Indomethacin was dissolved in 10% $NaHCO_3$; all other drugs were dissolved in physiological salt solution.

Bioassays were performed on the guinea pig ileum and rat colon muscularis mucosae preparations as previously described[15,16] but with the following modifications: (i) atropine (1 μM), mepyramine (1 μM), methysergide (1 μM), and indomethacin (1 μM) were included in the bathing media for all experiments; and (ii) the time cycle for administration of agonist doses was arranged such that there was a 7-min interval between doses in the guinea pig ileum and 10 min in the rat colon. Pilot experiments had demonstrated that between-dose interactions were minimized for SP when these schedules were used.

Formal 3 + 3 assays (guinea pig ileum) or 2 + 2 assays (rat colon) were performed on all analogues by using a randomized block design. Results were analyzed by analysis of variance, and only those assays that satisfied tests for parallelism, linearity, difference curvature, and regression were accepted for estimates of relative activities. In the presence of the antagonists described above, the EC_{50} for SP is 2×10^{-9} M in the guinea pig ileum and 2×10^{-7} M on the rat colon muscularis mucosae. All results are expressed relative to the SP EC_{50}, taking SP_{1-11} as 1.0.

Samples of peptides were prepared as stock solutions of 1–10 mM. SP_{6-11} was dissolved in Me_2SO. All other peptides were dissolved in 0.01 M acetic acid. The stock solution was divided into aliquots, which were stored at −30 °C until required for use. A sample of solution was dried under nitrogen and prepared for amino acid analysis to provide an accurate estimate of peptide concentration. A further sample of each compound was subjected to FAB mass spectrometry to confirm its structure. Thus, molecular ions (M^+) were observed at m/z 724, 815, 745, and 721 for the new peptides 14–17, respectively.

During the experiments, dilutions were made in physiological salt solution. Control experiments with Me_2SO showed that, at the concentrations used in the present experiments, this compound did not affect the response of the tissue to any of the peptides tested.

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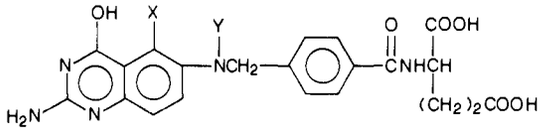
Synthesis of 5-Chloro-5,8-dideaza Analogues of Folic Acid and Aminopterin Targeted for Colon Adenocarcinoma[1]

J. B. Hynes,*† A. Kumar,†‡ A. Tomažič,† and W. L. Washtien§,‖

Department of Pharmaceutical Sciences, College of Pharmacy, Medical University of South Carolina, Charleston, South Carolina 29425, and Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611. *Received November 25, 1986*

Several classical quinazoline analogues of folic acid bearing chloro or methyl substituents at position 5 were evaluated as inhibitors of the growth of four human gastrointestinal adenocarcinoma cell lines in vitro. The preparation of two of these, 5-chloro-5,8-dideazaisofolic acid, **1e**, and 5-chloro-5,8-dideazaisoaminopterin, **2a**, is reported for the first time. In addition, a new synthetic route to 5-chloro-5,8-dideazaaminopterin, **2b**, is described. For compounds having a 2,4-diamino configuration, the presence of chlorine at position 5 afforded superior growth inhibitory potency. However, compound **1e** was substantially less effective than its 5-methyl counterpart.

The folate analogue 5,8-dideazaisofolic acid (IAHQ), **1a**, has generated considerable interest as a potential agent for treating solid tumors that are unresponsive to methotrexate (MTX). Recent studies revealed that IAHQ was an effective inhibitor of the growth of human colon adenocarcinoma cells (HCT-8) in vitro.[2] Significant activity



	X	Y
1a	H	H
b	CH_3	H
c	H	CH_3
d	CH_3	CH_3
e	Cl	H

against colon tumor 38 in mice was also demonstrated, while MTX was not effective in this model.[2,3] It was also found that IAHQ protected newborn hamsters from mortality due to transplantable human osteosarcoma cells, whereas MTX was without effect against this xenograph at maximally tolerated dose.[4] Recently, we reported the syntheses of the 5-CH_3, **1b**, 9-CH_3, **1c**, and 5,9-$(CH_3)_2$, **1d**, modifications of IAHQ.[5] Each of these derivatives was found to have a modest level of growth inhibitory activity similar to that of IAHQ toward four human gastrointestinal adenocarcinoma cell lines in vitro.[5]

It was of interest, therefore, to prepare the 5-Cl analogue of IAHQ, 5-chloro-5,8-dideazaisofolic acid, **1e**, in order to assess the effect of an electron-withdrawing lipophilic substituent located at position 5 upon antitumor activity. In the case of 5,8-dideaza analogues of aminopterin, the 5-Cl modifications were found to display greater inhibitory activity toward the growth of L1210 leukemia cells in vitro than their corresponding 5-CH_3 or 5-H counterparts.[6] It

† Medical University of South Carolina.
‡ Present address: Department of Chemistry, Ohio State University, Columbus, OH 43210.
§ Northwestern University Medical School.
‖ Present address: Department of Pharmaceutical Chemistry, University of California Medical School, San Francisco, CA 94143.

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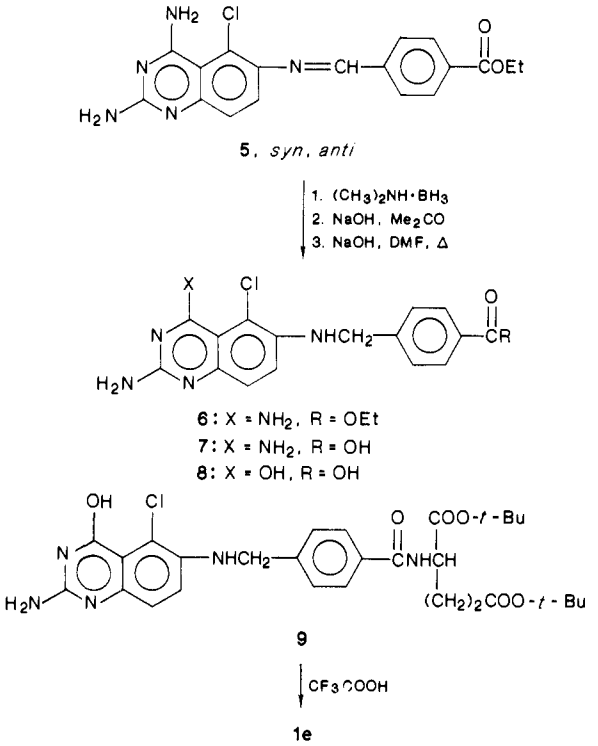
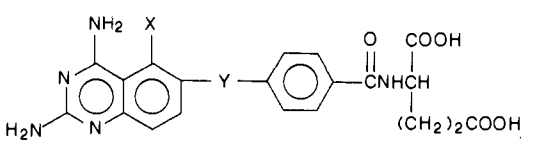
Scheme I. Synthesis of 5-Chloro-5,8-dideazaisofolic Acid



was anticipated that this lipophilic alteration might improve uptake into colon tumor cells, since it was recently shown that IAHQ has a significantly slower rate of influx into HCT-8 cells than MTX.[7] The 4-NH_2 counterpart of compound **1e**, 5-chloro-5,8-dideazaisoaminopterin, **2a**, was also prepared, since the routes to each proceeded through

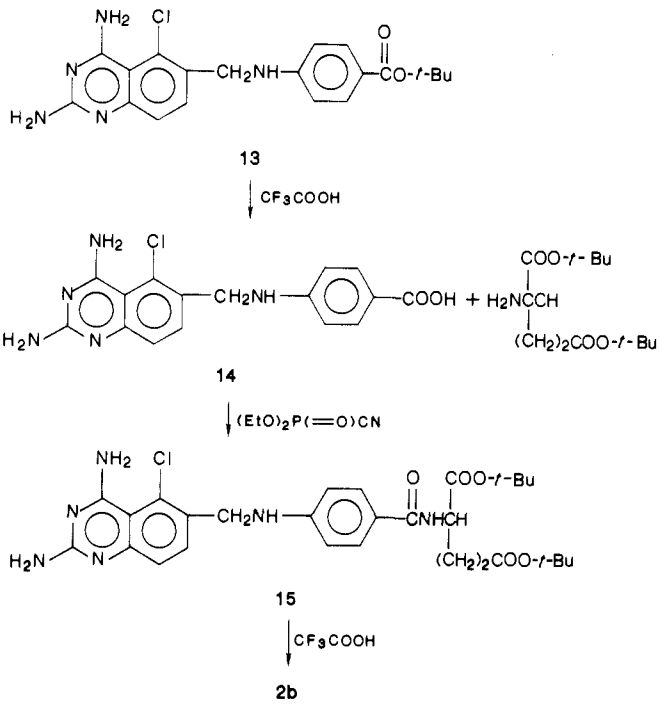


	X	Y
2a	Cl	$NHCH_2$
b	Cl	CH_2NH
c	H	$CH_2N(CH_3)$
d	CH_3	$NHCH_2$
e	CH_3	CH_2NH

a common intermediate, **7** (Scheme I). This compound was required for the current study, the goal of which is the identification of new agents having potential in the treatment of colorectal cancer. Compound **2a** was recently reported to be approximately 70-fold more inhibitory than MTX against a MTX-insensitive dihydrofolate reductase (DHFR) obtained from a MTX-resistant line of L5178Y mouse leukemia cells.[8] In addition, the related lipophilic analogue, 5,8-dideazamethopterin, **2c**, was reported to be approximately 3-fold more potent than MTX against the growth of three of the same four human gastrointestinal cell lines employed in this work.[9]

Chemistry. Earlier attempts to prepare compounds such as **1e** and **2a** by reductive condensation of the requisite 6-aminoquinazoline with diethyl *p*-formylbenzoyl-L-glutamate were unsuccessful, yielding extremely complex reaction mixtures. While this approach was successful in the case of 6-aminoquinazolines possessing either hydrogen or methyl at position 5,[5,10] the presence of the electron-withdrawing chlorine atom at position 5 apparently renders the 6-amino function insufficiently nucleophilic to effect clean reductive condensation in the presence of Raney nickel.

Scheme II. Synthesis of 5-Chloro-5,8-dideazaaminopterin



Therefore, compound **1e** was prepared in a stepwise fashion as depicted in Scheme I. The key intermediate, 5-chloro-2,4,6-triaminoquinazoline, **3**, was prepared in four steps, commencing with 2,3-dichloronitrobenzene according to procedures described earlier.[11] In conducting this synthetic sequence it was found that partial hydrolysis of the 4-amino group of 5-chloro-2,4-diamino-6-nitroquinazoline can occur under the originally described conditions.[11] Therefore, a modified nitration procedure was developed, which involved the use of reduced amounts of sulfuric acid, as well as neutralization of the reaction mixture with sodium bicarbonate rather than ammonium hydroxide.

The condensation of **3** with ethyl *p*-formylbenzoate, **4**, was effected by prolonged heating of the substrates under reflux in ethanol. The resulting anil **5** (syn, anti mixture) was then reduced with dimethylamine–borane, which is a selective reagent for reducing this functionality, yielding the ethyl ester **6**. This compound was reported earlier in very low yield from similar procedures, and the intermediate **5** was not characterized.[12] Careful saponification of **6** then gave the free acid **7**, which was employed in the synthesis of **2a** as well as being hydrolyzed to the pteroic acid analogue, **8**. Compound **8** was then reacted with di-*tert*-butyl L-glutamate by using diethyl phosphorocyanidate[13] as the coupling reagent to yield **9**. Removal

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Table I. Inhibition of Growth of Human Gastrointestinal Adenocarcinoma Cells in Vitro

	inhibition: IC_{50}, μM							
tumor	**2a**	**2d**	**2b**[a]	**2e**	1e	1a[b]	**1b**[b]	MTX[b]
HuTu80	0.022	0.047	0.0019	0.0034	8.5	4.5	4.0	0.009
HT29	0.025	0.060	0.0023	0.0030	6.7	1.7	1.1	0.018
SW480	0.020	0.056	0.0020	0.0050	38.0	4.5	10.1	0.015
WIDR	0.024	0.071	0.0021	0.0050	11.0	4.2	4.0	0.025

[a] Sample of **2b** employed in this study was prepared as described earlier.[14] [b] Reported previously; cf. ref 5.

of the *tert*-butyl protecting groups was performed by using anhydrous trifluoroacetic acid to yield the target compound, 5-chloro-5,8-dideazaisofolic acid, **1e**. The 4-amino counterpart, **2a**, was prepared in a similar fashion by condensing **7** with di-*tert*-butyl L-glutamate to yield di-*tert*-butyl 5-chloro-5,8-dideazaisoaminopterin, **10**. This compound was subsequently deprotected to yield the target compound **2a** by treatment with anhydrous trifluoroacetic acid.

The compound **2b** was first prepared in very low yield by the reductive condensation of diethyl *p*-aminobenzoyl-L-glutamate with 5-chloro-6-cyano-2,4-diaminoquinazoline, **11**, followed by saponification in dilute base.[11] An alternative approach to **2b** was developed as shown in Scheme II, which precluded the possibility of racemization during the final deprotection step. In addition, two of the intermediates, **13** and **14**, were required for related studies involving MTX-resistant tumor lines. The reductive condensation of **11** with *tert*-butyl *p*-aminobenzoate, **12**, gave the *tert*-butyl ester, **13**, which was deprotected to yield the key intermediate 6-[(*p*-carboxyanilino)methyl]-5-chloro-2,4-diaminoquinazoline, **14**. Peptide-bond formation with di-*tert*-butyl L-glutamate using diethyl phosphorocyanidate afforded the di-*tert*-butyl ester **15**, which upon treatment with anhydrous trifluoroacetic acid gave **2b**.

Biological Evaluation. The target compounds **1e** and **2a** were evaluated as inhibitors of the growth of four established gastrointestinal adenocarcinoma cell lines in vitro. In order to develop structure–activity patterns, three related known 2,4-diaminoquinazoline analogues prepared earlier were included in this study. These compounds were the normal-bridged isomer of **2a**, 5-chloro-5,8-dideazaaminopterin, **2b**, together with the corresponding methyl analogues, 5-methyl-5,8-dideazaisoaminopterin, **2d**, and 5-methyl-5,8-dideazaaminopterin, **2e**.[10,11,14] The last of these has been studied extensively with a variety of biological evaluation systems.[15,16] The results obtained are presented in Table I. For reference purposes we have also included the ED_{50} values reported earlier for MTX as well as 5-methyl-5,8-dideazaisofolic acid, **1b**, and IAHQ, 1a.[5]

Discussion

The results obtained with the 2-amino-4-hydroxyquinazoline derivative **1e** toward the growth of human gastrointestinal adenocarcinoma cells are puzzling, since it is 2- to 8-fold less effective than IAHQ.[5] Compound **2a**, on the other hand, is nearly as effective as MTX and is 2- to 3-fold more active than the 5-methyl analogue **2d**. The order of potency for classical 2,4-diaminoquinazolines having a bridge-reversed configuration at positions 9 and 10 appears to be as follows: 5-Cl ≅ 9-CH_3 > 5-CH_3 > 5-H.[9]

For 2,4-diaminoquinazoline analogues having a normal folate configuration at positions 9 and 10, the 5-chloro modification is also the most potent, with **2b** being from 5- to 10-fold more effective than MTX. When these values are combined with those reported recently,[9] the order of activity for 5,8-dideazaaminopterin analogues is 5-Cl > 5-H ≅ 5-CH_3 > 10-CH_3 > MTX.

On the basis of the results obtained, additional antitumor testing is indicated for certain of these compounds using in vivo colon tumor models. However, it should be noted that **2b** and **2e** were found to be extraordinarily toxic to mice bearing L1210 leukemia cells.[17] The preparation of additional 5,8-dideazaaminopterin analogues also appears warranted, particularly those possessing other substituents at positions 5 and/or 10. Synthetic efforts in this area are currently in progress.

Experimental Section

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. All analytical samples were dried under vacuum at 100 °C unless stated otherwise and gave combustion values for C, H, and N within ±0.4% of the theoretical values. Solvation due to water was confirmed by the presence of a broad peak centered at ca. 3.4 ppm in the ^1H NMR spectra. All intermediates were free of significant impurities on TLC using silica gel media (Baker 1B2-F). Free acids **1e**, **2a**, and **2b** were assayed on cellulose (0.1 M phosphate buffer, pH 7.4) and had R_f values of 0.64, 0.56, and 0.57, respectively. Column chromatographic separations were performed on Kieselgel 60 (70–230 mesh) obtained from E. Merck and Co. Compounds **5–10**, **2a**, and **1e** were analyzed by HPLC on a Du Pont 8800 liquid chromatograph with a UV (254 nm) detector and either a Bondapak C_{18} reverse-phase column (4.6 × 250 mm), for free acids, or a Partisil-10 normal-phase column (4.6 × 250 mm), for esters. The following systems were used to elute the compounds: reverse-phase column, 25-min linear gradient (0–25% v/v) of MeCN in H_2O at pH 4.5, and a flow rate of 2 mL/min (**1e**); reverse-phase column, 10-min linear gradient (0–25% v/v) of MeCN in H_2O containing 0.007 M Et_3N and 0.017 M HOAc, pH 6.5, and a flow rate of 2 mL/min (**2a**, **7**, **8**); normal-phase column, 25-min linear gradient (0–20% v/v) of MeOH in $CHCl_3$, and a flow rate of 1 mL/min (**5**, **6**, **9**, **10**). The compounds **13–15** and **2b** were analyzed by using a Waters Associates HPLC system consisting of Solvent Programmer Model 441, UV (254 or 302 nm) detector. A reverse-phase Bondapak C_{18} (3.9 mm × 30 cm) column and a two-solvent system (A and B) were used: A, 0.1% CF_3COOH in H_2O; B, 0.1% CF_3COOH in 1-propanol.[18] The following gradient conditions were employed: 0–100% B, 140 min, flow rate 0.5 mL/min (**13**); 0–30% B, 100 min, flow rate 1 mL/min (**14**); 0–30% B, 30 min, flow rate 1 mL/min (**15**, **2b**). Samples for HPLC were dissolved in Me_2SO just prior to injection. The UV spectra were determined by using a Cary 219 spectrophotometer in 0.1 M phosphate buffer, pH 7.0. The ^1H and ^{19}F NMR spectra were determined by using a Varian EM 390 spectrometer. High-resolution ^1H NMR spectra were acquired on a Bruker AM-300 or Bruker WH-400 spectrometer at the South Carolina Magnetic Resonance Laboratory, University of South Carolina, Columbia, SC. NMR chemical shifts are presented in parts per million

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downfield from Me_4Si as the internal standard. The relative peak areas are given to the nearest whole number. Fast-atom-bombardment mass spectra (FAB/MS) were obtained on a Finnigan MAT 212 spectrometer using argon bombardment. Di-*tert*-butyl L-glutamate was prepared by the reaction of L-glutamic acid and $(CH_3)_2C{=}CH_2$ under pressure as described earlier.[19] The free ester, which was found to be stable when stored at 4 °C, was employed in subsequent reactions as an oil rather than being converted into a salt derivative. The *tert*-butyl *p*-aminobenzoate was synthesized according to the literature procedure.[20] Ethyl *p*-formylbenzoate was prepared from ethyl *p*-cyanobenzoate (Aldrich Chemical Co.) by a modification of the method of Slotta and Kethur.[21] The methods of determination of the inhibition of growth of four human gastrointestinal adenocarcinoma cell lines were described recently.[5]

6-[(*p*-Carbethoxybenzylidene)amino]-5-chloro-2,4-diaminoquinazoline, 5 (Syn, Anti). A mixture of 1.0 g (4.5 mmol) of 5-chloro-2,4,6-triaminoquinazoline, **3**,[11] and 1.2 g (6.7 mmol) of ethyl *p*-formylbenzoate, **4**, in 60 mL of EtOH was heated under reflux for 35 h. The yellow solid was separated by filtration and washed successively with 1 mL of EtOH and 2 × 10 mL of *n*-hexane. Recrystallization from $CHCl_3$–*n*-hexane gave 1.1 g (66%) of a yellow crystalline solid: mp 224–227 °C; TLC (DMF–MeCN, 1:4); HPLC; NMR (90 MHz, CF_3COOD) δ 9.4 (s, 1, N=CH), 8.43 (s, 4, aromatic), 8.26 (d, 1, H_7, J_o = 9 Hz), 8.10 (d, 1, H_8, J_o = 9 Hz), 4.6 (q, 2, OCH_2), 1.56 (t, 3, CH_3), major isomer, and 10.01 (s, 1, N=CH), 8.28 (d, 1, H_7, J_o = 9 Hz), 8.11 (d, 1, H_8, J_o = 9 Hz), 7.73 (q, 4, aromatic), 4.53 (q, 2, OCH_2), 1.53 (t, 3, CH_3), minor isomer. Anal. ($C_{18}H_{16}N_5O_2Cl·0.4H_2O$) C, H, N.

6-[(*p*-Carbethoxybenzyl)amino]-5-chloro-2,4-diaminoquinazoline, 6. To a suspension of 0.60 g (1.6 mmol) of **5** in 8 mL of HOAc was added slowly 0.12 g (2 mmol) of $(CH_3)_2NH·BH_3$ in 3 mL of HOAc with stirring and cooling. After the mixture was stirred at room temperature for 5 h, it was diluted with 25 mL of H_2O and basified to pH 9 with 3 N NH_4OH with vigorous stirring and cooling in an ice bath. After 0.5 h, the precipitate was separated by filtration and washed successively with 3 × 25 mL of H_2O, 1 × 25 mL of *n*-hexane, and 2 × 10 mL of Et_2O to yield 0.50 g (78%): mp 172–178 °C (with sublimation) [lit.[12] mp 172–175 °C (anhydrous)]; TLC (DMF–MeCN, 1:4); HPLC; NMR (90 MHz, CF_3COOD) δ 8.16 (d, 2, aromatic, J = 8 Hz), 7.86 (d, 1, H_7, J_o = 9 Hz), 7.55 (d, 2, aromatic, J = 8 Hz), 4.93 (s, 2, NCH_2), 4.53 (q, 2, OCH_2), 1.50 (t, 3, CH_3). Anal. ($C_{18}H_{18}N_5O_2Cl·1.25H_2O$) C, H, N.

6-[(*p*-Carboxybenzyl)amino]-5-chloro-2,4-diaminoquinazoline, 7. A 0.45-g (1.2 mmol) sample of **6** was suspended in 45 mL of 0.2 N NaOH and 10 mL of $(Me)_2CO$. After the reaction mixture was stirred at room temperature for 18 h, the solution was filtered, diluted with 20 mL of H_2O, and acidified to pH 4.0 with 0.5 N HCl with external cooling. The resulting yellow solid was separated by filtration and washed successively with 5 × 4 mL of H_2O, 10 mL of *n*-hexane, 10 mL of Et_2O, and 10 mL of $(Me)_2CO$. There was obtained 0.30 g (58%) of yellow crystals: mp 286–288 °C dec; TLC (DMF–MeCN, 1:4); HPLC; NMR (90 MHz, CF_3COOD) δ 8.20 (d, 2, aromatic, J = 8 Hz), 7.90 (d, 1, H_7, J_o = 9 Hz), 7.60 (d, 1, H_8, J_o = 9 Hz), 7.56 (d, 2, aromatic, J = 8 Hz), 4.93 (s, 2, NCH_2). Anal. $C_{16}H_{14}N_5O_2Cl·2H_2O·HCl$) C, H, N.

2-Amino-6-[(*p*-carboxybenzyl)amino]-5-chloroquinazoline-4(3*H*)-one, 8. A mixture of 0.30 g (0.72 mmol) of **7** in 10 mL of 1 N NaOH containing 5 mL of DMF was heated under reflux for 1.5 h. Next, 35 mL of H_2O was added and the pH was adjusted to 4.5 with cooling by using 1 N HCl. The light yellow solid was separated by filtration and washed with 4 × 10 mL of H_2O, 5 mL of $(Me)_2CO$, and 10 mL of Et_2O. After drying, there was obtained 0.17 g (61%) of cream colored solid: mp 278–280 °C dec; TLC (*n*-BuOH–HOAc–H_2O, 6:2:2); HPLC (retention time 12.31 min; compound **7**, retention time 11.99 min, was not detected in this sample); NMR (90 MHz, CF_3COOD) δ 8.2 (d, 2, aromatic), 7.75 (d, 1, H_7), 7.75 (d, 1, H_7), 7.55 (m, 3, H_8 + aromatic), 4.96 (s, 2, NCH_2). Anal. ($C_{16}H_{13}N_4O_3Cl·H_2O·HCl$) C, H, N.

Di-*tert*-butyl 5-Chloro-5,8-dideazaisofolate, 9. To a stirred solution of $(Et)_3N$ (0.99 g, 0.9 mmol) and diethyl phosphorocyanidate (0.14 g, 0.9 mmol) in 5 mL of DMF was added a solution of **8** (0.12 g, 0.29 mmol) in 10 mL of DMF. After the reaction mixture was warmed at 60–65 °C for 0.25 h, a solution of $(Et)_3N$ (0.025 g, 0.29 mmol) and di-*tert*-butyl L-glutamate (0.073 g, 0.29 mmol) in 5 mL of DMF was added, and the resulting mixture was stirred at 60–65 °C for 18 h. The solvent was removed under vacuum and the residue suspended in 20 mL of H_2O and extracted with 3 × 25 mL of $CHCl_3$. The $CHCl_3$ extract was washed with 2 × 20 mL of H_2O, dried over Na_2SO_4, and then evaporated to dryness. Purification by flash column chromatography over silica gel using a 1% stepwise elution gradient of $CHCl_3$ to $CHCl_3$–MeOH, 94:6, gave a homogeneous fraction (96:4 to 95:5), which after solvent removal yielded 90 mg (48%) of yellow crystals: mp 164 °C; TLC ($CHCl_3$–MeOH, 4:1); HPLC; NMR (90 MHz, Me_2SO-d_6) δ 8.6–6.03 (m, 11, 5 NH + 6 aromatic), 4.73–4.10 (m, 3, NCH_2 + NCH), 2.3–1.73 (m, 4, $(CH_2)_2$), 1.36 (s, 9, $C(CH_3)_3$). 1.33 (s, 9, $C(CH_3)_3$). The elemental analysis was not acceptable and indicated a high degree of solvation by H_2O. This compound was employed in the succeeding step in this condition.

5-Chloro-5,8-dideazaisofolic Acid, 1c. A 60-mg (0.092 mmol) sample of **9** was stirred in 2 mL of CF_3COOH at ambient temperature for 1 h. The reaction mixture was added to 45 mL of cold Et_2O, and the resulting precipitate was separated by filtration and washed with 6 × 10 mL of Et_2O. After drying, there was obtained 38 mg (57%) of yellow solid: mp 258–270 °C dec; TLC; HPLC; UV λ_{max} 240 (ϵ 49.8 × 10^3), 275 (28.5 × 10^3), 370 (12.3 × 10^3) nm; NMR (300 MHz, Me_2SO-d_6) δ 8.53 (d, 1, CONH, J = 7.69 Hz), 7.80 (d, 2, 2′, 6′, J_o = 8.22 Hz), 7.41 (d, 2, 3′, 5′, J_o = 8.22 Hz), 6.96 (d, 1, H_7 or H_8, J_o = 9.01 Hz), 6.88 (d, 1, H_7 or H_8, J_o = 9.01 Hz), 6.17 (t, 1, N*H*CH_2, J = 4.96 Hz), 6.14 (br s, 2, NH_2), 4.50 (d, 2, NCH_2, J = 4.96 Hz), 4.34–4.41 (m, 1, glu α CH), 2.43 (t, 2, glu γ CH_2, J = 7.38 Hz), 1.86–2.13 (m, 2, glu β CH_2). The FAB/MS spectrum showed no pseudo molecular ion. Anal. ($C_{21}H_{20}N_5O_6Cl·1.5CF_3COOH·4.0H_2O$) C, H, N. The presence of CF_3COOH in this material was confirmed by ^{19}F NMR.

Di-*tert*-butyl 5-Chloro-5,8-dideazaisoaminopterin, 10. To a solution of $(Et)_3N$ (0.10 g, 1.0 mmol) and diethyl phosphorocyanidate (0.17 g, 1 mmol) in 5 mL of DMF was added a solution of **7** (0.137 g, 0.33 mmol) in 8 mL of DMF with stirring. After the reaction mixture was heated at 60–65 °C for 0.25 h, a solution containing $(Et)_3N$ (0.33 g, 0.33 mmol) and di-*tert*-butyl L-glutamate (0.83 g, 0.33 mmol) in 5 mL of DMF was added. The resulting mixture was stirred at 50–60 °C for 20 h, after which the solvent was removed under vacuum. The semisolid residue was suspended in 50 mL of H_2O and extracted with 4 × 20 mL of $CHCl_3$. The extract was washed with 2 × 50 mL of H_2O and dried over Na_2SO_4, and the solvent was removed under vacuum. The product was purified by flash chromatography using a 1% stepwise gradient of $CHCl_3$ to $CHCl_3$–MeOH, 95:5, to yield a yellow solid (95:5 eluate), which after crystallization from $CHCl_3$–*n*-hexane, 1:4, gave 0.12 g (58%) of yellow crystals: mp 112–116 °C; TLC ($CHCl_3$–MeOH, 4:1); HPLC; NMR (300 MHz, Me_2SO-d_6) δ 8.52 (d, 1, CON*H*, J = 7.61 Hz), 7.80 (d, 2, 2′, 6′, J_o = 8.14 Hz), 7.50 (br s, 2, NH_2), 7.42 (d, 2, 3′, 5′, J_o = 8.14 Hz), 7.06 (d, 1, H_7 or H_8, J_o = 9.15 Hz), 7.00 (d, 1, H_7 or H_8, J_o = 9.15 Hz), 6.25 (br s, 1, N*H*CH_2 + br s, 2, NH_2), 4.52 (br s, 2, NCH_2), 4.25–4.35 (m, 1, glu α CH), 2.32 (t, 2, glu γ CH_2, J = 7.4 Hz), 1.84–2.10 (m, 2, glu β CH_2), 1.39 (s, 9, $C(CH_3)_3$), 1.37 (s, 9, $C(CH_3)_3$). Anal. ($C_{29}H_{37}N_6O_5Cl·2.4H_2O·0.1Et_3N$) C, H, N. The presence of Et_3N was indicated by ^1H NMR.

5-Chloro-5,8-dideazaisoaminopterin, 2a. A 70-mg (0.11 mmol) sample of **10** was stirred in 3 mL of CF_3COOH for 1 h at ambient temperature. The reaction mixture was then added to 60 mL of cold Et_2O, and the resulting yellow solid was separated by filtration and washed with 8 × 10 mL of Et_2O. After drying, there was obtained 45 mg (67%): mp 186–191 °C; TLC; HPLC; UV λ_{max} 250 (ϵ 35.8 × 10^3), 277 (15.4 × 10^3) nm; NMR (300 MHz, Me_2SO-d_6) δ 8.52 (d, 1, CON*H*, J = 7.38 Hz), 8.29 (br s, 2, NH_2), 7.82 (d, 2, 2′, 6′, J_o = 8.01 Hz), 7.56 (br s, 2, NH_2), 7.42 (d, 2, 3′, 5′, J_o = 8.01 Hz), 7.19 (d, 1, H_7 or H_8, J_o = 8.98 Hz), 7.11 (d, 1, H_7 or H_8, J_o = 8.98 Hz), 6.65 (br s, 1, N*H*CH_2), 4.56 (br s, 2, NCH_2),

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4.36–4.39 (m, 1, glu α CH), 2.33 (t, 2, glu γ CH_2, J = 7.37 Hz), 1.85–2.16 (m, 2, glu β CH_2); FAB/MS, 473 (M + 1). Anal. ($C_{21}H_{21}N_6O_5Cl·CF_3COOH·H_2O$) C, H, N. The presence of CF_3COOH of solvation was confirmed by ^{19}F NMR.

6-[(*p*-Carboxyanilino)methyl]-5-chloro-2,4-diaminoquinazoline, *tert*-Butyl Ester, 13. 5-Chloro-6-cyano-2,4-diaminoquinazoline (6.5 g, 0.029 mol) was dissolved in 70% AcOH (300 mL). After the reaction mixture was warmed until complete dissolution occurred, *tert*-butyl *p*-aminobenzoate (6.6 g, 0.034 mol) and 2 g of Raney Ni slurry (50% catalyst by volume) were added. The reaction mixture was shaken at room temperature in a low-pressure Parr hydrogenator until H_2 uptake ceased. After 3 g of charcoal was added, the reaction mixture was filtered through a Celite bed. Evaporation of the solvent under reduced pressure gave the crude product, which was dissolved in $CHCl_3$ and applied to a silica gel column. The product was isolated by gradient elution ($CHCl_3$–MeOH, 99:1, to $CHCl_3$–MeOH, 92:8). Pooled TLC-homogeneous fractions were evaporated to obtain 5.3 g (45.7%) of bright-yellow powder: mp 236–237 °C; TLC ($CHCl_3$–MeOH, 9:1); HPLC; NMR (90 MHz, Me_2SO-d_6) δ 7.55 (d, 2, 2′, 6′, J_o = 9 Hz), 7.37 (d, 1, H_8, J_o = 8 Hz + br s, 4, NH_2), 7.08 (d, 1, H_7, J_o = 8.0 Hz), 6.51 (d, 2, 3′, 5′, J_o = 9 Hz), 6.05 (br s, 1, CH_2NH), 4.30 (br s, 2, CH_2N), 1.42 (s, 9, $C(CH_3)_3$). Anal. ($C_{20}H_{22}N_5O_2Cl$) C, H, N, Cl.

6-[(*p*-Carboxyanilino)methyl]-5-chloro-2,4-diaminoquinazoline, 14. Compound **13** (2 g, 5 mmol) was dissolved in CF_3COOH (13 mL) and $CHCl_3$ (27 mL). After the reaction mixture was stirred at room temperature for 3 h, 200 mL of cold Et_2O was added to it. The precipitate was filtered and washed with cold Et_2O. The crude product was dissolved in 500 mL of H_2O, and the resulting suspension was basified to pH 12 with 30% NH_4OH. The solution was slowly brought to pH 6 with concentrated HCl and then 2 N HCl. The precipitate was separated by filtration and washed with H_2O and then Et_2O. After drying in high vacuum at 100 °C, 1.6 g (93%) of yellow crystalline powder was obtained: mp 228–235 °C (lit.[12] mp 232–235 °C); TLC ($CHCl_3$–MeOH, 9:1); HPLC; NMR (400 MHz, Me_2SO-d_6) δ 7.65 (d, 2, 2′, 6′, J_o = 8.71 Hz), 7.43 (d, 1, H_8, J_o = 8.69 Hz + br s, 2, NH_2), 7.13 (d, 1, H_7, J_o = 8.69 Hz), 7.02 (t, 1, CH_2NH, J = 5.69 Hz), 6.57 (d, 2, 3′, 5′, J_o = 8.71 Hz), 6.19 (br s, 2, NH_2), 4.36 (d, 2, CH_2N, J = 5.54 Hz). Anal. ($C_{16}H_{14}N_5O_2Cl·1.5H_2O$) C, H, N.

Di-*tert*-butyl 5-Chloro-5,8-dideazaaminopterin, 15. To a solution of 14·1.5H_2O (0.25 g, 0.67 mmol) in dry DMF (20 mL) were added Et_3N (0.205 g, 2 mmol) in 2 mL of DMF and diethyl phosphorocyanidate (0.33 g, 2 mmol) in 2 mL of DMF. After 30 min of heating at 65 °C, the reaction mixture was cooled to room temperature and a mixture of L-glutamic acid di-*tert*-butyl ester hydrochloride (Sigma Chemical Co.) (0.204 g, 0.69 mmol) and Et_3N (0.070 g, 0.69 mmol) in dry DMF (10 mL) was added. A second portion of Et_3N (0.068 g, 0.67 mmol) was added, and stirring at 65 °C was continued for 18 h under N_2. The reaction mixture was evaporated under reduced pressure, 30 mL of H_2O was added to the residue, and the H_2O phase was extracted with EtOAc. The organic layer was washed with H_2O and saturated solution of NaCl, dried over $MgSO_4$, and evaporated. The crude product was applied to a silica gel column. Very mobile impurities were eluted from the column with $CHCl_3$, and the product **15** was eluted with $CHCl_3$–MeOH (9:1). Evaporation of pooled TLC-homogeneous fractions gave an orange solid, which was recrystallized from $CHCl_3$–*n*-hexane, 1:4, to yield 0.297 g (63%): melting point poorly defined; TLC ($CHCl_3$–MeOH, 4:1); HPLC; NMR (400 MHz, Me_2SO-d_6) δ 8.13 (d, 1, CONH, J = 7.46 Hz), 7.64 (d, 2, 2′, 6′, J_o = 8.47 Hz), 7.57 (d, 1, H_8, J_o = 8.50 Hz), 7.26 (d, 1, H_7, J_o = 8.50 Hz + br s, 2, NH_2), 6.91 (br s, 1, CH_2NH), 6.56 (d, 2, 3′, 5′, J_o = 8.47 Hz + br s, 2, NH_2), 4.39 (br s, 2, CH_2N), 4.26–4.28 (m, 1, glu α CH), 2.28 (t, 2, glu γ CH_2, J = 7.35 Hz), 1.87–1.98 (m, 2, glu β CH_2), 1.38 (s, 9, $C(CH_3)_3$), 1.36 (s, 9, $C(CH_3)_3$). Anal. ($C_{29}H_{37}N_6O_5Cl·4.5H_2O·0.22Et_3N$) C, H; N: calcd, 12.66; found, 11.64. The presence of Et_3N was indicated by ^1H NMR.

5-Chloro-5,8-dideazaaminopterin, 2b. Compound **15** (0.160 g, 0.22 mmol) was dissolved in CF_3COOH (7 mL). After the solution was stirred at room temperature under N_2 for 1 h, it was evaporated under reduced pressure. Next, 15 mL of Et_2O was added to the dry residue, and the precipitate was separated by filtration and washed with 10 mL of Et_2O and 10 mL of Me_2CO. The crude product was dissolved in 15 mL of H_2O, and the resulting suspension was basified to pH 10–11 with 1 N NaOH. Traces of insoluble material were removed by filtration. The solution was brought to pH 3.5 with 1 N HCl. After 15 min of stirring at room temperature, the precipitate was filtered, washed with 3 × 10 mL of cold H_2O, and dried under vacuum, at 100 °C, yielding 0.074 g (66%) of crystalline powder: mp 195–200 °C (lit.[11] mp 220–222 °C); TLC (1-propanol–H_2O–HOAc, 3:1:1); HPLC; NMR (400 MHz, Me_2SO-d_6) δ 8.09 (d, 1, CONH, J = 7.49 Hz), 7.64 (d, 2, 2′, 6′, J_o = 8.60 Hz), 7.51 (br s, 2, NH_2), 7.44 (d, 1, H_8, J_o = 8.67 Hz), 7.14 (d, 1, H_7, J_o = 8.67 Hz), 6.83 (t, 1, CH_2NH, J = 5.61 Hz), 6.56 (d, 2, 3′, 5′, J_o = 8.60 Hz), 6.29 (br s, 2, NH_2), 4.37 (d, 2, CH_2N, J = 5.61 Hz), 4.28–4.34 (m, 1, glu α CH), 2.30 (t, 2, glu γ CH_2, J = 7.33 Hz), 1.84–2.05 (m, 2, glu β CH_2). Anal. ($C_{21}H_{21}N_6O_5Cl·1.2H_2O$) C, H, N.

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Lysosomotropic Agents. 7.[1] Broad-Spectrum Antifungal Activity of Lysosomotropic Detergents

Raymond A. Firestone,* Judith M. Pisano, George M. Garrity, Robert A. Fromtling, and Sheldon B. Zimmerman

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065. *Received March 13, 1987*

Lysosomotropic detergents, which kill mammalian cells by disrupting lysosomal membranes, have now been found to be antifungals also. All strains in our assay are susceptible. The mode of action is as yet undetermined, but intracellular vacuoles may be the primary targets.

We report on a new class of broad-spectrum antifungals. Lysosomotropic detergents (LD's)[2] are amines of intermediate pK (5.5–7.6) that bear long alkyl chains. As free bases they are lipophilic and nonsurface-active, but when protonated they become powerful detergents. Thus they do not harm the outer membranes of cells, which reside in a neutral medium where LD's are hardly protonated. In the acidic milieu of lysosomes, however, LD's are largely

* Address correspondence to this author at Bristol-Myers Co., Pharmaceutical Research, Wallingford, CT 06492-7660.

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