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# Synthesis and pharmacological evaluation of some amino-acid-containing cyproheptadine derivatives as dual antagonists of histamine $H_1$- and leukotriene $D_4$-receptors

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**Summary** — A novel series of cyproheptadine derivatives, in which an amino acid or a dipeptide moiety was introduced at the piperidine nitrogen, have been synthesized. The amino acid and dipeptide moieties were taken as part of leukotriene $D_4$ ($LTD_4$) pharmacophore. This modification reduced the $H_1$-antihistamine activity (100–1000-fold) but elevated the anti-$LTD_4$ activity (10–100-fold) of the compounds, as compared with cyproheptadine. As a result, some of the new compounds, especially the α-amino-propionic acid derivatives **4**, are well-balanced dual antagonists of histamine and $LTD_4$ with both activities at micromolar range. Radioligand binding studies have confirmed that the new compounds, but not cyproheptadine for $LTD_4$, exert their action through competetive occupation of the receptors. One compound, (*S*)-2-benzyloxycarbonyl-amino-3-[4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclo-hepten-5-yloxy)piperidin-1-yl]propionic acid (**4c**), was tested in an in vitro guinea-pig asthma model. It exhibits much more potent inhibition ($IC_{50}$ = 1.5 μM) against antigen-induced contraction than either terfenadine or FPL55712, the reference drugs. As indicated by an ex vivo binding assay, the drug **4c** does not readily pass the blood–brain barrier, and therefore is unlikely to cause sedating side-effects at a therapeutic dose.

**antiasthmatic activity / antihistamine activity / drug design / dual antagonist / $H_1$-receptor / in vitro asthma model / leukotriene $D_4$ antagonist**

Histamine is probably the first chemical recognized as a mediator of immediate allergic response in humans. Despite the compelling evidence that suggests histamine's playing an important pathological role in asthma [1, 2], the effectiveness of antihistamines (or more precisely $H_1$-receptor antagonists) in the treatment of asthma is still a matter of debate [3, 4]. Interestingly, almost all $H_1$-antihistamines found moderately effective in asthma are reported to possess other 'beneficial' activities in addition to $H_1$-receptor antagonism [3–5]. In fact, asthma is such a complex disease that several chemical mediators are involved in its pathogenesis. It is unlikely, therefore, any drug with antagonism of a single mediator would be sufficiently effective for the management of asthma. As a consequence, increasing effort has been made to develop $H_1$-antihistamines with additional activities, for example, dual antagonists of histamine/PAF [6], histamine/$TXA_2$ [7], histamine/$LTD_4$ [8] and combined inhibitors of histamine action and phosphodiesterase IV [9]. The effectiveness of these multifaceted drugs in the treatment of asthma has yet to be proved by clinical investigation.

As part of our continuing effort in search for more effective antiasthmatic agents, we decided to develop dual antagonists of histamine $H_1$- and leukotriene $D_4$ ($LTD_4$)-receptors [8], not only because $LTD_4$ is probably the most important bronchoconstrictor in asthma as indicated by the clinical studies of $LTD_4$ antagonists, but also because $LTD_4$ and histamine complement each other during inflammatory and allergic responses. Histamine is preformed in cells, has a rapid onset of action and is therefore mainly responsible for the early phase of allergic reactions. In contrast, $LTD_4$ is synthesized 'on demand', has a slow onset and is believed to play a major role in the late phase reactions. Recent studies have even suggested that histamine and leukotrienes orchestrate an additive and synergetic effect in contracting airway smooth muscles and increasing vascular permeability after exposure of animals to allergen [10, 11].

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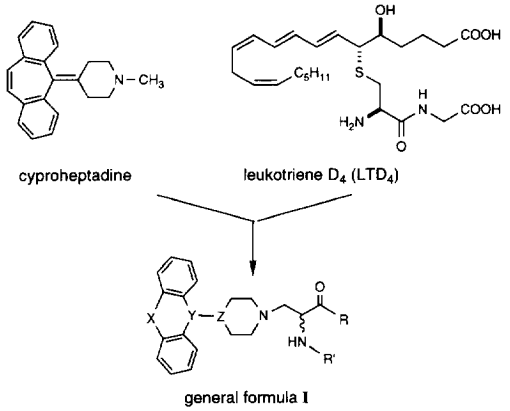
In this paper, we describe the synthesis and pharmacological evaluation of a series of cyproheptadine derivatives having an amino acid or a dipeptide moiety attached to the piperidine nitrogen (general formula I). Cyproheptadine was selected as the prototype of this series from an in vitro anti-$LTD_4$ screening of 22 known $H_1$-antihistamines. These antihistamines have diverse types of chemical structures (Zhang and Timmerman, unpublished results). (The 22 $H_1$-antihistamines tested are: acrivastine, astemizole, azelastine, cetirizine, *d*- and *l*-chlorpheniramine, *d*- and *l*-cicletanide, clemastine, cyproheptadine, *d*- and *l*-dimethindene, ebastine, flezelastine, levocabastine, loratadine, *d*- and *l*-MDL16,455 (terfenadine metabolites), temelastine, *d*- and *l*-terfenadine, triprolidine.) It was found that cyproheptadine exhibited about 50% inhibition at the concentration of 10 μM against 10 nM $LTD_4$-induced contraction of guinea-pig ileum. Since the drug also possesses many other pharmacological activities, we reasoned we could increase the selectivity as well as potency of the compounds towards $LTD_4$-receptor by incorporating some structural characteristics of $LTD_4$ itself (fig 1). This design strategy is based on the following two observations: i) many structural analogues of $LTD_4$ show antagonistic activity, implying $LTD_4$ and its antagonists may bind to the same site of the receptor [12]; and ii) two phenyl groups and the basic nitrogen of cyproheptadine compose the pharmacophore of $H_1$-antagonistic activity, indicating that substitution at the nitrogen may not influence the antihistamine activity too seriously [13]. It was also considered that some of the derivatives would exist as zwitterions so that they may not easily cross the blood–brain barrier, therefore avoiding the potential CNS side effects [14].

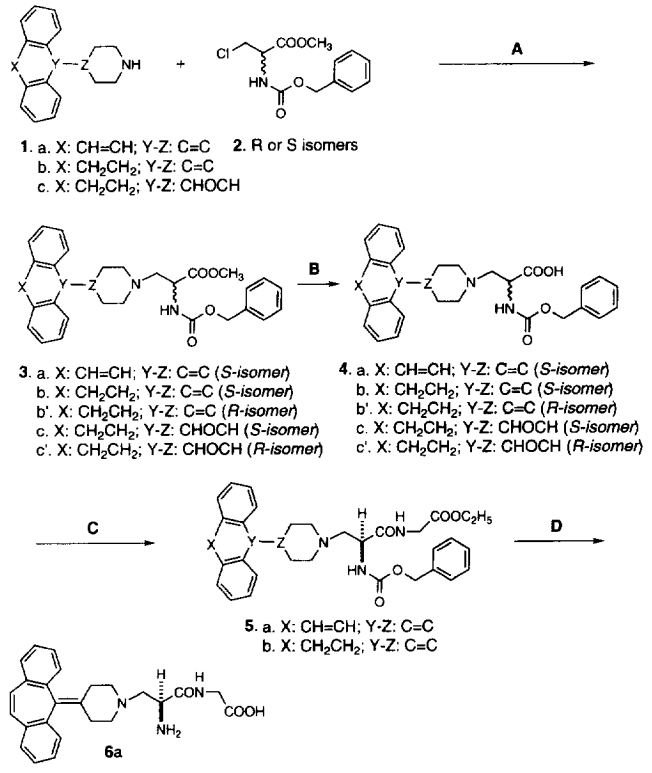



X: CH=CH or $CH_2CH_2$; Y-Z: C=C or CHOCH; R: $OCH_3$, OH, $NHCH_2COOC_2H_5$ or $NHCH_2COOH$; R': $OCOOCH_2Ph$ or H

**Fig 1.** Design of the target structures. Cyproheptadine pharmacophore is combined with an α-amino-acid residue typically found in $LTD_4$.

## Chemistry

The synthesis of the target compounds is illustrated in scheme 1. Alkylation of 4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidine **1a** [15], 4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidine **1b** [15] or 4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-yloxy)piperidine **1c** [16] with optically pure 3-chloropropionate **2** [17] afforded the tertiary amines **3** in reasonably good yield (> 50%). After purification with column chromatography, the esters **3** were hydrolyzed under mild conditions to the corresponding acids **4**. The peptide bond of the esters **5** was constructed by reacting glycine ethyl ester with the acids **4**, after the latter was typically activated with ethyl chloroformate. Since the α-amino group was protected by carbobenzoxy function throughout the above reactions, no racemization was observed although no direct measurement of optical purity was made. Hydrolysis of **5** first under basic, then acid conditions afforded **6a**.




**Scheme 1.** Synthesis of cyproheptadine derivatives. **A**: $NaI/Na_2CO_3$/acetone, reflux, overnight; **B**: 1 N NaOH aq/EtOH, rt, 3 h; **C**: $ClCOOEt/CH_2Cl_2/Et_3N/H_2NCH_2CO_2Et$ HCl, 0 °C, 1.5 h; **D**: i) 1 N NaOH/EtOH, rt, 3 h; ii) 2 N HCl, rt, 1 h.

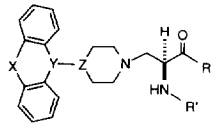
## Results and discussion

Compounds **3–6** were tested in vitro for their inhibitory activity against histamine ($H_1$) and $LTD_4$ induced contraction of guinea-pig ileum. As shown in table I, the antihistamine activity of the tested compounds were some 100–1000 times lower than that of cyproheptadine, but comparable to the still very effective $H_1$-antagonist terfenadine [18]. There is no apparent preference between the free bases **3** and the zwitterions **4** in antihistamine potency. Surprisingly, however, there is a significant difference of antihistamine potency between the enantiomeric pairs **4b/4b'** and **4c/4c'** (see table II). This is inconsistent with the general concept of $H_1$-antihistamine stereoselectivity, in which a chiral center away from the benzhydryl pharmacophore gives minor influence on the antihistamine activity [19].

Although most compounds from the present series are not very active against $LTD_4$-induced contraction, the propionic acids **4** are quite potent, with **4c** being more than 100 times more active than the prototype cyproheptadine (table II). Most importantly, **4b** and **4c** showed also relatively high affinity to $LTD_4$-receptors when assayed in a radioligand binding experiment. The $K_D$ values of the two compounds (table II) are comparable to that of FPL55712, a standard $LTD_4$ antagonist [20]. Cyproheptadine was totally inactive in the same assay and it should also be mentioned that the inhibitory activity of cyproheptadine against $LTD_4$ in the functional tests is not dose-dependent. These results indicate **4b** and **4c** exert their anti-$LTD_4$ activity by the occupation of $LTD_4$-receptors whereas cyproheptadine exhibits anti-$LTD_4$ activity by an unknown mechanism. The two derivatives **4b** and **4c**, which have the same configuration as that of the cysteine moiety in $LTD_4$, are also much more potent than their corresponding D-isomers **4b'** and **4c'**. In fact **4b'** and **4c'** did not displace the receptor bound [$^3$H]$LTD_4$ up to the concentration of 100 μM (table II). In an in vivo assay in guinea pigs [8], compound **4c** showed 95 and 30% inhibition, respectively, against histamine- and $LTD_4$-induced vascular permeability increase when administered intraperitoneally at a dose of 10 mg/kg.

The additional $LTD_4$-receptor antagonistic activity of this series of antihistamines, especially **4c**, as compared with cyproheptadine offers some advan-

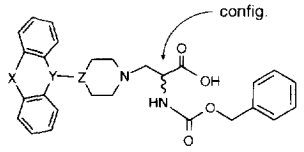
**Table I.** Novel cyproheptadine derivatives **3–6**[a].




| *Compound* | *X* | *Y-Z* | *R* | *R'* | *Antihistaminic*[b] *activity, $K_b$ (μM)* | *Anti-$LTD_4$*[c] *activity, $IC_{50}$ (μM)* |
|---|---|---|---|---|---|---|
| **3a** | CH=CH | C=C | $OCH_3$ | $C(O)OCH_2Ph$ | 0.85 ± 0.18 | >100 |
| **3b** | $CH_2CH_2$ | C=C | $OCH_3$ | $C(O)OCH_2Ph$ | 0.014 ± 0.008 | 95 ± 11 |
| **3c** | $CH_2CH_2$ | CHOCH | $OCH_3$ | $C(O)OCH_2Ph$ | 0.50 ± 0.23 | 98 ± 9 |
| **4a** | CH=CH | C=C | OH | $C(O)OCH_2Ph$ | 0.43 ± 0.12 | 65 ± 10 |
| **4b** | $CH_2CH_2$ | C=C | OH | $C(O)OCH_2Ph$ | 0.033 ± 0.010 | 2.58 ± 0.23 |
| **4c** | $CH_2CH_2$ | CHOCH | OH | $C(O)OCH_2Ph$ | 0.11 ± 0.05 | 0.89 ± 0.15 |
| **5a** | CH=CH | C=C | $NHCH_2C(O)OC_2H_5$ | $C(O)OCH_2Ph$ | 0.35 ± 0.20 | >100 |
| **5b** | $CH_2CH_2$ | C=C | $NHCH_2C(O)OC_2H_5$ | $C(O)OCH_2Ph$ | 0.18 ± 0.08 | >100 |
| **6a** | CH=CH | C=C | $NHCH_2C(O)OH$ | H | 0.24 ± 0.11 | >100 |
| Cyproheptadine | | | | | $6.31 \pm 0.25 \times 10^{-4}$ | 49 ± 15%[d] |
| Terfenadine | | | | | 0.25 ± 0.06 | >100 |
| FPL55712 | | | | | >100 | 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]Inhibition percentage of 10 nM $LTD_4$-induced contraction of guinea-pig ileum at the drug concentration of 10 μM.

**Table II.** Receptor binding affinity and stereoselectivity of the propionic acids **4**[a].




| *Compound* | *X* | *Y-Z* | *Configuration* | *Receptor affinity, $K_D$ (μM)* | |
|---|---|---|---|---|---|
| | | | | *$H_1$-receptor[b] vs [$^3$H]mepyramine* | *$LTD_4$-receptor[c] vs [$^3$H]$LTD_4$* |
| **4a** | CH=CH | C=C | *S* | 0.15 ± 0.07 | >100 |
| **4b** | $CH_2CH_2$ | C=C | *S* | 0.87 ± 0.21 | 6.80 ± 0.35 |
| **4b'** | $CH_2CH_2$ | C=C | *R* | 0.31 ± 0.05 | >100 |
| **4c** | $CH_2CH_2$ | HCOCH | *S* | 0.41 ± 0.08 | 1.55 ± 0.12 |
| **4c'** | $CH_2CH_2$ | HCOCH | *R* | 0.027 ± 0.010 | >100 |
| Cyproheptadine | | | | $5.4 \pm 0.8 \times 10^{-4}$ | >100 |
| Terfenadine | | | | 0.35 ± 0.18 | >100 |
| FPL55712 | | | | >100 | 1.12 ± 0.20 |

[a]All $K_D$ data are the means of of two independent binding assays performed in triplicate in guinea-pig cerebellum ($H_1$) or lung ($LTD_4$) membranes. [b](−)-Dimethindene (100 μM) was used to define the non-specific 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 0.3 mL ([$^3$H]mepyramine concentration: 0.5 nM). [c]$LTD_4$ (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).

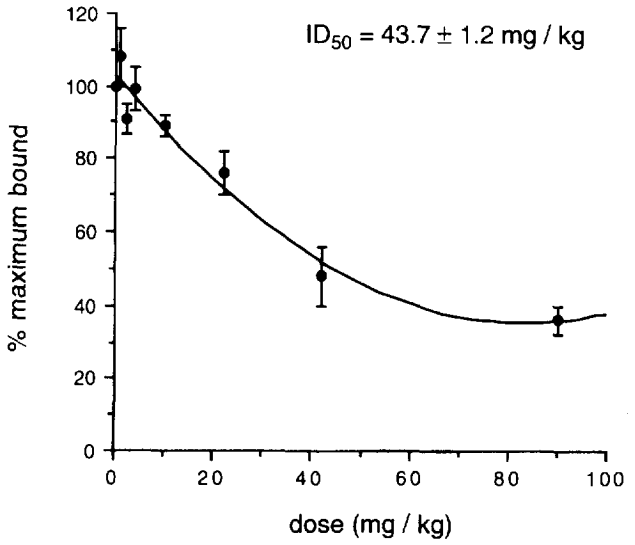



**Fig 2.** Ex vivo displacement of [$^3$H]mepyramine from mouse cortex by **4c**. Each point represents a mean ± SD of three measurements. In the identical experimental conditions, cyproheptadine and terfenadine showed $ID_{50}$ values of 0.02 and 26 mg/kg, respectively.

tages for systematic application, especially in avoiding excessive dosage which may cause sedating side effects. In fact when analyzed in an ex vivo binding assay with mouse cortex (fig 2), **4c** showed an $ID_{50}$ of 43.7 mg/kg, a little higher than that of terfenadine (26 mg/kg), a proven non-sedating $H_1$-antihistamine, and more than 2000 times higher than that of cyproheptadine (0.02 mg/kg), a sedating $H_1$-antihistamine. Therefore if a drug like **4c** was to be used at an anti-asthmatic dose, it would be very unlikely to cause sedation.

To prove that a dual antagonist of $H_1$- and $LTD_4$-receptors, such as **4c**, is more effective in releaving allergic conditions than a drug with single action, we have tested **4c** for its inhibitory activity against antigen-induced contraction of guinea-pig trachea. The single-acting agents terfenadine and FPL55712 were used to characterize the antigen-induced contraction. Since the two drugs possess a similar potency to the individual potency of **4c** (table II), they are the proper agents for comparison. As shown in figure 3A, treatment of the trachea with terfenadine (10 μM) before antigen challenge produced a delay in the onset of the response compared with the control, as well as a decrease in the rate of contraction and a decrease in height of the initial phase (the first 6 min) of the response. Interestingly, the contraction after 6 min is

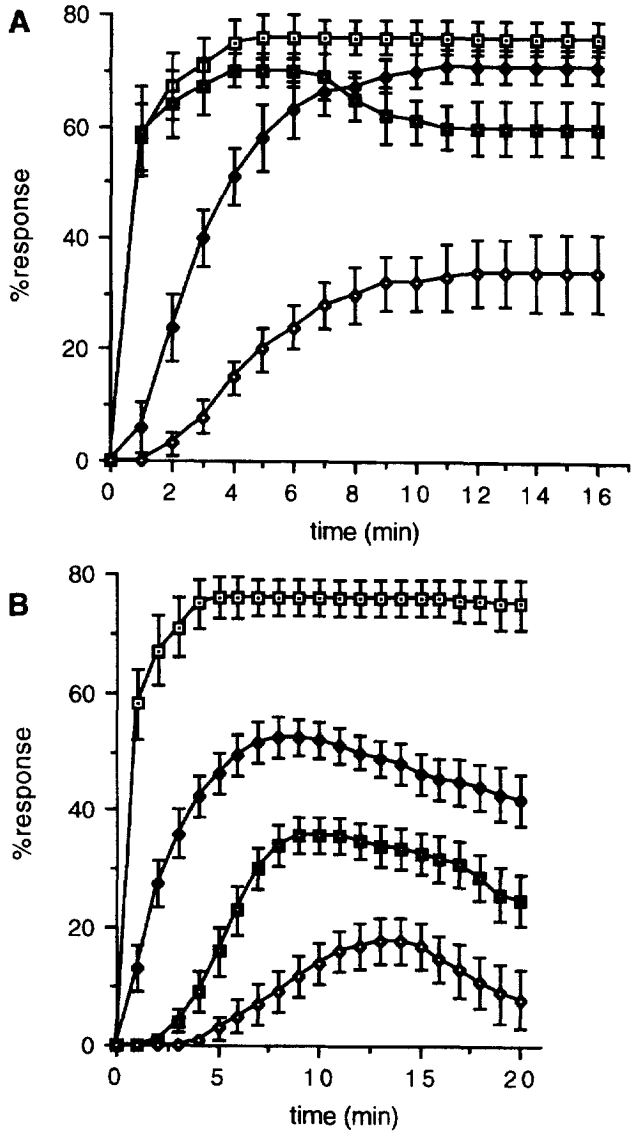



**Fig 3.** In vitro inhibition of antigen-induced contraction of guinea-pig trachea by terfenadine, FPL55712 (**A**) and **4c** (**B**). All points are means ± SD of four independent measurements. In graph **A**, the concentrations of terfenadine and FPL55712 are 10 μM, and the combination is a mixture of the two drugs, both in 10 μM. In graph **B**, increasing concentrations of **4c** were employed. All measurements were performed in a constantly air-bubbled Krebs buffer containing 3 μM indomethacin. Trachea were incubated with or without the drug 30 min before the challenge with antigen. ⊡ : control; ◆ terfenadine; ■ : FPL55712; ◇ : combination.

essentially unaffected by terfenadine. In contrast, treatment of the trachea with FPL55712 (10 μM) produced a significant decrease in the height of the later phase of the contraction (after 6 min), although it had little effect on the onset and initial phase of the contraction. These results indicate that the contractile response to antigen in guinea-pig trachea is mainly due to the release of histamine and leukotrienes, with histamine being responsible for the early contraction and leukotrienes for the later.

When the trachea was treated with a combination of terfenadine (10 μM) and FPL55712 (10 μM), an inhibition in both the early and late phases of the contraction was observed. Moreover the inhibiton is significantly greater than the additive effects of the two individual compounds, indicating a synergistic action (fig 3A). It is intriguing that the combination of terfenadine and FPL55712 did not completely abolish the contraction. Since both drugs are competitive antagonists, it is possible that the failure to achieve complete inhibition reflects a rapid phase of mediator release during which tissue concentrations of endogenous chemicals are extremely high [10]. Treatment of the trachea with **4c** (20 μM) produced a similar but stronger inhibition than that of the combination of terfenadine and FPL55712 (fig 3B). It delays the onset of the contraction and decreases both the rate and amplitude of the contraction in both early and late phases. The inhibition by **4c** is dose-dependent with an $IC_{50}$ of ± 1.5 μM.

In conclusion, we have synthesized a novel series of tricyclic antihistamines in which the traditional antihistamine pharmacophore was incorporated with some structural charateristics of leukotriene $D_4$. A few compounds from this series, especially **4c**, possess additional activity against $LTD_4$. The dual action of **4c** is well balanced. As the contractile response of airway smooth muscles to antigen appears to be a result of synergism of histamine and leukotrienes, a dual antagonist of histamine and leukotriene, such as **4c**, would be more effective than a single-acting antagonist in treating asthma, even if the dual antagonist had lower individual activity against different mediators. Because **4c** does not penetrate the brain easily, it is fairly reasonable to suggest that it will not cause sedating side-effects when applied at a therapeutic dose. The new compound **4c** (coded VUF4876) has been selected for further pharmacological evaluation.

## Experimental protocols

*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 MAT90 mass spectrometer with electron impact (EI) ionization, ion source temperature 200 °C, source pressure 2.2 x $10^{-6}$ 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 Kiesegel 60 F254 (Merck) thin-layer chromatography (TLC) aluminum sheets and all purified compounds showed a single spot.

*Methyl (S)-2-benzyloxycarbonylamino-3-[4-(5H-dibenzo[a,d]-cyclohepten-5-ylidene)piperidin-1-yl]propionate* ***3a***
A mixture of 4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidine [15] (2.73 g, 10 mmol), L-methyl 2-benzyloxycarbonylamino-3-chloropropionate [17] (2.71 g, 10 mmol), NaI (1.50 g, 10 mmol) and $Na_2CO_3$ (1.06, 10 mmol) in 250 mL dry acetone was refluxed overnight. After evaporating acetone, the residue was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ solution was dried over $Na_2SO_4$ and evaporated to dryness. The resulting light brown oil was put on a silica gel column and eluted with ether. The fractions with $R_f$ (TLC) = 0.85 ($Et_2O$/EtAc 1:1) were collected and evaporated to dryness. The title compound was thus obtained as a slightly yellow solid. Yield: 2.49 g (49%). Mp 116.0–118.8 °C. $[\alpha]_D^{25}$ – 2.1° (*c* = 1, $CHCl_3$). $^1$H-NMR ($CDCl_3$) δ (ppm): 2.0–2.7 (m, 10H, piperidine H + piperidine-NC$H_2$CH), 3.6 (s, 3H, $OCH_3$), 4.35 (m, 1H, piperidine-NC$H_2$C*H*), 5.1 (s, 2H, $OCH_2$Ph), 5.8 (d, 1H, *J* = 6 Hz, NH), 6.9 (s, 2H, dibenzocycloheptene $C_{10,11}$-H), 7.15–7.4 (m, 13H, aromatic H).

*Methyl (S)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro 5H-dibenzo[a,d]cyclohepten-5-ylidene)piperidin-1-yl]propionate* ***3b***
The title compound, a white crystalline product, was prepared in a similar way as described under **3a** with 4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidine [15] (2.75 g, 10 mmol) instead of 4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidine. Yield: 2.55 g (50%). Mp. 131.7–132.3 °C (EtOH). $[\alpha]_D^{25}$ –0.5° (*c* = 1, $CHCl_3$). $^1$H-NMR ($CDCl_3$) δ (ppm): 2.3–2.7 (m, 10H, piperidine H + piperidine-NC$H_2$CH), 2.85 and 3.5 (two m, 4H, dibenzocycloheptene $C_{10,11}$-H), 3.75 (s, 3H, $OCH_3$), 4.4 (m, 1H, piperidine-NC$H_2$C*H*), 5.15 (s, 2H, $OCH_2$Ph), 5.75 (d, 1H, *J* = 6 Hz, NH), 7.1–7.4 (m, 13H, aromatic H).

*Methyl (R)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)piperidin-1-yl]propionate* ***3b'***
The title compound, a white crystalline product, was prepared in a similar way as described for **3b** using D-methyl 2-benzyloxycarbonylamino-3-chloropropionate in the place of L-methyl 2-benzyloxy-carbonylamino-3-chloropropionate. Yield: 54%. Mp. 135.7–136.6 °C (EtOH). $[\alpha]_D^{25}$ + 0.7° (*c* = 1, $CHCl_3$). $^1$H-NMR ($CDCl_3$) δ (ppm): identical to those of **3b**.

*Methyl (S)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yloxy)piperidin-1-yl]propionate* ***3c***
The title compound, a light yellow oil, was prepared in a similar way as described under **3a** with 4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-yloxy)piperidine [17] (2.93 g, 10 mmol) instead of 4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidine. $R_f$ (TLC) = 0.95 (EtAc). Yield: 4.12 g (78%). $[\alpha]_D^{25}$ –1.5° (*c* = 1, $CHCl_3$). $^1$H-NMR ($CDCl_3$) δ (ppm): 7.75–2.65 (m, 10H, piperidine H + piperidine-NC$H_2$CH), 3.05 and 3.4 (two m, 4H, dibenzocycloheptene $C_{10,11}$-H), 3.55 (m, 1H, piperidine C4'-H), 3.65 (s, 3H, $OCH_3$), 4.25 (m, 1H, piperidine-NC$H_2$C*H*), 5.05 (s, 2H, $OCH_2$Ph), 5.5 (s, 1H, dibenzocycloheptene $C_5$-H), 5.6 (d, 1H, *J* = 6 Hz, NH), 7.1–7.4 (m, 13H, aromatic H).

*Methyl (R)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yloxy)piperidin-1-yl]propionate* ***3c'***
The title compound, a slightly brown oil, was prepared in a similar way as described under **3c** using D-methyl 2-benzyloxycarbonylamino-3-chloropropionate in the place of L-methyl 2-benzyloxycarbonylamino-3-chloropropionate. Yield: 71%. $[\alpha]_D^{25}$ + 1.5° (*c* = 1, $CHCl_3$). $^1$H-NMR ($CDCl_3$) δ (ppm): identical to those of **3c**.

*(S)-2-Benzyloxycarbonylamino-3-[4-(5H-dibenzo[a,d]cyclohepten-5-ylidene)piperidin-1-yl]-propionic acid* ***4a***
To a solution of methyl (*S*)-2-benzyloxycarbonylamino-3-[4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidin-1-yl]propionate (2 g, 3.9 mmol) in 30 mL ethanol was added 10 mL of 1 N NaOH aqueous solution. The mixture was then stirred at room temperature for 3 h. After removing most of the ethanol under reduced pressure, the remaining mixture was diluted with ice-water and neutralized with acetic acid. The mixture was then extracted with chloroform (2 x 30 mL). The combined chloroform extract was washed with saline and dried over $Na_2SO_4$. After evaporating to dryness, the solid residue was recrystallized from ethanol to afford the title compound as a white crystalline product. Yield: 1.77 g (91%). Mp 178.3–179.4 °C. $[\alpha]_D^{25}$ –26.3° (*c* = 1, DMF). $^1$H-NMR ($CDCl_3$) δ (ppm): 2.1–2.8 (m, 10H, piperidine H + piperidine-NC$H_2$CH), 4.3 (m, 1H, piperidine-NC$H_2$C*H*), 5.15 (s, 2H, $OCH_2$Ph), 5.25 (br s, 1H, NHCOO), 6.45 (br s, 1H, piperidine-N$^+$H), 6.95 (s, 2H, dibenzocycloheptene $C_{10,11}$-H), 7.2–7.4 (m, 13H, aromatic H).

*(S)-2-Benzyloxycarbonylamino-3-[4-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)piperidin-1-yl]propionic acid* ***4b***
The title compound, a white crystalline product, was prepared in a similar way as described for **4a** from methyl (*S*)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5*H*-dibenzo-[*a,d*]cyclohepten-5-ylidene)piperidin-1-yl]propionate. $R_f$ (TLC) = 0.65 (EtAc/MeOH 1.25:1). Yield: 95%. Mp 149.9–152.3 °C (EtOH). $[\alpha]_D^{25}$ –28.6° (*c* = 1, DMF). $^1$H-NMR ($CDCl_3$) δ (ppm): 2.4–2.85 (m, 10H, piperidine H + piperidine-NC$H_2$CH), 2.9 and 3.4 (two m, 4H, dibenzocycloheptene $C_{10,11}$-H), 4.15 (m, 1H, piperidine-NC$H_2$C*H*), 4.9 (br s, 1H, NHCOO), 5.05 (s, 2H, $OCH_2$Ph), 6.4 (br s, 1H, piperidine-N$^+$H), 6.9–7.15 (m, 13H, aromatic H). MS *(m/z)*: 496 $[M]^+$, 387 $[M - H - PhCH_2OH]^-$.

*(R)-2-Benzyloxycarbonylamino-3-[4-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)piperidin-1-yl]propionic acid* ***4b'***
The title compound, a white crystalline product, was prepared in a similar way as described for **4b** starting with methyl (*R*)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)piperidin-1-yl]propionate. Yield: 92%. Mp 149.9–152.3 °C (MeOH). $[\alpha]_D^{25}$ + 28.5° (*c* = 1, DMF). $^1$H-NMR ($CDCl_3$) δ (ppm): identical to those of **4b**.

*(S)-2-Benzyloxycarbonylamino-3-[4-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yloxy)piperidin-1-yl]propionic acid* ***4c***
The title compound, a slightly yellow crystalline product, was prepared in a similar way as described under **4a** from methyl (*S*)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5-yloxy)piperidin-1-yl]propionate. $R_f$ (TLC) = 0.10 (EtAc). Yield: 83%. Mp 131.5–133.0 °C (EtOH). $[\alpha]_D^{25}$ –7.1° (*c* = 1, DMF). $^1$H-NMR ($CDCl_3$) δ (ppm): 1.8–2.9 (m, 10H, piperidine H + piperidine-NC$H_2$CH), 3.05 and 3.5 (two m, 5H, dibenzocycloheptene $C_{10,11}$-H + piperidine $C_4$-H), 4.25 (m, 1H, piperidine-NC$H_2$C*H*), 4.95 (s, 2H, $OCH_2$Ph), 5.35

(br s, 1H, dibenzocycloheptene $C_5$-H), 5.7 (d, 1H, $J$ = 5.3 Hz, NHCOO), 6.55 (br s, 1H, piperidine-N$^+$H), 7.05–7.4 (m, 13H, aromatic H). MS *(m/z)*: 514 $[M]^+$, 405 $[M - H - PhCH_2OH]^-$, 208 [dibenzosuberone]$^{-\bullet}$.

*(R)-2-Benzyloxycarbonylamino-3-[4-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-yloxy)piperidin-1-yl]propionic acid* ***4c'***
The title compound, a white crystalline product, was prepared in a similar way as described for **4c** starting with methyl (*R*)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5*H*-dibenzo-[*a,d*]cycloheptene-5-yloxy)piperidin-1-yl]propionate. Yield: 85%. Mp 128.0–129.5 °C (MeOH/$Et_2O$). $[\alpha]_D^{25}$ + 7.8° (*c* = 1, DMF). $^1$H-NMR ($CDCl_3$) δ (ppm): identical to those of **4c**.

*Ethyl α-{(S)-2-benzyloxycarbonylamino-3-[4-(5H-dibenzo[a,d]-cyclohepten-5-ylidene)piperidin-1-yl]propionylamino}acetate* ***5a***
A solution of (*S*)-2-benzyloxycarbonylamino-3-[4-(5*H*-dibenzo[*a,d*]cycloheptene-5-ylidene)piperidin-1-yl]propionic acid (1.0 g, 2 mmol) and triethylamine (0.2 g, 2 mmol) in 20 mL dry $CH_2Cl_2$ was cooled to 0 °C on a salt–ice bath. To this solution was added dropwise 0.22 g (2 mmol) of ethyl chloroformate in 5 mL dry $CH_2Cl_2$. After the mixture was stirred at 0 °C for 30 min, a solution of glycine ethyl ester hydrochloride (0.28 g, 2 mmol) and triethylamine (0.2 g, 2 mmol) in 10 mL dry ether was added and the mixture was stirred at 0 °C for another 30 min. The mixture was consequently stirred at room temperature for 15 min and refluxed for 30 min. After cooling, the solution was washed with saline and the organic layer was separated, dried over $Na_2SO_4$ and evaporated to dryness. The oily residue was put on a silica gel column and eluted with a mixture of petroleum ether (40–60 °C)/EtAc 1:1. The fractions with $R_f$ = 0.44 (petroleum ether (40–60 °C)/EtAc 1:1) was collected and evaporated to dryness. To the oily residue was added a solution of oxalic acid in ether to afford the title compound as a white crystalline oxalate. Yield: 0.83 g (71%). mp. 95.0–97.6 °C. $[\alpha]_D^{25}$ –7.9° (*c* = 1, DMF). $^1$H-NMR ($CDCl_3$) δ (ppm): 1.3 (t, 3H, $J$ = 6.7 Hz, $CH_2CH_3$), 2.2–2.9 (m, 10H, piperidine H + piperidine-$NCH_2CH$), 3.95 (m, 1H, piperidine-$NCH_2CH$), 4.15 (m, 2H, $CONHCH_2$), 4.2 (q, 2H, $J$ = 6.7 Hz, $CH_2CH_3$), 5.1 (s, 2H, $OCH_2Ph$), 5.95 (br s, 1H, NHCOO), 6.9 (s, 2H, dibenzocycloheptene $C_{10,11}$-H), 7.15–7.35 (m, 13H, aromatic H), 8.9 (br s, 1H, $CONHCH_2$).

*Ethyl α-{(S)-2-benzyloxycarbonylamino-3-[4-(10, 11-dihydro-5H-dibenzo[a,d]cycloheptene-5-ylidene)piperidin-1-yl]propionylamino}acetate* ***5b***
The title compound, a white crystalline product, was prepared in a similar way as described under **5a** from (*S*)-2-benzyloxycarbonylamino-3-[4-(10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene-5-ylidene)piperidin-1-yl]propionic acid. Yield: 58%. Mp 125.6–126.4 °C. $[\alpha]_D^{25}$ –1.0° (*c* = 1, $CHCl_3$). $^1$H-NMR ($CDCl_3$) δ (ppm): 1.25 (t, 3H, $J$ = 6.7 Hz, $CH_2CH_3$), 2.35 (m, 6H, piperidine H + $CH_2CHNH$), 2.65 (m, 4H, piperidine H), 2.8 and 3.35 (two m, each 2H, dibenzocycloheptene $C_{10,11}$-H), 3.95 (d, 2H, $J$ = 7 Hz, $CONHCH_2$), 4.15 (q, 2H, $J$ = 6.7 Hz, $CH_2CH_3$), 4.25 (m, 1H, $CH_2CHNH$), 5.1 (s, 2H, $CH_2Ph$), 5.7 (br s, 1H, $CH_2CHNH$), 5.85 (br s, 1H, $CONHCH_2$), NHCOO), 7.05–7.3 (m, 13H, aromatic H).

*α-{(S)-2-Amino-3-[4-(5H-dibenzo[a,d]cyclohepten-5-ylidene)-piperidin-1-yl]propionylamino}acetic acid* ***6a***
To a solution of ethyl α-{(*S*)-2-benzyloxycarbonylamino-3-[4-(5*H*-dibenzo[*a,d*]cycloheptene-5-ylidene)piperidin-1-yl]propionyl-amino}acetate (1 g, 1.7 mmol) in 20 mL ethanol was added 20 mL of 1 N NaOH solution. The mixture was stirred at room temperature for 3 h and acidified with conc HCl. The mixture was stirred at room temperature for another hour. After neutralization with $NaHCO_3$, the white precipitate was collected by filtration and dried in vacuum. Recrystallization from methanol afforded the title compound as a white crystalline product. Yield: 0.5 g (69%). Mp 175.3–176.2 °C. $[\alpha]_D^{25}$ –8.9° (*c* = 1, DMF). $^1$H-NMR (DMSO-$d_6$) δ (ppm): 2.05–2.8 (m, 10H, piperidine H + piperidine-$NCH_2CH$), 3.2 (s, 2H, $NH_2$), 3.7 (d, 2H, $J$ = 5.5 Hz, $CONHCH_2$), 4.15 (m, 1H, piperidine-$NCH_2CH$), 6.45 (d, 1H, $J$ = 5.5 Hz, $CONHCH_2$), 6.55 (br s, 1H, piperidine-N$^+$H), 7.0 (s, 2H, dibenzocycloheptene $C_{10,11}$-H), 7.2–7.4 (m, 8H, aromatic H).

*Pharmacology*

*In vitro inhibition of histamine- or $LTD_4$-induced contraction of guinea-pig ileum*
The method used was similar to that described previously [8]. Briefly, a piece of ileum (about 2 cm 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 is 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 [18]. Briefly, a mixture of 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 ice-cold phosphate buffer and followed by immediate filtration through Whatman GF/C filters. The filters were washed twice with about 20 mL cold buffer. The retained radioactivity was determined by a liquid scintillation counter after addition of 5 mL scintillation liquid.

In the saturation experiment, $10^{-4}$ M *R*(–)dimethindene was used to define the non-specific binding. A single, saturable binding site with $B_{max}$ = 278 ± 24 fmol/mg protein was found from the saturation experiment. The $K_D$ of [$^3$H]mepyramine was found to be 3.30 ± 0.26 x $10^{-9}$ 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 very similar to that described previously [8]. Briefly, a mixture of 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 a 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 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 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 non-specific binding. A single, saturable binding site with $B_{max}$ = 988 fmol/mg protein was found from the saturation experiment. The $K_d$ of [$^3$H]-$LTD_4$ was found to be 2.16 x $10^{-10}$ 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 [8]. Briefly, Swiss mice (20–23 g) were given a certain dose of the testing compound via ip injection. One hour after 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 wet weight). Triplicate aliquots (900 μL) of homogenate were mixed with 100 μL [$^3$H]mepyramine solution (final conc 0.5 nM). Incubation was continued for 50 min at 37 °C. After addition of 5 mL ice-cold phosphate buffer, the mixture was filtered (Whatman GF/C filters) and washed twice with 20 mL cold buffer. The radioactivity retained on the filters was determined by a scintillation counter.

*In vitro inhibition of antigen-induced contraction of guinea-pig trachea*

*Sensitization.* Male Hartley guinea pigs (450–500 g) were given, via ip injection, 2 mg ovalbumin (OA) in 200 μL saline (0.9% NaCl). The procedure was performed at least 2 weeks before the experiments.

*Antigen-induced contraction.* The animals were sacrificed by a sharp blow to the head and tracheae were isolated, carefully trimmed of excess fatty and connective tissue and cut into strips of equal width (one full cartilage ring, ~ 2 mm). The preparation was performed under 50 mM $Na^+/K^+$ phosphate buffer. Each preparation was placed in a 20 mL water-jacketed organ bath containing Krebs buffer which was maintained at 37 °C and constantly aerated with 95% $O_2$/5% $CO_2$. The Krebs buffer contained, besides the usual composites, 3 μM indomethacin. The tissues were placed under 0.5 g of passive tension and equilibrated for 60 min, during which they were washed every 15 min with fresh indomethacin-containing Krebs solution. Before the challenge with ovalbumin, the tissues were incubated with or without the testing drug for 30 min. Tissues were contracted with a single concentration of ovalbumin (5 ng/mL) for an indicated time (20–60 min). After this period, carbachol (10 μM) was added to the preparations, and the antigen-induced response, which was recorded continuously, was expressed as a percentage of this reference carbachol contraction. No significant difference was found in carbachol-induced contraction with or without the tested compound. Only one OA-induced contraction was generated per preparation.

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