Notes

(Piperidinylalkoxy)chromones: Novel Antihistamines with Additional Antagonistic Activity against Leukotriene D_4

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Received February 15, 1995[®]

A series of novel chromone derivatives, in which the chromone moiety is connected to a (diphenylmethylene)-, (diphenylmethyl)-, or (diphenylmethoxy)piperidine via an alkyloxy spacer, were synthesized as antiallergic and antiasthmatic agents. In addition to their potent antihistaminic activity, the compounds also inhibit contraction in guinea pig ileum induced by leukotriene D_4. When analyzed by radioligand binding assays in guinea pig lung membranes, one of the compounds, 7-[[3-[4-(diphenylmethylene)piperidin-1-yl]propyl]oxy]-2-(5-tetrazolyl)-4-oxo-4*H*-1-benzopyran, showed dissociation constants (K_D) of 5.62 nM and 2.34 μM for H_1- and LTD_4-receptors, respectively. In vivo at the dose of 10 mg/kg, the compound inhibited the histamine- and LTD_4-induced increase of vascular permeability in guinea pigs by 95 and 30%, respectively. The inhibition of LTD_4-induced increase in vascular permeability by the compound was increased to 56% when a dose of 50 mg/kg was employed. Similar to terfenadine, the compound does not readily occupy the brain H_1-receptors when given intraperitoneally to mice, implying no sedating side effects.

Histamine produces various complex biological actions via interaction with specific receptors in the membranes of cell surfaces. Action of histamine on H_1-receptors stimulates many smooth muscles to contract, such as those in the bronchi. Histamine also increases the permeability of the capillary walls so that more of the constituents of the plasma can escape into the tissue spaces, leading to an increase in the flow of lymph and its protein content and formation of edema.[1,2] As such, histamine H_1-receptor antagonists are useful therapeutic agents for many allergic disorders, e.g., allergic rhinitis, dermatosis, urticaria, etc. The recent success in reducing side effects of antihistamines, mainly those related to the central nervous system depression and muscarinic receptor blocade, has made it possible to use these drugs in relatively high doses on pateints with severe allergic diseases such as asthma.[3,4] Unfortunately the efficacy of antihistamines in the treatment of asthma remains limited because of the involvement of many other allergic/inflammatory mediators in the disease.[5]

Peptidoleukotrienes (pLTs), a group of arachidonic acid metabolites including leukotriene C_4, D_4, and E_4, are one of these important mediators. They have powerful spasmogenic activity particularly in airway smooth muscles. They are more than 100 times more potent than histamine or methacholine as bronchoconstrictors in man when administered by aerosol.[6] Moreover, asthmatic patients show enhanced sensitivity to the bronchoconstricting effects of pLTs. Thus normal volunteers are 40 times more sensitive to leukotriene D_4 (LTD_4) than platelet-activating factors (PAF), but in asthmatics LTD_4 is 1000 times more potent than PAF.[6] Peptidoleukotrienes also stimulate mucus secretion and enhance vascular permeability, which all contribute to airway obstruction characteristic of asthma. Currently there is an intensive research effort to develop pLTs antagonists,[7] and clinical trials of some LTD_4 antagonists have shown promising results for the treatment of asthma.[8,9]

Peptidoleukotrienes and histamine complement each other during inflammatory and allergic responses. Histamine is preformed in the cell and has a rapid onset of action. Histamine is thought to be mainly responsible for the early phase of allergic reactions. In contrast, pLTs are synthesized on demand with a slow onset and are believed to play a major role in the late phase reactions. The action of pLTs is also of more prolonged duration than histamine, and LTC_4, LTD_4, and LTE_4 collectively account for all the biological actions previously ascribed to "slow-reacting substance of anaphylaxis (SRS-A)". Thus an agent which inhibits the actions of pLTs in addition to those of histamine may be clinically more effective. In this paper we describe the synthesis and biological activities of a series of compounds (**1**) which antagonize the action of both histamine and LTD_4, the most potent spasmogen among pLTs. Well-characterized H_1-antagonist terfenadine and LTD_4-antagonists FPL55712 and ONO-1078 (see Chart 1 for structures) were used as reference drugs during our series of experiments, and they were obtained by synthesis according to literature methods.[10–12]

Chemistry

All compounds in Table 1 were synthesized according to the method outlined in Scheme 1. The carboxyl derivatives **1a–j** were obtained by hydrolysis of the

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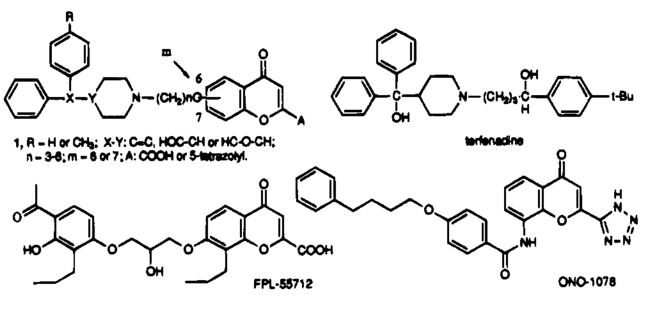
[®] Abstract published in *Advance ACS Abstracts*, May 15, 1995.

Table 1. Novel Chromone Derivatives **1**[a]

no.	R	X–Y	n	sub positn at chromone	A	mp (°C)	antihistaminic[b] activity, K_b (nM)	anti-LTD_4[c] activity, IC_{50} (μM)
1a	H	C=C	3	7	COOH	173–174	16.22 ± 0.51	20.4 ± 1.3
b	H	C=C	3	6	COOH	167–168	5.75 ± 0.76	0.65 ± 0.06
c	H	HOC-CH	3	7	COOH	157–160	5.25 ± 0.58	22.8 ± 1.7
d	H	HOC-CH	3	6	COOH	158–160	3.80 ± 0.65	>100
e	H	HC-O-CH	3	7	COOH	150–153	9.12 ± 0.60	1.45 ± 0.11
f	H	HC-O-CH	4	7	COOH	153–154	7.08 ± 0.50	9.89 ± 0.14
g	H	HC-O-CH	5	7	COOH	167–170	16.98 ± 0.60	18.3 ± 2.1
h	H	HC-O-CH	6	7	COOH	154–155	5.13 ± 0.56	48.2 ± 7.4
i	(+)-CH_3	HC-O-CH	3	7	COOH	156–158	12.30 ± 0.63	16.6 ± 1.9[d]
j	(−)-CH_3	HC-O-CH	3	7	COOH	144–145	2.24 ± 0.54	212 ± 25[d]
k	H	C=C	3	7	tetrazole	180–181	3.63 ± 0.72	0.19 ± 0.08
l	H	HOC-CH	3	7	tetrazole	174–176	1.70 ± 0.63	56.7 ± 4.2
terfenadine							24.55 ± 0.58	NA[e]
FPL55712							NA[e]	0.12 ± 0.02

[a] All activity data are means ± SD of at least three independent experiments. Antagonistic activity of the compounds was measured as inhibition of histamine- or LTD_4-induced contraction in the isolated guinea pig ileum. The measurement was performed in a constantly air-bubbled Krebs buffer at 37 °C. [b] The K_b values were calculated according to Cheng and Prusoff from a typical cumulative dose–response experiment (histamine concentrations ranging from 10 nM to 10 μM). [c] Concentration of the antagonist for 50% maximal inhibition of the contraction induced by LTD_4 (10 nM). [d] Concentration inhibiting 50% of [^{3}H]LTD_4 binding in guinea pig lung membrane fragments. [e] Not active.

Chart 1



corresponding ethyl esters **4a**, whereas the tetrazolyl analogues **1k**,**l** were prepared by the condensation between the nitrile **4b** and NaN_3 in the presence of NH_4Cl. The precursors **4a**,**b** were synthesized by the alkylation of an appropriate piperidine with [(ω-chloroalkyl)oxy]chromones **3** which were obtained by the alkylation of hydroxychromones **2**.[13] As the reactions do not involve any bond around the chiral center, the configuration and optical purity of **1i**,**j** are assumed to be the same as their corresponding precursors (+)- and (−)-**7**.

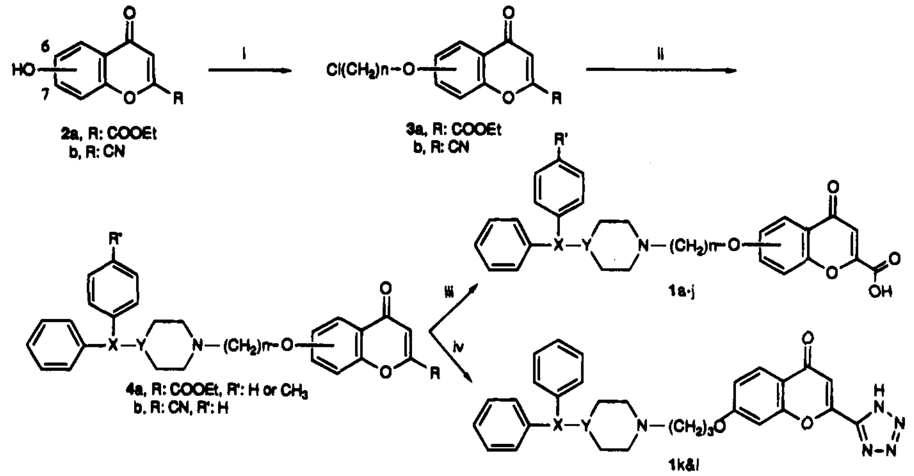
α,α-Diphenyl-4-piperidinemethanol for the preparation of **1c**,**d**,**l** was a commercial product from Janssen Chimica, Tilburg, The Netherlands. 4-(Diphenylmethylene)piperidine for the preparation of **1a**,**b**,**k** was obtained by an acid-catalyzed dehydration of α,α-diphenyl-4-piperidinemethanol. 4-(Diphenylmethoxy)piperidine (**6**) and racemic 4-[α-(4-methylphenyl)-α-phenylmethoxy]piperidine (**7**) were synthesized by condensation between 4-hydroxylpiperidine (**5**) and the corresponding benzhydrol in the presence of p-toluenesulfonic acid (Scheme 2). In the literature all (diphenylmethoxy)piperidines are prepared by Williamson ether formation, either between a diphenylmethanol and a halopiperidine or between a diphenylmethyl halide and a hydroxypiperidine.[14] The disadvantage of this type of reaction is the undesired formation of piperidine N-substituted side products. Although occassionally the yield of ether formation can be increased by the addition of a tertiary amine,[15] in general, it is necessary to protect the piperidine nitrogen, e.g., as a urethane which has to be removed under rather harsh conditions. In the present method, the desired ethers **6** and **7** were obtained in yields of about 90%, and the reaction was completed within 3 h. The much more rapidly formed benzhydryl carbocation and its steric bulkiness disfavored formation of symmetric ethers. Resolution of racemic **7** was achieved by diastereoisomeric salt formation with (+)-dibenzoyl-D-tartaric acid or (−)-dibenzoyl-L-tartaric acid.[16] The optical purity of the free bases (+)- and (−)-**7** was more than 96% ee as determined by chiral HPLC, and the assignment of absolute configuration was based on the circular dichroism method.[16]

The 2-cyanochromone **10** was prepared by the dehydration of the 2-carbamoylchromone **9** with trifluoroacetic anhydride and pyridine in DMF (Scheme 3). The reaction was carried out at room temperature, and the yield was more than 90%. No acetate was formed during the reaction as detected by NMR and MS spectra. The dehydrating reagent $(CF_3CO)_2O$/pyridine[17] offers advantages over other dehydrating reagents, e.g., $POCl_3$ and $SOCl_2$, in that it smoothly converts the amide to the nitrile under mild conditions and avoids the potential substitution of the hydroxyl.

Results and Discussion

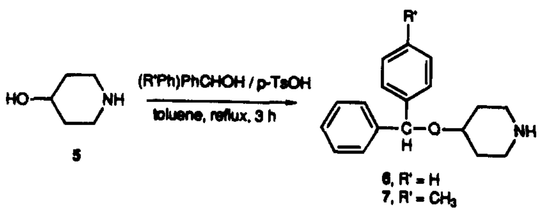
The compounds were tested for their inhibitory effects on both histamine- and LTD_4-induced contraction in isolated guinea pig ileum (Table 1). All compounds showed more potent antihistaminic activity than the reference drug terfenadine. The structural linkage between the benzhydryl group and the piperidine ring does not significantly alter the antihistaminic activity of the compounds. Nevertheless introduction of a *p*-methyl into the benzhydryl moiety produces a pair of enantiomers (**1i**,**j**) which exhibit different antihistaminic potency. This result indicates that the benzhydryl part of the molecule may participate in the specific interaction with the receptor. Structural variations on the substituent of the piperidine nitrogen do not affect the antihistaminic activity significantly. This is evidenced in terms of both the length of a spacer between the chromone and the piperidine moieties and the substitution position at the chromone system. Thus the antihistaminic activity of the propyl derivative **1e** is almost as potent as the butyl, pentyl, and hexyl congeners **1f**–**h**. These structure–activity relationship data support the general structural requirements for histamine H_1-

Scheme 1[a]

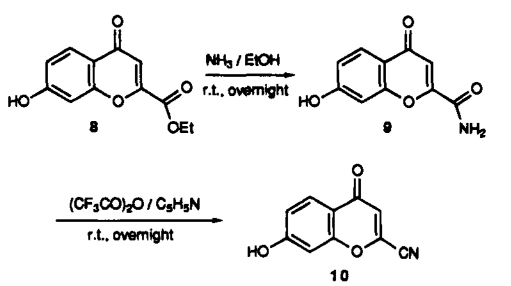


[a] Reagents: (i) $Cl(CH_2)nBr/K_2CO_3$/acetone, reflux 6 h; (ii) $Ph_2X\text{-}Y(CH_2CH_2)_2NH/NaI/K_2CO_3$/acetone, reflux, overnight; (iii) 10% NaOH/EtOH, reflux, 4 h; (iv) NaN_3/NH_4Cl/DMF, 120 °C, overnight.

Scheme 2



Scheme 3



receptor antagonists, in which three elements, the basic nitrogen and two aromatic rings (e.g., benzhydryl), constitute the pharmacophore.[18]

The compounds of this series also showed moderate anti-LTD_4 activity with a few members being equipotent to the reference agent FPL55712 (Table 1). From the data within the group of 4-(benzhydryloxy)piperidines (**1e–h**), it appears that the LTD_4 antagonizing activity is increased along with the shortening of the alkyl spacer between the chromone and piperidine moieties. Unfortunately attempts to synthesize the ethylene derivative within this group were not successful. Replacing the chromone 2-carboxylic acid group with a tetrazole, a common approach to enhance LTD_4 antagonizing activity,[19] gave different effects for diphenylmethylene derivatives (**1a** vs **1k**) and diphenylmethyl derivatives (**1c** vs **1l**). Thus the tetrazole **1k** is more than 100 times more active than the carboxylic acid **1a**, whereas the tetrazole **1l** is almost 2.5-fold less active than its carboxylic counterpart **1c**. Interestingly LTD_4-receptors seem to have the opposite stereoselectivity toward the benzhydryl chiral center as compared with H_1-receptors. For LTD_4-receptors, the (+)-isomer of the 4-(methylbenzhydryloxy)piperidine **1i** is more potent than the (−)-isomer **1j**, whereas for H_1-receptors the latter is more potent than the former.

Table 2. Receptor Binding Affinity of **1b,i** in Comparison with Terfenadine and FPL55712[a]

drugs	H_1-receptor affinity K_D (nM)[b]	K_b (nM)[c]	LTD_4-receptor affinity K_D (μM)[d]	IC_{50} (μM)[e]
1b	5.98 ± 1.41	5.75 ± 0.76	4.27 ± 0.19	0.65 ± 0.06
1k	5.89 ± 0.88	3.63 ± 0.72	2.34 ± 0.22	0.19 ± 0.08
terfenadine	35.48 ± 2.30	24.55 ± 0.58	f	NA[g]
FPL55712	f	NA[g]	1.12 ± 0.21	0.12 ± 0.02

[a] All K_D data are the means of two independent binding assays performed in triplicate in guinea pig lung membranes. [b] (−)-Dimethindene (100 μM) was used to define the nonspecific binding. The K_D of [^{3}H]mepyramine was found to be 3.30 nM, and the slope of Hill plots was 1.005. Incubation was performed at 37 °C for 50 min in a total volume of 1.0 mL ([^{3}H]mepyramine concentration: 0.5 nM). [c] See footnote b of Table 1. [d] LTD_4 (2 μM) was used to define the nonspecific binding. The K_D of [^{3}H]LTD_4 was found to be 0.21 nM, and the slope of Hill plots was 0.99. Incubation was performed at 22 °C for 30 min in a total volume of 0.3 mL ([^{3}H]LTD_4 concentration: 0.2 nM). [e] See footnote c of Table 1. [f] Not tested. [g] Not active.

For the assessment of receptor affinity, radioligand binding assays on guinea pig lung membranes were used (Table 2). Whereas the binding affinities of **1b,k** to H_1-receptors are consistant with those obtained from functional assays, the dissociation constants of the two compounds for LTD_4-receptors are somewhat lower than the potency observed in functional assays. Such a difference in anti-LTD_4 potency measured by the two assays was also observed with the standard LTD_4-antagonist FPL55712. This discrepancy between binding and functional potency might result from the different tissue bioavailablability and the unstable chemical nature of LTD_4 which is easily hydrolyzed to the less potent congener LTE_4. Therefore, the differences in experimental conditions (e.g., incubation temperature and time) in the functional and binding assays can give rise to the different affinity values of the antagonists. In the literature many known LTD_4-antagonists were actually found to exhibit 10–100-fold lower affinity potency measured by binding assays than by functional assays.[7]

To confirm the in vitro antihistaminic and antileukotriene activity of the compounds, we have also performed in vivo testing of the most promising compound, **1k**. In Figure 1, the inhibitory effect of **1k** on the

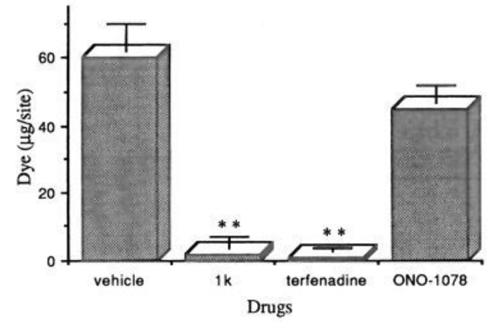


Figure 1. Inhibitory effect of **1k** (10 mg/kg), terfenadine (10 mg/kg), and ONO-1078 (10 mg/kg) ip on histamine-induced increase of vascular permeability in guinea pigs. One hour after ip administration of the drugs, Evans blue dye (50 mg/kg) was injected intravenously to the animal followed immediately by the intradermal injection of histamine (300 ng/site). Thirty minutes later, Evans blue dye was extracted from the isolated dorsal skin and the concentration was determined. $n = 5-7$; mean ± SE; $^{**}p < 0.01$ (Dunnet's analysis).

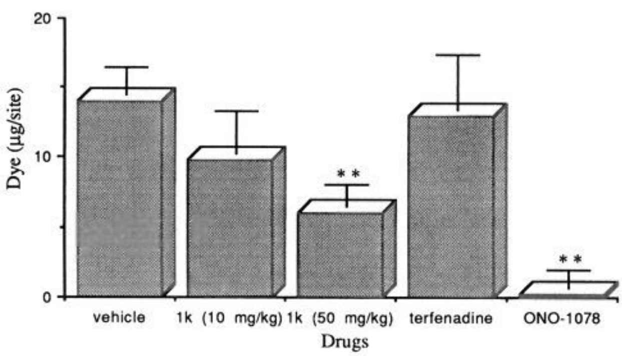


Figure 2. Inhibitory effect of **1k** (10 and 50 mg/kg), terfenadine (10 mg/kg), and ONO-1078 (10 mg/kg) ip on leukotriene D_4-induced increase of vascular permeability in guinea pigs. The same method as described under Figure 1 was employed except LTD_4 (50 ng/site) was used after the injection of Evans blue dye. $n = 5-7$; mean ± SE; $^{**}p < 0.01$ (Dunnet's analysis).

increased vascular permeability induced by histamine is shown together with those of terfenadine and ONO-1078, a potent LTD_4 antagonist.[12] Administered intraperitoneally at a dose of 10 mg/kg, the chromone **1k**, like terfenadine, almost completely blocked the effect of histamine whereas ONO-1078 has little effect. The chromone **1k** also exerts inhibition, in a dose-dependent manner, to the LTD_4-induced increase in vascular permeability (Figure 2). Thus at a dose of 10 mg/kg, **1k** inhibited the effect of LTD_4 by about 30%, whereas at a dose of 50 mg/kg the inhibition was increased to 56%. Terfenadine at a dose of 10 mg/kg had little effect, while ONO-1078 at the same dose blocked completely the effect of LTD_4. Although the LTD_4-receptor antagonism of **1k** and its analogues is much lower than their histamine H_1-receptor antagonism, this additional anti-LTD_4 activity is likely to increase their efficacy against complicated allergic conditions like asthma. This is evidenced by the much improved efficacy of azelastine, a novel H_1 antihistaminic with weak LTD_4-antagonism (IC_{50} 1.1×10^{-5} M),[20] in the treatment of asthma compared with other conventional antihistamines.[21] Furthermore it has recently been observed that the LTD_4-antagonism reaches a certain level of plateau, implying a very high receptor affinity may not be necessary, for it is unlikely to increase much the efficacy in the clinical conditions.[22]

In conclusion we have synthesized a series of novel chromone derivatives. In addition to their potent antihistaminic activity, the compounds also antagonize the effect of LTD_4. One compound of this series (**1k**) has been identified to inhibit both in vitro and in vivo action of histamine and LTD_4. At a dose of 12 mg/kg, ip, **1k** exhibits weak inhibition (20%) of [^{3}H]mepyramine binding in mouse brain determined by ex vivo assay.[23] Under identical conditions, terfenadine (a nonsedating antihistamine) exhibits 18% inhibition of [^{3}H]mepyramine binding and cyproheptadine (a sedating antihistamine) shows 50% inhibition at a dose of 0.1 mg/kg. We conclude that **1k** does not readily penetrate the blood–brain barrier, thus unlikely to cause sedation. Coupled with its lower acute toxicity (LD_{50} 492 mg/kg, ip) than that of terfenadine (LD_{50} 43 mg/kg, ip) and negligible anticholinergic activity, **1k** is a potencially useful drug for the management of allergic disorders in general and asthma in particular.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane, and coupling constants are in hertz (Hz). Mass spectral data were registered on a Finnigan MAT 90 mass spectrometer with electron impact (EI) ionization, ion source temperature 200 °C, source pressure 2.2×10^{-6} Torr. Melting points were determined on a Mettler FP-5 melting point apparatus and are uncorrected. Specific rotations were measured on a Perkin-Elmer 241 MC polarimeter. Thin-layer chromatography was performed on a Kiesegel 60 F254 (Merck) thin-layer chromatography (TLC) aluminum sheets.

2-Carbamoyl-7-hydroxy-4-oxo-4*H*-1-benzopyran (9). A solution of 2.34 g (10 mmol) of ethyl 7-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate[13] in 100 mL of anhydrous ethanol was bubbled with dry ammonia for 15 min. The solution was then allowed to stand at room temperature overnight. The yellow precipitate was collected by filtration, washed with water, and dried in vacuum. Yield: 97%. Mp: 270–271 °C. ^{1}H NMR (DMSO-d_6): δ 6.66 (s, 1H, C_4-H), 6.87 (m, 2H, $C_{6,8}$-H), 7.80 (d, 1H, $J = 8.3$ Hz, C_5-H), 8.08 and 8.48 (two s, 2H, NH_2), 11.01 (s, 1H, OH).

2-Cyano-7-hydroxy-4-oxo-4*H*-1-benzopyran (10). To a solution of 2.05 g (10 mmol) of 2-carbamoyl-7-hydroxy-4-oxo-4*H*-1-benzopyran in 50 mL of dry *N*,*N*-dimethylformamide at 0 °C were added 4.62 g (22 mmol) of trifluoroacetic anhydride and 3.16 g (40 mmol) of pyridine. The mixture was then stirred at room temperature overnight. After removing the solvent, water was added to the residue and the mixture was allowed to stand at room temperature for 2 h. The off-white precipitate was collected by filtration, washed with water, and dried in vacuum. Recrystallization from methanol afforded the title compound as a white crystalline product. Yield: 98%. Mp: 251–252 °C. ^{1}H-NMR (DMSO-d_6): δ 6.91 (d, 1H, $J = 2.2$ Hz, C_8-H), 6.95–7.01 (m, 1H, C_6-H), 7.21 (s, 1H, C_3-H), 7.88 (d, 1H, $J = 8.8$ Hz, C_5-H), 11.16 (s, 1H, OH).

4-(α,α-Diphenylmethoxy)piperidine (6). A solution of 1.84 g (10 mmol) of α,α-diphenylmethanol, 1.01 g (10 mmol) of 4-hydroxypiperidine, and 2.09 g (11 mmol) of *p*-toluenesulfonic acid monohydrate in 500 mL of toluene was refluxed with a Dean–Stark condenser for 3 h. After cooling to room temperature, the toluene solution was washed with 5% sodium hydroxide solution and water and dried with sodium sulfate. Removing the solvent afforded the title compound as a thick colorless oil. Yield: 90%. ^{1}H NMR ($CDCl_3$): δ 1.55 (m, 2H, piperidine), 1.9 (m, 3H, piperidine and piperidine NH), 2.5 (m, 2H, piperidine), 3.0 (m, 2H, piperidine), 3.5 (m, 1H, piperidine), 5.55 (s, 1H, diphenyl-CH), 7.4–7.2 (m, 10H, diphenyl).

2-Cyano-7-[(3-chloropropyl)oxy]-4-oxo-4*H*-1-benzopyran (3b). A mixture of 2.43 g (13 mmol) of 2-cyano-7-hydroxy-4-oxo-4*H*-1-benzopyran (**10**), 2.04 g (13 mmol) of 3-chlorobromopropane, and 1.80 g (13 mmol) of potassium carbonate in 250 mL of dry acetone was refluxed overnight. After evaporating to dryness, the residue was extracted with chloroform (3 × 100 mL). After drying with sodium sulfate, the chloroform solution was evaporated to one-third of the original volume to which *n*-hexane was added. The white precipitate was collected by filtration, washed with *n*-hexane, and dried in vacuum. Yield: 51%. Mp: 124–125 °C. ^{1}H NMR ($CDCl_3$): δ 2.31 (m, 2H, $CH_2CH_2CH_2$), 3.77 (t, 2H, J = 6.1 Hz, $ClCH_2$), 4.24 (t, 2H, J = 5.9 Hz, CH_2O), 6.77 (s, 1H, C_3-H), 6.90 (d, 1H, J = 2.3 Hz, C_8-H), 7.04 (m, 1H, C_6-H), 8.09 (d, 1H, J = 8.9 Hz, C_5-H).

Ethyl 7-[(3-Chloropropyl)oxy]-4-oxo-4*H*-1-benzopyran-2-carboxylate or Ethyl 6-[(3-Chloropropyl)oxy]-4-oxo-4*H*-1-benzopyran-2-carboxylate (3a). The title compounds were prepared from ethyl 7-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate[13] or ethyl 6-hydroxy-4-oxo-4*H*-1-benzopyran-2-carboxylate[14] in the same manner as described for **3b**. Yield: 90–93%.

General Procedure for the Preparation of (Piperidinylalkoxy)chromones 4 Exemplified by the Preparation of 2-Cyano-7-[[3-[4-(diphenylmethylene)piperidin-1-yl]propyl]oxy]-4-oxo-4*H*-1-benzopyran (4k). A mixture of 0.4 g (1.5 mmol) of 2-cyano-7-[(3-chloropropyl)oxy]-4-oxo-4*H*-1-benzopyran (**3b**), 0.38 g (1.5 mmol) of 4-(diphenylmethylene)piperidine, 0.225 g (1.5 mmol) of sodium iodide, and 0.21 g (1.5 mmol) of potassium carbonate in 200 mL of dry acetone was refluxed for 48 h. After removing the solvent, the solid residue was extracted with chloroform. The chloroform solution was evaporated to dryness, and the residue was put on a silica gel column and eluted with a mixture of diethyl ether/ethyl acetate (5:1) (TLC R_f = 0.39). Removing the solvents of the collected fractions afforded the title compound as a thick colorless oil. Yield: 79%. ^{1}H NMR ($CDCl_3$): δ 2.02 (m, 2H, $CH_2CH_2CH_2$), 2.36–2.58 (m, 10H, piperidine H & $CH_2CH_2CH_2N$), 4.10 (t, 2H, J = 6.1 Hz, CH_2O), 6.76 (s, 1H, chromone $C_{3'}$-H), 6.89 (d, 1H, J = 2.3 Hz, chromone $C_{8'}$-H), 7.01 (m, 1H, chromone $C_{6'}$-H), 7.10–7.30 (m, 10H, phenyl H), 8.06 (d, 1H, J = 9.0 Hz, chromone $C_{5'}$-H).

General Procedure for the Preparation of (Piperidinylalkoxy)chromonecarboxylic Acids 1a–j Exemplified by the Preparation of 6-[[3-[4-(Diphenylmethylene)piperidin-1-yl]propyl]oxy]-4-oxo-4*H*-1-benzopyran-2-carboxylic Acid (1b). A mixture of 0.52 g (1 mmol) of ethyl 6-[[3-[4-(diphenylmethylene)piperidin-1-yl]propyl]oxy]-4-oxo-4*H*-1-benzopyran-2-carboxylate (**4b**) in a 1:1 mixture of saturated sodium bicarbonate and ethanol was refluxed for 5 h. The solution was then evaporated to remove ethanol, and the remaining mixture was neutralized with acetic acid. The white precipitate was collected by filtration, washed with water, and dried in vacuum. Yield: 83%. The oxalate salt was prepared by dissolving the product in a methanol solution of oxalic acid and precipitating it by adding diethyl ether. Mp: 237–238 °C. ^{1}H NMR (free base, $CDCl_3$): δ 2.00 (m, 2H, $CH_2CH_2CH_2$), 2.40–2.56 (m, 10H, piperidine H & NCH_2), 4.10 (t, 2H, J = 6.4 Hz, CH_2O), 6.89–7.50 (m, 14H, aromatic H), 12.56 (s, 1H, COOH). HRMS: 495.5738 found for $C_{31}H_{29}NO_5$ (calculated, 495.5737).

7-[[3-[4-(Diphenylmethylene)piperidin-1-yl]propyl]oxy]-2-(5-tetrazolyl)-4-oxo-4*H*-1-benzopyran (1k). A mixture of 0.57 g (1.2 mmol) of 2-cyano-7-[[3-[4-(diphenylmethylene)piperidin-1-yl]propyl]oxy]-4-oxo-4*H*-1-benzopyran (**4k**), 0.47 g (7.2 mmol) of sodium azide, and 0.39 g (7.2 mmol) of ammonium chloride in 50 mL of dry *N*,*N*-dimethylformamide was stirred at 120 °C under nitrogen overnight. After evaporating to dryness, to the residue was added water, and the mixture was extracted with chloroform. The combined chloroform solution was dried with sodium sulfate and evaporated to dryness. Further purification on a silica gel column eluted with a mixture of ethyl acetate/methanol (2:1) (TLC R_f = 0.20) afforded the title compound as a yellow crystalline solid. Yield: 77%. Mp: 180–181 °C. ^{1}H NMR (DMSO-d_6): δ 1.96 (m, 2H, $CH_2CH_2CH_2$), 2.29–2.52 (m, 10H, piperidine H & $CH_2CH_2CH_2N$), 4.21 (t, 2H, J = 6.0 Hz, CH_2O), 6.80 (s, 1H, chromone $C_{3'}$-H), 7.02–7.35 (m, 12H, phenyl H & chromone $C_{6',8'}$-H), 7.94 (d, 1H, J = 8.9 Hz, chromone $C_{5'}$-H). FABMS: 519 found for $C_{31}H_{29}N_5O_3$ (calculated, 519.6018).

Pharmacology. In Vitro Inhibition of Histamine- or LTD_4-Induced Contraction of Guinea Pig Ileum. A piece of ileum (about 2 cm in length) isolated from guinea pigs was trimmed, tied at both ends, and mounted in a 20 mL organ bath containing Krebs buffer (37 °C, constantly bubbled with 95% O_2–5% CO_2). The first three dose–response experiments were performed by adding histamine or leukotriene D_4 cumulatively to the organ bath. After adequate washing, the ileal strip was incubated with the testing compound for 30 min. The dose–response experiment was then conducted again. The dissociation constant (K_b) of the receptor–antagonist complex was used as the parameter to indicate the potency of the testing compound and was calculated according to the Cheng–Prusoff equation.

In Vitro Inhibition of [^{3}H]Mepyramine Binding to Guinea Pig Lung Membranes. The method is based on that described previously.[10] Briefly, a mixture of a total volume of 1.0 mL containing 0.5 nM [^{3}H]mepyramine (specific activity 21 Ci/mmol), guinea pig lung membrane proteins (±370 μg/mL), and the testing compound in 50 mM Na-K phosphate buffer (pH 7.5) was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 5 mL of ice-cold phosphate buffer and followed by immediate filtration through Whatman GF/C filters. The filters were washed twice with about 20 mL of cold buffer. The retained radioactivity was determined by a liquid scintillation counter after addition of 5 mL of scintillation liquid.

In the saturation experiment, 10^{-4} M (*R*)-(−)-dimethindene was used to define the nonspecific binding. A single, saturable binding site with B_{max} = 278 ± 24 fmol/mg of protein was found from the saturation experiment. The K_D of [^{3}H]mepyramine was found to be (3.30 ± 0.26) × 10^{-9} M, and no cooperativity was detected when the data were analyzed by Hill plots (slope = 1.005).

In Vitro Inhibition of [^{3}H]LTD_4 Binding to Guinea Pig Lung Membranes. The method is a modification of that described in the literature.[24] Briefly, a mixture of a total volume of 0.3 mL containing 0.2 nM [^{3}H]LTD_4, guinea pig lung membrane proteins (±170 μg/mL), and the testing compound in 10 mM piperazine–*N*,*N'*-bis(2-ethanesulfonic acid) buffer (pH 7.5) was incubated at 22 °C for 30 min. The piperazine-*N*,*N'*-bis(2-ethanesulfonic acid) buffer contained 10 mM $CaCl_2$, 10 mM $MgCl_2$, 50 mM NaCl, 2 mM cysteine, and 2 mM glycine. The reaction was terminated by the addition of 5 mL of ice-cold Tris-HCl/NaCl buffer (10 mM/100 mM, pH 7.5). The mixture was immediately filtered under vacuum (Whatman GF/C filters), and the filters were washed once with 20 mL of ice-cold buffer. The retained radioactivity was determined by a liquid scintillation counter.

In the saturation experiment, 2 μM LTD_4 was used to define the nonspecific binding. A single, saturable binding site with B_{max} = 988 fmol/mg of protein was found from the saturation experiment. The K_D of [^{3}H]LTD_4 was found to be 2.16 × 10^{-10} M, and no cooperativity was detected when the data were analyzed by Hill plots (slope = 0.99).

Ex Vivo Inhibition of [^{3}H]Mepyramine Binding in Mouse Cortex. The method is based on that described in the literature.[23] Briefly, Swiss mice (20–23 g) were given a certain dose of the testing compound via ip injection. One hour after the administration, the mouse was killed by decapitation. The brain was dissected and homogenized in 50 mM Na-K phosphate buffer (pH 7.5) (40 mL/g of wet weight). Triplicate aliquots (900 μL) of homogenate were mixed with 100 μL of [^{3}H]mepyramine solution (final concentration 0.5 nM). Incubation was continued for 50 min at 37 °C. After addition of 5 mL of ice-cold phosphate buffer, the mixture was filtered (Whatman GF/C filters) and washed twice with 20 mL of cold buffer. The radioactivity retained on the filters was determined by a scintillation counter.

In Vivo Inhibition of Vascular Permeability Increase Induced by Histamine or LTD_4 in Guinea Pigs. Male Hartley guinea pigs (~250 g body weight) were deprived of food for 24 h but allowed free access to water. A solution of the testing compound (10 mg/mL) in DMSO was injected interaperitoneally at a dose of 10 mg/kg. One hour later, Evans blue dye was injected intravenously at a dose of 50 mg/kg (50 mg/mL of saline), and immediately histamine and LTD_4 were injected intradermally to the backs of the animals. Histamine and LTD_4 were dissolved in 0.1 mL of Tyrode solution and injected at doses of 300 and 50 ng/site, respectively. Thirty minutes later, the dorsal skin was removed and Evans blue dye was extracted. The concentration of the dye was then determined.

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JM950125S