injection of 0.1 mL of a 1% aqueous suspension of carrageenan into the left hind paw. Paw volume measurements were taken immediately after and 3 h after the carrageenan injection by means of mercury displacement. Drug activity was expressed as the percent difference between the test and control groups edema. Dose ranges included at least three doses of the drug.

Phenylquinone-Induced Writhing (PQW). The procedure employed was a modification of the method of Siegmund et al.[12] A 0.125% concentration of phenylquinone (phenyl-*p*-benzoquinone, Eastman) in a 5% aqueous ethanol solution was injected into male CD-1 Charles River mice, weighing 18–24 g, at 10 mL/kg ip. Animals were fasted overnight (water ad libitum). Groups of five mice were treated with test drug orally at various time intervals prior to phenylquinone injection. Control mice were treated with an equal volume of vehicle. After phenylquinone injection, the mice were placed individually in 1000-mL beakers, and 5 min later, the number of writhes was recorded for a 10-min period. The peak time of test drug activity was thereby determined. A dose–response study was performed in a similar manner, except that 8 to 10 animals per group were used at the peak time of activity. Animals were dosed and tested in a randomized manner using four drug doses and one control group. Drug activity is expressed as the percent inhibition of the control group per number of writhes.

Gastric Irritation (GI). Groups of 10 male Wistar rats weighing 150–175 g were fasted 48 h (water ad libitum) prior to administration of the test drug orally (10 mL/kg). Control rats received vehicle only (10 mL/kg). For a time response, animals were treated with a highly active antiinflammatory dose of the test drug and then sacrificed at 3, 5, and 7 h after drug administration. Stomachs and intestines were removed and examined for the presence of lesions. The presence of single or multiple lesions (erosion, ulcer, or perforation) was considered an ulcerogenic effect. A dose–response was performed at the peak time using four doses of the test drug.

Acknowledgment. The authors express their appreciation to Marc N. Agnew and Anastasia Rizwaniuk for spectral data, to Karen McCormack and Janis Little for performing pharmacological assays, and to Rose Marie Boysen and Grace M. Naumovitz for assistance in the preparation of the manuscript.

Registry No. 1, 59961-15-8; **2a**, 86223-05-4; **2b**, 62889-58-1; **3a**, 86223-06-5; **3b**, 88358-29-6; **4a**, 86223-07-6; **4b**, 86223-08-7; **4c**, 86223-09-8; **5a**, 88358-30-9; **5b**, 88358-31-0; **5c**, 88358-32-1; **5d**, 88358-33-2; **5e**, 88376-64-1; **6**, 61727-71-7; **7**, 88358-34-3; **8a**, 88358-35-4; **8b**, 88358-36-5; **8c**, 88358-37-6; **9**, 88358-38-7; **10**, 88358-39-8; 11, 88358-40-1; 2-amino-2-methyl-1-propanol, 124-68-5.

(12) Siegmund, E.; Cadmus, R.; Lu, G. *Proc. Soc. Exp. Biol. Med.* **1957**, *95*, 729.

Synthesis and Antifolate Properties of 10-Alkyl-8,10-dideazaminopterins

J. I. DeGraw,*[†] P. H. Christie,[†] E. G. Brown,[†] L. F. Kelly,[†,⊥] R. L. Kisliuk,[‡] Y. Gaumont,[‡] and F. M. Sirotnak[§]

Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025, Department of Biochemistry, Tufts University Medical School, Boston, Massachusetts 02111, and Sloan Kettering Cancer Center, New York, New York 10021.
Received March 21, 1983

The synthesis of 10-alkyl analogues of the potent antitumor agent 8,10-dideazaminopterin is described. Alkylation of appropriate α-alkyl homoterephthalate esters with 2,4-diamino-6-(bromomethyl)-8-deazapteridine afforded 10-alkyl-10-carboxy-4-amino-4-deoxy-8,10-dideazapteroic acid diesters. Ester cleavage and decarboxylation at C-10 were accomplished by heating with sodium cyanide in Me_2SO at 170–180 °C to afford the 2,4-diamino-10-alkyl-8,10-dideazapteroic acids. The acids were coupled with diethyl glutamate, followed by saponification, to give the 10-alkyl-8,10-dideazaminopterins. The compounds were potent inhibitors of growth in folate-dependent bacteria, *Streptococcus faecium* and *Lactobacillus casei*. The 10-methyl and 10-ethyl analogues gave the highest percent increases in life span for mice infected with L1210 leukemia with ILS values of +203 and +235%, respectively.

In previous papers we have reported the synthesis and antifolate activities of 10-deazaminopterin[1,2] and its 10-alkyl analogues.[3] These compounds were found to be powerful antifolates with transport and pharmacokinetic properties that made them prime candidates as antitumor agents for human use.[4-6] As an extension of this research program, we have also investigated the synthesis of ring deazapteridines. In a recent paper we described the synthesis and biological activity of 8,10-dideazaminopterin (**6a**).[7] This compound was found to be a very potent inhibitor of dihydrofolate reductase and encouraged us to investigate the 10-alkyl analogues **6b–d**. We report the synthesis and properties of these analogues in this article.

Chemistry. We have previously reported[7,8] two independent syntheses of **6a** that proceeded through 9,10-didehydrofolate intermediates. However, neither of these routes was considered to be convenient for the synthesis of the 10-alkyl analogues **6b–d**.

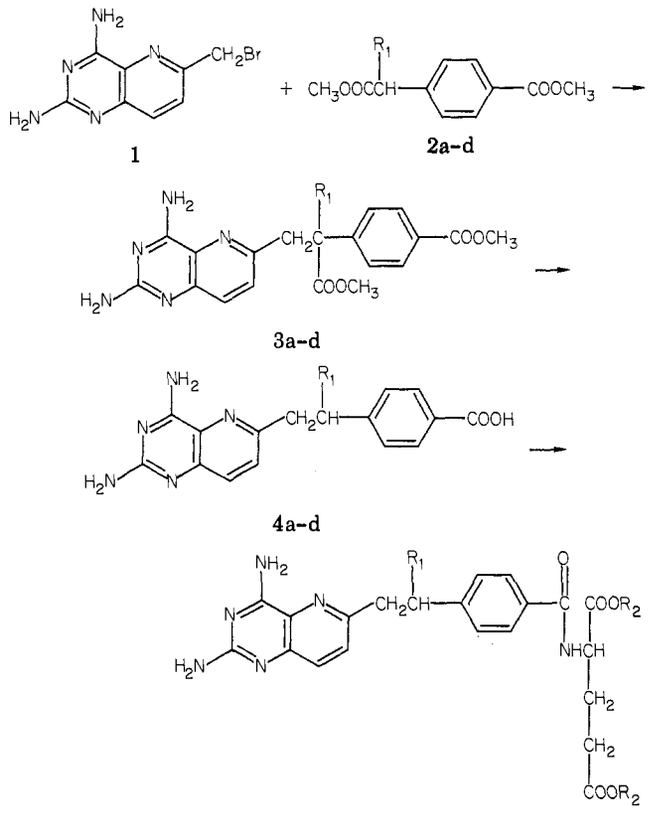
Dimethyl homoterephthalate (**2a**) was converted (Scheme I) to its anion by treatment with potassium hydride in dimethylformamide. The ester anion was alkylated with 2,4-diamino-6-(bromomethyl)-8-deazapteridine

[†] SRI International.
[‡] Tufts University Medical School.
[§] Sloan Kettering Cancer Center.
[⊥] Present address: Department of Chemistry, Australian National University, Canberra, Australia.

(1) J. I. DeGraw, R. L. Kisliuk, Y. Gaumont, C. M. Baugh, and M. G. Nair, *J. Med. Chem.*, **17**, 552 (1974).
(2) J. I. DeGraw, V. H. Brown, R. L. Kisliuk, and F. M. Sirotnak. "Chemistry and Biology of Pteridines", R. Kisliuk and G. Brown, Eds., Elsevier/North Holland, New York, 1979, p 225.
(3) J. I. DeGraw, V. H. Brown, H. Tagawa, R. L. Kisliuk, Y. Gaumont, and F. M. Sirotnak, *J. Med. Chem.*, **25**, 1227 (1982).
(4) F. M. Sirotnak, J. I. DeGraw, R. Donsbach, and D. M. Moccio, *Proc. Am. Assoc. Cancer Res.*, 18, 37 (1977).
(5) F. M. Sirotnak, J. I. DeGraw, D. Moccio, and D. M. Dorick, *Cancer Treat. Rep.* **62**, 1047 (1978).
(6) F. M. Sirotnak, P. Chello, J. I. DeGraw, J. R. Piper, and J. A. Montgomery, in "Molecular Actions and Targets for Cancer Chemotherapeutic Agents", A. Sartorelli, Ed., Academic Press, New York, 1981, pp 349–384.
(7) J. I. DeGraw, L. F. Kelly, R. L. Kisliuk, Y. Gaumont, and F. M. Sirotnak, *J. Heterocycl. Chem.*, **19**, 1587 (1982).
(8) J. I. DeGraw, L. F. Kelly, R. L. Kisliuk, Y. Gaumont, and F. M. Sirotnak, "Chemistry and Biology of Pteridines", DeGruyter: Berlin and New York, 1983; pp 457–461.

0022-2623/84/1827-0376$01.50/0

Scheme I[a]



1

2a-d

3a-d

4a-d

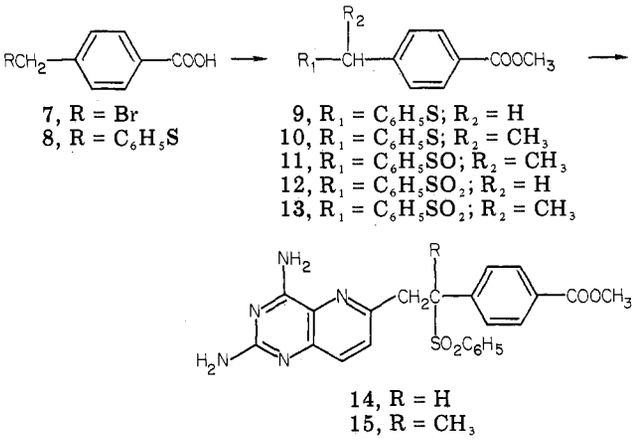
5a-d, R_2 = Et
6a-d, R_2 = H

[a] Series: a, R_1 = H; b, R_1 = CH_3; c, R_1 = C_2H_5; d, R_1 = n-C_3H_7.

(1)[9] at −40 °C to afford the 10-carbomethoxy ester **3a**. Since the bromide is isolated as a hydrated hydrobromide salt, best results were obtained when 3–3.5 equiv of ester anion was used. It was perceived that the product represented an extended "malonate" system that could undergo decarboxylation of the 10-carboxyl group. Treatment of the diester with nucleophilic agents in dimethyl sulfoxide at 170–180 °C caused cleavage of the ester groups and effected decarboxylation[10-12] to yield 4-amino-4-deoxy-8,10-dideazapteroic acid (**4a**). The use of 3 equiv of sodium cyanide was found to cause ester cleavage and decarboxylation after heating for 1–2 h. The starting diester, **2a**, could be alkylated with lower alkyl halides to afford the dimethyl α-alkylhomoterephthalates **2b–d**. These were alkylated with the bromide 1 in the manner described above and similarly decarboxylated to give the desired 4-amino-4-deoxy-10-alkyl-8,10-dideazapteroic acid analogues **4b–d**. Coupling with diethyl L-glutamate, followed by saponification of the glutamate diesters **5b–d**, afforded the target 10-alkyl-8,10-dideazaminopterins **6b–d** (Table I).

We initially sought to alkylate a *p*-alkylbenzoate (Scheme II) containing a carbanion at the benzylic position with 2,4-diamino-6-(bromomethyl)-8-deazapteridine (**1**). We had observed that attempts to alkylate *p*-toluic acid dianion with 2,4-diamino-6-(bromomethyl)pterins gave none of the expected alkylation products. It appeared that a charge-stabilizing group was necessary to soften the anion character to prevent decomposition of the bromide and allow alkylation to take place. The methyl *p*-[(phenylthio)methyl]benzoate (**9**) and methyl *p*-[(phenylsulfinyl)methyl]benzoate (**11**) were prepared; however, their anions were likewise unsuitable. The methyl *p*-[(phenylsulfonyl)methyl]benzoate (**12**) anion could be readily alkylated by the bromide **1**. The phenylsulfonyl ester **12** allowed facile abstraction of a proton, and careful treatment of the anion with methyl iodide gave the monomethyl ester **13**. Further alkylation of the anions of the esters **12** and **13** gave the 2,4-diamino-10-(phenylsulfonyl)-8,10-dideazapteroic esters **14** and **15**. However, all attempts to remove the sulfonyl moiety (Al/Hg, Na/Hg, Raney nickel, chromous chloride) were unsuccessful in either case. We are unable to account for this lack of reactivity but noted that α-methyl-*p*-carbomethoxybenzylphenyl sulfone (**13**) could be readily desulfonylated under the above conditions.

Scheme II

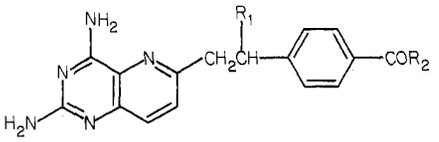


7, R = Br
8, R = C_6H_5S

9, R_1 = C_6H_5S; R_2 = H
10, R_1 = C_6H_5S; R_2 = CH_3
11, R_1 = C_6H_5SO; R_2 = CH_3
12, R_1 = $C_6H_5SO_2$; R_2 = H
13, R_1 = $C_6H_5SO_2$; R_2 = CH_3

14, R = H
15, R = CH_3

Table I. Physical Data for 8,10-Dideazaminopterin Analogues



compd	R_1	R_2	UV λ_{max}, nm (ϵ), at pH 13	formula[a]
4a	H	OH	238 (33 763) 343 (5209)	$C_{16}H_{15}N_5O_2 \cdot 0.25(CD_3)_2SO$
4b	CH_3	OH	238 (36 399) 343 (7280)	$C_{17}H_{17}N_5O_2 \cdot 1.5H_2O$
4c	C_2H_5	OH	238 (35 374) 343 (5134)	$C_{18}H_{19}N_5O_2$
4d	n-C_3H_7	OH		$C_{19}H_{21}N_5O_2$
6b	CH_3	Glu	238 (37 395) 343 (5521)	$C_{22}H_{24}N_6O_5 \cdot 1.5H_2O$
6c	C_2H_5	Glu	238 (37 652) 343 (5298)	$C_{23}H_{26}N_6O_5 \cdot 1.25H_2O$
6d	n-C_3H_7	Glu	238 (36 869) 343 (5048)	$C_{24}H_{28}N_6O_5 \cdot 1.5H_2O$

[a] Compounds analyzed for C, H, and N within ±0.4% of theory.

Biological Results

In Table II, we have presented data for the relative potencies of compounds **6a–d** for inhibition of growth of

(9) A. Srinivasan and A. Broom, *J. Org. Chem.*, **46**, 1777 (1981).
(10) A. P. Krapcho and A. J. Lovey, *Tetrahedron Lett.*, 957 (1973).
(11) C. L. Liotta and F. L. Cook, *Tetrahedron Lett.*, 1095 (1974).
(12) We noted that LiI or NaCl could also be used, as can HMPA as solvent, and that decarboxylation begins at temperatures lower than those used here. However, NaCN/Me_2SO appears to give optimal results. Use of NaCl at lower temperatures gave substantial retention of the benzoate ester. The reactions are much slower if an ethyl ester is used; see P. Müller and B. Siegfried, *Tetrahedron Lett.*, 3565 (1973).

Table II. Bacterial Growth Inhibition by 8,10-Dideazaminopterin Analogues

	concn, ng/mL,[a] for 50% inhibn of growth				
	S. faecium		*L. casei*		
compd	ATCC8043	MTX resist	ATCC7469	MTX resist	*P. cerevisiae*
6a	0.04	1200	0.005	>2000	9.0
6b	0.04	700	0.004	>2000	3.0
6c	0.17	1300	0.003	>2000	11.0
6d	0.24	500	0.017	>2000	20.0
MTX	0.15	5000	0.005	$>5 \times 10^5$	190

[a] Folate concentration = 1 ng/mL.

Table III. Enzyme Inhibitory Activity of 8,10-Dideazaminopterin Derivatives

	concn, M, for 50% inhibn			
	DHFR		TS	
compd	*S. faecium*	*L. casei*	*S. faecium*	*L. casei*
6a	1.0×10^{-9}	1.0×10^{-8}	7.2×10^{-5}	1.1×10^{-4}
6b	1.2×10^{-9}	1.1×10^{-8}	3.8×10^{-5}	1.2×10^{-4}
6c	1.0×10^{-9}	1.6×10^{-8}	7.0×10^{-5}	2.2×10^{-4}
6d	1.3×10^{-9}	1.3×10^{-8}	1.2×10^{-4}	$>2.5 \times 10^{-4}$
MTX	1.2×10^{-9}	1.0×10^{-8}	1.5×10^{-4}	0.8×10^{-4}

Table IV. Antitumor Effects against L1210 Leukemia in Mice

compd[a]	LD_{10}, mg/kg	e2d×5:[b] MST, days	ILS, %
MTX	15	20.4 ± 2.1	+187
6a	0.75		+189[c]
6b	0.6	21.5 ± 1.5	+203
6c	1.5	23.8 ± 1.9	+235
6d	6.0		+176[c]
control		7.1 ± 0.8	

[a] Drug given sc 24 h after 10^6 cells implanted intraperitoneally. [b] Ten animals per run. [c] Compounds 6a and 6d were reported earlier;[7,13] MTX comparison was +163% in those assays.

the folate-dependent bacteria *Streptococcus faecium*, *Lactobacillus casei*, and *Pediococcus cerevisiae*. In *S. faecium* it was found that the 10-methyl analogue (**6b**) was equipotent to the powerful inhibitor **6a**,[7] but the potencies of the 10-ethyl and 10-propyl analogues declined considerably, although they still compared favorably with methotrexate (MTX). In contrast, the 10-ethyl analogue **6c** was the most potent compound against *Lactobacillus casei*. The compounds were not exceptional inhibitors of *P. cerevisiae*, although they were considerably more potent than MTX in that regard. In the *S. faecium* and *L. casei* system, the 10-alkyl analogues **6b–d** were more potent than their relatives in the 10-alkyl-10-deazaminopterin series.[3]

In Table III, data are shown for inhibition of the enzymes, dihydrofolate reductase (DHFR) and thymidylate synthase (TS), derived from both *S. faecium* and *L. casei*. As expected, compounds **6a–d** are potent inhibitors of the DHFR from both bacterial sources. While the low order of activity against TS from *L. casei* was predictable, the inhibition of TS from *S. faecium* was greater than expected. However, these values are in the range of only moderate potency.

In Table IV are shown the relative antitumor properties of compounds **6a–d** in murine L1210 leukemia. The 8,10-dideazaminopterins were considerably more potent than their counterparts in the pteridine series[3] and were about as effective in extending life spans for the tumor-bearing mice. The comparisons for both series of analogues are relative to the active standard, methotrexate. As in the pteridine series, the 10-methyl and 10-ethyl analogues were clearly the most efficacious, with ILS values above **200%**. It is interesting that efficacy and potency decline significantly with the *n*-propyl analogue, **6d**, also observed for the pteridine case.[13] Further evaluation of these compounds in other tumor systems will be reported elsewhere.

Experimental Section

Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN. The ^1H NMR spectra were taken on a Varian EM 360A or a 300-MHz Nicolet spectrometer. Mass spectra were run on a LKB 9000 GC–MS spectrometer. Ultraviolet spectra were taken on a Perkin-Elmer 552 spectrophotometer. TLC was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Melting points were determined on a Thomas Hoover Uni-melt apparatus.

Alkylation of Dimethyl Homoterephthalate (2a). α-Alkylhomoterephalates (2b–d). A solution of dimethyl homoterephthalate (**2a**; 4.16 g, 20 mmol) in 15 mL of dry THF was added dropwise over 15 min to a stirred mixture of KH (2.5 g of 35% oil suspension, 22 mmol) in 75 mL of THF at 0 °C. After 30 min, a homogeneous yellow suspension had formed. Methyl iodide (2.79 g, 22 mmol) was then added over 5 min, and the mixture was stirred for another 30 min. The mixture was quenched with 2 mL of 50% HOAc, diluted with 500 mL of water, and extracted with 3 × 75 mL portions of Et_2O. The Et_2O extract was washed with water, dried over $MgSO_4$, and evaporated to leave an oil. The material was chromatographed on silica gel with elution by Et_2O–hexane (1:9) to afford 3.15 g (71%) of dimethyl α-methylhomoterephthalate (**2b**) as a clear oil: mass spectrum, *m/e* 222; NMR ($CDCl_3$) δ 1.50 (3 H, d, CH_3), 3.65 (3 H, s, OCH_3), 3.93 (3 H, s, benzoate OCH_3).

Dimethyl α-ethylhomoterephalate (**2c**) was obtained in 72% yield: mass spectrum, *m/e* 236; NMR ($CDCl_3$) 0.9 (3 H, t, CH_3), 2.0 (2 H, m, CH_2CH_3), 3.5 (1 H, m, CHC_2H_5), 3.70 (3 H, s, OCH_3), 3.90 (3 H, s, benzoate OCH_3).

Dimethyl α-propylhomoterephthalate (**2d**) was obtained in 74% yield: mass spectrum, *m/e* 250; NMR ($CDCl_3$) δ 0.91 (3 H, t, CH_3), 1.26 (2 H, m, CH_2CH_3), 1.75–2.05 (2 H, m, $CH_2C_2H_5$), 3.62 (1 H, t, CHC_3H_7), 3.66 (3 H, s, OCH_3), 3.91 (3 H, s, benzoate OCH_3).

Alkylation of Homoterephthalates with 2,4-Diamino-6-(bromomethyl)-8-deazapteridine (1). 4-Amino-4-deoxy-10-alkyl-10-carbomethoxy-8,10-dideazapteroates (3a–d). To a stirred mixture of 5.24 g of a 22.3% oil suspension of KH (29.2 mmol) and 24 mL of dry DMF at 0 °C was slowly added 6.48 g (29.2 mmol) of dimethyl α-methylhomoterephthalate (**2b**). After 30 min, the yellow solution was cooled to −40 °C, and 3.6 g (9.2 mmol) of the bromomethyl compound **1** in 48 mL of DMF was added over a 10-min period. The mixture was brought to ambient temperature and stirred for 2 h. The solvent was removed in vacuo, and the residue was partitioned between 100 mL of $CHCl_3$ and 25 mL of water. The $CHCl_3$ solution was dried ($MgSO_4$), concentrated in vacuo, and chromatographed on silica gel with initial elution by $CHCl_3$ to remove unreacted ester, followed by elution of product by $CH_3OH/CHCl_3$ (1:6) to afford 2.85 g (78%) of a yellow solid (**3b**): mass spectrum, *m/e* 395 (M^+); NMR (Me_2SO-d_6) δ 1.50 (3 H, s, CH_3), 3.53 (1 H, d, C-9 H), 3.59 (3 H, s, OCH_3), 3.72 (1 H, d, C-9 H), 3.86 (3 H, s, benzoate OCH_3), 7.32 (1 H, d, C-7 H), 7.47 (1 H, d, C-8 H), 7.54 (2 H, d, 3′,5′-H's), 7.96

(13) F. M. Sirotnak and J. I. DeGraw, unpublished results.

(2 H, d, 2',6'-H's). Anal. ($C_{22}H_{21}N_5O_4 \cdot H_2O$) C, H, N.

The 10-ethyl diester **3c** was obtained in 74% yield: mass spectrum, m/e 409 (M^+); NMR (Me_2SO-d_6) δ 0.73 (3 H, t, CH_3), 2.20 (2 H, m, CH_2CH_3), 3.67 (5 H, m, 9-CH_2, $COOCH_3$), 3.90 (3 H, s, benzoate OCH_3).

The 10-propyl diester **3d** was obtained in 52% yield: mass spectrum, m/e 423 (M^+); NMR (Me_2SO-d_6) δ 0.72 (3 H, t, CH_3), 1.05 (2 H, m, CH_2CH_3), 1.92 (2 H, m, $CH_2CH_2CH_3$), 3.57 (3 H, s, $COOCH_3$), 3.63 (2 H, m, C-9 H's), 3.85 (3 H, s, benzoate OCH_3).

The 10-unsubstituted diester **3a** was obtained in 83% yield: mass spectrum, m/e 381 (M^+).

Decarboxylation of 10-Carbomethoxypteroates. 4-Amino-4-deoxy-10-alkyl-8,10-dideazapteroic Acids (4a–d). The 10-methyl diester (**3b**; 2.85 g, 7.22 mmol) and 1.06 g (21.7 mmol) of NaCN in 50 mL of Me_2SO were stirred at 175–180 °C under N_2 for 2.5 h. The dark mixture was cooled, and the solvent was removed in vacuo. The residue was dissolved in 45 mL of water, the solution was filtered, and the filtrate was acidified with HOAc. The precipitate was collected, washed with water, and dried to leave 2.28 g (96%) of **4b**: mass spectrum, m/e 323 (M^+); NMR (Me_2SO-d_6) δ 1.23 (3 H, d, CH_3), 3.06 (2 H, m, 9-CH_2), 3.50 (1 H, m, 10-H), 7.27 (1 H, d, 7-H), 7.39 (3 H, m, 8-H, 3',5'-H's), 7.82 (2 H, d, 2',6'-H's).

The 10-unsubstituted (**4a**), 10-ethyl (**4c**), and 10-propyl (**4d**) compounds were similarly obtained and are shown in Table I. Analytical samples for **4b–d** were obtained by recrystallization from Me_2SO. Compound **4a** prepared by this method was equivalent to material previously[7,8] prepared, as shown by NMR.

Coupling of 4a–d with Diethyl L-Glutamate. 10-Alkyl-8,10-dideazaminopterin Diethyl Esters (5a–d). A mixture of 2.38 g (7.3 mmol) of 4-amino-4-deoxy-10-methyl-8,10-dideazapteroic acid (**4b**), 2.03 mL (14.7 mmol) of triethylamine, and 54 mL of dry DMF was treated dropwise with 1.9 mL (14.7 mmol) of isobutyl chloroformate. The mixture was stirred at room temperature for 1 h, and a mixture of 3.52 g (14.7 mmol) of diethyl L-glutamate hydrochloride, 2.03 mL (14.7 mmol) of triethylamine, and 18 mL of DMF was added. The mixture was stirred at ambient temperature for 24 h, and the solvent was removed in vacuo. The residue was partitioned between 50 mL of saturated $NaHCO_3$ and 50 mL of $CHCl_3$, followed by two additional $CHCl_3$ extractions. The $CHCl_3$ extract was dried ($MgSO_4$) and evaporated. The crude material was chromatographed on 110 g of silica gel with initial elution by $CHCl_3$ and then removal of the product by MeOH/$CHCl_3$ (2.5:97.5) to give 2.01 g (54%) of the diester **5b** as a yellow gum: mass spectrum, m/e 508 (M^+); NMR ($CDCl_3$) δ 1.23 (9 H, m, 10-CH_3, $COOCH_2CH_3$), 2.4 (4 H, m, $CH_2CH_2COOCH_2CH_3$), 3.1–3.5 (3 H, m, 10-H, 9-CH_2), 4.3 (4 H, q, $COOCH_2CH_3$), 4.8 (1 H, d, CONHCH), 7.2–7.4 (4 H, m, 3'-H, 5'-H, 7-H, 8-H) 7.8 (2 H, d, 2'-H, 6'-H).

The 10-unsubstituted (**5a**) [mass spectrum, m/e 494 (M^+)], 10-ethyl (**5c**) [mass spectrum, m/e 522 (M^+)], and 10-propyl (**5d**) [mass spectrum, m/e 536 (M^+)] glutamate esters were similarly obtained in 25, 34, and 38% yields, respectively.

10-Alkyl-8,10-dideazaminopterin (6a–d). A solution of 0.43 g of the 10-methyl diester **5b** in 6.5 mL of 2-methoxyethanol was treated with 4.3 mL of 1 N NaOH. The solution was maintained at ambient temperature for 3 h and diluted with 35 mL of H_2O. The solution was acidified with HOAc to precipitate the product. After refrigeration of the solution for 24 h, the precipitate was collected, washed with H_2O, and dried to leave 0.265 g (70%) of **6b** as a pale yellow microcrystalline solid: mass spectrum, m/e (as tetratrimethylsilyl derivative) 740 (M^+).

The other 10-alkyl analogues, **6c–d**, were prepared in a similar manner, and their properties are shown in Table I. Compound **6a** was previously obtained from hydrolysis of the diester **5a**, prepared by a different route.[7]

***p*-[(Phenylthio)methyl]benzoic Acid (8).** A solution of 12.1 g (0.11 mol) of benzenethiol in 350 mL of 10% Na_2CO_3 was treated with 18.6 g (0.087 mol) of 4-(bromomethyl)benzoic acid (**7**) in 4 portions over 10 min. The mixture was stirred under N_2 for 3 h and slowly acidified with 3 N HCl. The white solid was collected, washed with H_2O, and dried to leave 20 g (95%). Recrystallization from 70% EtOH gave an analytical sample, mp 174–175 °C. Anal. ($C_{14}H_{12}O_2S$) C, H, S.

Methyl *p*-[(Phenylthio)methyl]benzoate (9). A mixture of 20 g of the phenylthio acid **8**, 15 mL of trimethyl orthoformate, 2 mL of concentrated H_2SO_4, and 300 mL of MeOH was stirred at reflux for 24 h. The mixture was chilled, and the white solid was collected by filtration and washed with MeOH to afford 17 g (80%). Sublimation in vacuo gave white crystals, mp 80–82 °C. Anal. ($C_{15}H_{14}O_2S$) C, H, S.

α-Methyl-*p*-carbomethoxybenzyl Phenyl Sulfoxide (11). To 0.10 g (4.2 mmol) of NaH in 5 mL of DMF at 0 °C was added 1.03 g (4 mmol) of the phenylthio ester **9** in 7 mL of DMF. The mixture was stirred for 20 min, and the orange–red solution was cooled to −20 °C, whereupon 0.25 mL (4 mmol) of methyl iodide was added by syringe. The solution was maintained at room temperature for 30 min (color was pale yellow), and the solvent was removed in vacuo. The residue was partitioned between 20 mL of Et_2O and 20 mL of H_2O. The Et_2O solution was washed with 20 mL of H_2O, dried over $MgSO_4$, and evaporated to give 0.90 g (83%) of crude methyl α-methyl-*p*-[(phenylthio)-methyl]benzoate (**10**). The structure was confirmed by NMR, which showed about 20% of unreacted **10** to be present, but none of the *gem*-dimethyl product.

A solution of 0.6 g of crude **10** in 10 mL of CH_2Cl_2 was cooled to −78 °C and treated with 0.38 g of *m*-chloroperbenzoic acid. After 15 min, the solution was diluted with 100 mL of Et_2O and washed with 100 mL of 10% Na_2SO_3. The Et_2O solution was washed with 50 mL of saturated $NaHCO_3$ and 50 mL of H_2O, dried over $MgSO_4$, and evaporated to leave 0.6 g of an oil. The material was treated with hexane to afford **11** as white crystals, mp 84–87 °C. Anal. ($C_{16}H_{16}O_3S$) C, H.

When a solution of the sulfoxide **11** in DMF was heated at 110 °C for 1 h, it was quantitatively converted to methyl *p*-vinylbenzoate.

***p*-Carbomethoxybenzyl Phenyl Sulfone (12).** To a solution of 10.0 g (38.7 mmol) of the phenylthio ester **9** in 400 mL of 80% MeOH at 65 °C was added 35.7 g (66 mmol) of 50% potassium persulfate (Oxone, Alfa Products) in 5-g portions over 35 min. The mixture was heated at reflux for another 30 min, and the solvent was removed in vacuo. The solid was washed with H_2O and stirred with saturated $NaHCO_3$. The material was collected by filtration, washed with H_2O, and dried. Sublimation in vacuo (180 °C, 3 mm) afforded 9.2 g (82%) of white crystals, mp 170–172 °C. Recrystallization from MeOH gave an analytical sample, mp 177–179 °C. Anal. ($C_{15}H_{14}O_4S$) C, H, S.

α-Methyl-*p*-carbomethoxybenzyl Phenyl Sulfone (13). To a mixture of 0.48 g (10 mmol) of 50% NaH in oil and 100 mL of DMF was added 2.90 g (10 mmol) of the sulfone **12**. The mixture was stirred for 15 min at room temperature, and the resulting yellow solution was cooled to −20 °C and treated with 1.42 g (10 mmol) of methyl iodide. The solvent was removed in vacuo, and the residue was washed with H_2O. The material was dried to leave 2.8 g.

The NMR spectrum indicated the presence of some *gem*-dimethyl product. Recrystallization from toluene–hexane (1:1) gave an analytical sample of white crystals, mp 108.5–110 °C. Anal. ($C_{16}H_{16}O_4S$) C, H, S.

Methyl 4-Amino-4-deoxy-10-(phenylsulfonyl)-8,10-dideazapteroate (14). A solution of the anion derived from 0.16 g (0.55 mmol) of the sulfone **12** and NaH as described above in 5 mL of DMF was cooled to −50 °C, and 0.13 g (0.39 mmol) of the bromide **1** in 4 mL of DMF was added. The mixture was stirred at ambient temperature for 2 h and diluted with 1 mL of H_2O. The solvent was evaporated in vacuo, and the residue was taken up in 5 mL of $CHCl_3$–MeOH (6:1). After filtration the solution was chromatographed on silica gel to give 0.15 g of semisolid product: mass spectrum, m/e 463 (M^+); UV λ_{max}, at pH 11, 235, 270 (sh), 338 nm; NMR (Me_2SO-d_6) δ 3.70 (2 H, d, J = 8 Hz, C-9 H's), 3.80 (3 H, s, OCH_3), 5.80 (1 H, t, C-10 H), 6.15 (2 H, s, NH_2), 7.35–7.70 (13 H, m, Ar H, NH_2).

The 10-methyl analogue **15** was similarly obtained from **13**: mass spectrum, m/e 477 (M^+); NMR (Me_2SO-d_6) δ 1.80 (3 H, s, CH_3).

Acknowledgment. This work was supported by NIH Grants CA-28783 (J.I.D.) CA-18856 (F.M.S.), and CA-10914 (R.L.K.). A preliminary communication describing a portion of this work was presented at the Symposium on Pteridines and Folic Acid, St. Andrews, Scotland, September 1982.[8]

Registry No. 1, 76807-56-2; **2a**, 52787-14-1; **2b**, 77959-48-9; **2c**, 88392-91-0; **2d**, 88392-92-1; **3a**, 88392-93-2; **3b**, 88392-94-3; **3c**, 88392-95-4; **3d**, 88392-96-5; **4a**, 88392-97-6; **4b**, 88392-98-7; **4c**, 88392-99-8; **4d**, 88393-00-4; **5a**, 85325-88-8; **5b**, 88393-01-5; **5c**, 88393-02-6; **5d**, 88393-03-7; **6a**, 85345-35-3; **6b**, 88393-04-8; **6c**, 88393-05-9; **6d**, 88393-06-0; **7**, 6232-88-8; **8**, 88382-49-4; **9**, 88393-07-1; **10**, 84851-57-0; 11, 88393-08-2; **12**, 59584-27-9; **13**, 88393-09-3; **14**, 88393-10-6; **15**, 88393-11-7; diethyl L-glutamate hydrochloride, 1118-89-4; benzenethiol, 108-98-5; dihydrofolate reductase, 9002-03-3; thymidylate synthase, 9031-61-2.

(Imidazolylphenyl)formamidines. A Structurally Novel Class of Potent Histamine H_2 Receptor Antagonists†

Arturo Donetti,* Enzo Cereda, Elio Bellora, Alberto Gallazzi, Cesare Bazzano, Piercarlo Vanoni, Piero Del Soldato, Rosamaria Micheletti, Ferdinando Pagani, and Antonio Giachetti

Departments of Medicinal Chemistry, Physical Chemistry, and Pharmacology, Istituto De Angeli, Via Serio 15, I-20139 Milan, Italy. *Received June 27, 1983*

Structure–activity considerations of N^{α}-guanylhistamine, the first compound found with detectable H_2-antagonist activity, led to the synthesis of a series of conformationally rigid guanylhistamine analogues, namely, (imidazolylphenyl)guanidines, imidazolylbenzamidines, and (imidazolylphenyl)formamidines. It was found that in the guanidine and benzamidine classes, the meta-substituted derivatives (**3**, **4**, **7**, and **8**) possessed H_2-antagonist activity, whereas in the class of formamidines, only the para-substituted derivative **10** was found active. A subsequent increase in the size of the substituent at the formamidino group of **10** led to compounds (**15–20**) of high H_2-antagonist affinity, which was related to the gastric antisecretory effect. Members of this structurally novel class of H_2 antagonists were 20- to 50-fold more potent than cimetidine both "in vitro" and "in vivo". Structure–activity relationships are discussed in terms of ionization properties, partitioning behavior, conformational aspects of the selected compound **17**, and of possible modes of interaction with the histamine H_2 receptor. It was found that the formamidine moiety was an important structural feature and that H_2-antagonist activity requires correct steric and electronic properties. Compound **17** (DA 4577), owing to its pharmacological profile and demonstrated safety in animals, was selected to be clinically investigated.

The development of cimetidine as a histamine H_2 receptor antagonist represents an excellent example of reasoned approach to the design of an antagonist modeled on an agonist effector molecule.

The therapeutic success of cimetidine stimulated the search for new histamine H_2 receptor antagonists following the knowledge of antagonist requirements accumulated during the research process on this therapeutic agent.

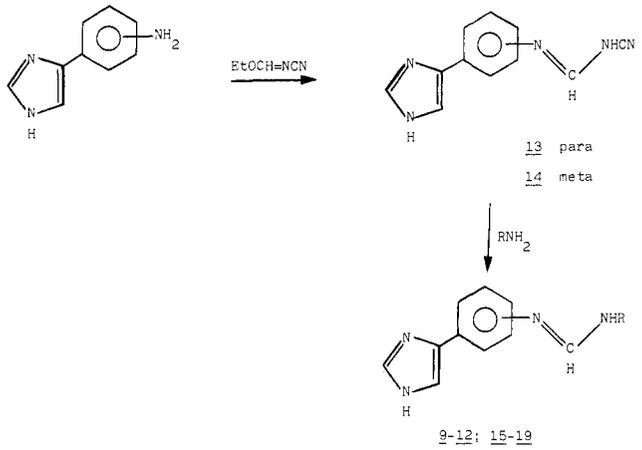
Ranitidine, tiotidine, etintidine, and oxmetidine can be considered as the first generation of H_2 antagonists. A second generation of newer compounds, e.g., SKF 93,479,[1] BL-6341A,[2] AH 22,216,[3] and YM 11,170,[4] claimed to be more potent or longer lasting than cimetidine, is now under study. Some of these compounds are nonimidazole structures, but all of them share structural features common to the prototype cimetidine molecule, i.e., a thiabutyl (or oxabutyl) side chain connecting a basic or basic substituted heteroaromatic or aromatic ring to a neutral moiety incorporating a 1,3 amidino system of NH groups.

This report describes a structurally novel class of potent H_2 receptor antagonists characterized by the presence of an amidino group positively charged at physiological pH (7.4), connected to an imidazole through a phenylene ring.

The importance of the basic amidino group and the involvement in receptor interaction of both the phenylene and the imidazole rings as reflected by H_2 antagonist activity in this series are also discussed.

Chemistry. The guanidine compounds (1–4, Table I) and the benzamidine compounds (**5–8**, Table I) were prepared by known procedures starting from the corresponding imidazolylanilines and imidazolylbenzonitriles,

Scheme I



respectively. The formamidines (**9–12** and **15–19**, Tables I and II) were prepared by reaction of the corresponding (imidazolylphenyl)cyanoformamidines (**13** and **14**, Scheme I) with the appropriate amine according to the route depicted in Scheme I. The formamidine derivative **20**, which could not be prepared by this route, was prepared by reacting 4(5)-(4-aminophenyl)-1*H*-imidazole with ethyl *N*-*tert*-butylformimidate tetrafluoroborate in dichloro-

† This paper has been presented in part. See: "Abstracts of Papers", 186th National Meeting of the American Chemical Society, Washington, DC, Sept 1, 1983; American Chemical Society: Washington, DC, 1983; Abstr MEDI 62.

(1) Blakemore, R. C.; Brown, T. H.; Durant, G. J.; Ganellin, C. R.; Parsons, M. E.; Rasmussen, A. C.; Rawlings, D. A. *Br. J. Pharmacol.* **1981**, *74*, 200 P.
(2) Algieri, A. A.; Luke, G. M.; Standridge, R. T.; Brown, M.; Partyka, R. A.; Crenshaw, R. R. *Abstr. Pap. Am. Chem. Soc.* **1981**, *181*, MEDI 142.
(3) Brittain, R. T.; Daly, M. J.; Humphray, J. M.; Stables, R.; Ware, U. K. *Br. J. Pharmacol.* **1982**, *76* (Suppl N 2), 195 P.
(4) Takeda, M.; Takagi, T.; Yashima, Y.; Maeno, H. *Arzneim.-Forsch.* **1982**, *32*, 734.

0022-2623/84/1827-0380$01.50/0