Synthesis and Antiallergy Activity of *N*-[2-(Dimethylamino)ethyl]-4-aryl-1-piperazinecarboxamide Derivatives

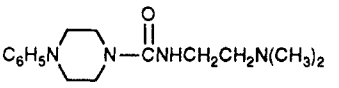
David A. Walsh,*,† Jerry B. Green,† Stephen K. Franzyshen,† Joseph C. Nolan,‡ and John M. Yanni‡

Departments of Chemical Research and Pharmacology, A. H. Robins Company, P.O. Box 26609, Richmond, Virginia 23261-6609. *Received December 20, 1989*

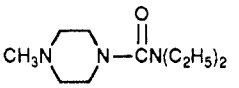
A series of *N*-[2-(dimethylamino)ethyl]-4-aryl-1-piperazinecarboxamides was synthesized and evaluated for antiallergy activity. Several derivatives had activity in the passive foot anaphylaxis (PFA) assay, an IgE-mediated model useful in the detection of compounds possessing antiallergic activity, but no derivative tested had activity at 10 mg/kg in the guinea pig anaphylaxis (GPA) assay. One analogue, *N*-[2-(dimethylamino)ethyl]-4-(4-fluorophenyl)-1-piperazinecarboxamide, had an IC_{50} = 310 nM for inhibition of tritiated mepyramine binding to H_1 histaminic receptors isolated from guinea pig cerebral cortex.

As part of a larger program to discover novel, potent, orally active drugs for the treatment of allergic disorders, file compounds were selected for testing in the passive foot anaphylaxis (PFA) assay in rats on the basis of their structural similarities to compounds already known to possess antiallergic properties. The PFA assay is useful as a primary screen for detecting compounds having antiallergic activity since it has been shown to be IgE mediated.[1] Compound **3**, one of the initial leads, has structural features in common with drugs such as diethylcarbamazine, reported[2] to be effective orally for the relief of asthmatic symptoms, and it also possesses a (dimethylamino)ethyl moiety, characteristic of many antihistamines (Chart I). Antiallergic activity was found for **3** at 31.6 mg/kg in the PFA assay (Table I), and a synthetic project was undertaken in an attempt to increase potency in this chemical series.

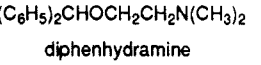
Chart I



3

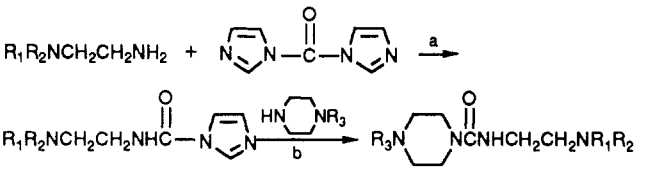


diethylcarbamazine



diphenhydramine

Scheme I[a]



R_1, R_2 = alkyl, aryl, aliphatic ring, heterocyclic ring
R_3 = aryl, benzyl, benzhydryl, benzoyl

[a] a. Tetrahydrofuran (THF), ambient temperature, 1.5 h; b. THF, Δ, 18 h.

Chemistry

Most of the compounds listed in Tables I–III were readily prepared in moderate yield by the coupling of a (dialkylamino)ethylamine derivative and a substituted piperazine using 1,1′-carbonyldiimidazole as depicted in Scheme I. All starting amines could be purchased or prepared by literature procedures.[3]

Results and Discussion

Table I shows the structure–activity data for compounds in which the structure of **3** was substantially modified. Simple urea derivatives of 1-phenylpiperazine, **1** and **2**, were inactive at 31.6 mg/kg, but when a (dimethylamino)ethyl group was added to the urea as in **3**, activity was observed at 31.6 mg/kg. Increasing the distance to three carbons (**4**) between nitrogen atoms destroyed activity; adding an additional methyl to the urea nitrogen (**5**) decreased activity. Benzyl (**6**), benzhydryl (**7**), and benzoyl (**8**) substituents attached to the basic nitrogen of the piperazine ring also rendered these compounds inactive. Opening the piperazine ring of **3** to give **9** resulted in a loss of activity in the PFA at 31.6 mg/kg. Table I shows that the only compound which has a potency comparable to that of **3** is **5**.

Compounds in which the (dimethylamino)ethylamino portion of **3** was varied are listed in Table II. Analogues **10–14**, where the dimethyl substituents of **3** were modified with other alkyl groups, all retained activity comparable to that of **3** at 31.6 mg/kg. Addition of a benzyl (**16**) substituent on the nitrogen resulted in loss of activity. Although including the basic nitrogen in a ring system (**11–14**) did not affect potency, adding an additional heteroatom such as oxygen (**17**, **18**) or nitrogen (**19–21**) to the ring did result in a loss of activity. The tertiary ureas **22** and **23** did not show any activity at 31.6 mg/kg. The results in Table II indicate that **3**, containing the dimethyl substituent, was equivalent in potency to compounds **10–14** containing other dialkyl substituents.

Table III lists PFA data for derivatives of **3** in which substituents on the aromatic ring were varied. The pyridyl derivative (**24**) was equipotent with **3**, but potency was increased when a 4-fluoro (**27**) or a 4-chloro (**30**) group was placed on the phenyl ring of **3**. Both of these derivatives showed activity at 10 mg/kg in the PFA assay. Other substituents gave compounds which were equipotent (**28**, **29**, **33–35**) or less potent (**25**, **26**, **31**, **32**, **36–47**) than **3**.

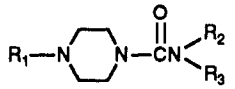
Secondary antiallergy testing was done in a classical model of immediate hypersensitivity, the guinea pig anaphylaxis (GPA) assay. Compounds **27** and **30**, derivatives of **3** which showed the greatest potency in the PFA assay, as well as diethylcarbamazine and diphenhydramine, were compared with aminophylline (Table IV). Only aminophylline showed significant antiallergic activity in the GPA model. It is not surprising that diphenhydramine had no activity in the GPA assay since the immediate hypersensitivity reaction involves the release of a variety of me-

† Department of Chemical Research.
‡ Department of Pharmacology.

(1) Martel, R. R.; Klicius, J. *Int. Arch. Allergy Appl. Immunol.* **1977**, *54*, 205–209.
(2) Salazar-Mallen, M. *Ann. Allergy* **1965**, *23*, 534–537.
(3) Jain, P. C.; Kapoor, V.; Anand, N.; Ahmad, A.; Patnaik, G. K. *J. Med. Chem.* **1967**, *10*, 812–818.

0022-2623/90/1833-2028$02.50/0

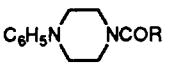
Table I. Oral Antiallergy Activity (1-h Pretreatment) for Various Amines in the Passive Foot Anaphylaxis (PFA) Assay

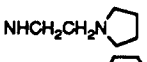
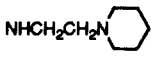
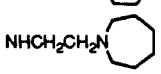
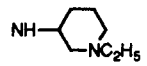
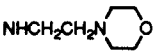
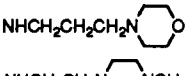
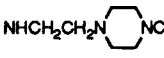
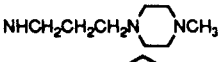
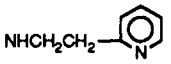
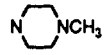
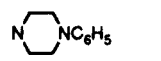


no.	R_1	R_2	R_3	formula[a]	mp, °C (solv)[b]	% yield	PFA[c] 31.6 mg/kg	PFA[c] 10 mg/kg
1[d]	C_6H_5	H	H	$C_{11}H_{15}N_3O$	151–153 (k)	50	10[e]/–74	
2[d]	C_6H_5	CH_3	CH_3	$C_{13}H_{19}N_3O$	62–63 (l)	32	–24[e]/–71	
3	C_6H_5	H	$CH_2CH_2N(CH_3)_2$	$C_{15}H_{24}N_4O$	88–90 (m)	43	–68/–90	–22[e]/–69
4	C_6H_5	H	$CH_2CH_2CH_2N(CH_3)_2$	$C_{16}H_{26}N_4O$	91–95 (n)	57	–13[e]/–74	
5[d]	C_6H_5	CH_3	$CH_2CH_2N(CH_3)_2$	$C_{16}H_{26}N_4O{\cdot}2HCl$	173–176 (op)	42	–38/–65	
6	$C_6H_5CH_2$	H	$CH_2CH_2N(CH_3)_2$	$C_{16}H_{26}N_4O$	72.5–74.5 (m)	30	–10[e]/–73	
7	$(C_6H_5)_2CH$[f]	H	$CH_2CH_2N(CH_3)_2$	$C_{22}H_{30}N_4O$	133–134 (m)	67	–30[e]/–66	
8	C_6H_5CO[g]	H	$CH_2CH_2N(CH_3)_2$	$C_{16}H_{24}N_4O_2{\cdot}C_2H_2O_4$[h]	143–144 (q)	60	–4[e]/–71	
9	$C_6H_5N(CH_3)CH_2CH_2N(CH_3)CONHCH_2CH_2N(CH_3)_2$[i]			$C_{15}H_{26}N_4O$[j]	oil	19	–23[e]/–79	

[a] All compounds wre analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values except as noted. [b] k = 2-propanol, l = petroleum ether (60–110 °C), m = isopropyl ether, n = cyclohexane, o = methanol, p = ethyl ether, q = absolute ethanol. [c] Percent change in volume of foot edema of test compound/percent change in volume of foot edema of positive control (aminophylline orally at 100 mg/kg). [d] Preparation described in Experimental Section. [e] Not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test. [f] Reference 10. [g] Reference 11. [h] Oxalate. [i] Reference 12. [j] C: Calcd, 64.72; found, 65.17.

Table II. Oral Antiallergy Activity (1-h Pretreatment) for *N*-Phenylpiperazine Derivatives in the Passive Foot Anaphylaxis (PFA) Assay



no.	R[a]	formula[b]	mp, °C (solv)[c]	% yield	PFA[d] 31.6 mg/kg
3	$NHCH_2CH_2N(CH_3)_2$	$C_{15}H_{24}N_4O$	88–90 (k)	43	–68/–90
10	$NHCH_2CH_2N(C_2H_5)_2$	$C_{17}H_{28}N_4O{\cdot}C_2H_2O_4{\cdot}H_2O$[f,g]	105–108 (l)	39	–47/–60
11		$C_{17}H_{26}N_4O$	81.5–84 (k)	59	–45/–68
12		$C_{18}H_{28}N_4O{\cdot}0.25H_2O$[g]	64–67 (m)	24	–49/–68
13		$C_{19}H_{30}N_4O{\cdot}2HCl$	190–193 (n)	53	–47/–76
14		$C_{18}H_{28}N_4O$	100–103 (k)	65	–58/–81
15	$NHCH_2CH_2N(CH_3)C_6H_5$	$C_{20}H_{26}N_4O{\cdot}2HCl{\cdot}0.5H_2O$[g]	157–160 (o)	21	–41/–63
16	$NHCH_2CH_2N(CH_3)CH_2C_6H_5$	$C_{21}H_{28}N_4O$	152–155 (p)	67	–35[e]/–50
17		$C_{17}H_{26}N_4O_2$	118–120 (m)	22	+10[e]/–74
18		$C_{18}H_{28}N_4O_2$	119–122 (n)	47	–28[e]/–69
19		$C_{18}H_{29}N_5O{\cdot}2C_2H_2O_4$[f]	195–197 (o,q)	37	–7[e]/–63
20		$C_{19}H_{31}N_5O$	124–125 (r,s)	57	–23[e]/–81
21		$C_{18}H_{22}N_4O$	97–99 (r,s)	65	–3[e]/–74
22[h]		$C_{16}H_{24}N_4O{\cdot}2HCl$	244–247 (k,n)	58	–7[e]/–68
23[h]		$C_{21}H_{26}N_4O$	171–172 (r,t)	12	–10[e]/–69

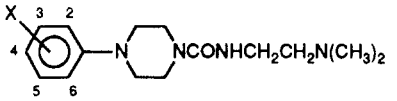
[a] All starting amines are commercially available. [b] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values. [c] k = isopropyl ether, l = acetone, m = ethyl ether, n = 2-propanol, o = methanol, p = toluene, q = water, r = cyclohexane, s = benzene, t = ethanol. [d] Percent change in volume of foot edema of test compound/percent change in volume of foot edema of positive control (aminophylline orally at 100 mg/kg). [e] Not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test. [f] Oxalate. [g] ^1H NMR confirms amount of water present. [h] Preparation described in Experimental Section.

diators in addition to histamine. However, antihistamines are known to be false positives in the PFA assay.[4] In order to assess the intrinsic H_1 antihistaminic activity of **27**, it was compared with diphenhydramine for inhibition of tritiated mepyramine binding to H_1 histamine receptors isolated from guinea pig cerebral cortex. Compound **27** (IC_{50} = 310 nM) was approximately equivalent to diphenhydramine (IC_{50} = 129 nM) as an inhibitor in this assay. The H_1 antihistaminic activity of **27** could account for the effects of this series of compounds in the PFA assay.

Further work on this chemical series as antiallergic agents[5] has been suspended in favor of other series[6] that

(4) Yanni, J. M.; Sancilio, L. F. *FASEB J.* **1981**, *40/311*, No. 4510, 1026.

Table III. Oral Antiallergy Activity (1-h Pretreatment) in the Passive Foot Anaphylaxis Assay for *N*-[2-(Dimethylamino)ethyl]-4-aryl-1-piperazinecarboxamide Derivatives



no.	X[a]	formula[b]	mp, °C (solv)[c]	% yield	PFA[d] 31.6 mg/kg	PFA[d] 10 mg/kg	PFA[d] 3.16 mg/kg
3	H	$C_{15}H_{24}N_4O$	88–90 (k)	43	−49/−53	−29/−69	
24	2-N[e]	$C_{14}H_{23}N_5O\cdot 2HCl$	208–210 (l)	11	−54/−65	−13[f]/−69	
25	2-F	$C_{15}H_{23}FN_4O\cdot 2HCl$	153–155 (m,n)	46	−37/−68		
26	3-F	$C_{15}H_{23}FN_4O\cdot 2HCl$	200–203 (m,n)	50	−31/−64		
27	4-F	$C_{15}H_{23}FN_4O$	99–101 (lo)	68	−87/−67	−63/−76	−35[f]/−84
28	2-Cl	$C_{15}H_{23}ClN_4O\cdot HCl$	185–188 (m,n)	33	−53/−65	−16[f]/−69	
29	3-Cl	$C_{15}H_{23}ClN_4O$	65–67	41	−78/−67	−32[f]/−77	
30	4-Cl	$C_{15}H_{23}ClN_4O$	125–128 (k,l)	66	−61/−65	−48/−62	+27[f]/−62
31	4-Br	$C_{15}H_{23}BrN_4O$	133–135	84	−31/−61		
32	2-OCH_3	$C_{16}H_{26}N_4O_2$	98–100 (pq)	49	−21[f]/−74		
33	3-OCH_3	$C_{16}H_{26}N_4O_2$	70–72 (r,s)	51	−67/−65	−36[f]/−62	
34	4-OCH_3	$C_{16}H_{26}N_4O_2$	78–82 (r,s)	24	−49/−65	−29[f]/−69	
35	4-CH_3	$C_{16}H_{26}N_4O$	85–88	47	−46/−65		
36	3-CF_3	$C_{16}H_{23}F_3N_4O\cdot 2HCl$	205–208 (m)	47	−18[f]/−62		
37	4-CN	$C_{16}H_{23}N_5O\cdot 2HCl$	161–164 (m,n)	20	−31/−61		
38	4-$CONH_2$	$C_{16}H_{25}N_5O_2$	193–195 (p,t)	20	0[f]/−80		
39	4-$CO_2C_2H_5$	$C_{18}H_{28}N_4O_3\cdot 2HCl$	179–182 (m,n)	71	−5[f]/−78		
40	4-$COCH_3$	$C_{17}H_{26}N_4O_2\cdot HCl$	221–223 (l,u)	19	−25[f]/−71		
41	4-NO_2	$C_{15}H_{23}N_5O_3$	115–120 (p)	54	−26[f]/−58		
42[g]	4-NH_2	$C_{15}H_{25}N_5O\cdot 3HCl$	114–116 (m,u)	20	−22[f]/−73		
43[g]	4-$N(CH_3)_2$	$C_{17}H_{29}N_5O\cdot 3HCl$	221–223 (m,n)	18	−29[f]/−77		
44	4-$NHCOCH_3$	$C_{17}H_{27}N_5O_2\cdot 2HCl$	216–217 (l)	6	+5[f]/−74		
45	3,4-Cl_2	$C_{15}H_{22}Cl_2N_4O$	80–83	75	−17[f]/−50		
46	3,4-$(OCH_3)_2$	$C_{17}H_{28}N_4O_3\cdot 2HCl$	208–210 (m,n)	57	−16[f]/−63		
47	3,4,5-$(OCH_3)_3$	$C_{18}H_{30}N_4O_4\cdot 2HCl$	205–208 (m,n)	56	+9[f]/−62		

[a] All arylpiperazine starting materials were commercially available or are known in the literature. [b] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values. [c] k = isopropyl ether, l = 2-propanol, m = methanol, n = ethyl ether, o = hexane, p = benzene, q = petroleum ether (30–60 °C), r = cyclohexane, s = benzene, t = ethanol, u = water. [d] Percent change in volume of foot edema of test compound/percent change in volume of foot edema of positive control (aminophylline orally at 100 mg/kg). [e] 2-Pyridyl. [f] Not significantly different from negative control group at $p < 0.05$ as determined by the Dunnett's t test. [g] Preparation described in Experimental Section.

Table IV. Oral Antiallergy Activity (1-h Pretreatment) of Selected Compounds in the Guinea Pig Anaphylaxis (GPA) Assay

no.	dose, mg/kg	GPA[a]
aminophylline	31.6	4/6
diphenhydramine	31.6	1/4
diethylcarbamazine	200	0/3
27	10	1/4
30	31.6	0/4

[a] Number of guinea pigs protected from collapse/number of guinea pigs challenged with antigen.

have shown greater therapeutic potential.

Experimental Section

Pharmacology. A. Primary Antiallergy Screen. A passive foot anaphylaxis model[1] in rats was used as the primary test for antiallergy activity. Fed, male, Sprague–Dawley rats were injected in the right hind paw with 0.2 mL of rat anti egg albumin serum at a dilution previously shown to produce significant edema upon antigen challenge. The animals were then fasted but allowed water ad libitum. The next day they were randomized into groups of six by means of tables generated by the IBM scrambler. Random-number tables were also used to determine the groups receiving the control, reference, and test compounds.

On the test day the right foot volume of each rat was determined plethysmographically; the hairline was used as the reference point. The volume of the foot was measured with a mercury filled tube that was connected to a P23A Grass pressure transducer that in turn was connected to a linear Cole-Parmer recorder (Model 255). The instrument was adjusted so that a pen deflection of 25 mm was equivalent to a 1-mL volume.

The reference, test, and control compounds were dissolved or suspended in 0.5% Tween 80 in distilled water. Sonification was used to facilitate solubilization or reduction in particle size. The animals were dosed orally (10 mL/kg) by gavage 1 h prior to the intravenous injection of the antigen: 2 mg of egg albumin in 0.2 mL of sterile saline. Thirty minutes later the foot volume of the right foot was measured again, and edema was determined by difference. Results were expressed as the average foot edema (mL) ± SD. A significant decrease ($p < 0.05$) in the edema of the treated group from that of the control group was considered as indicative of antiallergic activity. The results were acceptable only if the group receiving the reference compound showed a significant decrease in foot edema. The data were analyzed with the Dunnett's t test that compares several treated groups with a control group. Differences between groups were determined by the studentized range test. Regression analysis was used to determine relative potency.

B. Secondary Antiallergy Screen. Secondary antiallergy testing of selected compounds was done in the guinea pig anaphylaxis (GPA) model. Guinea pigs were actively sensitized to egg albumin (EA, Sigma Chemical Co., St. Louis, MO) at least 20 days prior to aerosol challenge by injecting 0.5 mL of EA-$AL(OH)_3$ conjugate (33 μg of EA/mL) intramuscularly in each hind leg.

On the test day, fasted, sensitized, Dunkin–Hartley guinea pigs were randomized by using random-number tables generated by an IBM scrambler into control ($N = 8$) and test ($N = 4$) groups. The control group was always labeled group 1. The order in which compounds were administered to the other groups was also determined by random-number tables from the IBM scrambler.

The reference (theophylline at 100 mg/kg, po), test, and control compounds were dissolved or suspended in 0.5% Tween 80 in distilled water, and the concentration was adjusted so that each animal received 10 mL/kg. Compounds were administered by

(5) Walsh, D. A.; Cale, A. D., Jr. Eur. Patent Appl. EP 277 725, 1988; *Chem. Abstr.* **1988**, *109*, 231074n.
(6) Walsh, D. A.; Franzyshen, S. K.; Yanni, J. M. *J. Med. Chem.* **1989**, *32*, 105–118.

gavage with syringes having rubber catheters attached to their tip.

At a specified time (1–24 h) following the administration of the test, reference, or control compound, each animal was placed in an aerosolization chamber. A 1% solution (w/v) of EA was aerosolized at a flow rate of 10 L of air/min into the chamber for a maximum of 5 min. An electronic timer was started when the aerosolization began. The anaphylactic response consisted of coughing, dyspnea, reeling, collapse, and death. Upon collapsing, the animals were removed from the chamber. The chamber was then flooded with air before the next animal was placed inside. Animals were considered protected if they did not collapse within 5 min of exposure to the aerosolized antigen. The number of animals that collapsed in each group was recorded. PD_{50} for collapse was calculated by the method of Litchfield and Wilcoxon[7] for evaluation of dose–effect experiments.

Comparisons of PD_{50}s from different experimental trials and determinations of relative potency are determined by the Litchfield and Wilcoxon method. The following conditions were met before an experiment was acceptable: (1) The control group showed collapse in 7/8 or 8/8 animals. (2) The theophylline reference group showed protection in 3/4 or 4/4 animals.

A compound was judged active if it showed protection in 3/4 or 4/4 animals.

The decision to accept a compound for further study was based on the Fisher's Exact 2 × 2 test. The χ^2 test was used to test similarity between control groups to allow pooling of data.

C. Tritiated Mepyramine Binding to H_1 Histamine Receptors in Guinea Pig Cerebral Cortex. 1. Preparation of Cerebral Cortical Membrane. The procedure was a modification of procedures reported by Wallace and Young[8] and Chang et al.[9] Dunkin–Hartley guinea pigs were killed by decapitation, and the cerebral cortex was quickly removed and weighed. The cerebral cortex was placed in 30 volumes of cold 50 mM sodium potassium phosphate (pH 7.5) buffer and then processed in a polytron homogenizer for 30 s at a power setting of 6. The homogenate was centrifuged at 48000*g* for 10 min at 4 °C, and the resultant pellet was resuspended in 30 volumes of fresh buffer. The centrifugation and suspension procedure was repeated twice, and the final pellet was suspended in 30 mL of buffer per g of wet tissue.

2. Procedure for Ligand-Binding Assay. Test and reference compounds were dissolved in buffer or the appropriate vehicles at a concentration of 1×10^{-3} M. The assay mixtures consisted of 100 μL of 15 nM tritiated mepyramine (1.5 nM final concentration); 100 μL of test, control, or reference compound; and 800 μL of membrane preparation for a total volume of 1.0 mL. The assay mixture was incubated for 20 min at 25 °C. The reaction was stopped when the assay mixture was washed (3 × 5 mL) with cold buffer and filtered through GF/B glass-fiber filters. The filters were then transferred to vials; scintillation cocktail was added; and the radioactivity in each vial was determined by liquid scintillation counting.

For the determination of an IC_{50} value, six concentrations (1×10^{-5} to 1×10^{-10} M) of the various compounds were tested in triplicate. An IC_{50} value was calculated by means of regression analysis of the logits of the percent of control binding vs the log of the molar compound concentration.

General Procedures. Melting points were determined in open capillary tubes in a Thomas-Hoover melting point apparatus and are uncorrected; ^1H NMR spectra were obtained in $CDCl_3$ or Me_2SO-d_6 with Me_4Si as internal standard on a Varian A-60 or Varian EM-360L spectrometer; ^{13}C NMR spectra were obtained in the same solvents on a Varian FT-80A spectrometer; mass spectra were determined on a Varian MAT-44 mass spectrometer; IR spectra were run as KBr pellets on a Beckman IR8 or Perkin-Elmer 297 IR spectrophotometer. Spectral data for all reported compounds were consistent with assigned structures. Purification was done by column chromatography on silica gel or Florisil and by high-pressure liquid chromatography with use of a Waters Prep LC-500A apparatus with a PrepPAK-500 silica cartidge. Analytical results for compounds followed by elemental symbols are within ±0.4% of theory and were determined on a Perkin-Elmer Model 240 CHN analyzer or on a Control Equipment Corporation 240-XHA CHN analyzer. Diethylcarbamazine was obtained from the American Cyanamid Co. Diphenhydramine was purchased from Aldrich Chemical Co.

General Procedure for the Preparation of *N*-[2-(Dialkylamino)ethyl]-4-aryl-1-piperazinecarboxamide Derivatives (3, 4, 6–21, 24–41, 44–47). To a solution of 5.7 g (0.035 mol) of 1,1′-carbonyldiimidazole in 75 mL of THF was added a solution of 0.034 mol of a (dialkylamino)alkylamine in 75 mL of THF, and the reaction mixture was stirred at ambient temperature for 1.5 h. A solution of 0.03 mol of a 1-arylpiperazine derivative in 50 mL of THF was then added, and the reaction mixture was heated at reflux for 18 h. The solution was concentrated, and the residue was dissolved in 150 mL of C_6H_6. The solution was washed three times with 50-mL portions of H_2O and once with brine, dried (Na_2SO_4), and concentrated. If the residue was a solid, it was triturated with petroleum ether (30–60 °C), collected by filtration, recrystallized, and characterized. If the residue was an oil, it was converted to a crystalline salt, and then this solid was recrystallized and characterized.

4-Phenyl-1-piperazinecarboxamide (1). A mixture of 8.1 g (0.05 mol) of 1-phenylpiperazine and 7.9 g (0.075 mol) of nitrourea in 200 mL of acetone was heated at reflux for 16 h. The mixture was concentrated under reduced pressure, and the solid residue was dissolved in 150 mL of CH_2Cl_2. The solution was washed twice with water and once with brine, dried (Na_2SO_4), and concentrated. The residue was recrystallized from 2-propanol to yield 5.1 g (50%) of **1** as a white powder, mp 151–153 °C. Anal. ($C_{11}H_{15}N_3O$) C, H, N.

***N*,*N*-Dimethyl-4-phenyl-1-piperazinecarboxamide (2).** To a solution of 8.1 g (0.05 mol) of 1-phenylpiperazine in 150 mL of C_6H_6 were added 6 mL of pyridine and 5.4 g (0.05 mol) of dimethylcarbamoyl chloride, and the mixture was heated at reflux overnight. The mixture was cooled, washed twice with H_2O and once with brine, dried (Na_2SO_4), and concentrated. The gummy solid was recrystallized from petroleum ether (60–100 °C) to yield 3.7 g (32%) of **2** as a white powder, mp 62–63 °C. Anal. ($C_{13}H_{19}N_3O$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-*N*-methyl-4-phenyl-1-piperazinecarboxamide Dihydrochloride (5).** A solution of 3.0 g (0.03 mol) of phosgene and 9.1 g (0.09 mol) of triethylamine in 50 mL of CH_2Cl_2 was treated dropwise with a solution of 4.9 g (0.03 mol) of 1-phenylpiperazine in 50 mL of CH_2Cl_2, and the mixture was stirred at ambient temperature for 4 h. A solution of 3.1 g (0.03 mol) of *N*,*N*,*N*-trimethylethylenediamine in 50 mL of CH_2Cl_2 was then added dropwise, and the mixture was stirred at ambient temperature for 16 h. The mixture was washed successively once with H_2O, three times with a 25% K_2CO_3 solution, once with brine, dried ($MgSO_4$), and concentrated. The oily residue was dissolved in ethyl ether and treated with ethereal HCl. The solid which precipitated was collected by filtration and recrystallized from $CH_3OH/(C_2H_5)_2O$ to yield 3.7 g (42%) of **5** as a white solid, mp 173–176 °C. Anal. ($C_{16}H_{26}N_4O \cdot 2HCl$) C, H, N.

1-[(4-Methyl-1-piperazinyl)carbonyl]-4-phenylpiperazine Dihydrochloride (22). According to the above procedure, 11.8 g (0.12 mol) of phosgene, 37.4 g (0.37 mol) of triethylamine, 20.0 g (0.12 mol) of 1-phenylpiperazine, and 12.4 g (0.12 mol) of 1-methylpiperazine in a total of 550 mL of CH_2Cl_2 gave 20.6 g (58%) of **22** as a white solid, mp 244–247 °C (2-propanol/isopropyl ether). Anal. ($C_{16}H_{24}N_4O \cdot 2HCl$) C, H, N.

1,1′-Carbonylbis-4-phenylpiperazine (23). According to the above procedure, 5.9 g (0.06 mol) of phosgene, 18.0 g (0.18 mol) of triethylamine, and 20.0 g (0.12 mol) of 1-phenylpiperazine in a total of 300 mL of CH_2Cl_2 gave 2.5 g (12%) of **23** as a white solid, mp 171–172 °C (cyclohexane/ethanol). Anal. ($C_{21}H_{26}N_4O$) C, H, N.

(7) Litchfield, J. T., Jr.; Wilcoxon, F. J. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.
(8) Wallace, R. M.; Young, J. M. *Mol. Pharmacol.* **1983**, *23*, 60–66.
(9) Chang, R. S. L.; Tran, V. T.; Snyder, S. H. *Eur. J. Pharmacol.* **1978**, *48*, 463–464.
(10) Hamlin, K. E.; Weston, A. W.; Fischer, F. E.; Michaels, R. J., Jr. *J. Am. Chem. Soc.* **1949**, *71*, 2731–2734.
(11) Cymerman-Craig, J.; Rogers, W. P.; Tate, M. E. *Aust. J. Chem.* **1956**, *9*, 397–405.
(12) Mull, R. P.; Tannenbaum, C.; Dapero, M. R.; Bernier, M.; Yost, W.; DeStevens, G. *J. Med. Chem.* **1965**, *8*, 332–338.

4-(4-Aminophenyl)-*N*-[2-(dimethylamino)ethyl]-1-piperazinecarboxamide Trihydrochloride (42). A solution of 8.0 g (0.02 mol) of **41** in 150 mL of CH_3OH was hydrogenated in a Parr apparatus over 0.25 tsp of 5% Pd/C for 1 h. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was dissolved in ethyl ether and treated with ethereal HCl. The resulting solid was collected by filtration and recrystallized from CH_3OH/H_2O to yield 2.0 g (20%) of **42** as a white solid, mp 114–116 °C. Anal. ($C_{15}H_{25}N_5O\cdot3HCl$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-4-[4-(dimethylamino)-phenyl]-1-piperazinecarboxamide Trihydrochloride (43).** A solution of 1.7 g (0.005 mol) of **41** and 0.80 mL (0.01 mol) of 37% formalin solution in 200 mL of CH_3OH was hydrogenated in a Parr apparatus over 0.25 tsp of 5% Pd/C until H_2 uptake ceased. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was dissolved in ethyl ether and treated with ethereal HCl. The resulting solid was collected by filtration and recrystallized from $CH_3OH/(C_2H_5)_2O$ to yield 0.4 g (18%) of **43** as a white solid, mp 221–223 °C. Anal. ($C_{17}H_{23}N_5O\cdot3HCl$) C, H, N.

Acknowledgment. We thank Dr. Larry W. Jaques, Ashby F. Johnson, John B. Forehand, Joanne E. Anderson, and Malcolm D. Stone who determined the spectral and analytical data; Marianne H. Foxwell and Laura L. Whitman provided technical assistance in conducting the pharmacological assays; Dr. Brian F. Kilpatrick and Robin D. Tabor determined the H_1 histamine binding data; Drs. William J. Welstead, Jr. and Mark C. Sleevi kindly proofread the manuscript.

Porphyrin Dimers as Photosensitizers in Photodynamic Therapy

Ravindra K. Pandey,* Kevin M. Smith,† and Thomas J. Dougherty‡

Oncologic Foundation of Buffalo, 225 Oak Street, Suite 200, Buffalo, New York 14203, Department of Chemistry, University of California, Davis, California 95616, and Division of Radiation Biology, Department of Radiation Medicine, Roswell Park Memorial Institute, Buffalo, New York 14263. *Received July 27, 1989*

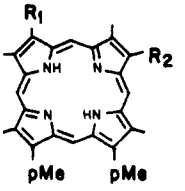
Porphyrin dimers **9** with ether linkages and possible isomers bis[1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphin-4-yl]ethyl] ether (**10**) bis[1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-4-vinylporphin-2-yl]ethyl] ether (**11**), and 1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphin-4-yl]ethyl 1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-4-vinylporphin-2-yl]ethyl ether (**12**) were synthesized from the corresponding (1-hydroxyethyl)vinyldeuteroporphyrin IX dimethyl esters (Hvd). The pure Hvd isomers 2-(1-hydroxyethyl)-4-vinyldeuteroporphyrin IX dimethyl ester (**7**) and 4-(1-hydroxyethyl)-2-vinyldeuteroporphyrin IX dimethyl ester (**8**) were obtained from 2-acetyl-4-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**3**) and 4-acetyl-2-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**4**). Porphyrins **3** and **4** were prepared either by partial reduction of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**2**) or by oxidation of hematoporphyrin IX dimethyl ester (**1**) by using tetra-*n*-propylammonium perruthenate (Pr^n_4N)(RuO_4) with *N*-methylmorpholine *N*-oxide as an oxidizing agent. The in vivo photosensitizing ability and therapeutic ratios of dimers **9–12** were compared with that of Photofrin II in the SMT-F tumor growing subcutaneously in DBA/2 Ha mice. These dimers were found to have better tumorcidal activity than Photofrin II with reduced skin phototoxicity.

Introduction

Photodynamic therapy (PDT) is a new procedure for the treatment of various types of malignant tumors and involves local photochemical activation following accumulation of the photosensitizers in the tumors.[1,2] Currently, Photofrin II (Quadralogic Technology, Vancouver, Canada, stored <–20 °C) enriched in the active components of hematoporphyrin derivative (Hpd) has been used worldwide for tumor photosensitization and more than 4000 patients have been treated so far. Upon light activation, generally delivered from lasers via fiber optics, the sensitizers generate singlet oxygen, which is apparently the cytotoxic agent, causing both vascular damage and injury to tumor cells.[3] Photofrin II is currently in phase III clinical trials for treatment of obstructive endobronchial tumors, tumors of the esophagus, and superficial bladder tumors.

Hematoporphyrin derivative is prepared in two steps by following Lipson's procedure,[4] as modified by Dougherty.[1] Dougherty et al.[5] isolated the active fraction in Hpd by gel-exclusion chromatography representing approximately 45% of the total mixture. This material was found to be responsible for the tumor-photosensitizing ability, was free from most of the monomers, and also appeared to provide a higher therapeutic ratio (tumor vs skin) than the Hpd mixture. Commercially available Photofrin II is chemically similar to the gel-purified Hpd by HPLC.

Chart I



1. $R_1 = R_2 = -CH(OH)CH_3$
2. $R_1 = R_2 = -COCH_3$
3. $R_1 = COCH_3$, $R_2 = -CH(OH)CH_3$
4. $R_1 = -CH(OH)CH_3$, $R_2 = -COCH_3$
5. $R_1 = -COCH_3$, $R_2 = -CH=CH_2$
6. $R_1 = -CH=CH_2$, $R_2 = -COCH_3$
7. $R_1 = -CH(OH)CH_3$, $R_2 = -CH=CH_2$
8. $R_1 = -CH=CH_2$, $R_2 = -CH(OH)CH_3$

pMe = $-CH_2CH_2CO_2CH_3$

* Address correspondence to Oncologic Foundation of Buffalo.
† University of California.
‡ Roswell Park Memorial Institute.

(1) Dougherty, T. J.; Kaufman, J. H.; Goldfarb, A.; Weishaupt, K. R.; Boyle, D.; Mittleman, A. *Cancer Res.* **1976**, *38*, 2628.
(2) Dougherty, T. J. *CRC Crit. Rev. Oncol. Hematol.* **1984**, *2*, 83.
(3) Weishaupt, K. R.; Gomer, C. J.; Dougherty, T. J., *Cancer Res.* **1976**, *36*, 2326.
(4) Lipson, R. L.; Baldes, E. J.; Olsen, A. M. *J. Natl. Cancer Inst.* **1961**, *26*, 1.

0022-2623/90/1833-2032$02.50/0