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Simplified analogues of ritanserin and their affinity at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} serotonin receptors

Francesco Claudi[a]*, Loredana Scoccia[a], Gianfabio Giorgioni[a], Gabriella Marucci[a], Antonio Di Stefano[b], Stefania Gessi[c], Anna Siniscalchi[c], Pier Andrea Borea[c]

[a]*Dipartimento di Scienze Chimiche, Università di Camerino, Via S. Agostino 1, 62032 Camerino (MC), Italy*
[b]*Istituto di Scienza del Farmaco, Università G. D'Annunzio, Via dei Vestini, 66013 Chieti Scalo (CH), Italy*
[c]*Dipartimento di Medicina Sperimentale e Clinica, Sezione di Farmacologia, Università di Ferrara, Via Fossato di Mortara 17–19, 44100 Ferrara, Italy*

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Abstract – *The 5-HT_2 serotonin antagonist ritanserin (6-{2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl}-7-methyl-5H-thiazole[3,2-a]pyrimidin-5-one, **2**) binds with high affinity to 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} serotonin receptors. With the aim of exploring how simplification of the thiazolepyrimidinone nucleus of **2** affects the affinity and selectivity for 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} subtypes, some derivatives of 4-[bis(4-fluorophenyl)methylene]piperidine were synthesized, and their 5-HT_{2A} and 5-HT_{2C} receptor binding affinities and 5-HT_{2B} antagonistic affinity evaluated. The new compounds bind the three 5-HT_2 subtypes with lower affinity than did **2**. Simplification of the thiazolepyrimidinone nucleus of ritanserin has only slight influence on the selectivity for 5-HT_2 subtypes. The results suggest that the thiazolepyrimidinone moiety participates in key binding interactions and is determinant for high affinity at 5-HT_2 receptor subtypes. Some derivatives showed antagonistic activity at 5-HT_{2A} receptor.* © Elsevier, Paris

ritanserin analogues / synthesis / 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} serotonin receptor affinity

I. Introduction

Currently, the 5-HT_2 family of serotonin (5-hydroxytryptamine, 5-HT) receptors includes three subtypes, namely 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, grouped in the same class on the basis of molecular structure, signal transduction characteristics and pharmacology [1]. They are linked to G-proteins, share the same secondary messenger system (phosphatidylinositol hydrolysis) and have similar gene sequences with 68–79% homology in the transmembrane domains [2, 3].

The 5-HT_{2A} subtype is the best characterized with respect to distribution and function. It is present in the brain (cortical regions) [4] and periphery (gastrointestinal tract, cardiovascular system) [5] and is involved in various cardiovascular and mental disorders, such as depression and schizophrenia [6].

The 5-HT_{2B} receptor is expressed in rat stomach fundus, where it mediates a contractile response to 5-HT. Its mRNA transcript is present in human brain [7], and it has been suggested that it could be involved in the pathophysiology of migraine [8]. The 5-HT_{2C} receptor was initially characterized in the choroid plexus, is widely distributed in the brain [4] and has been suggested as playing a role in migraine, obsessive compulsive disorders and anxiety.

Since the three subtypes show a close structural similarity, it is possible that some of the functional effects previously attributed to 5-HT_{2A} (previously 5-HT_2) receptor activation may be mediated by 5-HT_{2C} or by 5-HT_{2B} subtypes.

To date, some antagonists selective for 5-HT_{2A} subtype are available [9], although, recently, antagonists that begin to differentiate among the 5-HT_{2B} and 5-HT_{2C} subtypes have been identified [10–15].

For some time 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2,4(1H,3H)quinazolinedione (ketanserin, **1**,

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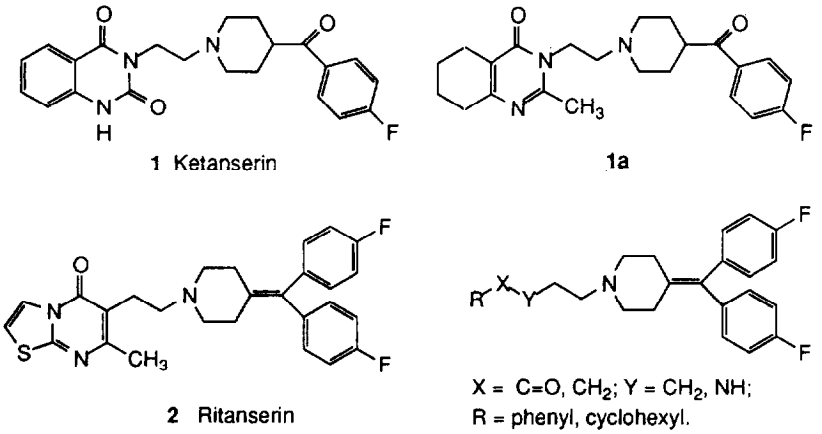


Figure 1. Structural formulas of the 5-HT_{2A} antagonists (ketanserin, ritanserin and **1a**) and ritanserin simplified analogues.

figure 1) and 6-{2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl}-7-methyl-5H-thiazole[3,2-a]pyrimidin-5-one (ritanserin, **2**) have been studied as 5-HT_2 antagonists. Ketanserin was once considered a prototypical 5-HT_{2A} selective antagonist, but it binds with little selectivity for 5-HT_{2A} versus 5-HT_{2C} [16]. Ritanserin has high affinity for all 5-HT_2 subtypes [16]. Both compounds were used to explain the molecular structural requirements for the 5-HT_2 antagonists binding [17,18] and to build the three-dimensional models of 5-HT_{2A} and 5-HT_{2C} receptors [19–21]. The structural elements affecting the affinity are: (a) the 4-(4-fluorobenzoyl)piperidine and 4-[bis(4-fluorophenyl) methylene]piperidine which seem to be essential for binding at 5-HT_2 receptors; (b) the quinazolinedione and the thiazolepyrimidinone ring systems. Replacement of these rings with different bicyclic systems affects 5-HT_{2A} affinity [22,23]. Some authors have suggested that the quinazolinedione contributes to 5-HT_{2A} affinity, but it is not essential for binding [17].

Andersen [24] and Mokrosz [25] developed a receptor-interaction model for 5-HT_{2A} antagonists by conformational analysis and superimposition studies of some serotonin 5-HT_{2A} ligands. This model '*is described by the distance between the centers of the two benzene rings and the distances from the centers of these rings to a point simulating the receptor site hydrogen bonding with the basic nitrogen atoms*' as illustrated in *figure 2*.

Structural parameters defining the topographic model of some 5-HT_{2A} ligands are held in the 4-[bis(4-fluorophenyl)methylene]piperidine **3** (*figure 2*), which seems to be essential for the antagonistic activity of **2**. On the other hand, **2** is not selective for the 5-HT_{2A} sites, but it binds with high affinity at 5-HT_{2B} and 5-HT_{2C} receptors [16]. This could suggest that the structural features of **3** facilitate the binding at the 5-HT_2 receptor subtypes. Moreover, the thiazolepyrimidinone nucleus of **2** contains an aromatic ring (thiazole) connected to a carbonyl group (*figure 1*). A similar structural feature is embedded in the quinazolinedione nucleus of **1** where the phenyl ring is connected to an amide group (*figure 1*). These strucural elements may play a role in binding at 5-HT_2 receptors.

Starting from these findings, we decided to explore how simplification of the thiazolepyrimidinone ring of **2** affects the affinity and selectivity for 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} subtypes. Thus, we have synthesized the derivatives of 4-[bis(4-fluorophenyl)methylene] piperidine **9a–12a** (*figure 3*). Glennon and co-workers in studies on ketanserin analogues suggested that there exists a receptor site that accomodates the benzene portion of the quinazolinedione ring [17], but it does not seem that the phenyl ring is critical for binding [18]. In a previous work, we found that 3-{2-[4-(-fluorophenyl)

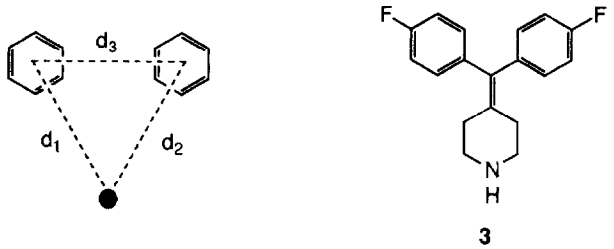


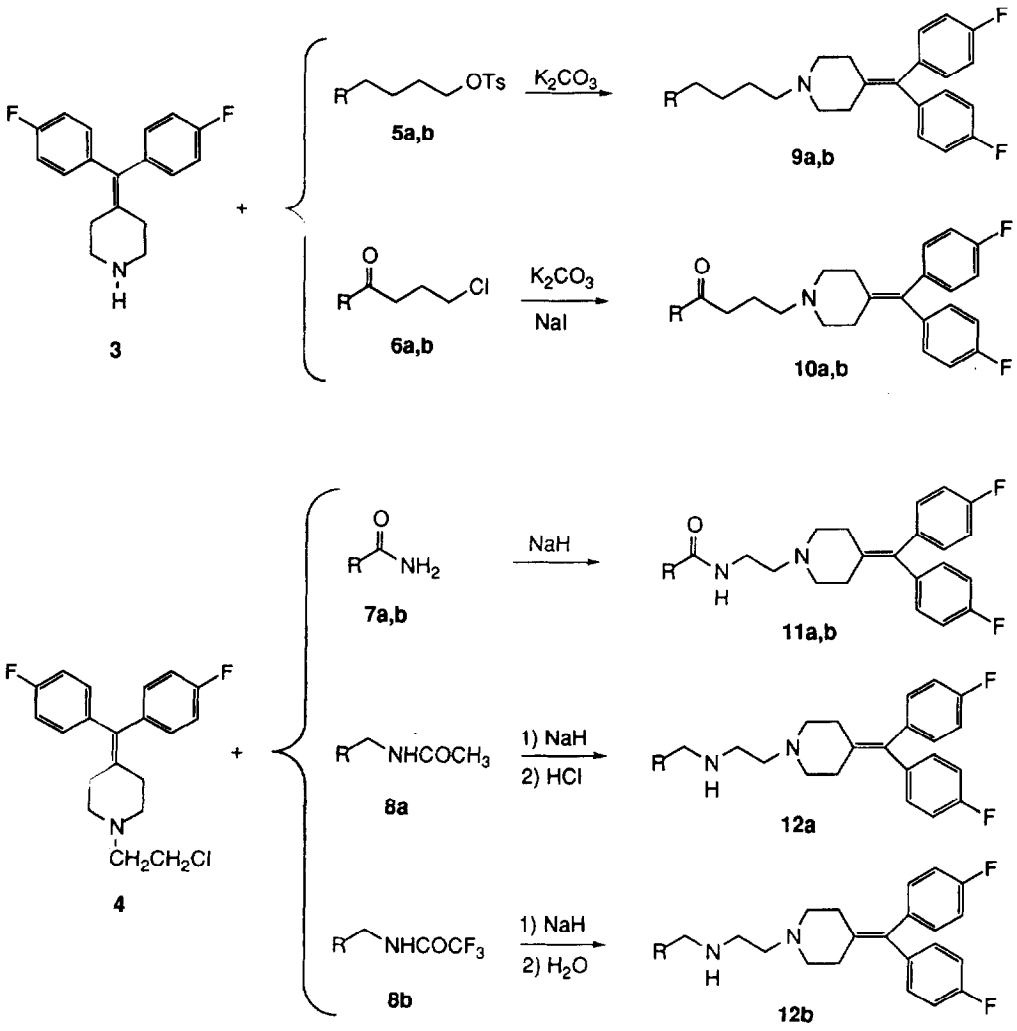
Figure 2. Andersen's and Mokrosz's model of 5-HT_{2A} antagonist–receptor interaction and 4-[bis(4-fluorophenyl) methylene]piperidine **3**.

piperidin-1-yl]ethyl}-5,6,7,8-tetrahydro-4(3H)quinazolinone (**1a**, *figure 1*), a ketanserin analogue, shows 5-HT_{2A} affinity ($pK_i = 8.58$) similar to that of ketanserin ($pK_i = 8.43$) [26]. This suggests that phenyl group reduction does not affect the 5-HT_{2A} affinity. In order to verify whether the phenyl ring is determinant for binding, we also prepared the cyclohexyl derivatives **9b–12b**.

2. Chemistry

The synthetic routes for the preparation of the new compounds are outlined in *figure 3*. The 4-[bis(4-fluorophenyl)methylene]piperidine **3** was used as starting material for the preparation of **9a,b–10a,b**. For the synthesis of **9a,b** the 4-phenylbutyl-p-toluenesulfonate **5a** or 4-cyclohexylbutyl-p-toluenesulfonate **5b** were used. Condensation of **3** with 1-phenyl-4-chloro-1-butanone **6a** or 1-cyclohexyl-4-chloro-1-butanone **6b** afforded the compounds **10a,b**.

The 4-[bis(4-fluorophenyl)methylene]-1-(2-chloroethyl)piperidine **4** was prepared in a two-step procedure by alkylation of **3** with 2-iodoethanol. Treatment of the alcohol with thionyl chloride afforded **4** which is described in a patent, but the synthetic procedure is not



a R = phenyl; **b** R = cyclohexyl

Figure 3.

readily available from the literature [27]. From this compound, the derivatives **11a,b–12a,b** were obtained. Amides **11a,b** were prepared from benzamide or cyclohexanecarboxamide. Secondary amines **12a,b** were prepared in a two-step procedure by alkylation of N-benzylacetamide or N-cyclohexylmethyltrifluoracetamide in presence of sodium hydride and subsequent hydrolysis of the intermediate tertiary amides.

3. Pharmacology

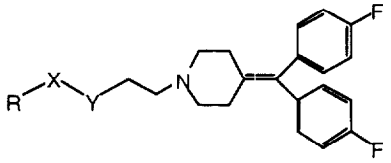
The affinity of compounds for 5-HT_{2A} and 5-HT_{2C} receptors was assessed in vitro in rat cerebral cortex preparations. [^{3}H]Ketanserin was used as radiolabeled ligand for the 5-HT_{2A} receptors and [^{3}H]mesulergine was used for the 5-HT_{2C} receptors. As no radiolabeled antagonists are available for the 5-HT_{2B} receptors, the antagonistic affinity was determined by the inhibition of 5-HT-induced contractions of rat stomach fundus [11]. The results are reported in *table I*. The 5-HT_{2B} receptor affinities were obtained from functional data and caution should be exercised when comparing them with data from binding assays.

Derivatives **10a,b–11a,b** were evaluated for their antagonist activity at central 5-HT_{2A} receptors by testing their ability to antagonize the facilitatory effect of 5-HT on basal acetylcholine release from guinea pig striatal slices [28]. Acetylcholine release induced by 5-HT could be attributed to 5-HT_{2A} receptor activation. In fact, it was concentration-dependently antagonized by 5-HT_{2A} antagonists, while the 5-HT_{2C} antagonist mesulergine was unable to counteract it, except at 10 μmol, a high concentration compatible with the 5-HT_{2A} block.

4. Results and discussion

The 5-HT_{2A} and 5-HT_{2C} receptor binding affinities and the 5-HT_{2B} antagonistic affinity of the new compounds are shown in *table I*. Removal of the 6-ethylthiazolepyrimidinone fragment of **2** to give **3** decreases the affinity for all 5-HT_2 receptor subtypes. This suggests that the thiazolepyrimidinone nucleus induces high 5-HT_2 affinities and the effect is more consistent for 5-HT_{2A}–5-HT_{2C} than for 5-HT_{2B} sites. Therefore, the thiazolepyrimidinone moiety participates in key binding interactions and is determinant for high affinity at 5-HT_{2A} and 5-HT_{2C} receptors.

Table I. 5-HT_{2A}, 5-HT_{2C} (rat cortex) receptor binding affinities and 5-HT_{2B} (rat stomach fundus) affinity, of compounds **3**, **9a,b–12a,b**. [a]



a: R = phenyl, **b**: R = cyclohexyl

Compound	X	Y	K_i (nM) [b]		K_b (nM) [c]	Ratio 2B/2A [d]
			5-HT_{2A}	5-HT_{2C}	5-HT_{2B}	
Mesulergine			40.0 ± 1.1	0.24 ± 0.01		
1 ketanserin			0.24 ± 0.005	1.76 ± 0.005		
2 ritanserin			1.2 ± 0.1	0.29 ± 0.17	17.3 ± 2.3	14.4
3			150 ± 28.86	588 ± 34	1120 ± 250	7.4
9a	CH_2	CH_2	220.0 ± 11.5	264.6 ± 17	720 ± 90	3.2
9b	CH_2	CH_2	100.0 ± 11.5	89.3 ± 8.1	670 ± 80	6.7
10a	C=O	CH_2	50.6 ± 2.7	82.0 ± 3.4	850 ± 160	17
10b	C=O	CH_2	26.0 ± 1.1	97.0 ± 1.7	360 ± 70	13.8
11a	C=O	NH	60.0 ± 2.3	62.6 ± 4	1100 ± 220	18.3
11b	C=O	NH	34.6 ± 3.5	98.3 ± 9.6	240 ± 40	6.9
12a	CH_2	NH	2400 ± 230	4366 ± 202	2700 ± 490	1.1
12b	CH_2	NH	700 ± 57	1773 ± 30	220 ± 50	0.3

[a] All values represent means ± SEM; $n \geq 3$ determinations. [b] Binding affinity (rat cortex; 5-HT_{2A} [^{3}H]Ketanserin, 5-HT_{2C} [^{3}H]Mesulergine). [c] Apparent antagonist dissociation constant, rat stomach fundus. [d] 5-HT_{2A} selectivity: K_b 5-HT_{2B} / K_i 5-HT_{2A}.

Binding data indicate that the nitrogen tied substituents off **3** influence the affinity in a different way. As concerns the 5-HT_{2A} affinity, the substituents containing a carbonyl group increase the affinity (compare **3** with **10a,b** and **11a,b**). Replacement of the carbonyl group of the ketones **10a,b** with a methylene gives the derivatives **9a,b** which show 5-HT_{2A} affinity similar to **3**. Reduction of the amides **11a,b** to the amines **12a,b** decreases affinities by 20- to 40-fold, and **12a,b** bind the 5-HT_{2A} sites even with lower affinity than does **3**. From these data it clearly appears that a carbonyl group facilitates the interaction with the 5-HT_{2A} receptor. The increased affinity of **10a,b** and **11a,b** in comparison with **3** could indicate that the carbonyl group participates in a hydrogen-bond interaction with the 5-HT_{2A} receptor.

An interesting result is that the cyclohexyl derivatives **9b–12b** show higher 5-HT_{2A} affinity than do the phenyl analogues **9a–12a**, and this suggests that the phenyl ring is not critical for 5-HT_{2A} binding.

Concerning the 5-HT_{2C} receptors binding, it is evident that the affinity is enhanced about six-fold when the piperidine nitrogen is substituted with a 4-cyclohexylbutyl group (compare **3** with **9b**). Moreover, compounds **10a,b–11a,b**, containing a carbonyl group, show higher affinity than does **3**. Comparing **9a** to **10a** and **11a**, it appears that the carbonyl group enhances the affinity in the phenyl series, but this effect is not evident in the cyclohexyl derivatives since the compounds with a carbonyl group (**10b**,**11b**) show the same affinity as **9b**. Reduction of the carbonyl group in amides **11a,b** results in a dramatic decrease in affinity at 5-HT_{2C} receptors, as previously observed also for binding at 5-HT_{2A} receptors. Compounds **12a,b** are isosteres of **9a,b** where a methylene is replaced by an amino group. This group, in the form of an ammonium ion, may bind the receptors and this indicates that the 5-HT_{2A} and 5-HT_{2C} receptors do not tolerate the presence of this polar group.

As concerns the affinity at 5-HT_{2B} receptors, the new compounds show micromolar affinities. Cyclohexyl derivatives **10b–12b**, containing a carbonyl, an amide or a secondary amino group, are more active than the phenyl derivatives **10a–12a**. Contrary to what was observed for 5-HT_{2A} and 5-HT_{2C} receptors, the antagonistic affinity for 5-HT_{2B} receptor seems to be more influenced by the cyclohexyl than by the carbonyl group and, in this case, also the secondary amine **12b** shows affinity comparable to carbonyl derivatives **10b** and **11b.** This could suggest that the cyclohexyl derivatives bind the 5-HT_{2B} receptor on sites which differ from those of 5-HT_{2A} and 5-HT_{2C} receptors. Compounds **9a,b–11a,b** bind the 5-HT_{2B} sites with lower affinity than for 5-HT_{2A} and 5-HT_{2C} receptors. Nevertheless, none of the new compounds is particularly selective for 5-HT_2 receptor subtypes. Moreover, from a comparison of **3** with **2**, it appears that the thiazolepyrimidinone increases the affinity for 5-HT_{2B} receptor.

Functional assays on rat stomach fundus indicate that **9a,b–12a,b** are non-surmountable antagonists of 5-HT, as reported also for **2** [29].

Derivatives **10a,b–11a,b** show antagonist activity at central 5-HT_{2A} receptors counteracting the release of acetylcholine induced by 5-HT (figure 4). They are less active than the standard **1** and the potency order, **1** > **10b** > **11b** > **11a** ~ **10a**, is in good agreement with the binding data. All the antagonists, when tested at the highest concentration, proved to be devoid of intrinsic activity, except **11b** which at 10 μM evoked a net increase in [^{3}H]choline efflux of 0.76 ± 0.25% (n = 3).

In conclusion, the simplification of the thiazolepyrimidinone moiety of ritanserin decreases the affinity for all the three subtypes and does not significantly influence the selectivity. The thiazolepyrimidinone system is determinant for binding at 5-HT_2 receptor subtypes. Moreover, a phenyl ring on the modified tails tied to 4-[bis(4-

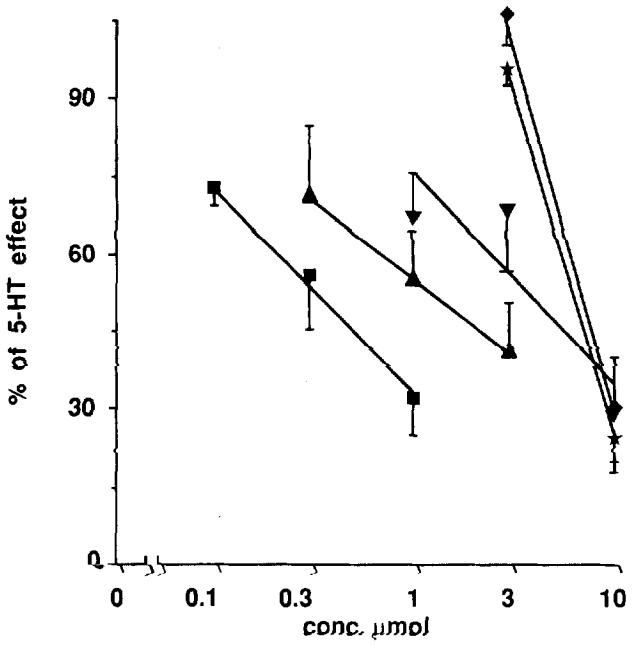


Figure 4. Basal tritium efflux from guinea pig striatal slices prelabelled with [^{3}H]choline. Relationship between 5-HT_{2A} antagonists concentrations (μmol, abscissa, log scale) and inhibition of the facilitatory effect of 5-HT 30 μmol (%, ordinate). Points represent the means ± SEM of 4–9 experiments. Squares: ketanserin (IC_{50} 0.369 μmol); triangles (pointing up): **10b** (IC_{50} 1.456 μmol); triangles (pointing down): **11b** (IC_{50} 4.489 μmol); diamonds: **10a**; stars: **11a**.

fluorophenyl)methylene]piperidine is not essential for binding and may be replaced by a cyclohexyl group. However, it must be considered that the modified tails tied to 4-[bis(4-fluorophenyl)methylene]piperidine may bind at a receptor site which differs from that of thiazolepyrimidinone.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Buchi 510 apparatus and are uncorrected. Microanalyses were performed on a 1106 Carlo Erba CHN Analyzer, and the results were within ± 0.4% of the calculated values. ^{1}H NMR spectra were recorded on a Varian VXR 200 MHz spectrometer. Chemical shifts are reported in parts per million (δ) downfield from the internal standard tetramethylsilane (Me_4Si). The identity of all new compounds was confirmed both by elemental analysis and NMR data; homogeneity was confirmed by TLC on silica gel Merck 60 F_{254}. Chromatographic purifications were performed by Merck-60 silica gel columns 70–230 mesh ASTM from Merck with a reported solvent.

5.1.1. *1-(2-Chloroethyl)-4-[bis(4-fluorophenyl)methylene]piperidine* **4**

To a solution of 4-[bis(4-fluorophenyl)methylene]piperidine hydrochloride (**3**) (10 g, 0.031 mol) in acetonitrile (100 mL), K_2CO_3 (11 g, 0.08 mol) and 2-iodoethanol (4.1 mL, 0.052 mol) were added. The mixture was heated at reflux for 16 h and another portion of 2-iodoethanol (2 mL, 0.025 mol) added. After reflux for 4 h, the solvent was evaporated and H_2O added to the residue. The solution was extracted with $CHCl_3$. The combined organic phases were dried (Na_2SO_4), filtered and evaporated. The oily residue was dissolved in 95% EtOH and concentrated HCl added. Evaporation of the solvents afforded the crude 1-(2-hydroxyethyl)-4-[bis(4-fluorophenyl)methylene]piperidine hydrochloride which was recrystallized from 2-propanol; yield 82%, m.p. 221–223 °C; NMR (DMSO-d_6) δ 10.62 (bs, 1H, OH), 7.14 (m, 8H, ArH), 5.31 (bs, 1H, NH^+), 3.80 (t, 2H, CH_2O), 3.63 (m, 2H, CH_2N), 3.12 (m, 4H, H_{pip}), 2.55 (m, 4H, H_{pip}).

To a solution of the crude alcohol (3.3 g, 0.01 mol) in $CHCl_3$ (40 mL), thionyl chloride (2.2 mL) was added dropwise. The mixture was stirred at room temperature for 6 h, and another portion of thionyl chloride (2 mL, 0.025 mol) was added. After stirring for 4 h, the solvent was evaporated and H_2O added to the residue. The solution was basified with 2 N NaOH and extracted with $CHCl_3$. The combined organic phases were dried (Na_2SO_4), filtered and evaporated. The oily residue was dissolved in $CHCl_3$ and the solution was filtered through a short pad of silica gel. The filtrate was evaporated and the oily residue recrystallized from hexane: yield 82%, m.p. 91–92 °C; NMR ($CDCl_3$) δ 7.0 (m, 8H, ArH), 3.61 (t, 2H, CH_2Cl), 2.78 (t, 2H, CH_2N), 2.57 (m, 4H, H_{pip}), 2.49 (m, 4H, H_{pip}). Anal. ($C_{20}H_{20}ClF_2N$) C, H, N.

5.1.2. *4-Phenylbutyl-p-toluenesulfonate* **5a**

p-Toluenesulfonyl chloride (4.2 g, 0.022 mol) was added dropwise to a stirred solution of 4-phenyl-1-butanol (3 g, 0.02 mol) in pyridine (6.4 mL, 0.08 mol). The reaction mixture was stirred for 4 h at room temperature, then treated with 2 N HCl (50 mL). The two-phase mixture was extracted with $CHCl_3$. The organic layers were dried (Na_2SO_4), filtered and evaporated to provide an oily reisidue that was used for the next step without further purification: yield 91%; NMR ($CDCl_3$) δ 7.78 (m, 2H, ArH), 7.25 (m, 7H, ArH), 4.05 (t, 2H, OCH_2), 2.55 (m, 2H, $ArCH_2$), 2.45 (s, 3H, CH_3), 1.68 (m, 4H, two CH_2). Anal. ($C_{17}H_{20}O_3S$) C, H, N.

5.1.3. *N-Cyclohexylmethyltrifluoracetamide* **8b**

A solution of trifluoroacetic anhydride (15.5 mL, 0.11 mol) in CH_2Cl_2 (20 mL) was added dropwise to a solution of N-cyclohexylmethylamine (5.66 g, 0.05 mol) and pyridine (12.11 mL, 0.15 mol) in CH_2Cl_2 (20 mL). The mixture was stirred at room temperature for 3 h, then poured into ice-water and extracted with CH_2Cl_2. The organic phase was washed with 2 N NaOH, H_2O and brine, dried (Na_2SO_4), filtered and evaporated to provide a white solid which was recrystallized from hexane: yield 71%; m.p. 70–71 °C. NMR ($CDCl_3$) δ 6.32 (bs, 1H, NH), 3.21 (t, 2H, CH_2N), 1.71 (m, 5H, H_{cycl}), 1.56 (m, 1H, H_{cycl}), 1.24 (m, 3H, H_{cycl}), 0.97 (m, 2H, H_{cycl}). Anal. ($C_9H_{14}F_3NO$) C, H, N.

5.1.4. *1-Phenyl-4-{4-[bis(4-fluorophenyl)methylene]piperidin-1-yl}butane hydrochloride* **9a**

A mixture of 4-phenylbutyl-p-toluenesulfonate (1.82 g, 0.006 mol), 4-[bis(4-fluorophenyl)methylene]piperidine (1.71 g, 0.006 mol) and K_2CO_3 (1.24 g, 0.009 mol) in acetone (25 mL) was heated at reflux for 20 h. The mixture was cooled at room temperature and the inorganics were filtered. The filtrate was concentrated in vacuo to a residue which was partitioned between $CHCl_3/H_2O$. The organic phase was dried (Na_2SO_4), filtered and evaporated. The liquid was dissolved in 95% EtOH and the solution acidified with ethanolic HCl. The white solid was filtered and recrystallized from 95% EtOH/MeOH: yield 72%, m.p. 223–224 °C. NMR ($CDCl_3$) δ 12.58 (bs, 1H, NH), 7.20 (m, 5H, ArH), 6.93 (m, 8H, ArH), 3.55 (m, 2H, CH_2N^+), 3.02 (m, 4H, H_{pip}), 2.65 (m, 6H, $ArCH_2$, H_{pip}), 1.93 (m, 2H, CH_2), 1.70 (m, 2H, CH_2). Anal. ($C_{28}H_{29}F_2N$•HCl) C, H, N.

5.1.5. *1-Cyclohexyl-4-{4-[bis(4-fluorophenyl)methylene]piperidin-1-yl}butane hydrochloride* **9b**

This compound was prepared following the procedure described for compound **9a**, starting from 4-cyclohexylbutyl-p-toluenesulfonate and 4-[bis(4-fluorophenyl)methylene]piperidine. The HCl salt was recrystallized from 2-propanol: yield 75%, m.p. 164–166 °C. NMR (DMSO-d_6) δ 10.48 (bs, 1H, NH^+), 7.20 (m, 8H, ArH), 3.50 (m, 2H, CH_2N^+), 2.98 (m, 4H, H_{pip}), 2.55 (m, 6H, CH_2, H_{pip}), 1.70 (m, 6H, CH_2, H_{pip}), 1.20 (m, 7H, H_{cycl}), 0.87 (m, 2H, CH_2). Anal. ($C_{28}H_{35}F_2N$•HCl) C, H, N.

5.1.6. *1-Phenyl-4-{4-[bis(4-fluorophenyl)methylene]piperidin-1-yl}butan-1-one hydrochloride* **10a**

1-Phenyl-4-chlorobutan-1-one (0.6 mL, 0.0035 mol) was added to a solution of sodium iodide (0.52 g, 0.0035 mol) in acetonitrile (15 mL). The solution was heated to reflux for 3 h. A precipitate formation was observed. K_2CO_3 (0.5 g, 0.0035 mol) and 4-[bis(4-fluorophenyl)-methylene]piperidine (1 g, 0.0035 mol) were added. The mixture was heated at reflux for 20 h, cooled at room temperature and the inorganics filtered. The filtrate was concentrated in vacuo. The residue was triturated with Et_2O, the insoluble filtered and the filtrate evaporated. The residue was purified by

column chromatography (EtOAc) to afford the title compound which was recrystallized from hexane: yield 62%; m.p. 94–95 °C. The HCl salt was prepared and recrystallized from 2-propanol: m.p. 216–218 °C. NMR (DMSO-d_6) δ 10.12 (bs, 1H, NH^+), 8.0 (m, 2H, ArH), 7.61 (m, 3H, ArH), 7.21 (m, 8H, ArH), 3.59 (m, 2H, CH_2N^+), 3.21 (t, 2H, $COCH_2$), 3.10 (m, 4H, H_{pip}), 2.53 (m, 4H, H_{pip}), 2.03 (m, 2H, $COCCH_2$). Anal. ($C_{28}H_{27}F_2NO$•HCl) C, H, N.

5.1.7. 1-Cyclohexyl-4-{4-[bis(4-fluorophenyl)methylene]piperidin-1-yl}butan-1-one hydrochloride ***10b***

This compound was prepared following the procedure described for compound **10a**, starting from 1-cyclohexyl-4-chlorobutan-1-one and 4-[bis(4-fluorophenyl)methylene]piperidine. The mixture was heated to reflux for 26 h. The residue was purified by column chromatography (EtOAc/cyclohexane/MeOH, 2.5:7:0.5) to afford the title compound which was recrystallized from hexane: yield 62%; m.p. 82–84 °C.

The HCl salt was prepared and recrystallized from acetone: m.p. 174–176 °C. NMR (DMSO-d_6) δ 10.40 (bs, 1H, NH^+), 7.15 (m, 8H, ArH), 3.50 (m, 2H, CH_2N^+), 2.98 (m, 4H, H_{pip}), 2.56 (t, 2H, $COCH_2$), 2.42 (m, 5H, H_{cycl}, H_{pip}), 1.75 (m, 8H, CH_2, H_{pip}), 1.20 (m, 4H, H_{cycl}). Anal. ($C_{28}H_{33}F_2NO$•HCl) C, H, N.

5.1.8. N-{2-[4-[bis(4-Fluorophenyl)methylene]piperidin-1-yl]ethyl}benzamide hydrochloride ***11a***

To a suspension of previously washed (with hexane) sodium hydride (0.3 g of 60% dispersion in mineral oil, 0.0075 mol) in anhydrous DMF (10 mL) benzamide (0.5 g, 0.004 mol) was added. The mixture was stirred, under nitrogen atmosphere, at room temperature for 1 h, then a solution of 4-[bis(4-fluorophenyl) methylene]-1-(2-chloroethyl)piperidine (1.42 g, 0.004 mol) in DMF (10 mL) was added. After heating for 5 h at 90 °C the solvent was evaporated and the residue partitioned between $CHCl_3/H_2O$. The organic phase was dried (Na_2SO_4), filtered and evaporated. The oily residue was purified by column chromatography (CH_2Cl_2/cyclohexane/MeOH, 5:4:1) to afford the title compound which was recrystallized from cyclohexane/EtOAc 9:1; yield 58%; m.p. 125–126 °C. NMR [$(CD_3)_2CO$] δ 7.86 (m, 2H, ArH), 7.63 (bs, 1H, NH), 7.45 (m, 3H, ArH), 7.12 (m, 8H, ArH), 3.52 (q, 2H, $CONCH_2$), 2.59 (m, 6H, CH_2N, H_{pip}), 2.32 (m, 4H, H_{pip}). Anal. ($C_{27}H_{26}F_2N_2O$) C, H, N. The HCl salt was prepared and recrystallized from 2-propanol: m.p. 182–184 °C. Anal. ($C_{27}H_{26}F_2N_2O$•HCl) C, H, N.

5.1.9. N-{2-[4-[bis(4-Fluorophenyl)methylene]piperidin-1-yl]ethyl}cyclohexanecarboxamide hydrochloride ***11b***

This compound was prepared following the procedure described for compound **11a**, using cyclohexanecarboxamide and 4-[bis(4-fluorophenyl)methylene]-1-(2-chloroethyl)piperidine as starting materials. The oily residue was purified by column chromatography ($CHCl_3$/EtOAc/cyclohexane/MeOH, 3:2:4:1) to afford the title compound which was recrystallized from cyclohexane/EtOAc 9:1; yield 64%; m.p. 130–132 °C. NMR [$(CD_3)_2CO$] δ 7.10 (m, 8H, ArH), 6.78 (bs, 1H, NH), 3.28 (q, 2H, $CONCH_2$), 2.47 (m, 6H, CH_2N, H_{pip}), 2.30 (m, 4H, H_{pip}), 2.06 (m, 1H, H_{cycl}), 1.79 (m, 5H, H_{cycl}), 1.32 (m, 5H, H_{cycl}). Anal. ($C_{27}H_{32}F_2N_2O$) C, H, N. The HCl salt was prepared and recrystallized from 2-propanol: m.p. 209–211 °C. Anal. ($C_{27}H_{32}F_2N_2O$•HCl) C, H, N.

5.1.10. N-{2-[4-[bis(4-Fluorophenyl)methylene]piperidin-1-yl]ethyl}benzylamine dihydrochloride ***12a***

To a suspension of previously washed (with hexane) sodium hydride (0.3 g of 60% dispersion in mineral oil, 0.0075 mol) in anhydrous DMF (10 mL) N-benzylacetamide (0.6 g, 0.004 mol) was added. The mixture was stirred, under nitrogen atmosphere, at room temperature for 1 h, then a solution of 4-[bis(4-fluorophenyl)methylene]-1-(2-chloroethyl)piperidine (1.42 g, 0.004 mol) in DMF (10 mL) was added. The mixture was heated for 5 h at 90 °C. After the solvent evaporation the residue was partitioned between $CHCl_3/H_2O$. The organic phase was dried (Na_2SO_4), filtered and evaporated. The residue was purified by column chromatography ($CHCl_3$/MeOH, 9.5:0.5). The fractions containing desired product were evaporated to provide an oil (1.04 g). This was dissolved in 95% EtOH (10 mL) and 6 N HCl (5 mL) was added. The mixture was heated to reflux for 2 h, cooled, diluted with H_2O (15 mL), basified with 6 N NaOH and extracted with $CHCl_3$. The organic phase was dried (Na_2SO_4), filtered and evaporated. To the dark oily residue hexane was added. The precipitate was filtered and the organic solution evaporated to afford a residue: yield 48%. NMR ($CDCl_3$) δ 7.29 (m, 5H, ArH), 7.0 (m, 8H, ArH), 3.82 (s, 2H, $ArCH_2$), 2.72 (t, 2H, NCH_2), 2.51 (m, 6H, NCH_2, H_{pip}), 2.32 (m, 4H, H_{pip}), 1.95 (bs, 1H, NH). The residue was dissolved in absolute EtOH (10 mL) and HCl gas was bubbled into the solution. The solvent was evaporated and the residue was triturated with hot 2-propanol. After cooling, the precipitate was filtered and recrystallized from absolute EtOH: m.p. 258–260 °C. Anal. ($C_{27}H_{28}F_2N_2$•2HCl) C, H, N.

5.1.11. N-{2-[4-[bis(4-Fluorophenyl)methylene]piperidin-1-yl]ethyl}cyclohexylmethylamine dihydrochloride ***12b***

This compound was prepared following the procedure described for compound **12a**, using N-cyclohexylmethyltrifluoroacetamide and 4-[bis(4-fluorophenyl)methylene]-1-(2-chloroethyl)-piperidine as starting materials. After evaporation of solvent H_2O was added and the solution extracted with $CHCl_3$. The organic phase was dried (Na_2SO_4), filtered and evaporated. The residue was purified by column chromatography ($CHCl_3$/cyclohexane/EtOAc/MeOH, 3:4:2:1). The fractions containing the desired product were evaporated to provide an oil. It was dissolved in n-hexane and the solution was cooled overnight. The precipitate was filtered and the solution evaporated to give an oil; yield 52%. NMR ($CDCl_3$) δ 7.0 (m, 8H, ArH), 2.69 (t, 2H, NCH_2), 2.50 (m, 8H, NCH_2, H_{pip}), 2.35 (m, 4H, H_{pip}), 1.72 (m, 5H, NH, H_{cycl}), 1.48 (m, 1H, H_{cycl}), 1.25 (m, 4H, H_{cycl}), 0.88 (m, 2H, H_{cycl}). The residue was dissolved in absolute EtOH (10 mL) and HCl gas was bubbled into the solution. The mixture was cooled, the precipitate was filtered and recrystallized from absolute EtOH: m.p. 270–271 °C. Anal. ($C_{27}H_{34}F_2N_2$•2HCl) C, H, N.

5.2. Pharmacology

5.2.1. Materials

Ketanserin tartrate and ritanserin were purchased from Research Biochemicals International (Natick, MA, USA). [^{3}H]Ketanserin (64.1 Ci/mmol) was purchased from New England Nuclear, Boston, MA, USA. [^{3}H]Mesulergine (76 Ci/mmol) and [^{3}H]choline (81 Ci/mmol) were purchased from Amersham Radiochemical Centre (Buckinghamshire, UK). All substances employed in the binding assays were dissolved in distilled water.

5.2.2. Animals

In the radioligand-binding studies rat cortex were obtained from male Wistar rats (250–300 g body weight) obtained from Nossan (Varese, Italy). Sections of stomach fundus were obtained from male CD Outbred rats (Charles River, Calco, Italy) weighing 125–150 g.

5.2.3. Binding assays

Cerebral cortices of male Wistar rats (150–200 g) were dissected on ice. The tissue was homogenized in 50 mmol Tris-HCl buffer (pH = 7.7 at 25 °C). The omogenate was centrifuged at 40000 *g* for 10 min. The supernatant was discarded and the pellet was resuspended in the same volume of Tris-HCl buffer and incubated at 37 °C for 10 min prior to a second centrifugation. Binding experiments [30] with [^{3}H]ketanserin (64.1 Ci/mmol) and [^{3}H]mesulergine (76 Ci/mmol) were performed in 250 µL of buffer which contained 1 nmol [^{3}H]ketanserin or [^{3}H]mesulergine, membranes from 10 mg (wet weight) of tissue and the compounds to be tested. After 30 min of incubation at 25 °C, separation of bound from free radioligand was performed by rapid filtration through Whatman GF/B glass fiber filters, which were washed three times with ice-cold buffer, dried and counted in 5 mL of Aquassure (Packard, Downers Grove, USA). Non-specific binding was measured in the presence of 10 µmol 5-HT for 5-HT_{2A} sites and 10 µmol cinanserin for 5-HT_{2C} sites with specific binding defined as the total binding minus the non specific binding. K_i values were calculated from the Cheng–Prusoff equation

$$K_i = IC_{50} / 1 + (\text{ligand} / K_d)$$

[31] where K_d = 0.8 nmol/L for [^{3}H]ketanserin and K_d = 1.9 nmol/L for [^{3}H]mesulergine [32].

5.2.4. Determination of apparent 5-HT_{2B} receptor antagonist dissociation constant

Experiments were performed as described by Nozulak et al. [11]. Male CD Outbred rats were sacrificed by CO_2, and longitudinal sections of the stomach fundus were prepared for in vitro examination. Strips were set up in organ baths of 10 mL containing Krebs solution (compositon in mmol: NaCl, 118; KCl, 4.7; $CaCl_2$, 1.25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11; $NaHCO_3$, 25) constantly bubbled with 5% CO_2 in oxygen. Contractions were measured isotonically under a resting tension of 1 g. Prior to testing, the strips were allowed to equilibrate for 1 h, during which time the bath was replaced every 15 min.

After control cumulative contractile responses to serotonin were obtained in the stomach fundus, the tissues were incubated with an appropriate concentration of antagonist for one hour. Contractile responses to serotonin were then repeated in the presence of the antagonist. Only one antagonist concentration was examined in each tissue. Apparent dissociation constants (K_b) were determined for each concentration of antagonist according to the following equation:

$$K_b = [B] / (\text{dose ratio} - 1)$$

where [B] is the concentration of the antagonist and the dose ratio is the ED_{50} of the agonist in the presence of the antagonist divided by the control ED_{50}.

5.2.5. Inhibition of acetylcholine release

Inhibition of the facilitatory effect of serotonin on basal acetylcholine release from guinea pig striatal slices was determined as previously described [28]. Caudate nucleus slices (400 µm thick) were incubated with 0.1 µmol [^{3}H]choline for 30 min and superfused at 0.25 mL/min with Krebs solution (compositon in mmol: NaCl, 118.5; KCl, 4.8; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11; hemicholinium 30.01) constantly bubbled with 5% CO_2 in oxygen. The radioactivity of the 5 min superfusate samples was determined by liquid scintillation. The effect of 5-HT, both in absence and in presence of antagonists, was quantified as the net increase of tritium efflux over the basal one, calculated as fractional rate (FR), i.e. as percent of tissue tritium content.

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