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Optically Active Analogues of Ebastine: Synthesis and Effect of Chirality on Their Antihistaminic and Antimuscarinic Activity

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ABSTRACT A series of optically active analogues of the H_1-antihistamine ebastine, with chiral center(s) at the benzhydryl and/or phenylbutyl part of the molecule, have been synthesized. Their in vitro antihistaminic and antimuscarinic activities were investigated, along with a molecular modelling study. It was found that introduction of the benzhydryl chiral center yielded significant stereoselectivity for both antihistaminic and antimuscarinic activities. The steric preferences of the benzhydryl chiral center for antihistaminic and antimuscarinic actions were mirror images of each other. The (−)-isomer of 4-methylebastine (**6d**) showed more than 10-fold higher in vitro antihistaminic potency than ebastine. Meanwhile the selectivity of **6d** for histamine H_1-receptors was also increased by more than 20 times in comparison with ebastine. The chirality at the phenylbutyl part of the molecule does not significantly alter the antihistaminic or antimuscarinic activity of the compounds although the (S)-isomers showed slightly but unanimously higher antihistaminic activity than the (R)-isomers. These results have been discussed with existing stereoselectivity data of antihistamines and an asymmetric pharmacophore model for H_1-antagonists has been described.

KEY WORDS: benztropine, histamine, H_1-receptors, molecular modelling, 4-methyldiphenhydramine, stereoselectivity, synthesis

Histamine H_1-receptor antagonists are effective therapeutic agents for the treatment of allergic disorders such as conjunctivitis, rhinitis, urticaria, etc. However, almost all classic histamine H_1-receptor antagonists suffer from troublesome side effects related to central nervous system depression and muscarinic receptor mediated dry mouth and visual disturbances. Several studies[1–3] have indicated that the CNS depression of histamine H_1-receptor antagonists is directly associated with the blockade of cerebral histamine H_1-receptors. In a study on the CNS effects of the enantiomers of chlorpheniramine and dimethindene in healthy human volunteers, it was found that only those enantiomers active as H_1-antagonists, i.e., (+)-chlorpheniramine and (−)-dimethindene, caused drowsiness and impaired performance.[4] Hence an H_1-antagonist incapable of crossing the blood–brain barrier would be devoid of such CNS side effects, as already claimed for many new nonsedating H_1-antagonists.

In contrast with the CNS studies, much less attention has been paid to those side effects associated with muscarinic receptor blockade of H_1-antagonists, although many so-called second generation H_1-antagonists do show, to different extents, affinity for the muscarinic receptors.[5,6] The discovery of selective H_1-antagonists devoid of antimuscarinic activity is therefore still a matter of trial and error. An earlier study[7] from this research group on an extended series of diphenhydramine derivatives suggested that the two phenyl rings of the benzhydryl moiety, found also in many other antihistamines, have "reverse" roles in the interaction with histamine and muscarinic receptors. For example, the unsubstituted phenyl group of (+)-(R)-4-methyldiphenhydramine (**1**, Scheme 1) was suggested to bind to a sterically hindered pocket of H_1-receptors and the 4-methylphenyl group was though to be involved in a π–π interaction with the H_1-receptor binding site. This orientation of the two phenyl rings is reversed when the drug binds to the muscarinic receptors.[8] This time, the 4-methylphenyl group binds to a sterically hindered pocket and the unsubstituted phenyl group participates in the π–π interaction. These proposed "reverse" modes of interaction at histamine and muscarinic receptors are further supported by the observation that the antihistaminic and antimuscarinic activity of 3-methoxycyproheptadine resides in the opposite antipodes.[9] Thus the (−)-pRapSb-isomer **3** (the designation of the absolute configuration is based on the Cahn–Ingold–Prelog conventions for planar chirality[10]) is the antihistaminic eutomer showing more than

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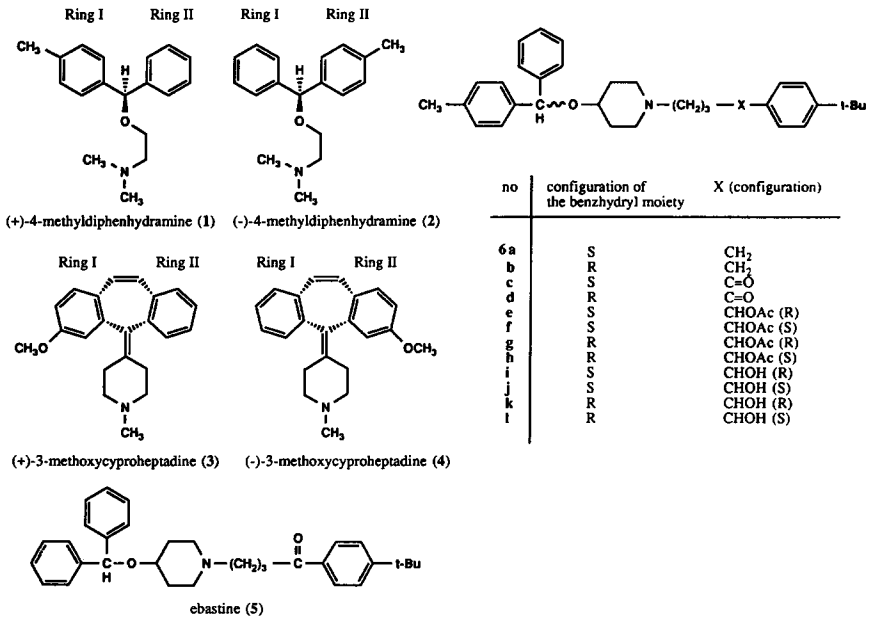


no	configuration of the benzhydryl moiety	X (configuration)
6a	S	CH_2
b	R	CH_2
c	S	C=O
d	R	C=O
e	S	CHOAc (R)
f	S	CHOAc (S)
g	R	CHOAc (R)
h	R	CHOAc (S)
i	S	CHOH (R)
j	S	CHOH (S)
k	R	CHOH (R)
l	R	CHOH (S)

Scheme 1. Structures of H_1 antihistamines studied in this article.

8-fold higher potency than (+)-pSapRb-isomer **4**, whereas **4** is the antimuscarinic eutomer with almost 40 times higher affinity than **3**[10] (see Scheme 1 for structures).

To validate further the hypothesis of the opposite steric preference for antihistaminic and antimuscarinic activity, we have synthesized a series of optically active analogues of ebastine **(5)**, a nonsedating H_1-antagonist, and tested them for antihistaminic and antimuscarinic activity. The different steric requirements for the compounds to interact with either of the receptors have been analyzed by a molecular modelling study. The results are discussed along with the previously known stereoselectivity of H_1-antagonists.

Chemistry

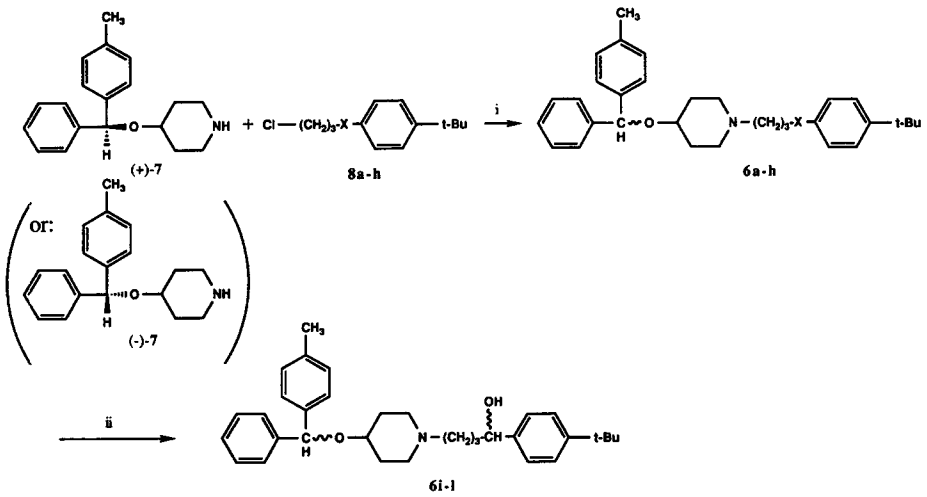
Optically pure analogues of ebastine **6a–h** were synthesized by the alkylation of (+)- or (−)-enantiomers of 4-[α-(4-methylphenyl)-α-phenylmethoxy]piperidine **(7)** with alkyl chlorides **8a–h** (Scheme 2). The butanol derivatives **6i–l** were obtained by the reduction of the corresponding acetates **6e–h** with lithium aluminium hydride in dry ether. The optical purity of **6i–l** (>95%) was determined by ^{31}P-NMR after derivatization with (+)-(4R,5R)-2-chloro-4,5-dimethyl-1,3,2-dioxaphospholane-2-oxide (Aldrich).[11] The preparation of alkyl chlorides **8a–h** as well as their stereochemistry has previously been reported.[11]

In the literature, diphenylmethoxypiperidines have been prepared by Williamson ether formation, either between a diphenylmethanol and a halopiperidine or between a diphenylmethyl halide and a hydroxypiperidine.[7,12] The disadvantage of this type of reactions is the undesired formation of piperidine N-substituted side products. Although occasionally the yield of ether formation can be increased by the addition of a tertiary amine,[13] in general it is necessary to protect the piperidine nitrogen, e.g., as urethane which has to be removed under rather harsh conditions. In the present study we have synthesized racemic diphenylmethoxypiperidine **7** by refluxing α-(4-methylphenyl)-α-phenylmethanol **(9)** and 4-hydroxypiperidine in toluene in the presence of *p*-toluenesulfonic acid (Scheme 3). The reaction was complete within 3 h and the yield of the desired ether **7** was about 90%. Obviously the much more rapidly formed benzhydryl carbocation and its steric bulkiness avoided formation of symmetric ethers.

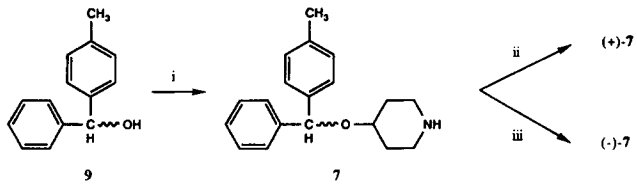
Resolution of racemic **7** was achieved by diastereoisomeric salt formation with (+)-dibenzoyl-D-tartaric acid or (−)-dibenzoyl-L-tartaric acid. The optical purity of the free bases (+)-**7** and (−)-**7** was more than 96% e.e..[14] The assignment of absolute configurations for (+)- and (−)-**7** was based on the comparison of characteristics between enantiomers of 4-methyldiphenhydramine (**1** and **2**) and (+)-**7**. As the chiral centers of compound **7** and 4-methyldiphenhydramine are closely related and the chromophores directly connected to their chiral centers are identical, the antipodes of the same absolute configuration should give rise to CD effects of identical sign and appearance. In Figure 1, their CD spectra are presented as plots of wavelength against ΔE, the differential molar absorptivity of left and right circularly polarized light. The close correspondence of the CD spectra of (+)-**7** and (−)-4-methyldiphenhydramine is evidence for the identical configuration of their benzhydryl chiral centers. Thus (+)-**7** was assigned the S-configuration and (−)-**7** was assigned the R-configuration.

Pharmacology

In vitro functional assay of ebastine derivatives **6a–l** was performed on isolated guinea pig ileum using either histamine or methacholine as the agonist. The usual cummulative concentration–response technique was employed for evaluating the antagonistic activity of the compounds. The negative log-



Scheme 2. Reagents: (i) Na_2CO_3/butanone-2, reflux, overnight; (ii) $LiAlH_4$/ether, rt, 4 h.



Scheme 3. Reagents: (i) 4-hydroxypiperidine/TsOH/toluene, reflux, 3 h; (ii) a. (+)-dibenzoyl-D-tartaric acid/methanol, b. 10% NaOH; (iii) a. (−)-dibenzoyl-L-tartaric acid/methanol, b. 10% NaOH.

arithm of the dissociation constant ($-\log K_B$) of the receptor–antagonist complex is used as the parameter to indicate the potency of the compound tested. This parameter is calculated by the following equation, assuming that the compound compete for the same receptor as the agonist:

$$-\log K_B = \log(EC'_{50}/EC_{50} - 1) - \log C_{compound}$$

wherein EC'_{50} and EC_{50}, respectively, represent the concentrations of the agonist (histamine or methacholine) giving 50% of the maximum contraction in guinea pig ileum in the presence or absence of the test compound at the concentration of $C_{compound}$. The results are summarized in Table 1.

Molecular Modelling

In order to gain insight into the different steric requirements of histaminergic (H_1) and muscarinic receptors[8] for interaction with this group of compounds, as well as the relationship between the stereoselectivity of these compounds with those of previously known antihistamines, we performed a molecular modelling study based on the cyproheptadine pharmacophore described by van Drooge et al.[15] This model was derived from a comformational analysis of cyproheptadine and fitting of two semirigid antihistamines triprolidine and phenindamine onto different conformers of cyproheptadine. The tentative active conformation of cyproheptadine was thus found to be that in which the piperidylene-ring assumes a boat conformation (see Fig. 2A).

For modelling of **6**, a structurally simpler analogue diphenylpyraline (**10**, Scheme 4) was used to avoid an unnecessary large number of possible conformers from the flexible phenylbutyl tail. This simplification is justified by the fact that diphenylpyraline (antihistamine pA_2 6.8 and antimuscarinic pA_2 7.2[7]) is the most likely pharmacophore of **6** for both antihistamine and antimuscarinic activities. From the compounds presented in Table 1, it is clear that chirality as well as structural variation in the phenylbutyl side chain does not alter the activity of **6** as significantly as those in the benzhydryl part.

To determine the conformational preferences of the piperidine ring system of **6**, a nortropine analogue benztropine (**11**, Scheme 4) (antihistamine pA_2 9.4 and antimuscarinic pA_2 9.7[7]) was incorporated in the modelling study. The rigidity of the nortropine ring system in combination with the potent antihistamine and antimuscarinic activity of benztropine allows easier identification of the active conformation of the piperidine ring of **6**. Enantiomers of 4-methyldiphenhydramine were also studied so that to identify the different putative roles of the two phenyl rings.

All structures were built in the nitrogen protonated form with the modelling package ChemX (Chemical Design Ltd., Oxford, UK) using crystal structure fragments. The structures were minimized with the AMBER force field as implemented in MacroModel.[16] The minimization was stopped when the RMS value was smaller than 0.100 kJ/Å-mol.

Conformational analysis was performed on the minimized structure using the MULTIC option in MacroModel. During the conformational analysis, all free rotatable bonds were rotated 360° in steps of 60° except the bonds connecting phenyl groups, which were rotated 180° in steps of 60° (see Scheme 4). All conformations were then minimized in batch mode using the BATCHMIN subprogram.[16] All duplicate conformers and conformers with energy higher than 20 kJ/mol com-

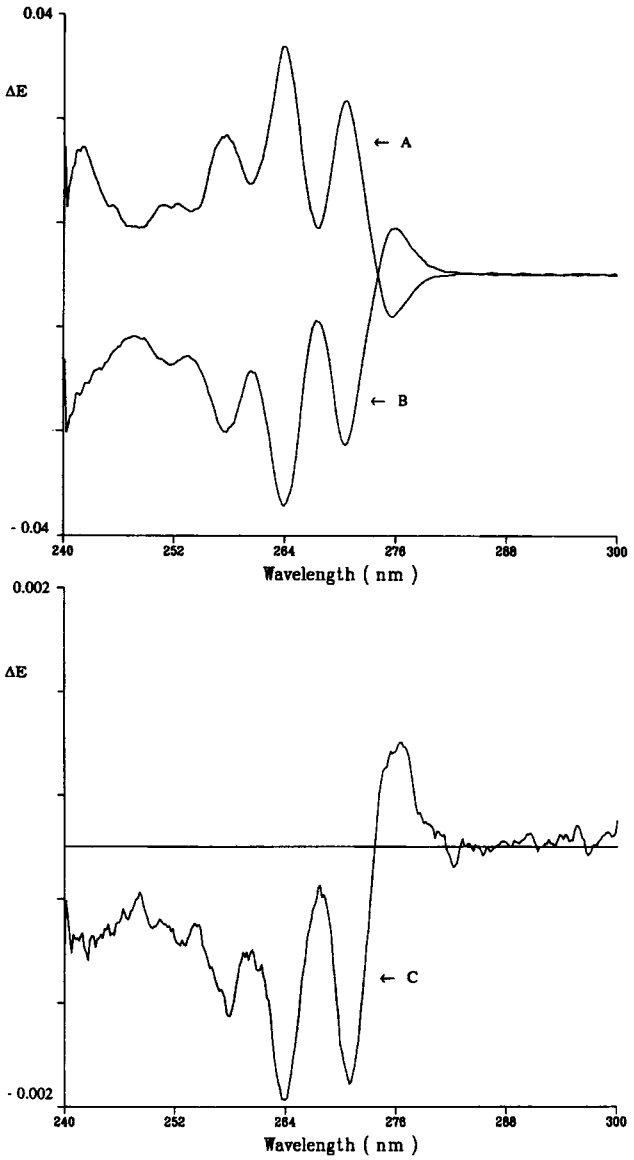


Fig. 1. The circular dichroism spectra of (+)-4-methyldiphenhydramine (**A**), (−)-4-methyldiphenhydramine (**B**), and (+)-**7** (**C**). The close correspondence of (+)-**7** CD spectrum with that of (−)-4-methyldiphenhydramine is the evidence for the identical configuration of their benzhydryl chiral centers.

pared with the lowest energy conformation were removed to yield a unique set of low energy conformations.

The semiempirical quantum mechanical method MNDO implemented in MOPAC 4.0[17] was used for a final optimization of all structures. MNDO optimizations were performed on internal coordinates using the default optimizer until Herbert's test was satisfied (using the keyword "PRECISE").

The molecular modelling program ChemX was used for both rigid and flexible fitting. The flexible fitting minimizer uses the ChemX nonbonded energy force field. This consists of a van der Waals term [Buckinham term (exp-6)[18]] and an electrostatic Coulomb term[18] using charges from an MNDO Mulliken population analysis. The flexible fitting minimizations

TABLE 1. Biological data of ebastine derivatives 6a-l

No.	C_{bz}[a]	X[b]	Antihistaminic activity, $-\log K_B$[c]	Anticholinergic activity, $-\log K_B$[d]
6a	S	CH_2	6.59 ± 0.07	5.55 ± 0.10
b	R	CH_2	7.10 ± 0.12	5.20 ± 0.05
c	S	C=O	7.81 ± 0.03	5.95 ± 0.21
d	R	C=O	8.75 ± 0.15	5.11 ± 0.11
e	S	CHOAc(R)	7.41 ± 0.09	5.67 ± 0.14
f	S	CHOAc(S)	7.68 ± 0.24	5.35 ± 0.07
g	R	CHOAc(R)	8.36 ± 0.19	5.10 ± 0.03
h	R	CHOAc(S)	8.60 ± 0.17	5.11 ± 0.08
i	S	CHOH(R)	6.90 ± 0.14	6.05 ± 0.14
j	S	CHOH(S)	7.11 ± 0.09	5.87 ± 0.17
k	R	CHOH(R)	8.17 ± 0.04	5.13 ± 0.15
l	R	CHOH(S)	8.28 ± 0.10	5.18 ± 0.08
Ebastine			7.71 ± 0.12	5.41 ± 0.17

[a] Configuration at the benzhydryl chiral center.
[b] Functional groups at the phenylbutanol side chain (configuration at this chiral center), see Scheme 1.
[c] Measured as inhibition of histamine-induced contraction in guinea pig ileum, means ± SD of three independent determinations.
[d] Measured as inhibition of metacholine-induced contraction in guinea pig ileum, means ± SD of three independent determinations.

were carried out using a quadratic gradient minimizer until the sum of gradients was smaller than 5.[18] Centroids of and planes through phenyl rings were defined by the corresponding six ring atoms (see Fig. 4).

MATERIALS AND METHODS

Chemistry

Structures of all compounds were checked by ^{1}H NMR and MS. The ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane and coupling constants are in 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 FP5 melting point apparatus. Specific rotations were measured on a Perkin-Elmer 241 MC polarimeter. Thin-layer chromatography was performed on a Kiesegel 60 F254 (Merck) TLC aluminum sheets. Circular dichroism (CD) spectra were recorded on a home-made spectropolarimeter (Department of Biophysics, Vrije Universiteit Amsterdam) in methanol (concentration: $\sim 2.5 \times 10^{-3}$ *M*) in a 1-cm cell from 300 to 240 nm.

Preparation of 6a–h as Examplified by (−)-1-{S-4-Acetoxy-4-[4-(1,1-dimethylethyl)phenyl]-butyl}-4-[R-α-(4-methylphenyl)-α-phenylmethoxy]-piperidine 6h

To a solution of 1.41 g (5 mmol) of (−)-4-chloro-1-[4-(1,1-dimethylethyl)phenyl]-1-butanol acetate[11] in 250 ml dry acetone was added 1.50 g (10 mmol) of sodium iodide (dried at 150°C overnight). The solution was refluxed for 6 h. After evaporating to dryness, the residue was extracted with petroleum ether (40–60°C) (100 ml × 3). The combined petroleum

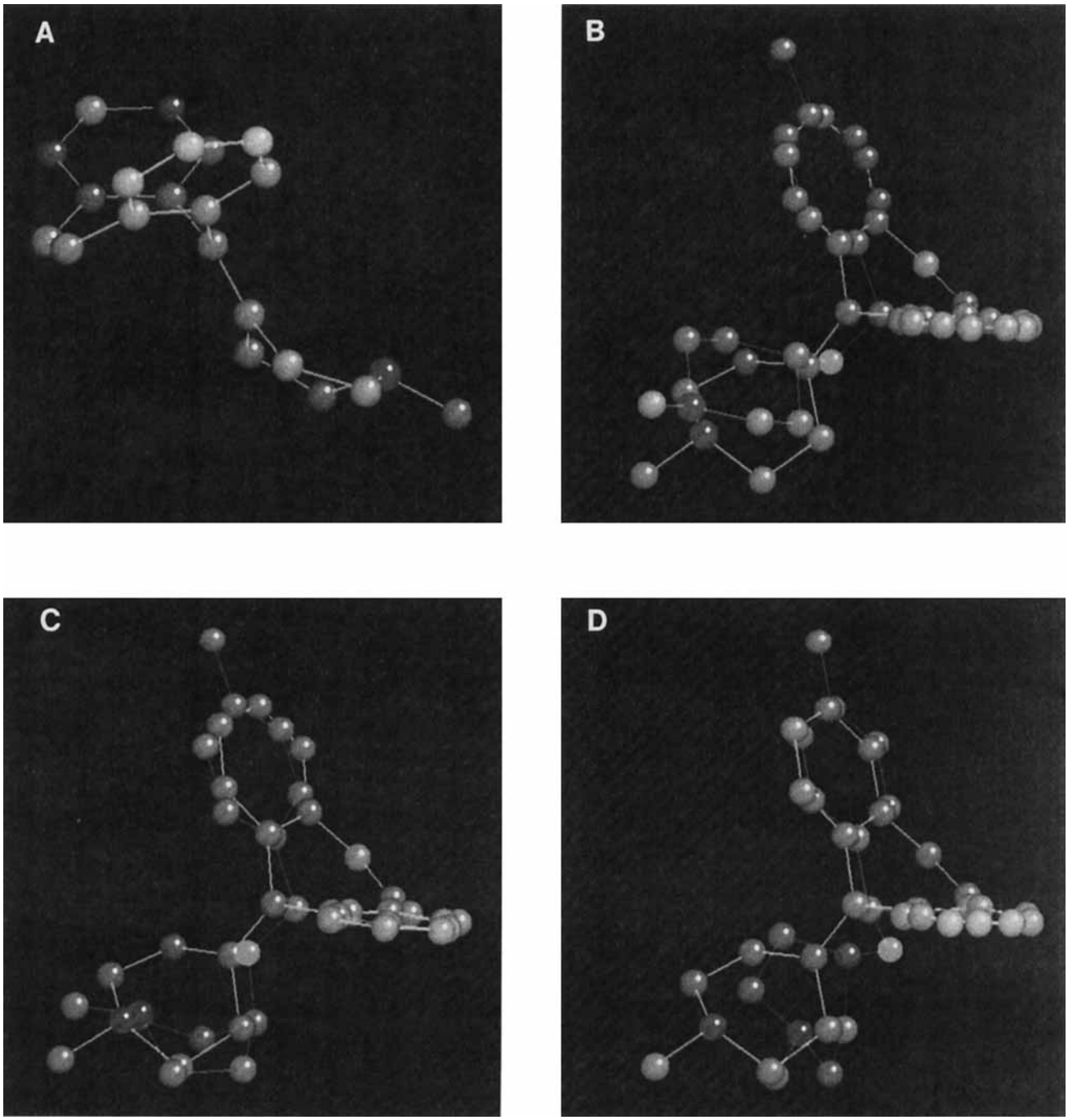


Fig. 2. Cyproheptadine template in which the piperidylene ring assumes the boat conformation **(A)** and the fit of (R)-diphenylpyraline chair conformation of the lowest energy **(B)**, (R)-diphenylpyraline boat conformation **(C)**, and (S)-diphenylpyraline boat conformation **(D)** onto the cyproheptadine template.

ether layer was dried with sodium sulfate and evaporated to dryness. The residue was then dissolved in 300 ml butanone-2. To this solution was added 1.40 g (5 mmol) of (−)-**7** and 0.69 g (5 mmol) of potassium carbonate. After refluxing overnight, the mixture was evaporated to dryness and the residue was taken up with dichloromethane. A slightly brown oil was obtained after evaporating the solvent. Purification by silica gel column chromatography (ethyl acetate/petroleumether 40–60°C 1:1) furnished the title compound as a thick colorless oil. Yield: 60%. The free base was converted to its oxalate salt by addition of an ethereal solution of oxalic acid. The white crystalline oxalate was obtained by filtration. mp. 151–152°C, $[\alpha]_D^{20}$ − 36.2° (c 0.5, MeOH); ^{1}H NMR for the free base ($CDCl_3$) δ: 1.31 (s, 9H, tBu-H), 1.50–1.90 [m,

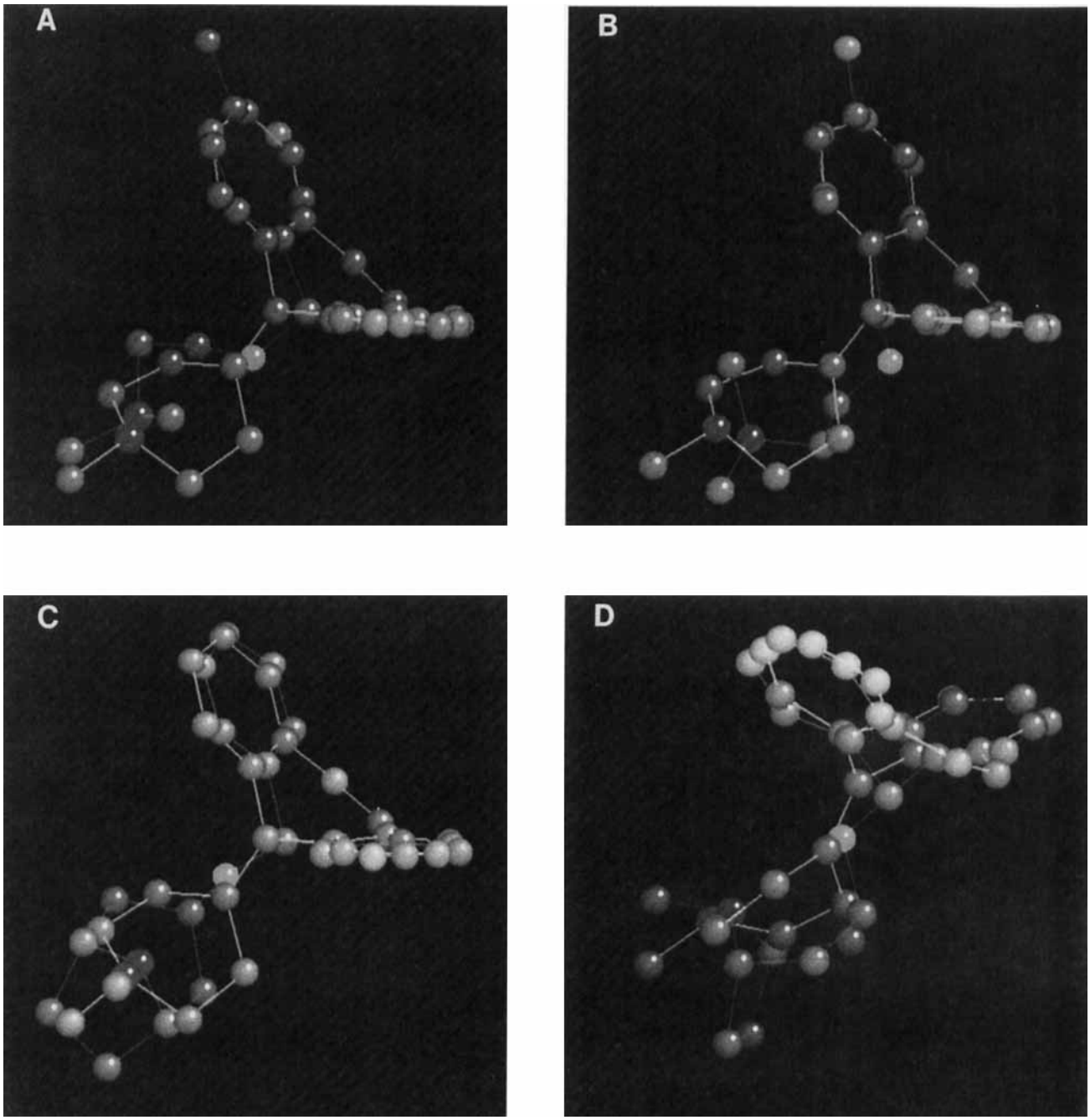


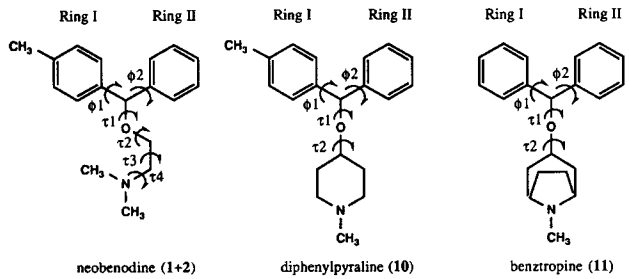
Fig. 3. Superimposition of the cyproheptadine template with (R)-4-methyldiphenhydramine **(A)**, (S)-4-methyldiphenhydramine **(B)**, and the boat 4 conformation of benztropine (front view: **C** and side view: **D**).

10H, $(CH_2)_3$ and piperidine H], 2.05 (s, 3H, $COCH_3$), 2.30 (s, 3H, $PhCH_3$), 2.20 (m, 2H, piperidine H), 2.28 (s, 3H, CH_3Ph), 2.65 (m, 2H, piperidine H), 3.40 (m, 1H, piperidine C_4-H), 4.93 (s, 1H, $CH_3Ph(Ph)CHO$), 5.68 (t, 1H, J = 6.7 Hz, $-CHOCOCH_3$), 7.05–7.40 (m, 13H, phenyl H).

Analogues **6a–g** were synthesized in the same way as described above. The results are summarized in Table 2.

Preparation of 6i–l as examplified by (−)-1-{S-4-Hydroxy-4-[4-(1,1-dimethylethyl)phenyl]-butyl}-4-[R-α-(4-methylphenyl)-α-phenylmethoxy]-piperidine 61

To a solution of 1.58 g (3 mmol) **6h** free base in 100 ml dry ether under nitrogen was added portionwise 0.12 g (3 mmol)



Scheme 4. Dihedral angles: τ1–4 were varied 360° in steps of 60°; ϕ1–2 were varied 180° in steps of 60° during the conformational analysis studies and flexible fitting procedures.

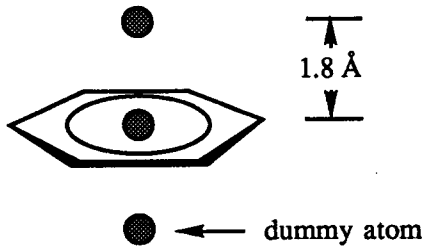


Fig. 4. Dummy atoms assigned for fitting aromatic rings. The dummy atoms are positioned on the axis perpendicular to the plane defined by the ring atoms, passing through the centroid of the same ring, and 1.8 Å above and below the plane.

of lithium aluminum hydride. The mixture was stirred at room temperature until the reaction was complete as detected by TLC (about 6 h). Water (50 ml) was added to the reaction mixture and the ether layer was separated. The water layer was extracted with dichloromethane. The combined organic solution was dried and evaporated to dryness. To the residue was added an ethereal solution of oxalic acid and the white oxalate precipitate collected by filtration and recrystallized from ethyl acetate/petroleum ether. Yield: 100%, $[\alpha]_D^{20}$ – 18.3° (c 0.5, MeOH). ^{1}H NMR for the free base ($CDCl_3$) δ (ppm): 1.23 (s, 9H, tBu-H), 1.60–1.90 [m, 10H, $(CH_2)_3$ and piperidine H], 2.30 (s, 3H, CH_3Ph), 2.35 (m, 2H, piperidine H), 2.70 (m, 2H, piperidine H), 3.45 (m, 1H, piperidine C_4-H), 4.55 (m, 1H, CHOH), 5.40 [s, 1H, $CH_3Ph(Ph)CHO$], 7.05–7.30 (m, 13H, phenyl H).

Analogues **6i–k** were synthesized in the same way as described above. The results are summarized in Table 2.

(±)-4-[α-(4-Methylphenyl)-α-phenylmethoxy]-piperidine 7

A solution of 19.8 g (0.1 mol) 4-methylbenzhydrol, 10.1 g (0.1 mol) 4-hydroxypiperidine, and 20.9 g (0.11 mol) *p*-toluenesulfonic acid monohydrate in 500 ml toluene was refluxed with a Dean-Stark condenser for 3 h. After cooling to the room temperature, the toluene solution was washed twice with 100 ml 5% aqueous NaOH solution, water, and dried with sodium sulfate. Removing the solvent afforded the title compound as thick colorless oil, Yield: 90%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.60 (m, 2H, piperidine H), 1.95 (m, 2H, piperidine H), 2.30 (s, 3H, CH_3), 2.60 (m, 2H, piperidine H), 3.10 (m, 3H, NH and piperidine H), 3.50 (m, 1H, piperidine C_4-H), 5.05 (s, 1H, CHO), 7.10–7.40 (m, 9H, phenyl H).

(+)-S-4-[α-(4-Methylphenyl)-α-phenylmethoxy]-piperidine (+)-7

(+)-Dibenzoyl-D-tartaric acid monohydrate (20.0 g) was added to the solution of 28.1 g (±)-**7** in methanol. The precipitate was collected by filtration to give 12.0 g of the crude (+)-dibenzoyl-D-tartrate [the mother liquor was used to obtain (−)-**7**, see below]. This crude (+)-dibenzoyl-D-tartrate was recrystallized twice more to yield 5.0 g of the pure (+)-dibenzoyl-D-tartrate as colorless needles. mp. 166–167°C. The tartrate was converted to the free base by extraction from 10% sodium hydroxide with chloroform. Removing chloroform afforded the title compound as colorless thick oil. $[\alpha]_D^{20}$ + 13.2 (c 0.5, $CHCl_3$), optical purity 96% e.e.[13]

(−)-R-4-[α-(4-Methylphenyl)-α-phenylmethoxy]-piperidine (−)-7

The mother liquor obtained after collecting (+)-**7** tartrate precipitate was evaporated to dryness. To the residue was added 10% sodium hydroxide and extracted with $CHCl_3$. The $CHCl_3$ solution was concentrated and to the residue was added a methanol solution of 18.8 g (−)-dibenzoyl-L-tartaric acid monohydrate. The precipitate was collected by filtration and recrystallized twice from methanol to yield 6.5 g of the pure (−)-dibenzoyl-L-tartrate as colorless needles. mp. 168–169°C. The free base was obtained as described for (+)-**7**. $[\alpha]_D^{20}$ – 13.4 (c 0.5, $CHCl_3$), optical purity 96% e.e.[13]

Pharmacology

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 (NaCl 117.5 m*M*; KCl 5.6 m*M*; $CaCl_2$ 2.5 m*M*, NaH_2PO_4 1.28 m*M*, $MgSO_4$ 1.18 m*M*; $NaHCO_3$ 25 m*M* and glucose 5.5 m*M*, pH 7.4). The buffer was constantly bubbled with 95% O_2–5% CO_2 at 37°C. The first three dose–response experiments were performed by adding histamine (from 1×10^{-8} to 1×10^{-5} *M*) or methacholine (from 1×10^{-9} to 1×10^{-6} *M*) cumulatively to the organ bath. After adequate washing, the ileal strip was incubated with the antagonist for 50 min. The dose–response experiment was then conducted again. Four different concentrations (3×10^{-8}, 1×10^{-7}, 3×10^{-7}, and 1×10^{-6} *M*) of each antagonist were used in rising order for each test following adequate washing and restoration of a stable baseline.

Molecular Modelling

The compounds were fitted on the cyproheptadine template (Fig. 2A) according to the procedure described by van Drooge.[15] The groups selected for superimposition were the basic nitrogen atom and the two aromatic rings. The aromatic rings were represented by two dummy atoms 1.8 Å above and below the centroids of the ring (see Fig. 4). During the superimposition, a restraint constant of 50 kcal/mol/Å^2 was used for the nitrogen atoms and the dummies representing the aromatic rings.

TABLE 2. Characteristics of ebastine derivatives 6a–l

No.	Formula[a]	Yield[b]	mp (°C)	$[\alpha]_D^{20}$ (c 0.5, MeOH)	R_f (TLC)[c]
6a	$C_{33}H_{43}NO \cdot C_2H_2O_4$	34	149.5–150.4	+10.3°	0.52
b	$C_{33}H_{43}NO \cdot C_2H_2O_4$	38	138.8–140.5	−11.1°	0.52
c	$C_{33}H_{41}NO_2 \cdot C_2H_2O_4$	30	155.0–156.0	+7.0°	0.36
d	$C_{33}H_{41}NO_2 \cdot C_2H_2O_4$	28	151.5–152.6	−7.1°	0.36
e	$C_{35}H_{45}NO_3 \cdot C_2H_2O_4$	25	145.7–146.6	+32.2°	0.47
f	$C_{35}H_{45}NO_3 \cdot C_2H_2O_4$	55	150.1–151.1	−25.7°	0.47
g	$C_{35}H_{45}NO_3 \cdot C_2H_2O_4$	25	143.1–146.0	+22.8°	0.47
h	$C_{35}H_{45}NO_3 \cdot C_2H_2O_4$	60	151.0–152.0	−36.2°	0.47
i	$C_{33}H_{43}NO_2 \cdot C_2H_2O_4$	100	110.2–111.0	+19.0°	0.15
j	$C_{33}H_{43}NO_2 \cdot C_2H_2O_4$	98	120.3–121.1	−4.5°	0.15
k	$C_{33}H_{43}NO_2 \cdot C_2H_2O_4$	99	90.2–91.0	+5.2°	0.15
l	$C_{33}H_{43}NO_2 \cdot C_2H_2O_4$	100	114.6–115.3	−18.3°	0.15

[a] All compounds are analyzed by high resolution mass spectra and they are in excellent agreement with the theoretical calculation.
[b] Yield of the free base after column chromatography purification and is not optimized.
[c] Eluent: ethyl acetate/petroleum ether (40–60°C) 1/1.

TABLE 3. Quality of the fits of diphenylpyraline enantiomers (representing 6) on the cyproheptadine model

	Chair [(R)-isomer)	Chair [(S)-isomer]	Boat [(R)-isomer]	Boat [(S)-isomer]
$\Delta\Delta Hf$ (kcal/mol)[a]	4.99	6.19	4.65	4.81
N-N (Å)[b]	1.069	1.325	0.394	2.558
Angle ring I (deg)[c]	1.67	1.78	1.78	2.19
Distance ring I (Å)[d]	0.058	0.057	0.293	0.328
Angle ring II (deg)[e]	1.78	1.67	1.79	1.31
Distance ring II (Å)[f]	0.058	0.058	0.291	0.332

[a] Heat of formation of fitted compound relative to its global minimum.
[b] Distance between the fitted nitrogen atoms.
[c] Angle between the fitted rings I, see Schemes 1 and 4 for indication.
[d] Distance between the centroids of the fitted rings I.
[e] Angle between the fitted rings II, see Schemes 1 and 4 for indication.
[f] Distance between the centroids of the fitted rings II.

To quantify the quality of the fit, the distance between nitrogens as well as the distance and angles between the aromatic rings of the fitted molecule and the template cyproheptadine was measured. Ideally, these distances and angles should be zero. The energy cost ($\Delta\Delta Hf$) for the molecule to adopt the fitted conformation is defined as the difference in the heat of formation (ΔHf) between the lowest energy conformation and the fitted conformation. They were calculated in MOPAC with a 1SCF calculation. This means that no geometry optimization is performed, only the electron distribution is optimized. A good fit should have a $\Delta\Delta Hf$ value not more than 5 kcal/mol which is generally available from drug-receptor interactions. The fitting results are presented in Tables 3 and 4.

RESULTS AND DISCUSSION

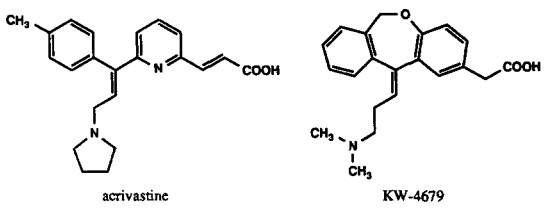
From Table 1, it is clear that methyl substitution in the benzhydryl moiety exerted a more profound influence on antihistamine activity than antimuscarinic activity of this series of compounds. This observation is in consistancy with the Pfeiffer's rule, i.e., with more potent drugs there is a bigger difference between the activities of the enantiomers.[19] Thus the antihistaminic eutomer of 4-methylebastine **6d** is more than 10 times more potent than ebastine in antagonizing histamine whereas the antimuscarinic eutomer **6c** is only less than 3.5-fold more potent than ebastine in antagonizing methacholine. In general this group of compounds showed 100- to 1000-fold higher potency as antihistamines than antimuscarinic agents. It is therefore more appropriate to consider these compounds as antihistamines. The chiral center in the benzhydryl part of the molecule has more influence than that in the phenylbutyl part on both antihistamine and antimuscarinic activities of the compounds. It is most likely that the benzhydryloxpiperidine is the pharmacophore for both antihistaminic and antimuscarinic actions of these compounds.

For antihistaminic activity, R is the preferred configuration at the benzhydryl part, with these isomers being 3- to 20-fold more potent than their S counterparts. This is in agreement with the general steric preference of known H_1-antagonists.[20] However, the influence of the chiral center in the phenylbutyl part is a little more complicated. On the one hand it seems that the chirality at this part of the molecule does not greatly alter the antihistaminic activity of the compound. This is true when comparing any pairs of isomers **6e,f, 6g,h, 6i,j,** or **6k,l.** A number of previous studies[11,20] have also showed that a chiral center far away from the benzhydryl moiety is of minor importance for stereoselectivity of antihistamines. However, the

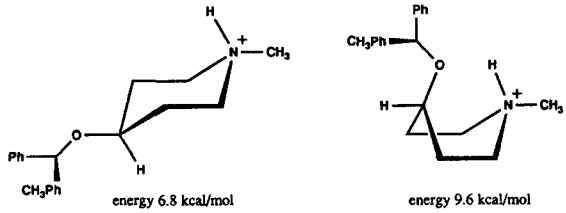
TABLE 4. Quality of the fits of 4-methyldiphenhydramine enantiomers and benztropine (boat 4) on the cyproheptadine model

	(R)-4-Methyldiphenhydramine	(S)-4-Methyldiphenhydramine	Benztropine (boat 4)
$\Delta\Delta Hf$ (kcal/mol)[a]	2.83	11.60	1.20
N-N (Å)[b]	0.098	0.108	0.425
Angle ring I (deg)[c]	3.63	3.47	1.52
Distance ring I (Å)[d]	0.093	0.094	0.296
Angle ring II (deg)[e]	1.80	1.79	2.41
Distance ring II (Å)[f]	0.092	0.094	0.296

[a-f]See footnotes to Table 3.



Scheme 5. Structures of antihistamines acrivastine and KW-4679. The aminomethyl and aminoethyl are at the *trans* position to the phenyl ring substituted with a hydrophilic group.

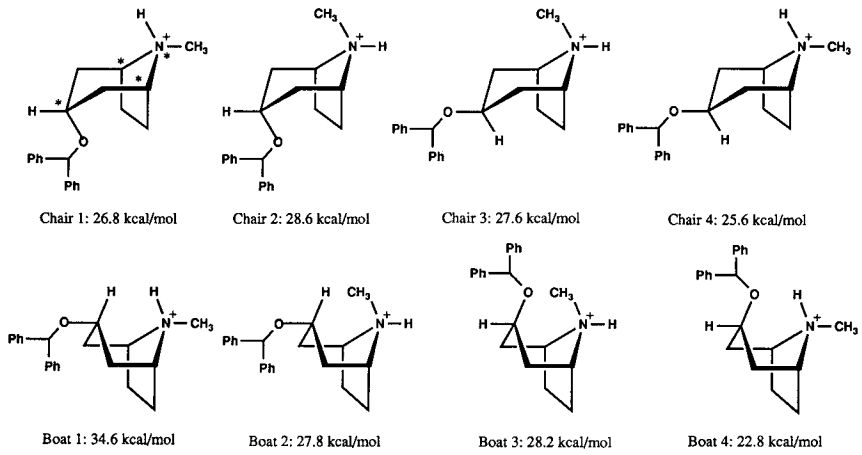


Scheme 6. Low energy conformers of (R)-diphenylpyraline. Energy data were obtained by calculation with MacroModel/AMBER force field using ECal function.

(S)-isomers at the phenylbutyl chiral center in this series all show slightly higher activity than those with the R configuration at the same chiral center. This might indicate that this part of the molecule also participates in the interaction with the receptors. Polymeropoulos et al.[21] suggested, through a modelling of 14 antihistamines, that there may be an additional interaction point at H_1-receptors for an electron rich atom (e.g., O or N) at a distance of 11–12 Å from one of the benzhydryl aromatic rings. Such an electron rich atom probably interacts with the receptor via a hydrogen bond. From our limited series of compounds, it appears that this electron rich atom acts most likely as a hydrogen bond acceptor. As such, the ketones **6c,d** are more active than the acetate **6e–h** which are more active than the alcohols **6i–l**, leaving the deoxy derivatives **6a,b** as the least active. Whether this putative hydrogen bonding site is stereoselective remains to be studied further. The present study remains inconclusive in this respect.

The picture of stereoselectivity for antimuscarinic activity is less clear. This might be due to their relatively low antimuscarinic activity and thus low stereoselectivity (Pfeiffer's rule).[19] It appears that both chiral centers in the benzhydryl and phenylbutyl parts influence the activity although the influence of the benzhydryl chiral center is apparently more prominent. The preferred configuration at the benzhydryl chiral center is S whereas the steric preference of the phenylbutyl chiral center is not obvious. The "reversed" steric preference of muscarinic receptors for the benzhydryl moiety compared with the histaminergic receptors results in the situation in which the antihistaminic eutomer happens to be the antimuscarinic distomer. Therefore the introduction of a chiral center into the benzhydryl moiety should increase the selectivity of the resulting isomer for either histaminergic or muscarinic receptors. This is at least true for the present series of ebastine derivatives. For example, the antihistaminic activity of **6d** is more than 4300 times higher than its antimuscarinic activity while achiral ebastine shows only about 200 times higher activity for antagonizing histamine than antagonizing methacholine. The selectivity of ebastine for histaminergic over muscarinic receptors has been increased by more than 20-fold.

For the correlation of our present findings with the existing stereoselectivity of H_1-antagonists, we performed a molecular modelling study based on cyproheptadine, the structurally most rigid antihistamine.[15] As the original cyproheptadine model is symmetric, we tried to fit the toluene ring of diphenylpyraline (representing **6**) on to either of the two phenyl rings of cyproheptadine template. The best fit for the antihistaminic eutomer (R)-diphenylpyraline, no matter which conformation the piperidine ring assumes, is always that with the toluene ring fitted onto the cyproheptadine phenyl corresponding to the unsubstituted phenyl of (−)-pRapSb-3-methoxycyproheptadine (see Table 3 and Fig. 2B and C). This phenyl group is defined as ring I (see Schemes 1 and 4 for indication). When (R)-4-methyldiphenhydramine was investigated in the same experiment the results are essentially the same, with the superimposion of the toluene on ring I giving the best fit (Fig. 3A and Table 4). As (R)-diphenylpyraline, (R)-4-methyldiphenhydramine, and pRapSb-3-methoxycyproheptadine are all the active isomers for antihistaminic action, these best fits likely represent the steric requirement of the H_1-antagonistic binding site. In a number of studies (see ref. 22 and literature cited therein) on geometric isomers of 1,1-diaryl-3-aminopropenes, 1,1-diaryl-4-aminobutenes, and 1,1-



Scheme 7. Low evergy conformers of benztropine. Energy data were obtained by calculation with MacroModel/AMBER force field using ECal function. The asterisks indicate the chiral centers in the nortropine system.

diaryl-3-pyrrolidinopropenes, it was found that *cis* dispositions of the substituted phenyl and the aminomethyl (or aminoethyl) tail around the double bond is optimal for antihistaminic activity.

The *cis* phenyl ring was suggested to be involved in a hydrophobic interaction with the receptor, as the antihistaminic activity could be quantitatively correlated with the fragmental hydrophobic parameter of this phenyl group.[22] Thus the substitution of a *para*-methyl or chlorine at this so-called *cis* ring increases the antihistaminic activity regardless of the substituents' electrostatic properties. The methoxy group in **4** decreases the hydrophobicity of the substituted phenyl (ring II). Therefore the unsubstituted phenyl (ring I) is favored to play the role of hydrophobic interaction (the role of the *cis* ring). This observation is supported by the finding that both acrivastine[23] and KW-4679[24] (Scheme 5) are more potent antihistamines than their Z-isomers. The phenyl ring substituted with a hydrophilic group in acrivastine and KW-4679 corresponds to the methoxyphenyl (ring II) of **4**. Our modelling results are consistent with these experimental findings, identifying the unsubstituted phenyl of **4** as the *cis* ring.

To determine the active conformation of diphenylpyraline, we constructed both chair and boat conformations of the piperidine ring with ChemX. After a series of minimization procedures, we obtained the corresponding lowest energy conformers for the chair and boat piperidines (Scheme 6). As usual, the chair conformer has an energy of 2.8 kcal/mol lower than the boat conformer. However, when the lowest chair conformer was fitted onto the cyproheptadine model the energy cost was 4.99 kcal/mol for the (R)-isomer and 6.19 kcal/mol for the (S)-isomer. The quality of the best fits for both isomers was also rather poor (see Table 3). In contrast, when the (R)-isomer with the boat conformation fitted onto the template, the energy cost was only 4.65 kcal/mol above the global minimum (lowest chair conformation). The quality of the fit was also improved in comparison with that of the chair conformer, especially the distance between the two nitrogen atoms was reduced to 0.39 Å from 1.07 Å in the chair fit.

To validate these results, we examined the active conformation of benztropine (**11**). The nortropine system of **11** contains 4 chiral centers including the nitrogen atom. Because of the common bond between three of the four chiral centers, the possible number of stereoisomers was reduced to 8 instead of the theoretical 16. Scheme 7 presents the 8 different conformers of the four stereoisomers and the other 8 possible conformers are the mirror images of those presented in Scheme 7. Molecular mechanical calculation shows that the lowest energy conformation is the boat with an intramolecular hydrogen bond between the protonated nitrogen and the ether oxygen (boat 4 in Scheme 7). This boat 4 conformer has an energy of 2.8 kcal/mol lower than the lowest energy of a chair conformer (chair 4 in Scheme 7). When the boat 4 conformer of benztropine was fitted onto the cyproheptadine, the energy cost for the best fit was merely 1.2 kcal/mol. The fit is also satisfactory in terms of other parameters such as the distance between the nitrogens as well as the distance and angles between the aromatic rings (Table 4 and Fig. 3C and 3D). Although the antihistaminic activities of benztropine stereoisomers are unknown, the lowest energy conformation boat 4 is definitely one of the most likely active conformations for H_1-receptors and it assumes a boat conformation for the piperidine ring.

It is interesting to note that when the toluene ring of diphenylpyraline or 4-methyldiphenhydramine was fitted on to the ring II of cyproheptadine, the best fits are those of the (S)-isomers. This is due to the mirror image relationship between the stereoisomers. For example when 3-methoxycyproheptadine enantiomers **3** and **4** are superimposed, the methoxyphenyl in **4** overlays on the unsubstituted phenyl in **3** and vice versa. As **3** and **4** are, respectively, the active isomers for antimuscarinic and antihistamine action, the roles of the two aromatic rings in **3** and **4** are reversed when interacting with muscarinic and histaminergic receptors. The same is true for **6** where the toluene and phenyl groups interact with histaminergic and muscarinic receptors in the "reverse" ways. The different steric preference of histaminergic H_1-receptors and

muscarinic receptors[8] for this series of compounds (6) has been quantitatively visualized in the present molecular modelling study.

In conclusion the absolute steric arrangement around the benzhydryl moiety (or other pharmacophores) is one of the key factors determining the selectivity of compounds for antihistamine action over antimuscarinic action, although other factors, especially electronic effects, are also important.[7] An asymmetric pharmacophoric model for H_1-antagonists has been derived from the present study. This new and asymmetric model should be valuable not only for the understanding of three-dimentional interaction of H_1-antagonists with their receptors but also for the design of novel antihistamines with low or no antimuscarinic activities.

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