C_6H_5-CH_2), 4.73 (d, J = 15.4 Hz, 1 H, C_6H_5-CH_2), 7.18 (s, 5 H, C_6H_5); ^{13}C NMR (TFA-*d*) 162.92 ppm (carbonyl carbon α to the nitrogen of the pyridine ring). Anal. ($C_{17}H_{18}N_6O$·2HCl), C, H, N, Cl.

3-Amino-9-benzyl-1-oxo-7,8,9,10-tetrahydro-2*H*-pyrimido-[4,5-*c*][2,6]naphthyridin-6(5*H*,9*H*)-one Hydrochloride (12). Compound **9** 4.00 g (12.4 mmol) was dissolved in 20% HCl (800 mL) and refluxed for 90 min. The solution was filtered hot and the filtrate left at room temperature to deposit 3.28 g (82%) of

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a yellow solid. TLC: *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose R_f = 0.77; mp >300 °C; IR (Nujol) 3480 (NH_2), 3320 (NH_2), 3160 (NH), 1780 (C=O), 1650 cm^{-1} (C=O); ^1H NMR (60 MHz) (TFA-*d*) δ 3.27 (m, 2 H, 7-CH_2), 3.60–4.10 (m, 2 H, 8-CH_2), 4.73 (m, 2 H, $C_6H_5CH_2$), 5.07 (br, s, 2 H, 10-CH_2), 7.59 (s, 5 H, C_6H_5). Anal. ($C_{17}H_{17}N_5O_2$·1HCl) C, H, N, Cl.

1,3-Diamino-9-benzyl-6-chloro-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,6]naphthridine (11). To a solution of DMF 6.9 mL (93.18 mmol) in dry $CHCl_3$ (30 mL) was added dropwise a solution of $SOCl_2$ (10.94 g, 93.18 mmol) in dry $CHCl_3$ (15 mL) such that the temperature of the reaction was maintained below 5 °C. After the reaction mixture was stirred for 20 min compound **9** 3.00 g (9.32 mmol) was added over a period of 10 min. The reaction was allowed to gradually warm to room temperature and then heated under gentle reflux for 5 h. The resulting mixture was cooled and concentrated in vacuo to leave a red-brown viscous residue. The residue was dissolved in 50% ethanol/water mixture (150 mL) and stirred at room temperature for 24 h. The mixture was then filtered and the filtrate neutralized by the addition of 50% NH_4OH. The brown precipitate which formed was collected by filtration, washed with water (250 mL), and recrystallized from DMSO/H_2O (1:1) to afford 1.27 g (40%) of 11 as a tan solid: TLC (a) *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.88, (b) CH_2Cl_2/MeOH (9:1)/silica gel, R_f = 0.70, (c) EtOH/H_2O/pyridine (4:1:1)/silica gel, R_f = 0.76; mp 230 °C dec; IR (KBr) 3365 (NH_2), 3325 (NH_2), 3175, 1625 cm^{-1}; ^1H NMR (300 MHz) (DMSO-d_6) δ 2.72 (s, 4 H, 7 and 8-CH_2), 3.72 (s, 2 H, 10-CH_2), 4.05 (s, 2 H, $C_6H_5CH_2$), 6.39 (s, 2 H, 1-NH_2), 6.85 (s, 2 H, 3-NH_2), 7.32 (s, 5 H, C_6H_5) (Addition of D_2O resulted in an exchange of the signals at 6.39 ppm and 6.85 ppm); ^1H NMR (300 MHz) (TFA-*d*) δ 3.04 (m, 2 H, 7-CH_2), 3.25 (m, 1 H, one of 8-CH_2), 3.83 (m, 1 H, one of 8-CH_2), 4.30 (d, 1 H, J = 12.5 Hz, 10-CH_2), 4.40 (d, 1 H, J = 12.5 Hz, 10-CH_2), 4.72 (d, 1 H, J = 15.0 Hz, $C_6H_5CH_2$), 5.00 (d, 1 H, J = 15.0 Hz, $C_6H_5CH_2$), 7.16 (s, 5 H, C_6H_5). Anal. ($C_{17}H_{17}N_6Cl$·1.2H_2O) C, H, N.

3-Amino-9-benzyl-6-chloro-1-oxo-7,8,9,10-tetrahydro-2*H*-pyrimido[4,5-*c*][2,6]naphthyridine Hydrochloride (13). Compound 11 5.00 g (14.7 mmol) was suspended in 20% aqueous HCl (900 mL), and the mixture was heated to dissolve the solid followed by further heating at reflux for 90 min. The solution was filtered hot and the filtrate set aside to deposit 4.21 g (84%) of a white solid: TLC (a) *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.83, (b) EtOH/CH_2Cl_2 (1:1)/cellulose, R_f = 0.92; mp, 235 °C dec; IR (KBr) 3325 (NH_2), 3100 (NH), 1700 (C=O), 1650 cm^{-1} (C=O); ^1H NMR (60 MHz) (DMSO-d_6) δ 3.20 (m, 2 H, 7-CH_2), 3.57 (m, 2 H, 8-CH_2), 4.60 (s, 2 H, C_6H_5-CH_2), 4.80 (s, 2 H, 10-CH_2), 7.67 (m, 5 H, C_6H_5); ^1H NMR (300 MHz) (TFA-d) δ 3.49 (m, 3H, 7-CH_2 and one of 8-CH_2), 4.22 (m, 1H, one of 8-CH_2), 4.69 (d, 1 H, J = 12.0 Hz, $C_6H_5HCH_2$), 4.78 (d, 1 H, J = 12.0 Hz, C_6H_5-CH_2), 4.83 (d, 1 H, J = 15.0 Hz, 10-CH_2),5.50 (d, 1 H, J = 15.0 Hz, 10-CH_2), 7.61 (s, 5 H, C_6H_5). Anal. ($C_{17}H_{16}N_5O$·1HCl·1H_2O) C, H, N, Cl.

3-Amino-1-oxo-7,8,9,10-tetrahydropyrimido[4,5-*c*][2,6]naphthyridine Hydrochloride (6). To a solution of 11 (4.00 g, 11.7 mmol), in a mixture of *i*-PrOH (8.00 mL), H_2O (100 mL), and concentrated HCl (300 mL), was added 10% Pd/C (8.00 g) and hydrogenolysis was carried out at room temperature and atmospheric pressure over a period of 6 h (H_2 consumption was 530 mL). The catalyst was filtered and washed with 70% AcOH (300 mL). The filtrate and washings were concentrated to dryness, and the residue was suspended in absolute ethanol (50 mL), stirred, and filtered to give 2.96 g (74%) of **6** as a yellow solid: TLC *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.55; mp >300 °C; IR (KBr) 3400 (-NH_2), 3255 (-NH), 1665 cm^{-1} (C=O); ^1H NMR (300 MHz) (TFA-*d*/D_2O), 2:1) δ 3.27 (t, 2 H, 7-CH_2), 3.62 (t, 2 H, 8-CH_2), 5.08 (d, 2 H, 10-CH_2), 8.57 (s, 1 H, C-6 aromatic proton); ^{13}C NMR (TFA-*d*/D_2O, 2:1) 153.2 ppm J = 185 Hz (C-6 aromatic carbon α to the nitrogen of the pyridine ring). Anal. ($C_{10}H_{11}N_5O$·2HCl) C, H, N, Cl.

(±)-*cis*-3-Amino-1-oxo-5,6,6a,7,8,9,10,10a-octahydro-2*H*-pyrimido[4,5-*c*][2,6]naphthyridine (19). A mixture of compound **6** 0.50 g, (1.97 mmol) in 50% aqueous TFA (20 mL) and PtO_2 (0.035 g) was hydrogenated on a Parr apparatus under 50 psi of hydrogen pressure at room temperature until the calculated amount of hydrogen was consumed (24 h). The catalyst was filtered and the filtrate concentrated under reduced pressure to a transparent syrupy residue. Absolute EtOH (10 mL) was added and the mixture stirred until a white solid separated which was filtered to afford 0.49 g (60%) of compound **19** as the TFA salt: TLC (a) iPROH/NH_4OH/H_2O (6:3:1)/cellulose, R_f = 0.48, (b) *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.55, (c) 3% NH_4Cl/cellulose, R_f = 0.52; mp 230 °C; IR (Nujol) 3400, 3240, 1840, 1730, 1695 cm^{-1}; ^1H NMR (300 MHz) (TFA-*d*) δ 2.14 (d, 1 H, 7-H), 2.47 (m, 2 H, 7-H and 6a-H), 3.43 (m, 1 H, 6-H), 3.57–3.75 (m, 6 H, 6-H, 10a-H, 8-CH_2, and 10-CH_2). Anal. ($C_{10}H_{15}N_5O$·1.5CF_3COOH·0.5H_2O) C, H, N.

(±)-*cis*-3-Amino-9-[4-(*tert*-butoxycarbonyl)phenyl]-1-oxo-5,6,6a,7,8,9,10,10a-octahydro-2*H*-pyrimido[4,5-*c*][2,6]naphthyridine (20). A mixture of *tert*-butyl 4-fluorobenzoate (0.39 g, 1.94 mmol), **19** (0.87 g, 1.94 mmol), and finely powdered anhydrous K_2CO_3 (1.3 g) in DMSO (10 mL) was heated at 120 °C (external temperature) with stirring under nitrogen for 10 h. The mixture was cooled to room temperature and poured into 100 mL of water, and the resulting precipitate was collected by filtration, washed with water, and air dried under vacuum over P_2O_5 for 24 h to give 0.604 g (78.5%) of **20** as a pale yellow solid. It was recrystallized by dissolving in absolute EtOH with the addition of drops of concentrated HCl and boiling. The solution was filtered hot and left at room temperature to deposit 0.56 g (72.5%) of a yellow-brown solid: TLC *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.95, mp 265 °C dec; IR (Nujol) 3400, 3320, 1700, 1290, 1120 cm^{-1}; ^1H NMR (60 MHz) (TFA- *d*) δ 1.67 (s, 9 H, *tert*-butyl), 2.57 (m, 3 H, 6a-CH and 7-CH_2), 3.80 (m, 7 H, 6-CH_2, 8-CH_2, 10-CH_2 and 10a-CH), 7.80 (d, 2 H, 3′5′-H), 8.40 (d, 2 H, 2′6′-H). Anal. ($C_{21}H_{27}N_5O_3$·1.5HCl) C, H, Cl, N*.

(±)-*cis*-3-Amino-9-(4-carboxyphenyl)-1-oxo-5,6,6a,7,8,9,10,10a-octahydro-2*H*-pyrimido[4,5-*c*][2,6]naphthyridine (21). Compound **20** (0.40 g, 1.01 mmol) was dissolved in TFA (8 mL). The solution was stirred at room temperature for 1.5 h. Following this period, the solution was concentrated to about half its volume, and H_2O (4 mL) was added. Concentrated NH_4OH solution was added to neutralize the solution to pH 7 while maintaining the temperature below 20 °C. The precipitated solid was collected by filtration, washed with water, and dried over P_2O_5 for 24 h to afford 0.34 g (98%) of a red-brown solid. An analytical sample was obtained by recrystallization from ethanol with the addition of drops of HCl: TLC *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.81; mp 253 °C; IR (Nujol) 3400, 3250, 1660 cm^{-1}; ^1H NMR (300 MHz) (DMSO-d_6) δ 1.52 (d, 1 H, 7-H), 1.96 (m, 2 H, 7-H and 6a-H), 2.84 (m, 2 H, 10-H and 10a-H), 3.04 (m, 2 H, 6-H and 8-H), 3.43 (m, 1 H, 6-H), 3.78 (d, 1 H, 8-H), 3.86 (d, 1 H, 10-H), 6.06 (s, 2 H, 3-NH_2), 6.44 (s, 1 H, 5-NH), 6.96 (d, 2 H, 3′,5′-H), 7.75 (d, 2 H, 2′,6′H), 8.31 (s, 0.2 H, 2-NH, not visible in all cases and when present it does not integrate for 1 H), 9.75 (br s, 1 H, COOH). Anal. ($C_{17}H_{17}N_5O_3$·1.4HCl·0.8H_2O) C, H, N, Cl.

***cis*-5,11-Methylenetetrahydro-5-deazahomofolate (5).** To a solution of (±)-21 (0.20 g, 0.59 mmol) in dry DMSO (3 mL) and dry DMF (3 mL), under nitrogen, was added 0.28 mL (2.06 mmol) of triethylamine. The mixture was stirred for 45 min, then 0.27 mL (2.06 mmol) of isobutyl chloroformate was added while maintaining the temperature of the reaction mixture between 0 and 5 °C. After 2 h at ambient temperature, a mixture of 0.31 g (1.28 mmol) of diethyl L-glutamate hydrochloride and 0.28 mL (2.06 mmol) of triethylamine in 1.5 mL of dry DMF were added and the mixture stirred for 24 h. Following this period, the mixture was concentrated under reduced pressure, and the residue was stirred with 5% $NaHCO_3$ (10 mL) and then with ether (10 mL) for 1 h. After filtration the solid was washed with water (50 mL) and dried under vacuum over P_2O_5 to give 0.105 g of the diester which was dissolved in 2-methoxyethanol (5 mL) and treated with 1 N NaOH (1.5 mL). The solution was kept at room temperature for 8 h and then diluted with water (10 mL). The mixture was neutralized to pH 7 by addition of HOAc, and the solvents were removed in vacuo. The residue was stirred with water (10 mL) and filtered to give 0.093 g (33.7%) of a tan solid: mp 251 °C dec; TLC (a) *n*-BuOH/H_2O/AcOH (3:3:1)/cellulose, R_f = 0.80, (b) *i*-PrOH/NH_4OH/H_2O (6:3:1)/cellulose, R_f = 0.54, (c) 0.1 M NaH_2PO_4 (pH 6.8)/cellulose, two spots of R_f = 0.20 and R_f = 0.42 were observed; UV max at pH 13, 223 nm (ε 13500), 227 nm (ε 16700), shoulder at 303 nm (ε 9950); UV max at pH 1, 204 nm (ε 18300), 227 nm (ε 11400), 275 nm (ε 1500); IR (Nujol)

3400 (-NH_2), 3175 (-NH), 1625 cm^{-1}; ^1H NMR (300 MHz) (DMSO-d_6) δ 1.53 (m, 1 H, 7-H), 1.96 (m, 4 H, H_{6a}, and 7-H, and β-CH_2) 2.34 (m, 2 H, γ-CH_2) 2.75–3.90 (m, 7 H, 6-H_2, 8-H_2, 10-H_2, and H_{10a}), 4.39 (m, 1 H, α-CH), 6.09 (s, 2 H, C-2 NH_2), 6.47 (s, 1 H, 8-NH), 6.98 (d, 2 H, 3′ and 5′-CH), 7.77 (d, 2 H, 2′ and 6′-CH), 8.26 (d, 1 H, amide NH) 9.85 (br s, 1 H, COOH of glutamate). Anal. ($C_{22}H_{26}N_6O_6 \cdot 1H_2O$) C, H, N.

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Registry No. (6a*R*,10a*S*)-L-**5**, 130985-82-9; (6a*S*,10a*R*)-L-**5**, 131064-26-1; **6**, 130985-83-0; **7**, 1004-38-2; **8**, 39514-19-7; **9**, 130985-84-1; 11, 130985-85-2; **12**, 130985-86-3; **13**, 131010-65-6; *cis*-(±)-**19**, 130985-88-5; *cis*-(±)-**20**, 130985-89-6; *cis*-(±)-**21**, 130985-90-9; (6a*R*,10a*S*)-L-**22**, 131100-23-7; (6a4S,10a*R*)-L-**22**, 131010-66-7; TS, 9031-61-2; 4-FC_6H_4COOBU-*t*, 58656-98-7; H-Glu(OEt)-OEt·HCl, 1118-89-4.

Novel Benzamides as Selective and Potent Gastrokinetic Agents. 2.[1] Synthesis and Structure–Activity Relationships of 4-Amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide Citrate (AS-4370) and Related Compounds

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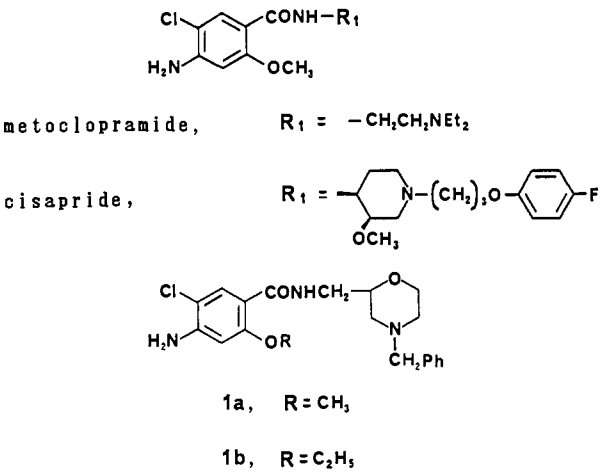
The title compounds (**19–55**) with a 4-substituted 2-(aminomethyl)morpholine group were prepared and evaluated for the gastrokinetic activity by determining their effect on gastric emptying of phenol red semisolid meal in rats. Introduction of chloro, fluoro, and trifluoromethyl groups to the benzyl group of the parent compounds **1a** and **1b** enhanced the activity. Among compounds tested, 4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide (**23b**) showed the most potent gastric emptying activity (effects on phenol red semisolid meal in rats and mice, and on resin pellets solid meal in rats). The gastrokinetic activity of **23b** citrate (AS-4370) compared very favorably with that of cisapride and was higher than that of metoclopramide. In contrast to metoclopramide and cisapride, AS-4370 was free from dopamine D_2 receptor antagonistic activity in both in vitro ([^3H]spiperone binding) and in vivo (apomorphine-induced emesis in dogs) tests.

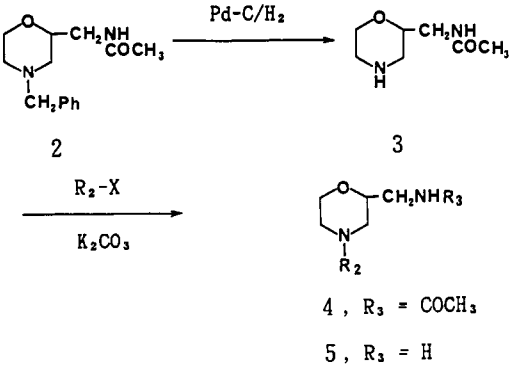
Metoclopramide as a stimulant of the upper gastrointestinal motility is limited in clinical use because of unfavorable side effects such as extrapyramidal symptoms arising from its dopamine D_2 receptor antagonistic property.[2] Substituted benzamides, which are structurally related to metoclopramide having a potent gastric prokinetic activity with minimized side effects, have recently been reported (Chart I).[3-9]

In a previous paper,[1] we reported that a series of benzamide derivatives appending a new amine moiety, 2-(aminomethyl)-4-benzylmorpholine, had a potent gastrokinetic activity with lack of the dopamine D_2 receptor antagonistic activity. In particular, 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide (**1a**) and its ethoxy analogue (**1b**) were of much interest to us, because their gastrokinetic activity was much higher than that of metoclopramide. In the present study, we focused on the introduction of various substituents to

Chart I



Scheme I



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