Synthesis, Affinity at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} Serotonin Receptors and Structure–Activity Relationships of a Series of Cyproheptadine Analogues

Maria Angeles HONRUBIA,[a] Jesús, RODRIGUEZ,[a] Rosa DOMINGUEZ,[a] Estrella LOZOYA,[b] Francesc MANAUT,[b] Julio A. SEIJAS,[a] Maria Carmen VILLAVERDE,[a] Jose M. CALLEJA,[a] Maria Isabel CADAVID,[a] Saul MAAYANI,[c] Ferran SANZ,[b] and Maria Isabel LOZA*,[a]

Departments of Pharmacology, Organic and Physical Chemistry, University of Santiago,[a] E-15706 Santiago de Compostela, Spain, Department of Medical Informatics, Institut Municipal d'Investigació M dica, Universitat Autènoma de Barcelona,[b] Doctor Aiguader 80, E-08003 Barcelona, Spain, and Department of Anesthesiology, Mount Sinai School of Medicine,[c] New York, New York 10029, U.S.A. Received August 5, 1996; accepted December 27, 1996

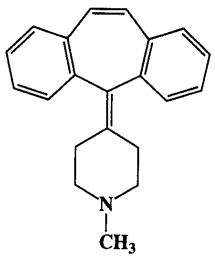
Cyproheptadine is a drug that shows high affinity for type 2 (5-HT_2) receptors. We studied a series of compounds obtained by modification of the tricyclic system of Cyp (dibenzocycloheptadiene): 2f (thioxanthene), 2g (xanthene), 2h (dihydrodibenzocycloheptadiene), 2j (diphenyl), 2i (fluorene), and 3b (phenylmethyl). Their activities at the rat cerebral cortex 5-HT_{2A} receptor were ($pK_i \pm$S.E.M.): 8.80±0.11 (Cyp), 8.60±0.07 (2f), 8.40±0.02 (2g), 8.05±0.03 (2h), 7.87±0.12 (2j), 6.70±0.02 (2i) and 6.45±0.02 (3b); those at the rat stomach fundus 5-HT_{2B} receptor ($pA_2 \pm$S.E.M.) were: 9.14±0.25 (Cyp), 8.49±0.07 (2f), 7.58±0.58 (2g), 7.02±0.14 (2h), 6.07±0.20 (2j), and undetectable (2i, 3b); and those at the pig choroidal plexus 5-HT_{2C} receptor ($pK_i \pm$S.E.M.) were: 8.71±0.08 (Cyp), 8.68±0.01 (2f), 8.58±0.20 (2g), 7.95±0.05 (2h), 7.57±0.04 (2j), 6.98±0.04 (2i) and 6.63±0.20 (3b). The slopes did not differ significantly from unity. The compounds exhibited the same order of activities at every type of receptor, and the most active molecules presented certain steric (butterfly conformation of the tricyclic system) and electrostatic (proton affinity on the top of the central rings) patterns. It is concluded that the activity of cyproheptadine derivatives at 5-HT_2 receptors is related to these molecular features, which make feasible a common disposition to interact with all three 5-HT_2 subtypes.

Key words cyproheptadine; 5-HT_2 receptor; molecular feature

Serotoninergic axon termini innervate virtually all brain regions, and serotonin-mediated mechanisms are involved in the regulation of sleep and sexual behaviour and in a number of pathological conditions. Many of the latter (including schizophrenia, anxiety, depression, anorexia nervosa, migraine, and hypertension and other cardiovascular disorders) have been related[1] to the group of serotonin receptors classified by Hoyer *et al.*,[2] as type 2 (5-HT_2 receptors), which consists of all receptors known to trigger intracellular hydrolysis of phosphoinositides and comprises the subtypes 5-HT_{2A} (5-HT_2 in earlier classifications), 5-HT_{2B} and 5-HT_{2C} (formerly 5-HT_{1C}).[3] Of these, 5-HT_{2B}, first found in rat stomach fundus[4] but now known to be present in many human tissues, including brain,[5,6] differs from the others in its greater sensitivity to serotonin in smooth muscle and in that its related G protein has as yet been only partially identified as $G_{\alpha Z}$-like.[7] 5-HT_{2A} receptors have been implicated[8] in the action of so-called atypical antipsychotic drugs such as clozapine (unlike classical neuroleptics, these atypical drugs relieve the "negative" symptoms of schizophrenia as well as the "positive" symptoms, and their antischizophrenic action at dopamine receptors is not accompanied by undesirable extrapyramidal effects). The 5-HT_{2C} receptor is implicated in a variety of disease processes, such as anxiety, schizophrenia and affective disorders, and also seems, to be a binding site for hallucinogenic drugs.[9,10]

Cyproheptadine antagonizes serotonin at all three of the above-mentioned receptors. It is well-tolerated and has been used for many years for clinical treatment of what are now recognized as 5-HT_2-related pathologies, including anorexia nervosa[11] and migraine; predisposition for migraine may be due to hypersensitive 5-HT_{2B} and/or 5-HT_{2C} receptors.[12,13] Like atypical antipsychotics, it also ameliorates the negative symptoms of schizophrenic patients,[11,14] although its antipsychotic activity is low. Cyproheptadine has been chosen by our group as the starting point of a search for compounds binding to 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors.

Like the subspecific 5-HT_{2A}-blocker ritanserin, the cyproheptadine molecule (Fig. 1) contains a diphenylmethylenepiperidine (DPMP) fragment. In cyproheptadine this fragment is rigidified by partial incorporation into the tricyclic system of the molecule, in contrast with ritanserin where the phenyl groups have no linking ring. In a previous study,[15] we found that DPMP analogues which had no central ring and in which the nitrogen atom bore a variety of substituents or was replaced by other atoms were less active than cyproheptadine at 5-HT_{2B}



cyproheptadine

Fig. 1. Chemical Structure of Cyproheptadine

* To whom correspondence should be addressed.

receptors. We have now investigated the activities of compounds in which the DPMP phenyl groups are linked by a central ring, as in cyproheptadine, and compared them with analogues lacking this ring. In this article we 1) describe the synthesis of a series of such compounds; 2) report their activities at 5-HT_{2A} receptors in rat frontal cortex (as measured by [^{3}H]ketanserin binding), 5-HT_{2B} receptors in rat stomach fundus (measured in terms of the inhibition of serotonin-induced contractions) and 5-HT_{2C} receptors in porcine choroid plexus (as measured by [^{3}H]mesulergin binding); 3) report the results of molecular modelling studies evaluating similarity between the conformation and molecular electrostatic potential distribution of the upper tricyclic portion of cyproheptadine and those of the synthesized molecules; and 4) discuss the conclusions to be drawn from comparison of the pharmacological and molecular modelling results.

Results and Discussion

Chemistry Our interest in the use of low-valent titanium (LVT) for organic synthesis led us to apply McMurry's method to generate the exocyclic double bond present in cyproheptadine and related compounds.[16] This method reduces the synthetic problem to the performance of a mixed dicarbonylic coupling between two suitable ketones. One of the advantages of mixed dicarbonylic coupling is that when one of the two ketones is aliphatic and the other biarylic, the difference between their secondary reduction potentials gives the mixed coupled product almost exclusively, instead of the symmetric ones.[17] This methodology has recently been applied to the synthesis of a series of diphenylmethylene analogs of cyproheptadine,[15] showing that it is suitable for use with heterocyclic aliphatic ketones; pharmacological evaluation of the prepared compounds indicated the necessity of a basic nitrogen in the aliphatic ring.

Following this general synthetic strategy (Chart 1), the cyproheptadine analogues **2a—e** and **3a** were synthesized, in moderate to good yields, by coupling between *N*-carbethoxy-4-piperidone and the corresponding ketone. Compounds **2f—j** and **3b** were obtained by further reduction with lithium aluminum hydride (LAH).

All coupling reactions were carried out by adding an equimolecular mixture of *N*-carbethoxy-4-piperidone and the appropriate biarylic ketone in 1,2-dimethoxyethane (DME) to a slurry of LVT at room temperature, stirring for 4 h, and then refluxing for 13 h. LVT was prepared by reduction of $TiCl_3$ with lithium in boiling DME. In the case of compound **3a** an excess of *N*-carbethoxy-4-piperidone was used for the coupling, in order to favor the mixed coupling product.

Pharmacology. Functional Experiments: Antagonism of Serotonin in Rat Stomach Fundus The antiserotoninergic 5-HT_{2B} activity of the compounds was determined as pA_2 for the inhibition of serotonin-induced contractions in isolated rat stomach fundus (Table 1). The 5-HT concentration–effect curves were displaced dose-dependently to the right without depression of their maxima (Fig. 2). The pA_2 of compound **2f** (8.49) was in the previously described range for cyproheptadine (7.5—8.5)[13] but less than the value measured for cyproheptadine in this work (9.14). Compounds **2g—j** had lower pA_2's (Table 1) and compounds **2i** and **3b** did not inhibit serotonin-induced contractions even at a concentration as high as 10 μM. The order of potency at the 5-HT_{2B} receptor in this series was thus cyproheptadine > **2f** > **2g** > **2h** > **2j** > **2i**=**3b**, in a series of four replicate experiments.

The competitive behavior reported above has not always been observed in similar studies: the antiserotoninergic activity of cyproheptadine analogues, like that of ergolines, has variously been described as competitive,[13,18] non-competitive[19,20] or mixed.[21] These discrepancies have been attributed either to differences between the preparations used in the various studies[19] or to the slowness of the dissociation of the antagonists from the receptor.

Binding Experiments In [^{3}H]ketanserine-binding 5-HT_{2A} assays, all the new compounds exhibited pK_i values of 6.45—8.6. Compounds **2f—h** all had values very close

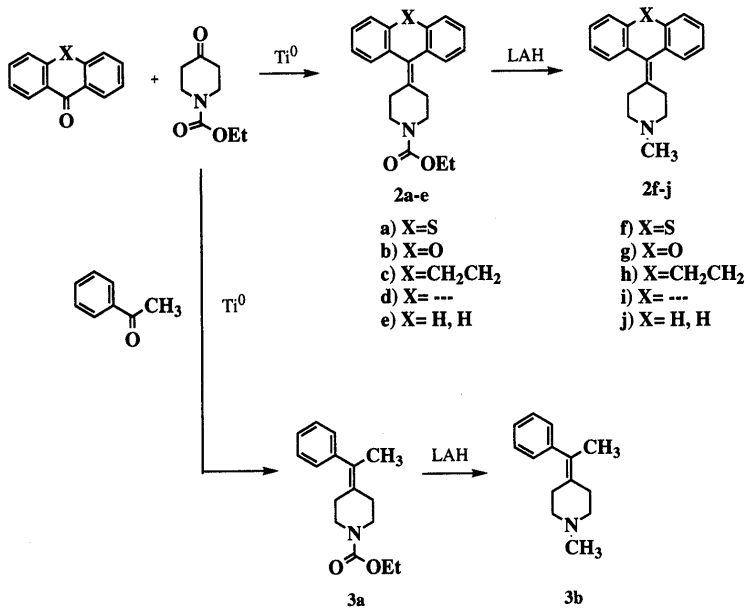


Chart 1

Table 1. Affinities for 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}

Compd.	5-HT_{2A} [a]		5-HT_{2C} [b]		5-HT_{2B} [c]	
	pK_i±S.E.M.	n±S.E.M.	pK_i±S.E.M.	n±S.E.M.	pA_2±S.E.M.	n±S.E.M.
Cypro.	8.80±0.11	0.98±0.05	8.71±0.08	0.93±0.05	9.14±0.25	0.86±0.14
2f	8.60±0.07	1.09±0.12	8.68±0.01	1.03±0.06	8.49±0.07	1.18±0.07
2g	8.40±0.02	0.99±0.09	8.58±0.20	0.85±0.05	7.58±0.58	1.12±0.07
2h	8.05±0.03	0.94±0.06	7.95±0.05	0.92±0.07	7.02±0.14	0.72±0.27
2j	7.87±0.12	0.92±0.09	7.57±0.04	0.89±0.01	6.07±0.20	0.79±0.17
2i	6.70±0.02	1.09±0.05	6.98±0.04	1.06±0.14	[d]	
3b	6.45±0.02	0.84±0.14	6.63±0.20	0.72±0.15	[d]	

The values represent the mean of 4—6 experiments. *a*) In rat cerebral cortex. *b*) In porcine choroid plexus. *c*) In rat stomach fundus. *d*) No detectable activity.

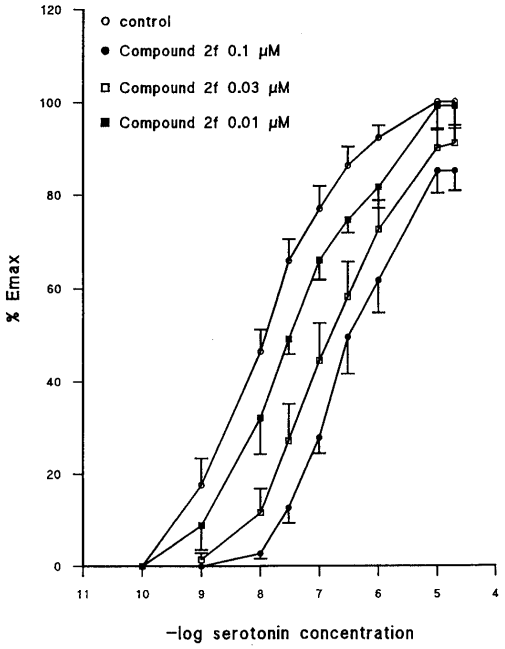


Fig. 2. 5-HT Concentration–Effect Curves Showing Antagonism by Compound **2f** in Rat Stomach Fundus

Vertical bars show the SEM of six individual experiments.

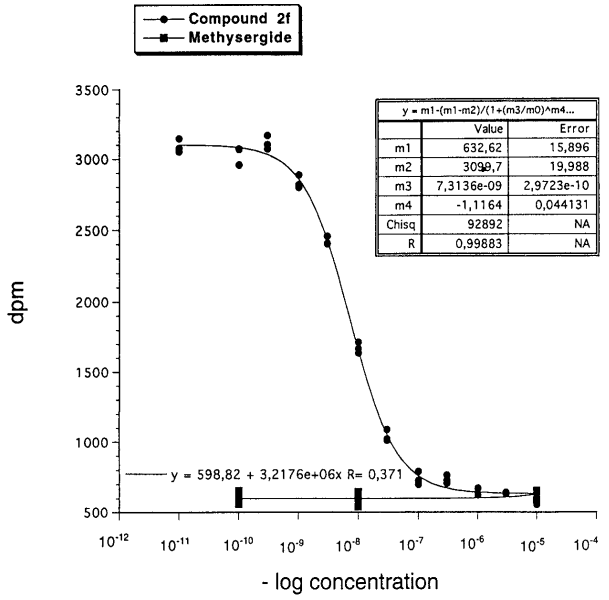


Fig. 3. [^{3}H]Ketanserine Binding in Rat Frontal Cortex Membranes in the Presence of Compound **2f**

The curve shown is from a representative experiment.

to that of cyproheptadine, 8.8 (Fig. 3; Table 1). For all the compounds, the pK_i values in [^{3}H]mesulergine binding assays (Fig. 4, Table 1) were similar to those measured in the [^{3}H]ketanserine binding 5-HT_{2C} assays.

The order of potency in both 5-HT_{2A} and 5-HT_{2C} receptors was the same as that found for 5-HT_{2B} receptors (see functional experiments). Thus, the differences among these compounds as regards their tricyclic system had no effect on their relative selectivities for the three receptors.

Molecular Modelling The geometries of the tricyclic moieties of all the considered molecules were fully optimized by *ab initio* calculations at the STO-3G level. Following geometric optimization, the molecular electrostatic potential (MEP) distributions were computed and their MEP minima were located and characterized. Computations were performed only for the tricyclic moieties of the compounds (including the exocyclic double bond) because previous calculations on cyproheptadine showed that the geometry and the MEP distribution of the tricyclic fragment are virtually independent of the geometry and the MEP distribution of the amino group of the piperidine ring.[15] For instance, the coordinates of the fully op-

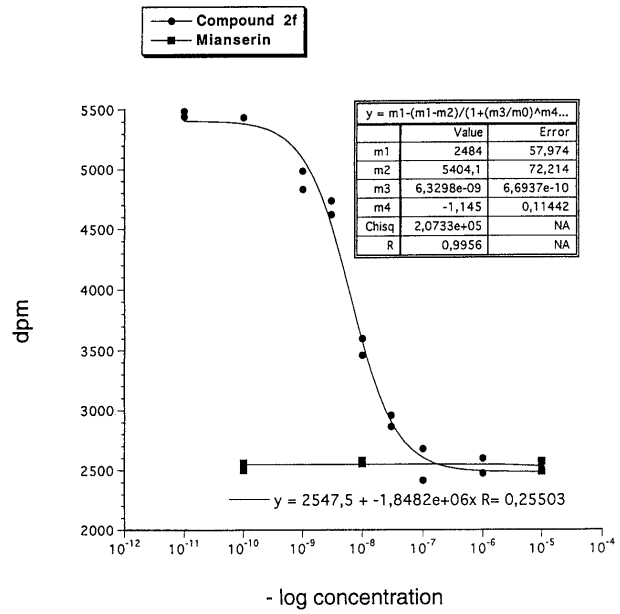


Fig. 4. [^{3}H]Mesulergine Binding in Porcine Choroid Plexus Membranes in the Presence of Compound **2f**

The curve shown is from a representative experiment.

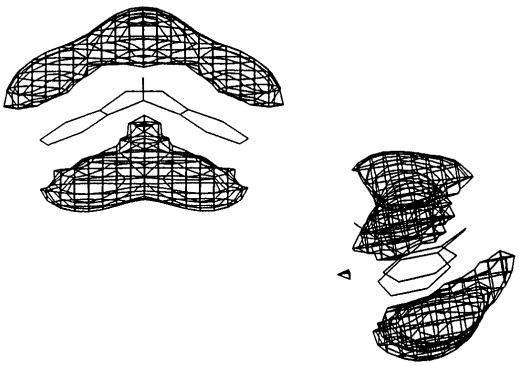


Fig. 5. Isopotential Surface (−5 kcal/mol) of the MEP Distribution of the Tricyclic Part of Cyproheptadine

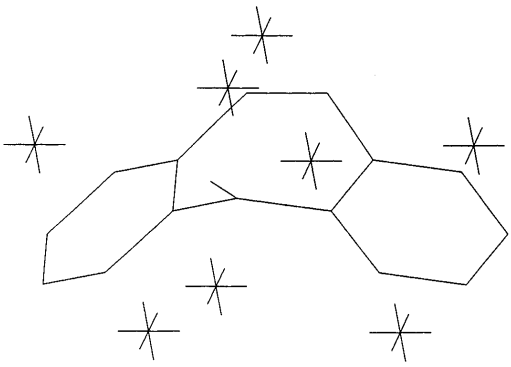


Fig. 6. MEP Minima of Cyproheptadine

timized tricyclic structure and the corresponding atoms of the fully optimized cyproheptadine show a root mean square (RMS) deviation of only 0.13 Å. Both structures show eight MEP minima, with as little as 0.27 Å RMS deviation between their coordinates and 1.7 kcal/mol RMS deviation between their values. The eight pairs of values of MEP minima show a correlation coefficient of 0.9463.

As previously reported,[22] the optimized geometry of the tricyclic system of cyproheptadine is a symmetric butterfly conformation. Figure 5 shows the −5 kcal/mol surfaces of its MEP (one on each side of the molecule), and Fig. 6 the locations of its eight MEP minima (one on either side of each aromatic ring and two symmetrically placed *versus* each of the two double bonds).

Figure 7 compares the optimized structure of the tricyclic fragment of cyproheptadine with those of compounds **2f**—**j** (**3b** is planar). Compounds **2f**, **2g** and **2i** are similar to cyproheptadine in possessing C_s-symmetry, and **2f** and **2g** also have butterfly conformations, albeit flatter ones than cyproheptadine, whereas **2i** (the least active tricyclic compound) is quite flat. Compound **2h** also has an almost symmetrical butterfly-like conformation. The optimal conformation of **2j** is neither C_s-symmetric nor butterfly, but since **2j** lacks a central ring, each phenyl ring is free to rotate about its exocyclic bond, and a C_s-symmetric, butterfly conformation can be adopted at an energy cost of only 2.8 kcal/mol. Thus, the four most active compounds (**2f**—**h**, **2j**) all have, or are capable of adopting, conformations similar to the symmetric butterfly conformation of cyproheptadine, and all have MEP minima in locations similar to those of six of the cyproheptadine minima (one on either side of each

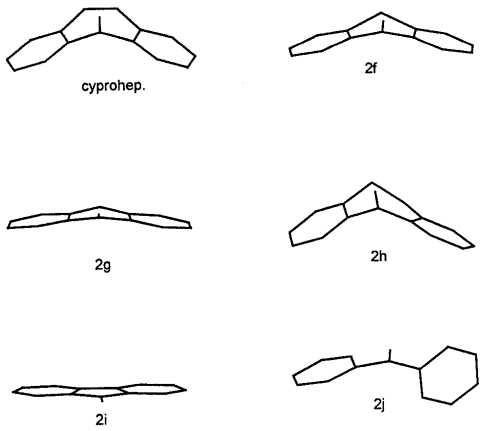


Fig. 7. Optimized Structures of the Studied Compounds

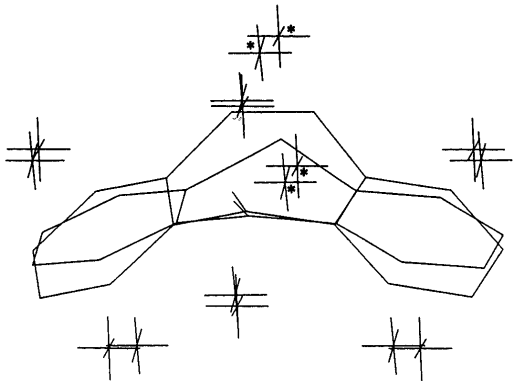


Fig. 8. Superimposition of Cyproheptadine with Compound **2f**

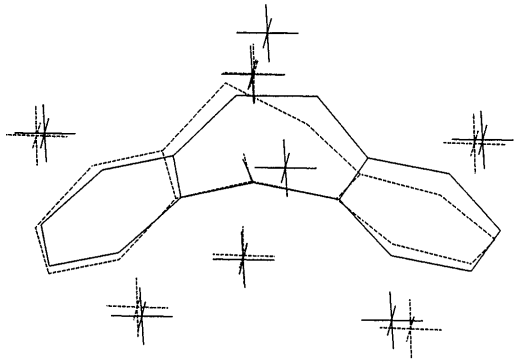


Fig. 9. Superimposition of Cyproheptadine with Compound **2h**

aromatic ring and two symmetrically placed *versus* their exocyclic double bond). Compound **2i**, which has very low activity, is flat; and **3b**, which has only one ring and hence only four MEP minima, is inactive.

Figures 8 and 9 show the best least-squares fits between the most active compounds and cyproheptadine when both nuclei positions and the locations of the six "essential" MEP minima are taken into account. According to our calculations, the most stable conformation of **2j** is clearly asymmetrical with dihedral angles very different from those of cyproheptadine (Fig. 10). However, the rotation of the phenyl rings allows a good superimposition of nuclei and MEP minima on those of cyproheptadine (Fig. 11). Although the best fitting compound, **2h**, is not as active as **2f** and **2g**, the latter are more like cyproheptadine in that, due to the lone pairs on their sulfur atom (**2f**) or

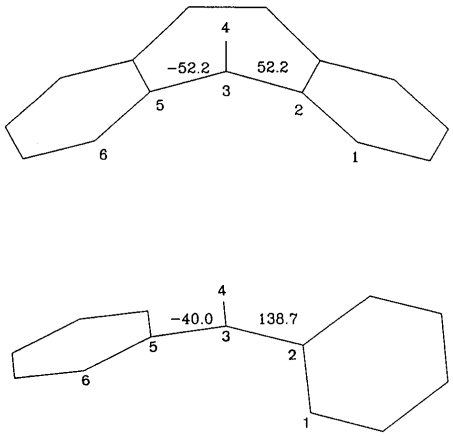


Fig. 10. Torsional Angles 1–2–3–4 and 4–3–5–6 of Cyproheptadine and Compound **2j**

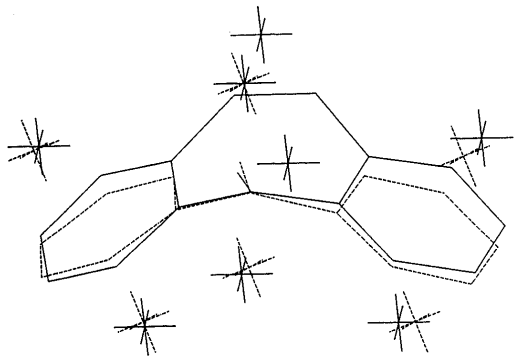


Fig. 11. Superimposition of Cyproheptadine with Bond-Rotate Compound **2j**

oxygen atom (**2g**), they have a supplementary pair of MEP minima (marked with asterisks in Fig. 8) that may play the role of those belonging to the double bond in the central ring of cyproheptadine. That the activity of **2f** is greater than that of **2g** is presumably due to its fitting cyproheptadine much better than the flatter **2g** if only the atomic positions are taken into account, which in turn derives from the C–S bonds of **2f** being longer than the C–O bonds of **2g**, and hence being able to compensate, to some extent, for the lack of the seventh bond featured by the central ring of cyproheptadine. The relatively low activity of **2j** may be due not only to the energy cost of rotating its phenyl rings into butterfly conformation, but also to the effective dilution of the active conformation as the result of its rotational flexibility.

In conclusion, the activities of the cyproheptadine analogues **2f**—**j** and **3b** at the 5-HT_{2A}, 5-HT_{2B} and 5-HT_2C receptors correlate well with their similarities to cyproheptadine when both molecular geometry and molecular electrostatic potential distributions are taken into account. Compounds resembling cyproheptadine show a range of activities and a lack of selectivity at 5-HT_2 receptors similar to those of cyproheptadine. The most different compounds (**2i**, **3b**) exhibit a lower activity than cyproheptadine, but the diminution of the activity is specially marked at the 5-HT_{2B} receptor; this may provide a clue to the development to more selective compounds.

Experimental

A. Chemistry. General Methods All melting points were recorded on a Kofler-Thermogeräte apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 1420 spectrometer. Proton NMR spectra (^{1}H-NMR) were obtained on a Bruker WM-250 (250 MHz) apparatus, using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard; all signals are expressed as δ values (ppm downfield from TMS). Mass spectra were recorded at 70 eV with a Kratos MS-50 spectrometer and with a Hewlett-Packard HP 59970 MS Chem Station. Dried solvents were distilled under argon from sodium benzophenone ketyl radical immediately prior to use. Column chromatography was performed using Merck 230—400 mesh silica gel.

General Procedure for Mixed Carbonyl Coupling Using $TiCl_3$/Li Lithium pieces (28 mmol) were added to a stirred slurry of $TiCl_3$ (8 mmol) in 30 ml of dry DME under an argon atmosphere and the mixture was refluxed for 2 h. The black slurry was then cooled to room temperature, and the two carbonyl compounds were added (1 mmol of each dissolved in 10 ml of DME). The mixture was stirred for 4 h at room temperature and then refluxed for 13 h. After cooling to room temperature, the reaction mixture was filtered. A saturated aqueous solution of K_2CO_3 was added to the filtrate, the organic layer was separated, and the aqueous layer was extracted with chloroform (3 × 50 ml). The pooled organic phase was dried (Na_2SO_4), and the solvent was evaporated to afford the crude product. This procedure (Li–$TiCl_3$–1-carbethoxy-4-piperidone–biarylic ketone mole ratio 28 : 8 : 1 : 1) was used for the syntheses of compounds **2a**—**e**; for compound **3a** the mole ratio used was 28 : 8 : 2 : 1.

4-(9*H*-Thioxanthen-9-ylidene)-1-piperidinecarboxylic Acid, Ethyl Ester (**2a**): Reaction of thioxanthen-9-one (658 mg, 3.1 mmol) with 531 mg (3.1 mmol) of 1-carbethoxy-4-piperidone gave a crude mixture that was purified by column chromatography (25 : 75, AcOEt–hexane) to yield 559 mg (51%) of compound **2a** as yellow crystals, mp 118—120 °C (hexane–ether). IR (NaCl) cm^{-1}: 1700 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.0 Hz, $OCH_2C\underline{H}_3$); 2.56—2.61 (4H, m, CH_2), 3.02—3.12 (2H, m, CH_2), 3.79 (2H, m, CH_2), 4.15 (2H, q, J=7.0 Hz, $OC\underline{H}_2CH_3$); 7.14—7.30 (6H, m, ArH), 7.49 (2H, dd, J=0.8, 6.7 Hz, ArH). MS m/z (rel. int. %): 352 (27), 351 (M^+, 99), 322 (27), 250 (21), 249 (100), 236 (21), 235 (71), 234 (55), 222 (19), 221 (71), 197 (28).

4-(9*H*-Xanthen-9-ylidene)-1-piperidinecarboxylic Acid, Ethyl Ester (**2b**): Reaction of xanthone (343 mg, 1.75 mmol) with 315 mg (1.75 mmol) of 1-carbethoxy-4-piperidone gave a crude mixture that was purified by column chromatography (85 : 15, AcOEt–hexane) to yield 161 mg (27%) of compound **2b** as yellow crystals, mp 126—128 °C (hexane–ether); lit.[23] 98—101 °C (ether). IR (NaCl) cm^{-1}: 1690 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.1 Hz, $OCH_2C\underline{H}_3$), 2.81 (4H, t, J=6.0 Hz, CH_2), 3.46 (4H, t, J=6.0 Hz, CH_2), 4.16 (2H, q, J=7.1 Hz, $OC\underline{H}_2CH_3$), 7.15—7.33 (8H, m, ArH). MS m/z (rel. int. %): 335 (M^+, 13), 233 (32), 219 (20), 218 (13), 205 (18), 197 (20), 196 (100), 181 (14), 180 (34), 168 (42), 149 (51), 139 (40).

4-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-iperidinecarboxylic Acid, Ethyl Ester (**2c**): Reaction of dibenzosuberone (625 mg, 3 mmol) with 500 mg (3 mmol) of 1-carbethoxy-4-piperidone gave a crude mixture that was purified by column chromatography (15 : 85, AcOEt–hexane) to yield 458 mg (44%) of compound **2c** as an oil. IR (NaCl) cm^{-1}: 1700 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.1 Hz, $OCH_2C\underline{H}_3$); 2.39 (4H, t, J=5.6 Hz, CH_2), 2.79—2.93 (2H, m, CH_2), 3.13—3.24 (2H, m, CH_2), 3.36—3.50 (2H, m, CH_2) 3.76—3.81 (2H, m, CH_2), 4.18 (2H, q, J=7.1 Hz, $OC\underline{H}_2CH_3$), 7.06—7.17 (8H, m, ArH). MS m/z (rel. int. %): 348 (43), 347 (M^+, 100), 245 (38), 231 (46), 217 (38), 216 (33), 215 (65), 202 (49), 191 (45), 102 (36).

4-(9*H*-Fluoren-9-ylidene)-1-piperidinecarboxylic Acid, Ethyl Ester (**2d**): Reaction of 9-fluorenone (315 mg, 1.75 mmol) with 300 mg (1.75 mmol) of 1-carbethoxy-4-piperidone gave a crude mixture that was purified by column chromatography (25 : 75, AcOEt–hexane) to yield 166 mg (52%) of compound **2d** as an oil.[16] IR (NaCl) cm^{-1}: 1700 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7.1 Hz, $OCH_2C\underline{H}_3$), 3.11 (4H, t, J=6.0 Hz, CH_2), 3.59—3.64 (4H, br s, CH_2), 4.22 (2H, q, J=7.1 Hz, OCH_2CH_3), 7.25—7.32 (4H, m, ArH), 7.64—7.72 (4H, m, ArH). MS m/z (rel. int. %): 319 (M^+, 4), 192 (20), 191 (15), 181 (100), 178 (25), 170 (55), 165 (25), 163 (16), 152 (62), 151 (25), 98 (18).

4-Diphenylmethylene-1-piperidinecarboxylic Acid, Ethyl Ester (**2e**): Reaction of benzophenone (319 mg, 1.75 mmol) with 300 mg (1.75 mmol) of 1-carbethoxy-4-piperidone gave compound **2e** in 93% yield as white crystals, mp 125 °C (methanol–hexane).[16] IR (NaCl) cm^{-1}: 1700 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.1 Hz, $OCH_2C\underline{H}_3$), 2.34 (4H, t, J=5.8 Hz, CCH_2), 3.49 (4H, t, J=5.8 Hz, NCH_2), 4.13 (2H, q, J=7.1 Hz,

OCH_2CH_3), 7.09—7.32 (10H, m, ArH). MS *m/z* (rel. int. %): 323 (15), 322 (M^+, 63), 206 (32), 191 (32).

4-Methylphenylmethylene-1-piperidinecarboxylic Acid, Ethyl Ester (**3a**): Reaction of acetophenone (210 mg, 1.75 mmol) with 600 mg (3.5 mmol) of 1-carbethoxy-4-piperidone gave a crude mixture that was purified by column chromatography (10:90, AcOEt–hexane) to yield 162 mg (36%) of compound **3a** as an oil. IR (NaCl) cm^{-1}: 1700 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.22—1.29 (3H, m, OCH_2CH_3), 1.99 (3H, s, CH_3), 2.12—2.14 (2H, m, CH_2), 2.39—2.43 (2H, m, CH_2), 3.31—3.36 (2H, m, CH_2), 3.51—3.56 (2H, m, CH_2), 4.09—4.18 (2H, m, OCH_2CH_3), 7.07—7.13 (3H, m, ArH), 7.21—7.35 (2H, m, ArH).

General Procedure for Reduction of *N*-Carbethoxy Compounds with LAH LAH (0.95 mmol) was added under argon to a stirred solution of an *N*-carbethoxy compound (0.44 mmol) in 25 ml of dry tetrahydrofuran (THF). The solution was refluxed for 2 h. After cooling to room temperature, a saturated aqueous solution of NH_4Cl was added, the THF was evaporated, and the mixture was extracted with dichloromethane. The organic extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure to give the final product. This procedure was used for the synthesis of compounds **2f—j** and **3b**.

1-Methyl-4-(9*H*-thioxanthen-9-ylidene)piperidine (**2f**): Reduction of compound **2a** (200 mg, 0.57 mmol) with 47 mg (1.25 mmol) of LAH yielded 149 mg (89%) of compound **2f** as an oil.[24,25] ^{1}H-NMR ($CDCl_3$) δ: 2.13—2.17 (2H, m, CH_2); 2.31 (3H, s, CH_3), 2.72—2.78 (6H, m, CH_2), 7.14—7.33 (6H, m, ArH); 7.47—7.50 (2H, m, ArH). MS *m/z* (rel. int. %): 293 (M^+, 73), 249 (63), 235 (36), 234 (26), 222 (36), 221 (100), 96 (30).

1-Methyl-4-(9*H*-xanthen-9-ylidene)piperidine (**2g**): Reduction of compound **2b** (118 mg, 0.34 mmol) with 29 mg (0.75 mmol) of LAH yielded 93 mg (98%) of compound **2g** as white crystals,[24,25] mp 166—168 °C (CH_2Cl_2); lit[24] 228—230 °C, as hydrochloride. ^{1}H-NMR ($CDCl_3$) δ: 2.20 (3H, s, CH_3), 2.33 (4H, t, J=5.7 Hz, CH_2), 2.82 (4H, t, J=5.7 Hz, CH_2), 7.09—7.34 (8H, m, ArH). MS *m/z* (rel. int. %): 277 (M^+, 55), 234 (22), 233 (100), 219 (39), 218 (24), 206 (29), 205 (76), 194 (37).

4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine (**2h**): Reduction of compound **2c** (209 mg, 0.6 mmol) with 50 mg (1.3 mmol) of LAH yielded 124 mg (72%) of compound **2h** as white crystals, mp 96 °C (CH_2Cl_2); lit[25] 273—274 °C (dec.), as hydrochloride. ^{1}H-NMR ($CDCl_3$) δ: 2.11—2.21 (2H, m, CH_2), 2.29 (3H, s, CH_3), 2.42—2.47 (4H, m, CH_2), 2.61—2.67 (2H, m, CH_2), 2.78—2.88 (2H, m, CH_2), 3.37—3.47 (2H, m, CH_2); 7.05—7.14 (8H, m, ArH). MS *m/z* (rel. int. %): 289 (M^+, 100), 288 (45), 217 (35), 216 (27), 215 (66); 203 (33), 202 (62), 96 (56).

4-(9*H*-Fluoren-9-ylidene)-1-methylpiperidine (**2i**): Reduction of compound **2d** (250 mg, 0.78 mmol) with 65 mg (1.7 mmol) of LAH yielded 183 mg (90%) of compound **2i** as a yellow oil.[16] ^{1}H-NMR ($CDCl_3$) δ: 2.23 (3H, s, CH_3), 1.99—2.64 (8H, m, CH_2), 7.20—7.74 (8H, m, ArH).

4-Diphenylmethylene-1-methylpiperidine (**2j**): Reduction of compound **2e** (141 mg, 0.44 mmol) with 36 mg (0.95 mmol) of LAH yielded 107 mg (93%) of compound **2j** as an oil.[15] ^{1}H-NMR ($CDCl_3$) δ: 2.45 (3H, s, CH_3), 2.57 (4H, t, J=5.9 Hz, CH_2), 2.68 (4H, t, J=5.9 Hz, CH_2), 7.09—7.32 (10H, m, ArH).

4-Methylphenylmethylene-1-methylpiperidine (**3b**): Reduction of compound **3a** (162 mg, 0.62 mmol) with 52 mg (1.36 mmol) of LAH yielded 100 mg (80%) of compound **3b** as white crystals, mp: 145—147 °C (CH_2Cl_2). ^{1}H-NMR ($CDCl_3$) δ: 1.99 (3H, s, CH_3), 2.47 (2H, br s, CH_2), 2.61 (3H, s, NCH_3), 2.77 (4H, br s, CH_2), 2.95 (2H, br s, CH_2), 7.07—7.09 (2H, m, ArH), 7.10—7.35 (3H, m, ArH). MS *m/z* (rel. int. %): 201 (M^+, 100), 200 (36), 129 (33), 128 (27), 115 (24), 96 (53).

B. Pharmacology. Drugs and Chemicals 5-HT hydrochloride and cyproheptadine hydrochloride were supplied by Sigma. Aqueous solutions of all drugs as their hydrochlorides were prepared daily using distilled water (compound **2j** was initially dissolved in a small quantity of absolute alcohol, which did not influence tissue response at its final concentration in the organ bath, <0.01%, v/v). All drug concentrations mentioned below are expressed as final molar concentrations in the tissue bath.

[^{3}H]Ketanserin (60.08 Ci/mmol) and [^{3}H]mesulergine (80 Ci/mmol) were obtained from New England Nuclear, Inc. (Boston, MA). Methysergide and cyproheptadine HCl were from Merck. Mianserine HCl was from Research Biochemicals, Inc. (Natick, MA). All other drugs and chemicals were of reagent grade from Sigma (St Louis, MO).

Functional Experiments Male Sprague-Dawley rats (250—300 g) were killed by cervical dislocation. The stomach was dissected free from the abdomen and immersed in modified Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.18; $NaHCO_3$, 25; glucose, 11. Strips of stomach fundus were prepared by Vane's method[26] and mounted in organ baths containing 10 ml of the same Krebs solution as above, maintained at 37 °C with aeration using carbogen (95% O_2, 5% CO_2). Before addition of drugs, the tissue strips were equilibrated for 1 h under a 1 g load. Isometric contractions were recorded during cumulative addition of serotonin using a Letica transducer and a Letica-Graph 1000-100 polygraph.

Concentration–response curves for serotonin were constructed as per Van Rossum.[27] In the initial control runs, stable contractions were achieved over the concentration range of 0.01 nM—10 μM. Following the initial control run, each tissue strip was run alternately with and without antagonist, the concentration of antagonist being increased in successive antagonist runs (see legends of figures). Between runs, the tissues were washed and allowed to rest for 60 min; if antagonist was to be used in the next run it was added at this point and the tissues were left in this solution for a further 45 min before the run. Antagonist potency was measured, following Arunlaksana and Schild,[28] in terms of pA_2 (−log concentration of antagonist required to maintain a constant response when the agonist concentration is doubled).

5-HT$_{2A}$ Receptor Binding Assay Male Sprague-Dawley rats (200—250 g, Charles River, Boston, MA) were asphyxiated with CO_2 and decapitated. The frontal cortex was dissected free on ice, frozen on dry ice and stored at −70 °C until assay (generally less than 1 week later), when the tissue was thawed on ice and homogenized in a Polytron (Brinkmann Instruments, Westbury, NY) with 10 volumes of 0.32 M sucrose. The homogenate was centrifuged twice at 4 °C (900 × *g* for 10 min followed by 40000 × *g* for 30 min). The supernatant fluid was discarded and the pellet resuspended in Tris HCl-buffer (pH=8.07). The homogenate was incubated at 37 °C for 15 min, to remove endogenous 5-HT[29] and centrifuged for 30 min at 40000 × *g*. The final pellet was suspended in Tris HCl-buffer of pH 8.07 containing 4 mM $CaCl_2$ and 0.1% ascorbic acid incubation buffer, and this suspension was used immediately.

Competition at [^{3}H]ketanserin binding sites was assayed in triplicate in assay mixtures (1 ml) consisting of 750 μl of membrane homogenate, 50 μl of assay buffer or masking ligand (1 μM methysergide), 50 μl of [^{3}H]ketanserin and 50 μl of either buffer or the compound under test (eleven different concentrations of test compound were used). Following initiation of the binding reaction by addition of the membrane preparation, the mixtures were incubated for 30 min at 37 °C. Membranes were harvested on Whatman GF/C filter strips (pre-soaked in 3% polyethylenylimine) in a Brandel cell harvester (Gaithersburg, MD). Unbound radioligand was removed with 3 × 5 ml of ice-cold incubation buffer. Radioactivity retained on filters was determined by liquid scintillation counting in Formula 963 (New England Nuclear, Boston, MA).

5-HT$_{2C}$ Receptor Binding Assay Porcine choroid plexus was brought fresh from a slaughterhouse on dry ice, thawed on ice and homogenized in assay buffer (50 mM HEPES, 2.5 mM $MgCl_2$, 2 mM EGTA, 0.1% ascorbic acid, 5 mM DL-dithiothreitol (DTT), adjusted to pH 7.4 with Tris HCl-buffer). The homogenate was centrifuged at 800 rpm for 10 min. The pellet was discarded and the supernatant was centrifuged for 10 min at 18000 rpm. The pellet was rehomogenized with aTeflon pestle in the same buffer, incubated for 15 min at 37 °C to remove endogenous 5-HT,[29] and centrifuged for 10 min at 18000 rpm. A suspension of the resulting pellet in the same buffer was stored on ice while not being manipulated. Competition at [^{3}H]mesulergine binding sites was determined by a protocol analogous to that described above for the 5-HT$_{2A}$ binding assay, using a final [^{3}H]mesulergine concentration of 2 nM and 1 μM mianserine as a masking ligand. The mixtures were incubated for 1 h at room temperature. Membranes were likewise harvested on a Whatman GF/B filter.

Expression of Results and Statistical Analysis All the data presented are the average values of 4—6 experiments. Pharmacological calculations were performed by the Pharmacological Calculation System Program.[30] In binding assays, non-linear regression calculations were carried out by using the curve-fitting program Kaleidagraph (Synergy Software, Reading, PA). The fitted equation was $E=E_{max}-(E_{max}-E_{basal}/(1+(IC_{50}/C)^n))$, where E_{max} and E_{basal} are dpm at the beginning and the end of the competition experiment, respectively, IC_{50} is the drug concentration required to inhibit 50% of the binding, C is the concentration of the compound and n is the slope of the sigmoid. Non-specific binding was determined independently in the presence of the respective masking

ligand. IC_{50} obtained from the fitted equation allowed estimation of pK_i using $K_i = IC_{50}/(1 + D/K_d)$, where K_d is the equilibrium dissociation constant from the radioligand and D is the total concentration of [^{3}H]ligand used.

Computational Tools The geometries of all the tricyclic fragments were optimized at the STO-3G level using the Gaussian 92 program.[31] Using the obtained STO-3G wavefunctions and the MEPSIM package,[32] MEP distributions and their minima were obtained. The plots of structures and MEP distributions as well as their fittings, were generated using the BIOSYM molecular modelling software (Biosym Technologies Inc., San Diego, CA, U.S.A.). The fittings of nuclei and MEP minima were carried out by RMS minimization of their coordinates.

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