N-Benzylpiperazino Derivatives of 3-Nitro-4-hydroxycoumarin with H_1 Antihistamine and Mast Cell Stabilizing Properties[1]

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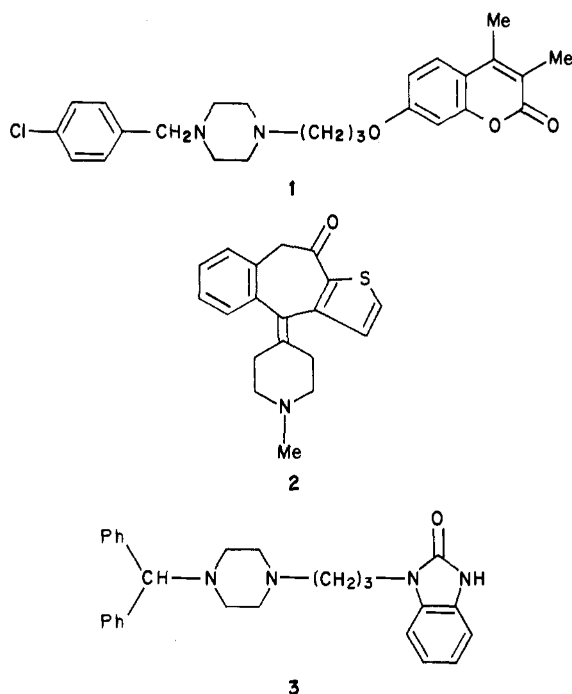
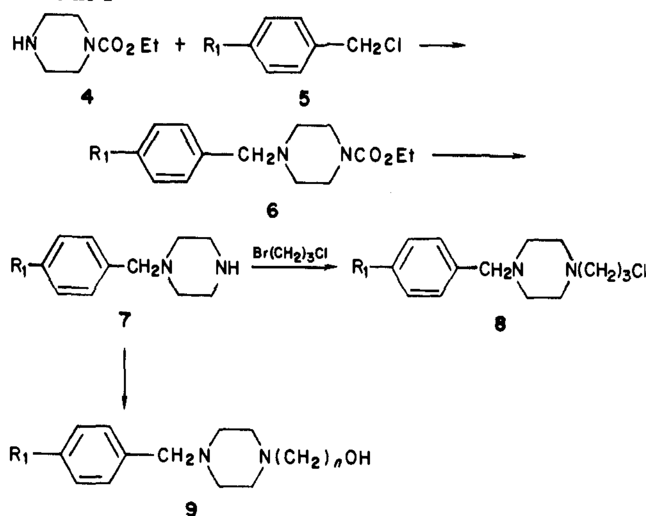
In a small range finding study a number of N-benzylpiperazino derivatives of 3-nitro-4-hydroxycoumarin have been shown to combine potent H_1-antihistamine activity with that of mast cell stabilization as demonstrated by their activity as antagonists of histamine on guinea pig ileum and by their inhibition of the release of histamine in rat passive peritoneal anaphylaxis (PPA). The most potent compound, 1-[2-hydroxy-3-[(4-hydroxy-3-nitrocoumarin-7-yl)oxy]propyl]-4-(4-chlorobenzyl)piperazine, **30**, had a pA_2 of 9.0 against histamine on guinea pig ileum and inhibited histamine release in the rat PPA test with a potency similar to that of disodium cromoglycate.

Histamine is implicated as a mediator of some of the symptoms of allergic rhinitis and other allergic diseases, but its importance in asthma is much less well understood. Indeed, it has been suggested that other spasmogens such as the slow-reacting substance of anaphylaxis (SRS-A), which is now known to be a composite of leukotrienes C, D, and E,[1] may be a major mediator of the allergic response.[2] Despite these arguments, however, recent studies have demonstrated the potential value of inhaled H_1 antihistamines in the treatment of asthma[3] and a number of groups have reported compounds of potential therapeutic benefit that were claimed to possess the combined properties of both histamine H_1 antagonism and mast cell stabilization. Notable among these latter compounds are BM 15100 (1),[4] ketotifen (2),[5] and oxatomide (3).[6]



Scheme I



We have been interested in potential antiasthmatic compounds with multiple activities for a number of years,[7] and in this paper we report our results on a series of nitrocoumarin derivatives **29–34** which combine potent H_1-antihistaminic properties with those of mast cell stabilization.

Chemistry. In previous studies we have shown that 4,7-dihydroxycoumarins may be selectively alkylated at the 7-position by the prior protection of the 4-hydroxy group as its benzyl ether followed by the subsequent cleavage of this protecting group under hydrogenolytic conditions.[7] Simpler ethers such as ethyl ether also function as suitable masking groups for the more reactive 4-hydroxy group, the cleavage of these being adequately effected hydrolytically, and we have used both of these derivatives as key intermediates in the synthesis of the title compounds by coupling, either directly or indirectly, with appropriately substituted piperazines.

The synthesis of the precursor piperazines was readily accomplished by the route shown in Scheme I. Thus, alkylation of ethyl piperazine-1-carboxylate (4) with either benzyl or 4-chlorobenzyl chloride **5** gave the corresponding esters **6**, which on alkaline hydrolysis and decarboxylation afforded the unprotected derivatives **7**. Reaction of **7** with 1-bromo-3-chloropropane or an appropriate ω-halo alcohol then gave the chloro or hydroxy compounds **8** and **9**, respectively.

Coupling of these piperazino derivatives with the protected coumarins could be effected in several ways, depending on the nature of the piperazine–coumarin bridge.

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(4) Roesch, A.; Roesch, E. *Br. J. Pharmacol.* **1976**, *57*, 438P.
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(6) Awouters, F.; Niemegeers, C. J. E.; Van der Berk, J.; Van Neuten, J. M.; Lenaerts, F. M.; Borgers, M.; Schellekens, K. H. L.; Broeckaert, A.; De Cree, J.; Janssen, P. A. J. *Experientia* **1977**, *33*, 1657.
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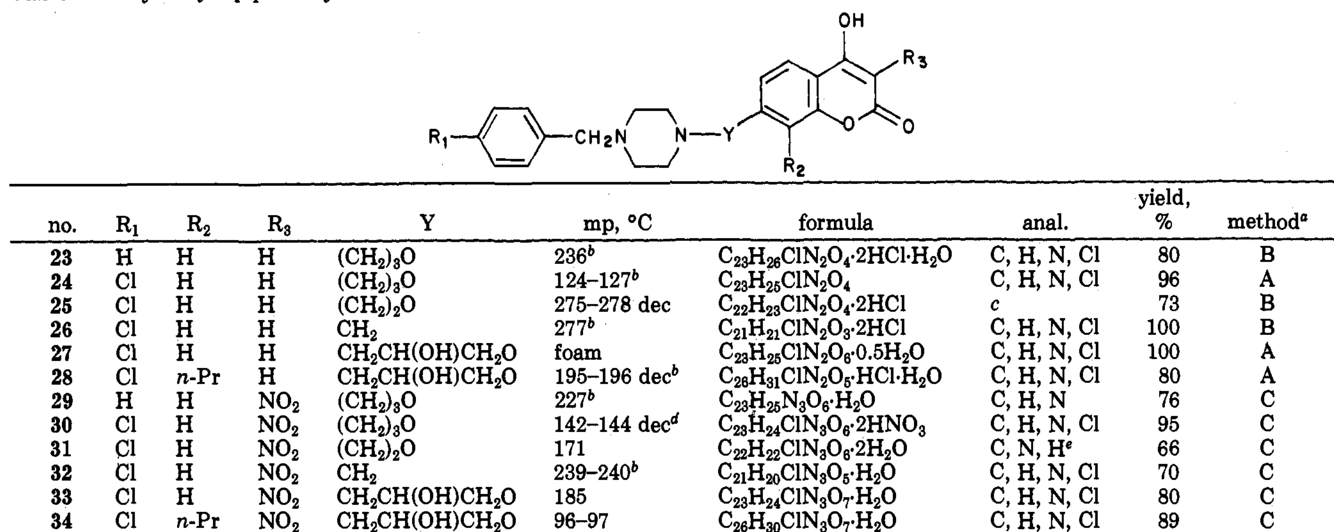
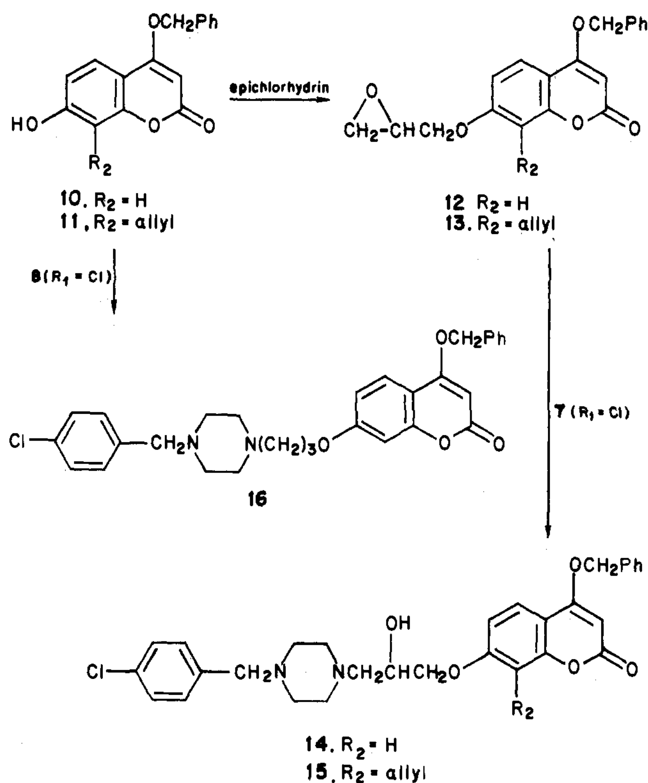
Table I. 4-Hydroxy-7-piperazinylcoumarins



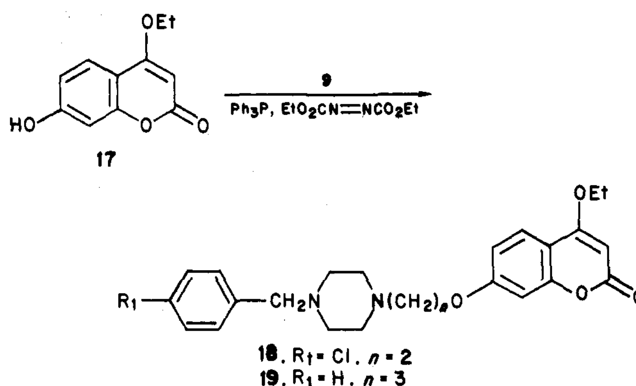
no.	R_1	R_2	R_3	Y	mp, °C	formula	anal.	yield, %	method[a]
23	H	H	H	$(CH_2)_3O$	236[b]	$C_{23}H_{26}ClN_2O_4 \cdot 2HCl \cdot H_2O$	C, H, N, Cl	80	B
24	Cl	H	H	$(CH_2)_3O$	124–127[b]	$C_{23}H_{25}ClN_2O_4$	C, H, N, Cl	96	A
25	Cl	H	H	$(CH_2)_2O$	275–278 dec	$C_{22}H_{23}ClN_2O_4 \cdot 2HCl$	*c*	73	B
26	Cl	H	H	CH_2	277[b]	$C_{21}H_{21}ClN_2O_3 \cdot 2HCl$	C, H, N, Cl	100	B
27	Cl	H	H	$CH_2CH(OH)CH_2O$	foam	$C_{23}H_{25}ClN_2O_6 \cdot 0.5H_2O$	C, H, N, Cl	100	A
28	Cl	*n*-Pr	H	$CH_2CH(OH)CH_2O$	195–196 dec[b]	$C_{26}H_{31}ClN_2O_5 \cdot HCl \cdot H_2O$	C, H, N, Cl	80	A
29	H	H	NO_2	$(CH_2)_3O$	227[b]	$C_{23}H_{25}N_3O_6 \cdot H_2O$	C, H, N	76	C
30	Cl	H	NO_2	$(CH_2)_3O$	142–144 dec[d]	$C_{23}H_{24}ClN_3O_6 \cdot 2HNO_3$	C, H, N, Cl	95	C
31	Cl	H	NO_2	$(CH_2)_2O$	171	$C_{22}H_{22}ClN_3O_6 \cdot 2H_2O$	C, N, H[e]	66	C
32	Cl	H	NO_2	CH_2	239–240[b]	$C_{21}H_{20}ClN_3O_5 \cdot H_2O$	C, H, N, Cl	70	C
33	Cl	H	NO_2	$CH_2CH(OH)CH_2O$	185	$C_{23}H_{24}ClN_3O_7 \cdot H_2O$	C, H, N, Cl	80	C
34	Cl	*n*-Pr	NO_2	$CH_2CH(OH)CH_2O$	96–97	$C_{26}H_{30}ClN_3O_7 \cdot H_2O$	C, H, N, Cl	89	C

[a] See text and Experimental Section. [b] Recrystallized from EtOH. [c] Inconsistent analytical results. [d] Melting point of free base 238 °C. [e] H: calcd, 5.28; found, 4.69.

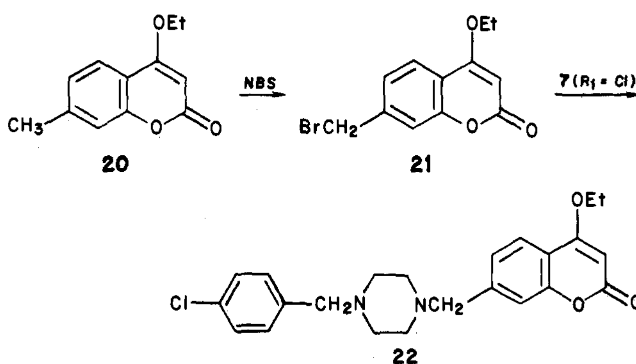
Scheme II



Scheme III



Scheme IV



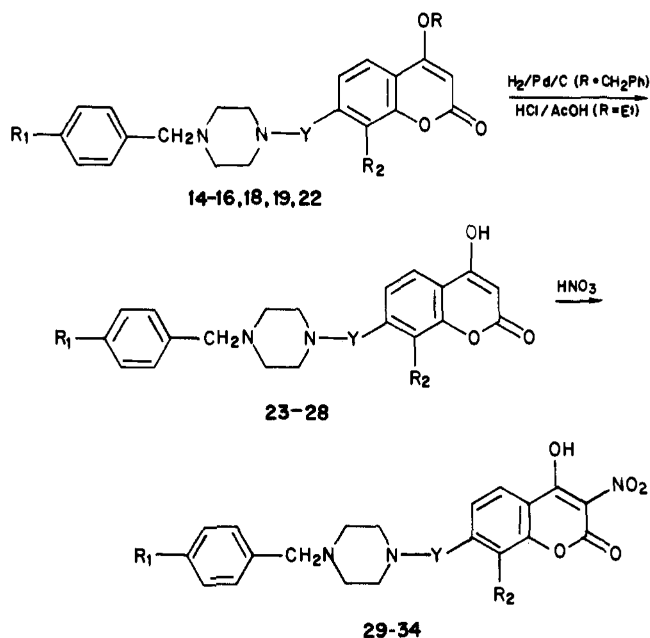
For those compounds having a 2-hydroxypropoxy linkage, the 7-hydroxycoumarins **10** or **11** were first condensed with epichlorohydrin to give the corresponding epoxides **12** and **13** (Scheme II), which reacted smoothly with **7** (R_1 = Cl) to give **14** and **15** in moderate yield. Those compounds linked via an unsubstituted alkylene chain were coupled by using two mutually interconvertible methods. Thus, the reaction of **10** with **8** (R_1 = Cl) was effected by using the anion of **10**, prepared in situ in *N*,*N*-dimethylformamide (DMF), and proceeded in 74% yield, whereas the ethoxycoumarin **17** (Scheme III) was coupled to the (hydroxyalkyl)piperazines **9** under diethyl azodicarboxylate–triphenylphosphine conditions[8] to give moderate yields of **18** and **19**.

The methylene-bridged intermediate **22** was prepared as shown in Scheme IV from the bromomethyl derivative **21**, which was itself made by bromination of the 7-methylcoumarin **20** using *N*-bromosuccinimide.

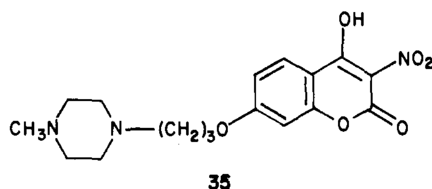
Unmasking of the 4-hydroxy group in these coupled products was effected catalytically (method A) for the benzyloxy compounds **14–16** and hydrolytically (method B) for the ethoxy compounds **18**, **19**, and **22** to give high yields of the coumarins **23–28** (Scheme V, Table I). In the case of compound **15**, the allyl group was simultaneously reduced to propyl during the hydrogenolysis of the *O*-benzyl group. Nitration of **23–28** with fuming nitric acid in chloroform (method C) furnished the 3-nitro derivatives **29–34** of Table I.

(8) Mitsunobu, O. *Synthesis* 1981, 1.

Scheme V

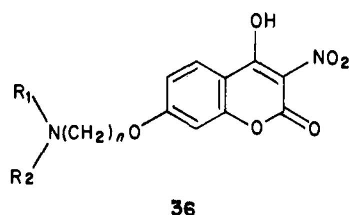


The *N*-methylpiperazino analogue **35** was prepared from compound **10** in a similar manner to that described for the chlorobenzyl compound **16**.[9]



Results and Discussion

In continuance of our program on 2-nitro-1,3-dicarbonyl compounds with potential antiasthmatic activity,[10] we were interested in preparing a series of 4-hydroxy-3-nitrocoumarin derivatives of general formula **36** in the hope that these would show improved pharmacokinetic properties over the simpler substituted compounds.[9] It was during



this work that the *N*-methylpiperazino derivative **35** was prepared, and it seemed to us that H_1-antihistaminic activity could be conferred on this type of derivative by the simple expedient of replacing the *N*-methyl substituent by that of benzyl. The compounds described in this paper form a series in which this modification has been made and in which the intention was to prepare an H_1 antihistamine with sufficient mast cell stabilizing activity for this to be relevant to its antianaphylactic activity in vivo.

Previous work on SRS-A antagonists with mast cell stabilizing activity[7] had suggested that derivatives of this type might retain their potent mast cell stabilizing properties, and this indeed was found to be the case. Furthermore, the occurrence of H_1-antihistaminic activity in these acidic derivatives represented an interesting finding since most, if not all, H_1 antihistamines are usually highly lipophilic compounds with little polar functionality.[11]

Compounds with H_1-antihistamine activity have been shown to inhibit histamine release in a variety of systems,[12,13] and H_1 antihistamines have been introduced with claims that mast cell stabilization might contribute to their therapeutic effects. Ketotifen (**2**)[5] and oxatomide (**3**)[6] are notable examples among these. The rat passive cutaneous anaphylaxis (PCA) test[14] is frequently used to assess compounds for their antiallergic potential and relies on the affinity of injected immunoglobulin E (IgE) for mast cells in the rat skin. Subsequent challenge with antigen results in reaction with the cell-bound IgE and the release of vasoactive materials such as histamine. This discharge produces extravasation at the antibody injection sites which is usually measured by prior labeling of plasma proteins with a blue dye. This test, however, is not specific to compounds that stabilize mast cells, and compounds that antagonize the mediators or stabilize the vasculature directly are also active.[15] Mast cells of the rat and mouse contain higher levels of 5-hydroxytryptamine (5-HT) than those in species such as man,[16,17] and rat PCA is completely inhibited by a mixture of 5-HT and histamine antagonists while each antagonist when given alone produces only a partial inhibition.[14] H_1 antihistamines with anti-5-HT activity will therefore be more effective inhibitors of rat PCA than those without. The anti-5-HT activity shown by the H_1 antihistamines oxatomide[18] and ketotifen[5] might contribute to their effectiveness in the rat PCA test.

Anaphylactic histamine release from mast cells is an energy-demanding process dependent on the viability of the cell,[19,20] and compounds that interfere with cell metabolism may therefore inhibit histamine release in in vitro systems without having a specific effect on the secretory process.[20,21] The H_1 antihistamines inhibit histamine release in in vitro systems at relatively high concentrations that are often close to those that release histamine,[12,13] and there is some evidence that they interfere with oxidative metabolism at inhibitory concentrations.[22] Doubt has been expressed as to the relevance of mast cell stabilization shown by the H_1 antihistamines in vitro to their antianaphylactic activity in vivo.[13]

Rat passive peritoneal anaphylaxis (PPA) provides a method for determining the mode of action of compounds with antiallergic activity in which it is possible to measure both the concentrations of histamine and other vaso-active materials released from the mast cell and the degree of extravasation that they produce. In this system it has been shown that disodium cromoglycate (DSCG) and compounds of a similar type only inhibit extravasation at doses

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(17) West, G. B. *J. Pharm. Pharmacol.* **1959**, *11*, 513.
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(19) Parratt, J. L. *C. R. Seances Soc. Biol. Ses Biol.* **1942**, *136*, 361.
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(21) Mota, I.; Ishii, T. *Br. J. Pharmacol.* **1960**, *15*, 82.
(22) Gushchin, I. S.; Kaminka, M. E.; Deryugin, I. C. *Bull. Exp. Biol. Med.* **1978**, *86*, 1367.

Table II. Rat Passive Peritoneal Anaphylaxis Data and H_1-Antihistamine Activities

compd	H_1-antihistamine activity, pA_2 on guinea pig ileum (method)[a]	rat PPA: inhibn of the release of histamine and extravasation, IC_{50}, M[b]	
		histamine	extravasation
mepyramine	9.1 (a)	$>2 \times 10^{-4}$	2.9×10^{-5} (6.1×10^{-5} to 1.6×10^{-5})
ketotifen	9.5 (a)	$>2 \times 10^{-4}$	9.1×10^{-6} (4.0×10^{-6} to 2.3×10^{-5})
DSCG	inactive	7.4×10^{-7} (4.0×10^{-7} to 1.2×10^{-6})	5.8×10^{-6} (2.5×10^{-6} to 2.6×10^{-5})
29	7.9 (b)	2.8×10^{-5} (1.2×10^{-5} to 4.5×10^{-5})	$>2 \times 10^{-4}$
30	9.0 (b)	1.9×10^{-6} (1.4×10^{-6} to 2.5×10^{-6})	5.8×10^{-6} (3.3×10^{-6} to 1.2×10^{-5})
31	6.8 (b)	8.2×10^{-6} (1.4×10^{-7} to 2.3×10^{-5})	$\approx 1 \times 10^{-4}$
32	5.6 (b)	1.4×10^{-5} (5.0×10^{-6} to 2.7×10^{-5})	8.3×10^{-5} (3.2×10^{-5} to 5.1×10^{-5})
33	8.3 (b)	$<2 \times 10^{-6}$	2×10^{-6}
34	8.4 (b)	2.6×10^{-6} (8.4×10^{-7} to 4.7×10^{-6})	1.6×10^{-5} (6.9×10^{-6} to 2.7×10^{-4})
35	4.0 (c)	$<2 \times 10^{-6}$	1.3×10^{-5} (6.7×10^{-6} to 2.3×10^{-5})

[a] The pA_2 value is the negative logarithm of the concentration of the compound producing a twofold shift to the right of the histamine dose–response curve as measured on the guinea pig ileum by methods a, b, or c; see Experimental Section. [b] This is the concentration of the compound in the 6 mL of fluid injected intraperitoneally that would reduce the concentrations of histamine and extravasated dye in the peritoneal fluids, 5 min later, by 50%. (95% confidence limits.)

at which they inhibit histamine release.[23] In contrast, the H_1 antihistamines inhibit extravasation at doses that have no effect on histamine release, showing that mast cell stabilization is not relevant to the antiallergic properties of the H_1 antihistamines in this in vivo system.[23]

Both mepyramine and ketotifen are potent H_1 antihistamines and will inhibit extravasation in the rat PPA system at doses that have no effect on histamine release (Table II), and it is evident, therefore, that their antiallergic effects are restricted to their ability to antagonize the effects of released mediators in this system. On the other hand, with the exception of the weakly potent nonhalogenated derivative **29**, all of the nitrocoumarins **30–34** show a similar profile of activity to that of DSCG in that they inhibit extravasation at doses that have a demonstrable inhibitory effect on histamine release. It is likely, therefore, that mast cell stabilization contributes to the antiallergic effects of this group in vivo.

Within the series **29–30**, the antihistaminic activity varies quite markedly with structural modification. The most potent compound is **30** with a pA_2 value similar to that of mepyramine. Removal of the chlorine atom, as in **29**, effects a noticeable reduction in potency as does shortening of the linkage between the piperazine and coumarin nuclei (compounds **31** and **32**). Hydroxylation of the alkyl bridge of **30** had a lesser effect on potency as seen in compounds **33** and **34** yet considerably improved the aqueous solubility. The *N*-methyl derivative **35**, however, is a very poor H_1 antihistamine and DSCG was inactive.

All compounds inhibited extravasation although **29** was of surprisingly low potency in this respect.

Experimental Section

Melting points were determined with a Büchi melting point apparatus and are recorded uncorrected. The structures of all compounds were consistent with their IR and ^1H NMR spectra, which were determined with a Perkin-Elmer 197 spectrophotometer and a Varian EM 390 90-MHz spectrometer, respectively. Where represented by elemental symbols, the analyses of these elements fall within ±0.4% of the calculated values.

Ethyl 4-(4-Chlorobenzyl)piperazine-1-carboxylate (6, R_1 = Cl). A solution of ethyl piperazine-1-carboxylate (25 g, 0.16 mol) and 4-chlorobenzyl chloride (26 g, 0.16 mol) in EtOH (250 mL) was gently refluxed for 5 h and the solvent then evaporated in vacuo. Recrystallization of the resulting solid from EtOH gave the dihydrochloride (29 g, 58%) of mp 208–210 °C, which formed the free base, mp (EtOH) 53.5 °C on neutralization with aqueous NaOH. Anal. ($C_{14}H_{19}ClN_2O_2$) C, H, Cl, N.

1-(4-Chlorobenzyl)piperazine (7, R_1 = Cl). A mixture of the above ester (7.4 g, 23 mmol), 20% w/v aqueous NaOH (300 mL), and 2-ethoxyethanol (75 mL) was stirred at reflux for 18 h and then cooled to 70 °C and acidified with concentrated HCl. When the vigorous gas evolution ceased, the mixture was cooled, rebasified with NaOH, and extracted well with Et_2O. The dried ($MgSO_4$) extracts were evaporated, and the residual oil was distilled to give 4.2 g (85%) of the title compound of bp 104–106 °C (0.1 mm) [lit.[24] bp 132–135 °C (2.0 mm)]. Anal. ($C_{11}H_{15}ClN_2$) C, H, Cl, N.

1-Chloro-3-[4-(4-chlorobenzyl)piperazin-1-yl]propane (8, R_1 = Cl). Anhydrous K_2CO_3 (6.25 g, 0.045 mol) was added to a solution of 1-(4-chlorobenzyl)piperazine (6.32 g, 0.03 mol) and 1-bromo-3-chloropropane (4.75 g, 0.03 mol) in butanone (75 mL), and the mixture was stirred at reflux for 3 h. Filtration of the cooled mixture and evaporation of the filtrate gave an oil, which was redissolved in Et_2O, washed with H_2O, and dried ($MgSO_4$). Evaporation afforded 6.86 g (80%) of essentially pure product, a small sample of which was distilled for analysis, bp 150 °C (0.5 mm). Anal. ($C_{11}H_{15}ClN_2$) C, H, Cl, N.

Similarly prepared were 3-(4-benzylpiperazin-1-yl)propan-1-ol (8, R_1 = H) in 61% yield after chromatography on silica and elution with $CHCl_3$ [mp [petroleum ether (bp 60–80 °C)] 59–60 °C (lit.[24] mp 49.5–51 °C); anal. ($C_{14}H_{22}N_2O$) C, H, N] and 2-[4-(4-chlorobenzyl)piperazin-1-yl]ethanol (9, R_1 = Cl, n = 2) in 34% yield of spectroscopically pure material after chromatography on silica and elution with $CHCl_3$.

4-(Benzyloxy)-7-(2,3-epoxypropoxy)coumarin (12). 4-(Benzyloxy)-7-hydroxycoumarin[7] (10.7 g, 41 mmol) was dissolved in EtOH (200 mL), and a solution of KOH (2.5 g) in H_2O (10 mL) was added with stirring. Epichlorohydrin (40 mL) was then added and the mixture was stirred at reflux for 2.5 h, after which time the solvent was removed in vacuo and the residue partitioned between water and AcOEt. Evaporation of the dried ($MgSO_4$) organic phase gave the product as a white crystalline solid. Recrystallization from EtOH afforded 11.1 g (83%) of **12**, mp 128–129 °C. Anal. ($C_{19}H_{16}O_5$) C, H.

8-Allyl-4-(benzyloxy)-7-(2,3-epoxypropoxy)coumarin (13). Reaction of 8-allyl-4-(benzyloxy)-7-hydroxycoumarin[7] (12.33 g, 40 mmol) with epichlorohydrin (25 mL) as described for compound **12** gave 11.97 g (82%) of **13**, mp (EtOH) 118–121 °C. Anal. ($C_{22}H_{20}O_5$) C, H.

4-Ethoxy-7-hydroxycoumarin (17). Dry HCl gas was passed through a cold solution of 4,7-dihydroxycoumarin[25] (17.8 g, 0.1 mol) in EtOH (300 mL) for 30 min and the resulting mixture heated to reflux for 30 min. Concentration to half the volume resulted in the precipitation of the title compound, which was filtered off and washed with cold EtOH after cooling to 0 °C. Recrystallization from EtOH gave 17 g (87%) of **17**, mp 266 °C. Anal. ($C_{11}H_{10}O_4$) C, H.

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4-Ethoxy-7-methylcoumarin (20). Dry HCl was passed through a solution of 4-hydroxy-7-methylcoumarin[26] (14.0 g, 80 mmol) in EtOH (30 mL) for 15 min and the resulting solution was heated to reflux for 30 min. After cooling, the solvent was evaporated in vacuo and the residue was recrystallized from EtOH to give **20** (11.7 g, 72%), mp 142–143 °C. Anal. ($C_{12}H_{12}O_3$) C, H.

7-(Bromomethyl)-4-ethoxycoumarin (21). *N*-Bromosuccinimide (9.59 g, 54 mmol) was added to a stirred solution of **20** (11 g, 54 mmol) in CCl_4 (600 mL) followed by a catalytic quantity of azobis(isobutyronitrile). After the mixture was refluxed for 1 h, the precipitated solid was removed by filtration and the solvent was removed in vacuo. The residue was recrystallized from EtOH to give 11.73 g (77%) of essentially pure **21** of mp 152–155 °C.

1-[3-[[4-(Benzyloxy)coumarin-7-yl]oxy]-2-hydroxypropyl]-4-(4-chlorobenzyl)piperazine (14). A solution of **12** (8.48 g, 26 mmol) and 1-(4-chlorobenzyl)piperazine (6.20 g, 29.5 mmol) in EtOH (50 mL) was stirred at reflux for 90 min and allowed to cool. Evaporation of the solvent in vacuo gave an oily residue, which crystallized on trituration with petroleum ether and was recrystallized from MeOH to give **14** (8.67 g, 62%): mp 130–133 °C; IR ν_{max} (mull) 1720 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (10 H, m, NCH_2), 3.20 (1 H, m, exchangeable, OH), 3.48 (2 H, s, NCH_2Ph), 4.07 (3 H, br s, OCH), 5.20 (2 H, s, OCH_2Ph), 5.68 (1 H, s, C-3 H), 6.89 (2 H, m, C-6 H, C-8 H), 7.30 (4 H, s, arom ClPh), 7.45 (5 H, s, Ph), 7.77 (1 H, d, J = 10 Hz, C-5 H). Anal. ($C_{30}H_{31}ClN_2O_5$) C, H, N.

1-[3-[[8-Allyl-4-(benzyloxy)coumarin-7-yl]oxy]-2-hydroxypropyl]-4-(4-chlorobenzyl)piperazine (15). Reaction of 1-(4-chlorobenzyl)piperazine (6.2 g, 30 mmol) with **11** (9.47 g, 26 mmol) as described for compound **14** afforded 7.85 g (52%) of the title compound of mp (MeOH) 155–157 °C. Anal. ($C_{33}H_{35}ClN_2O_5$) C, H, Cl, N.

1-[3-[[4-(Benzyloxy)coumarin-7-yl]oxy]propoxy]-4-(4-chlorobenzyl)piperazine (16). To a solution of **10**[7] (9.4 g, 35 mmol) in dry DMF (35 mL) was added NaH (0.84 g, 35 mmol), and the mixture was stirred for 1 h at 100 °C to complete formation of the sodium salt. To this was added a solution of 1-chloro-3-[4-(4-chlorobenzyl)piperazin-1-yl]propane (9.3 g, 35 mmol) in dry DMF (40 mL) over 1 h, and the resulting mixture was stirred for 4 h at 100 °C. The precipitated NaCl was filtered from the cooled mixture and the filtrate evaporated in vacuo to a red oil, which was extracted with dry Et_2O. Concentration of the extracts afforded 13.5 g (74%) of **16** as a white crystalline solid, mp (EtOH) 115–116 °C. Anal. ($C_{30}H_{31}ClN_2O_4$) C, H, Cl, N.

1-(4-Chlorobenzyl)-4-[2-[(4-ethoxycoumarin-7-yl)oxy]ethyl]piperazine (18). A suspension of **17** (6.47 g, 31.4 mmol) in dry THF (1 L) containing Ph_3P (9.07 g, 34.6 mmol) was stirred at 20 °C while diethyl azodicarboxylate (6.02 g, 34.6 mmol of 86% pure) in dry THF (25 mL) was added dropwise over 5 min. To this mixture was added 2-[4-(4-chlorobenzyl)piperazin-1-yl]ethanol (8.0 g, 31.4 mmol) in dry THF (100 mL) over 15 min and the total was stirred at ambient temperature for 30 min. Evaporation of the solvent in vacuo (<25 °C) gave an oil, which was dissolved in a minimum of EtOH, and the dihydrochloride salt of **18** precipitated by the addition of concentrated HCl. Filtration and drying gave 9.30 g (58%) of salt of mp 245–247 °C. Anal. ($C_{24}H_{27}ClN_2O_4\cdot 2HCl$) C, H, Cl, N.

1-Benzyl-4-[3-[(4-ethoxycoumarin-7-yl)oxy]propyl]piperazine (19). Condensation of **17** (11.0 g) with 3-(4-benzylpiperazin-1-yl)propan-1-ol (12.5 g) as described for compound **18** afforded 15.6 g (59%) of the title compound as its dihydrochloride of mp (EtOH) 240–241 °C. Anal. ($C_{25}H_{30}N_2O_4\cdot 2HCl$), C, H, Cl, N.

1-(4-Chlorobenzyl)-4-[(4-ethoxycoumarin-7-yl)methyl]piperazine (22). A mixture of 1-(4-chlorobenzyl)piperazine (4.21 g, 20 mmol), anhydrous K_2CO_3 (4.20 g, 30.5 mmol), and **21** (5.80 g, 20.5 mmol) in chlorobenzene (50 mL) was stirred for 20 h at 140 °C and the cooled product was treated with excess dilute HCl. The precipitated dihydrochloride was separated and dried to give 5.00 g (50%) of material of mp 260–264 °C, which was converted into the free base with 2 M NaOH. Recrystallization gave **22** of mp (EtOH) 122–124 °C. Anal. ($C_{23}H_{25}ClN_2O_3$) C, H, Cl, N.

4-Hydroxycoumarins 23–28 (Table I). Method A. Hydrogenolysis of the 4-Benzyloxy Derivatives. A solution of the benzyloxy compounds **14–16** in DMF was hydrogenated at atmospheric pressure in the presence of 10% palladium on charcoal until 1 equiv (2 equiv for compound **15**) of hydrogen was absorbed. Removal of the catalyst by filtration and evaporation of the solvent in vacuo gave **24**, **27**, and **28**.

Method B. Hydrolysis of the 4-Ethoxy Derivatives. A mixture of the ethoxy compound **18**, **19**, **or 22** (ca. 10 g), glacial AcOH (200 mL), and concentrated HCl (50 mL) was refluxed for 30 min, cooled, and then evaporated to dryness in vacuo. Trituration of the residual solid with EtOH gave **23**, **25**, and **26** in reasonable purity.

General Nitration Procedure (Method C). Fuming HNO_3 (10 mL, *d* 1.52) was added dropwise over 15 min to a cold (0 °C), vigorously stirred suspension of hydroxycoumarins **23–28** (2.0 g) in $CHCl_3$ (200 mL), and the mixture was stirred for a further 1 h at 0 °C before dilution with water (80 mL). Removal of the $CHCl_3$ in vacuo (T < 5 °C) gave a yellow solid, which was filtered off and washed with water to give **29–34** as their dinitric acid salts. Careful neutralization with 2 equiv of alkali furnished the free bases of Table I.

H_1-Antihistamine Activity: Determination of pA_2 Values. The terminal portion of guinea pig ileum was removed and suspended in a 4-mL organ bath and bathed in Tyrode solution, aerated and maintained at 35 °C. The tissue was washed by upward displacement of the bathing fluid. A tension of ca. 1 g was applied to the ileum, and the contractions were recorded by using a Devices isotonic photoelectric transducer and a Servoscribe flat bed recorder.

A dose–response curve was obtained for histamine, using a 3 × 3 Latin square design for dosing.

Method a. Increasing concentrations of antagonists were added to Tyrode solution and the dose–response curves for histamine were repeated at each concentration of the antagonists.

The results were calculated from the dose–response lines for histamine at each drug concentration. These were parallel. The doses of histamine required to produce the same size of contraction of the gut in the presence of each concentration of the antagonists were obtained from the graph.

The dose ratio of histamine at a given concentration of the antagonist was the dose of histamine required to produce the same, less than maximal, response in the presence of this concentration of the compound over that in its absence. The log of the dose ratios minus one (CR-1) were plotted against the negative log of molar concentration of the compound. The pA_2 value was read from where this line intersected the abscissa.[27]

Methods b and c. A dose–response curve to histamine was obtained as before. A dose of histamine was chosen that produced about 50% of the maximum response and this dose was added to the organ bath at least three times to confirm that the same size of contraction was obtained. The antagonist was then added to the organ bath and after 10 min, method b, or 30 s, method c, twice the selected dose of histamine was added to the bath and the response recorded. The tissue was washed until the response to the selected dose of histamine was reestablished. Three doses of the antagonist were evaluated in this way, the doses being chosen so that twice the selected dose of histamine produced less than a maximum response. Each dose of the compound was tested three times, using a Latin square design. A graph was plotted of the log dose of the antagonist against the ratio of the response to a double dose of histamine in the presence of the antagonist over the mean response to a single dose of histamine in the absence of the antagonist. The dose producing a ratio of 1 was obtained from the graph and the pA_2 value was the negative logarithm of the molar concentration of the antagonist at this dose.

For compounds evaluated by the different methods, methods a or b produced little difference in the measured pA_2 values, but method c could produce up to a 10-fold reduction in potency.

Passive Peritoneal Anaphylaxis (PPA). PPA was carried out and the antiserum was raised as previously described.[23]

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Briefly, rats were given intraperitoneal injections of 2 mL of a 1:5 dilution of the rat antiserum in isotonic saline. Two hours later 0.3 mL of a 5% solution of Pontamine Sky Blue (Raymond A. Lamb, London) in isotonic saline was injected intravenously, and followed 30 s later by an intraperitoneal injection of 5 mL of Tyrode solution containing 50 μg/mL of heparin and 0.4 mg/mL of ovalbumin. Exactly 5 min after challenge, the rats were stunned and bled and their peritoneal fluids were collected. Animals in control groups received a dilution of normal rat serum instead of antiserum at the time of sensitization (not sensitized) or were given Tyrode solution free of antigen at the time of challenge (not challenged). Compounds were given intraperitoneally in 1 mL of saline, 30 s before the antigen in 5 mL of Tyrode solution. Doses of the compounds are quoted as their concentrations in the 6 mL of fluid injected intraperitoneally.

Assay of Peritoneal Fluids. Collected peritoneal fluids were immediately cooled to 0 °C and centrifuged and the supernatant fluids assayed for dye within 2 h. The supernatant, 0.5 mL, was added to 1 mL of 12% trichloroacetic acid and stored at −20 °C and used to assay for histamine.

Dye Assay. The optical densities (OD) at 625 nm of the supernatants were determined.

Histamine Assay. Histamine was assayed by using an automated spectrofluorimetric system (Technicon Autoanalyser) as described.[23] At the concentrations used, the compounds tested did not interfere with the assay.

The concentrations of histamine and extravasated dye in the peritoneal fluids collected from nondrug-treated control rats were similar to those described;[23] i.e., the mean values obtained ± SEM (n = 19–36) were for passively sensitized and challenged rats 2.03 ± 0.08 μg/mL of histamine and 0.88 ± 0.06 OD (625 nm) for dye. For negative control rats, passively sensitized and no challenge or not sensitized and challenged, the mean values were up to 0.2 μg/mL for histamine and 0.12 OD for dye. For each drug studied, each dose was given to five to seven animals, and at least two doses were given that produced some but a less than maximum inhibition. The percentage inhibition in each animal was calculated from the concentration in that animal × 100 over the mean concentration in five to seven positive control animals treated at the same time from the same group. Negative controls were not taken into account. Regression lines were fitted to each data set plotted against the log of the dose. The median effective dose and associated confidence limits were then estimated as the doses corresponding to an inhibition of 50%, as calculated from the equations of the regression line and the 95% confidence limits of the mean response to any dose.

Registry No. **4**, 120-43-4; **6**, 55037-87-1; **7** (R_1 = Cl), 23145-88-2; **8** (R_1 = Cl), 39577-03-2; **8** (R_1 = H), 23253-99-8; **9** (R_1 = Cl, n = 2), 91860-37-6; **10**, 63360-23-6; 11, 69076-27-3; **12**, 75590-39-5; **13**, 75590-43-1; 14, 75590-40-8; **15**, 75590-44-2; **16**, 75590-35-1; **17**, 75590-48-6; 18, 91860-38-7; **18**·2HCl, 75590-49-7; **19**·2HCl, 75590-59-9; **20**, 75590-53-3; **21**, 75590-54-4; **22**, 75590-64-6; **22**·2HCl, 75590-55-5; **23**·2HCl, 75590-60-2; **24**, 75590-36-2; **25**·2HCl, 75590-50-0; **26**·2HCl, 75590-56-6; **27**, 75590-41-9; **28**·2HCl, 91860-39-8; **29**, 75590-61-3; **30**, 75590-37-3; **30**·$2HNO_3$, 75590-38-4; **31**, 75590-51-1; **32**, 75590-57-7; **33**, 75590-42-0; **34**, 75590-46-4; **35**, 70744-27-3; 4-chlorobenzyl chloride, 104-83-6; 1-bromo-3-chloropropane, 109-70-6; 4,7-dihydroxycoumarin, 1983-81-9; 4-hydroxy-7-methylcoumarin, 18692-77-8.

Bispyridinamines: A New Class of Topical Antimicrobial Agents as Inhibitors of Dental Plaque

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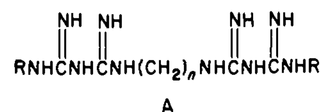
A series of N,N'-polyalkylenebis[4-(substituted-amino)pyridines] has been prepared, and members have been evaluated as potential anti-dental plaque agents. From among the most active members of the series, one compound, N,-N'-[1,10-decanediyldi-1(4H)-pyridinyl-4-ylidene]bis(1-octanamine) dihydrochloride, octenidine, was selected as a candidate for clinical study.

Periodontal disease constitutes the leading cause of tooth loss in man and is present in almost all persons who retain natural teeth.[1] It is widely accepted that dental plaque, a dense bacterial matrix that adheres avidly to tooth surfaces, plays an important role in the initiation of caries and periodontal diseases.[2,3] The control of dental plaque by prudent application of chemotherapeutic agents therefore constitutes a potentially effective means of controlling these common disease states.[4–6]

An effective antiplaque chemotherapeutic agent should possess a number of important characteristics. It should (1) be active at low minimal inhibitory concentrations against pathogenic plaque-forming species, (2) be substantive to tooth surfaces, and (3) retain efficacy in the presence of saliva.

Much attention has been directed toward the antimicrobial bisbiguanides A, as potential agents for controlling and/or preventing the formation of dental plaque,[7–18] and a number of structural studies have been published.[19–24]



Chlorhexidine, (A, R = 4-ClC_6H_4, n = 6) and alexidine (A, R = 2-ethylhexyl, n = 6) salts are the most studied com-

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