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Preliminary communication

4-(4-Fluorobenzoyl)-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine and its derivatives: synthesis and affinity at 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} serotonin receptors

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Abstract – 4-(4-Fluorobenzoyl)-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine (**7**) and its derivatives modified at the carbonyl group of the fluorobenzoyl moiety were prepared and evaluated for affinity at 5-HT_{2A}, 5-HT_{2C} (rat cortex) and 5-HT_{2B} (rat stomach fundus) serotonin receptors. Compound **7** bound the 5-HT_{2A} sites with higher affinity (K_i = 8.2 nM) than the 5-HT_{2B} (K_b = 1 290 nM) and 5-HT_{2C} ones (K_i = 54.2 nM). Modification of the benzoyl carbonyl group decreased the 5-HT_{2A} and 5-HT_{2C} affinities but did not significantly influence 5-HT_{2B} affinity. This suggests that the carbonyl group is the determinant for the interaction with 5-HT_{2A} and 5-HT_{2C} receptor subtypes. Compound **7** was found to be a 5-HT_{2A} receptor antagonist. © 1999 Éditions scientifiques et médicales Elsevier SAS

4-(4-Fluorobenzoyl)-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine derivatives / synthesis / 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} serotonin receptor affinity / 5-HT_{2A} antagonistic activity

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) mediates a number of neuronal processes both in the central nervous system and peripheral tissues. During the last decade multiple 5-HT receptor subtypes have been characterized and grouped in seven classes (5-HT_1–5-HT_7) [1]. The 5-HT_2 class includes the subtypes 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} which are grouped together considering their high degree of transmembrane sequence homology and second messenger coupling system. The 5-HT_{2A} subtype is present in the brain (cortical regions) [2] and periphery (gastrointestinal tract, cardiovascular system) [3], and is involved in various cardiovascular and mental disorders, such as depression and schizophrenia [4]. 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 the human brain [5], and it has been suggested to be involved in the pathophysiology of migraine [6]. The 5-HT_{2C} receptor was initially characterized in the choroid plexus, is widely distributed in the brain [2], and has been suggested as playing a role in migraine, obsessive compulsive disorders, and anxiety [7].

To date, few antagonists discriminating between the 5-HT_2 subtypes are available [8–14]. With the lack of selective agents has come the realization that many pharmacological and physiological effects once attributed to 5-HT_{2A} receptors (previously 5-HT_2) may, in fact, involve more than one receptor subtype. Hence, there is a need to discover new agents able to bind with high selectivity at one of the three subtypes.

The 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2,4(1H,3H)-quinazolinedione (ketanserin, **1**, *figure 1*) is a prototypical 5-HT_{2A} antagonist reported to bind with as little as 15-fold [9] to as much as 140-fold selectivity for 5-HT_{2A} versus 5-HT_{2C} receptors [10]. It was used to explain the molecular structural prerequisites for the 5-HT_{2A} antagonist binding [9, 10], and to build the

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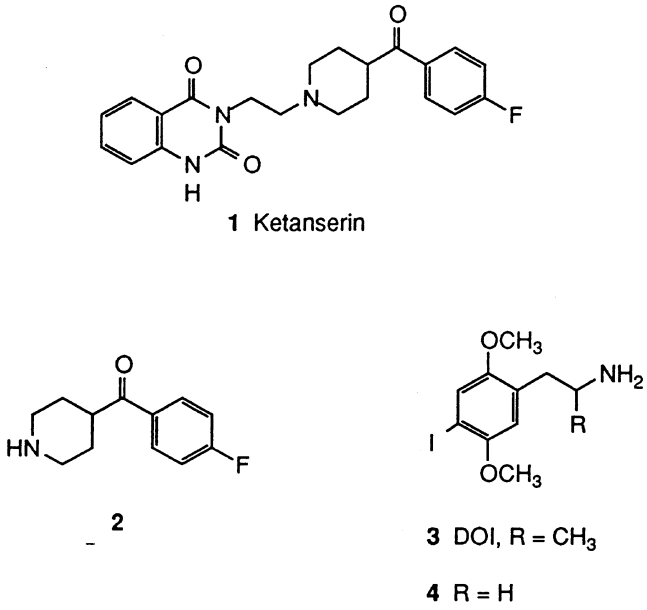

Figure 1. Structures of ketanserin, 4-(4-fluorobenzoyl) piperidine (**2**), DOI, and 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminoethane (**4**).

three-dimensional models of 5-HT_{2A} and 5-HT_{2C} receptors [15–17].

Several studies on ketanserin analogues showed that the 4-(4-fluorobenzoyl)piperidine (**2**) fragment is endowed with 5-HT_{2A} antagonistic activity and seems to be essential for binding at the 5-HT_{2A} receptor [9, 18].

Among the 5-HT_2 agonists, the most extensively studied is the 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane (**3** R(-)-DOI), currently reported as a 5-$HT_{2C/2A}$ agonist [19]. Investigations on 1-(2,5-dimethoxyphenyl)-2-aminopropane derivatives have established that the methyl group α to the amine in **3** does not influence the in vitro receptor affinity. Thus, the 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminoethane **4** binds with high affinity (K_i = 1.53 nM) the 5-HT_2 receptors labelled by R-[^{125}I]-DOI [20], and has been reported to act as agonist at 5-HT_2 receptors [21].

Site-directed mutagenesis and molecular modelling studies indicate that the piperidine nitrogen atom of **1** and the amino group of **3** have a strong elecrostatic interaction with the carboxylate anion of the aspartate residue Asp 155 in transmembrane helix 3 (TM 3) of the 5-HT_{2A} receptor [15, 17]. Therefore, it would appear that Asp-155 is a common binding site for agonists **3** and **4**, and for antagonist **1**.

Some time ago, Ariens suggested that an agonist can be turned into a competitive antagonist by appending to the agonist structure a hydrophobic bulky group able to bind the accessory binding sites of the receptor [22].

On the basis of the Ariens strategy and of the above mentioned observations, we decided to connect the 4-(4-fluorobenzoyl)piperidine with the 2-(4-iodo-2,5-dimethoxyphenyl)ethyl fragment of **4** to obtain 4-(4-fluorobenzoyl)-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine **7** *(figure 2)*. This compound was chosen in order to obtain a new 5-HT_2 antagonist and to investigate how a modified tail tied to 4-(4-fluorobenzoyl)piperidine could influence selectivity for the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} subtypes.

Studies with ketanserin and related 5-HT_2 antagonists have suggested that the carbonyl group of 4-(4-fluorobenzoyl)piperidine is involved in a hydrogen-bonding interaction with the 5-$HT_{2A/2C}$ receptors [23] and may have a prominent role in anchoring ketanserin to 5-HT_{2A} receptors [10, 15]. Thus, in order to obtain further information about the role of the carbonyl group in the affinity and selectivity for the three 5-HT_2 subtypes, two series of compounds were synthesized. The first series keeps an sp^2 carbon atom placed between the piperidine and the fluorophenyl ring (**8**–**11,** *figure 3*), while in the other (**12**–**16**, *figure 4*) the same carbon has an sp^3 hybridization.

2. Chemistry

The desired 4-(4-fluorobenzoyl)-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine **7** was synthesized by condensation of 4-(4-fluorobenzoyl)piperidine **2** with the tosyl ester of 2-(4-iodo-2,5-dimethoxyphenyl)ethanol **6** *(figure 2)*. Iodination of 2-(2,5-dimethoxyphenyl)ethanol with iodine in the presence of silver trifluoroacetate gave the 2-(4-iodo-2,5-dimethoxyphenyl)ethanol **5** which was esterified with tosyl chloride. Oxime **8** and the O-alkyloximes **10** and **11** were obtained by reaction of the ketone **7** in pyridine and absolute ethanol with hydroxylamine or O-ethylhydroxylamine or O-benzylhydroxylamine *(figure 3)*. The oxime acetate **9** was obtained from **8** by reaction with acetic anhydride. Oxime isomers were not separated. Ketone **7** was also reduced by sodium borohydride to alcohol **12** or transformed into the dioxolane **13**. From **12**, by reaction with acetic anhydride or benzoyl chloride, the esters **14** and **15** were obtained *(figure 4)*.

In order to obtain the derivative **16** with a methylene replacing the carbonyl group, the tosylate ester **6** was reacted with 4-(4-fluorobenzyl)piperidine.

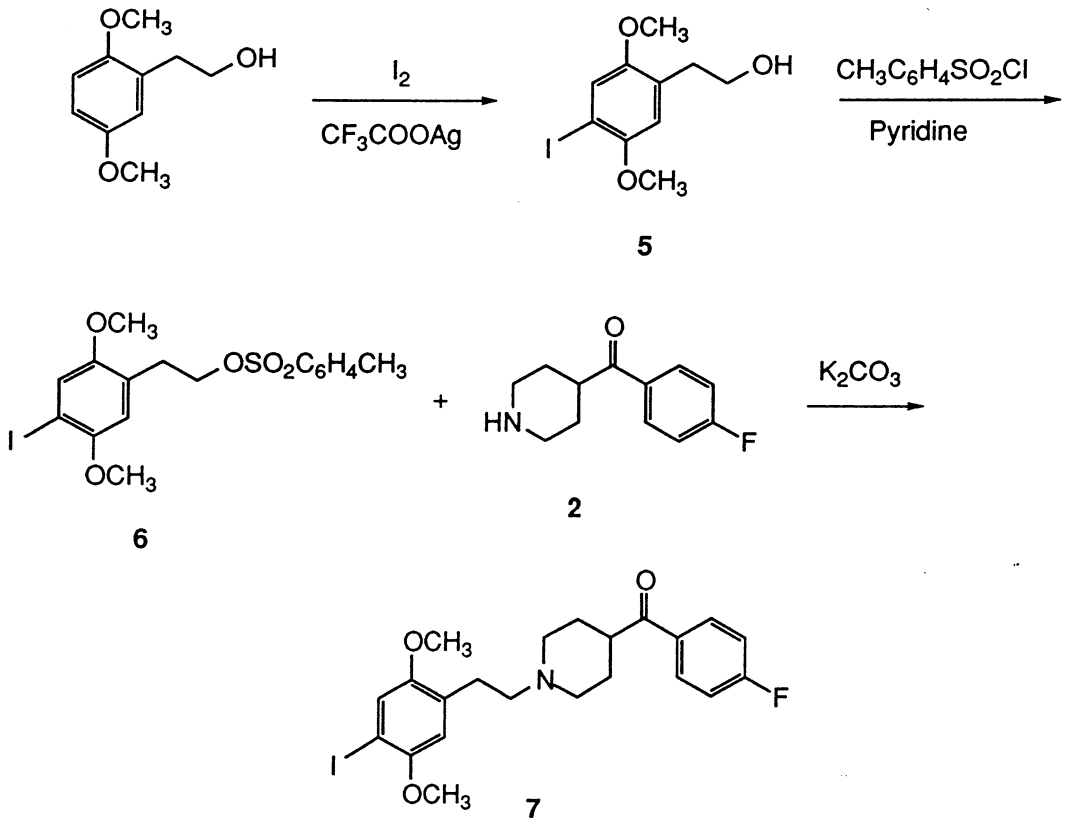

Figure 2. Synthesis of **7**.

3. Pharmacology

The affinity of compounds for 5-HT_{2A} and 5-HT_{2C} receptors was assessed in vitro in the cerebral cortex preparations. [^{3}H]Ketanserin as radiolabelled ligand for the 5-HT_{2A} receptors, and [^{3}H]mesulergine for the 5-HT_{2C} receptors were used. The antagonistic affinity at the 5-HT_{2B} receptors was determined by the inhibition of 5-HT-induced contractions of rat stomach fundus. The results are reported in *table I*. The 5-HT_{2B} receptor affinities were obtained from functional data; however, caution should be exercised when comparing them with data from binding assays.

Derivatives **7**, **9**, **10**, and **13** 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 [24]. 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 added to the superfusion medium from the beginning of the experiment, while the 5-HT_{2C} antagonist mesulergine was unable to counteract it, except at 10 μmol, a high concentration able to block 5-HT_{2A} receptors as well.

4. Results and discussion

The data in *table I* indicate that the 4-(4-fluorobenzoyl)piperidine **2** binds with the same affinity at 5-HT_{2A} and 5-HT_{2C} sites. Moreover, 2-(4-iodo-2,5-dimethoxyphenyl)ethylamine **4** binds with lower affinity to the 5-HT_{2A} than to the 5-HT_{2C} sites (Ki = 300 ± 33 nM vs. 2.5 ± 0.14 nM). Accordingly, in the assays of acetylcholine release from guinea-pig striatal slices, its 5-HT_{2A} agonistic action is negligible (net [^{3}H]choline efflux increase at 30 μM = 0.11 ± 0.03%, n = 5), as is the antagonistic one (5 HT 30 μM-induced net [^{3}H]choline efflux increase in the presence of **4** = 0.55 ± 0.06%, not significantly different from 5-HT 30 μM alone (0.89 ± 0.11%). Functional assays on rat stomach fundus indicate that **4** acts as a full agonist (pD_2 = 6.54 ± 0.12, less potent

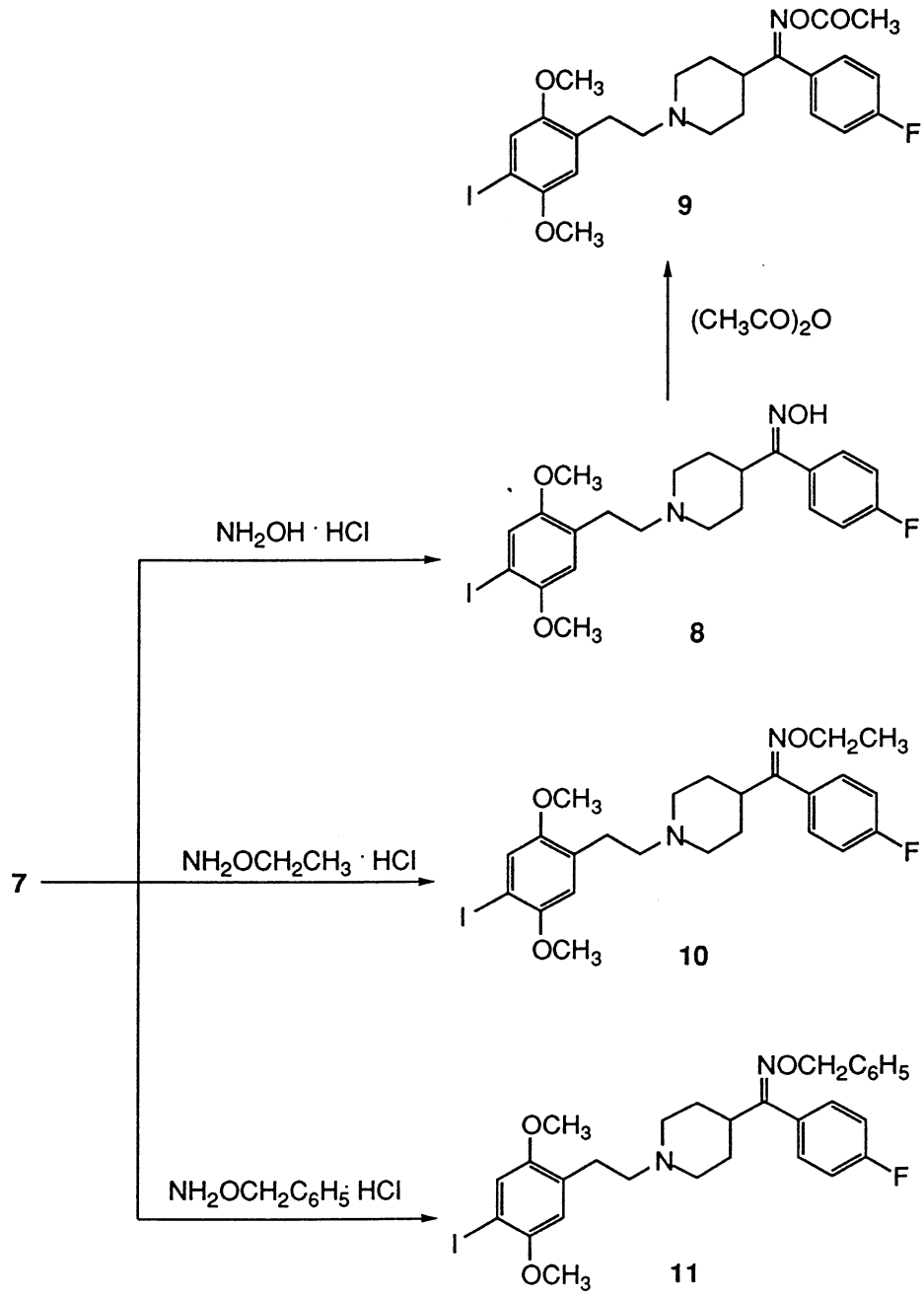

Figure 3. Synthesis of compounds **9–11**.

than 5-HT: pD_2 = 8.20 ± 0.09). Introduction on the piperidine nitrogen of the 2-(4-iodo-2,5-dimethoxyphenyl)ethyl fragment increases the affinity for all 5-HT_2 receptor subtypes, and this effect is more pronounced for 5-HT_{2A} sites (80-fold) than for 5-HT_{2B} and 5-HT_{2C} (13-fold) ones. On the other hand, when the amino group of **4** is substituted by the 4-(4-fluorobenzoyl)piperidine moiety, the 5-HT_{2A} and 5-HT_{2B} affinities increase and the 5-HT_{2C} affinity decreases. Compound **7** binds the 5-HT_{2A} sites with lower affinity than does the reference com-

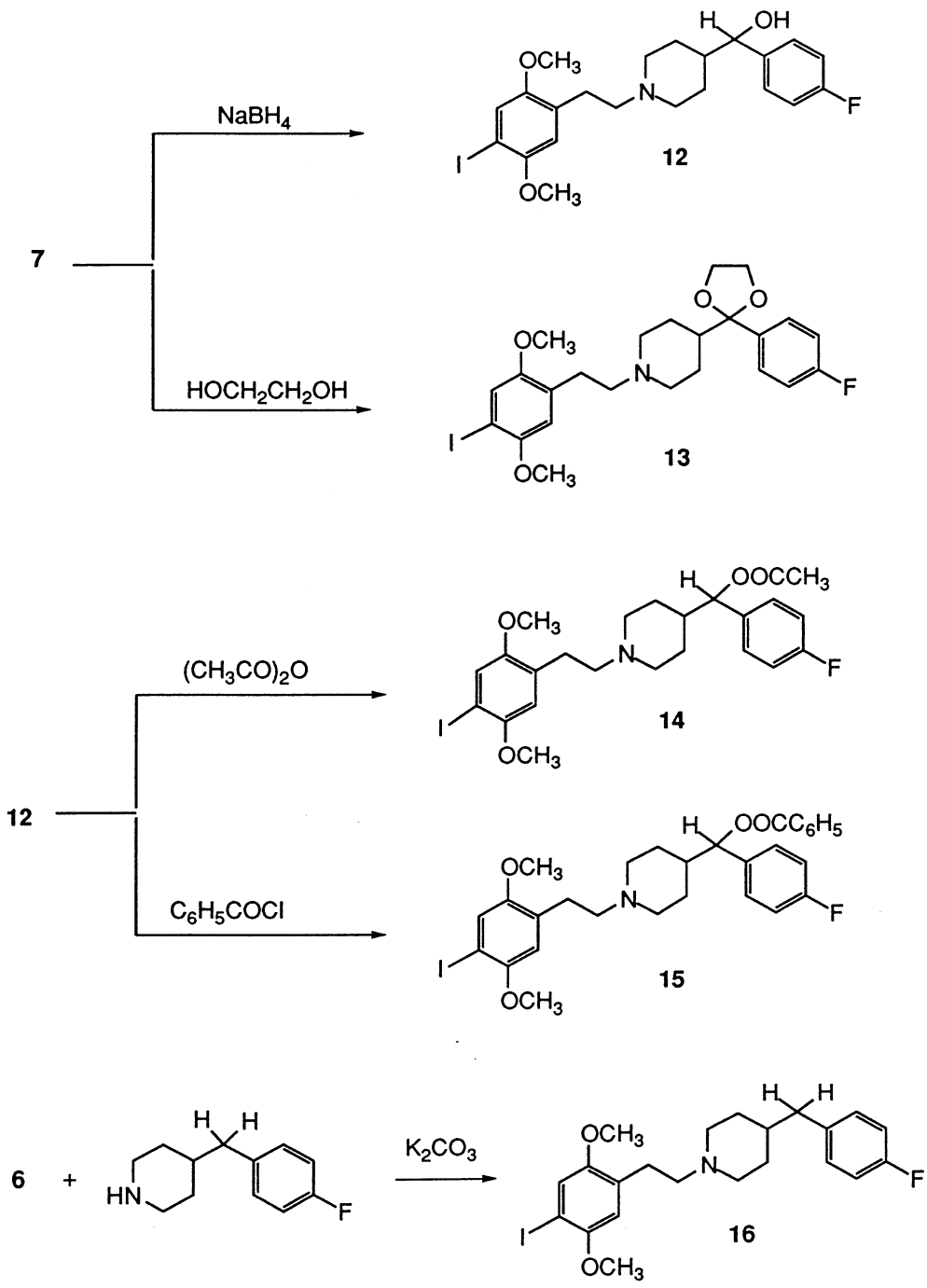

Figure 4. Synthesis of compounds **12–16**.

pound **1** (8.2 nM vs. 0.24 nM) and, under our assay conditions, displays the same selectivity for 5-HT_{2A} versus 5-HT_{2C} receptors (about 7-fold). Compound **7** is also 157-fold more selective for 5-HT_{2A} versus 5-HT_{2B} receptors. These results suggest that the 2-(4-iodo-2,5-dimethoxyphenyl)ethyl fragment enhances to a greater extent the affinity for 5-HT_{2A} than that for 5-HT_{2B} or 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 **1**, **2**, **4**, **7–16**[a].

Compound	K_i (nM)[b]		K_b (nM)[c]
	5-HT_{2A}	5-HT_{2C}	5-HT_{2B}
1	0.24 ± 0.01	1.76 ± 0.05	8 660 ± 810
2	667 ± 44	710 ± 59	> 10 000
4	300 ± 33	2.5 ± 0.14	> 10 000[d]
7	8.2 ± 0.4	54.2 ± 2.1	1 290 ± 60
8	666 ± 44	2 270 ± 176	1 300 ± 150
9	100 ± 6	1 450 ± 87	1 350 ± 170
10	150 ± 6	887 ± 59	1 000 ± 130
11	275 ± 14	598 ± 37	ND[e]
12	616 ± 60	1 220 ± 78	807 ± 115
13	118 ± 14	432 ± 19	1 080 ± 170
14	325 ± 14	687 ± 59	ND
15	275 ± 14	1 660 ± 137	7 140 ± 980
16	238 ± 7	497 ± 20	ND

[a]All values represent means ± SEM; $n \geq 3$ determinations. [b]Binding affinity (rat cortex; 5-HT_{2A} [^{3}H]keranserin, 5-HT_{2C} [^{3}H]mesulergine). [c]Apparent antagonist dissociation constant, rat stomach fundus. The compounds were tested at 10^{-5} M and behave as competitive antagonists. [d]Affinity constant (Kb) determined as reported in [28]. [e]Not determined.

The modification of the carbonyl group in **7** always decreases the 5-HT_{2A} and 5-HT_{2C} affinities and does not affect significantly the 5-HT_{2B} affinity. In the compounds having an sp^2 carbon atom (**8–11**), the carbonyl substitution with the oxime group decreases the 5-HT_{2A} and 5-HT_{2C} affinities. Both acetylation and etherification of the oxime hydroxyl group increases the affinities. These are influenced in an opposite way: the 5-HT_{2A} affinity increases and 5-HT_{2C} affinity decreases following the order **9**, **10**, **11**. This could suggest a different interaction of **9–11** with the 5-HT_{2A} and 5-HT_{2C} sites. It can also be seen from *table I* that the replacement of the carbonyl group with an sp^3 carbon atom (compounds **12**–**16**) decreases the 5-HT_{2A} and 5-HT_{2C} affinities. In the series with an sp^3 carbon atom between the piperidine and the fluorophenyl ring, only the dioxolane **13** presents moderate 5-HT_{2A} affinity. The highest decrease in affinity is observed when the carbonyl group is reduced to alcohol **12**. Of importance is that the 5-HT_{2A} and 5-HT_{2C} affinities decrease to a greater extent in derivatives **8** and **12** bearing a hydroxyl group. The results indicate that the carbonyl oxygen participates in a key binding interaction and that a hydrogen-bond acceptor seems to be required by the 5-HT_{2A} and 5-HT_{2C} receptors. Moreover, it should be observed that also the carbonyl group reduction to methylene (compound **16**) decreases the affinities. With regard to the 5-HT_{2B} receptor, it is evident that the carbonyl group modification does not significantly influence the affinity.

Derivatives **7**, **9**, **10**, and **13** show antagonist activity at central 5-HT_{2A} receptors counteracting the release of acetylcholine induced by 5-HT *(figure 5)*. They behave as competitive antagonists and are less active than the standard **1**. The potency order was **1** > **7** > **9** > **10** > **13**, in good agreement with the binding data. All the antagonists, when tested at the highest concentration, proved to

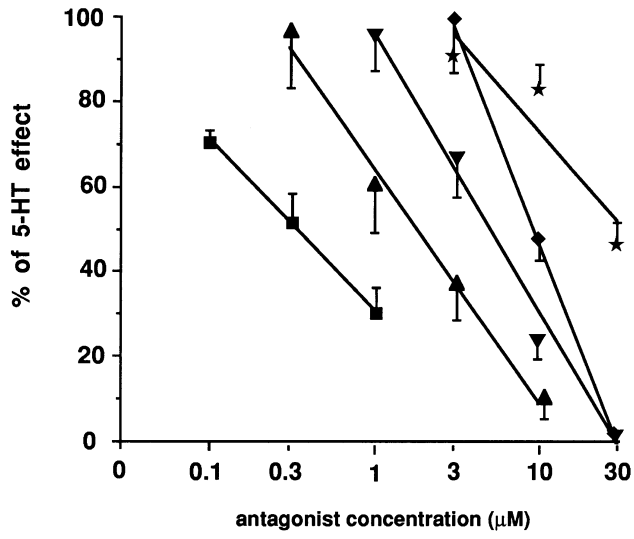

Figure 5. Basal tritium efflux from guinea-pig striatal slices prelabelled with [^{3}H]choline. Relationship between 5-HT_{2A} antagonist concentrations (μmol, abscisses, log scale) and inhibition of the facilitatory effect of 5-HT 30 μmol (%, ordinate). Points represent the means ± SEM of 4–9 experiments. ■ Ketonserin, ▲ **7**, ▼ **9**, ◆ **10**, ★ **13**.

be devoid of intrinsic activity, except **13**, which at 30 μM evoked a net increase in [^{3}H]choline efflux of 0.67 ± 0.11% (n = 3).

In conclusion, compound **7** represents a successful application of the Ariens strategy in the design of a 5-HT_{2A} antagonist by substituting the amino group of 2-(4-iodo-2,5-dimethoxyphenyl)ethylamine with the bulky lipophilic moiety of 4-(4-fluorobenzoyl)piperidine. Comparing **1** with **7**, it is evident that the 3-ethyl-2,4-quinazolinedione moiety gives higher 5-HT_{2A} and 5-HT_{2C} affinities than does the 2-(4-iodo-2,5-dimethoxyphenyl)ethyl fragment.

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 theoretical 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. 2-(4-Iodo-2,5-dimethoxyphenyl)ethanol **5**

A solution of iodine (0.92 g, 3.6 mmol) in chloroform (20 mL) was added dropwise, under stirring at room temperature, to a slurry of silver trifluoroacetate (0.89 g, 3.6 mmol) and 2-(2,5-dimethoxyphenyl)ethanol (0.66 g, 3.6 mmol) in chloroform (5 mL). The mixture was stirred for 30 min. The insoluble material was filtered. The filtrate was washed with aqueous 10% Na_2SO_3, brine and dried (Na_2SO_4). The solvent was evaporated and the residue was crystallized from isopropyl ether, m.p.: 93–94 °C; yield 76%. ^{1}H-NMR ($CDCl_3$) δ 1.59 (1H, bs, OH), 2.88 (2H, t, J = 6.3 Hz, $ArCH_2$), 3.80 (8H, m, OCH_3, CH_2O), 6.68 (1H, s, ArH), 7.21 (1H, s, ArH). Anal. $C_{10}H_{13}IO_3$ (C, H).

5.1.2. 2-(4-Iodo-2,5-dimethoxyphenyl)ethyl-p-toluensulfonate **6**

p-Toluenesulfonyl chloride (0.63 g, 3.3 mmol) was added portionwise to a solution of **5** (0.92 g, 3 mmol) in dry pyridine (1 mL). After stirring for 5 h at room temperature, 2 N HCl (9 mL) was added to yield a precipitate. This was filtered and crystallized from cyclohexane, m.p.: 110–112 °C; yield 82%. ^{1}H-NMR ($CDCl_3$) δ 2.46 (3H, s, CH_3), 2.89 (2H, t, J = 6.3 Hz, $ArCH_2$), 3.63 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.22 (2H, t, J = 6.3 Hz, CH_2O), 6.57 (1H, s, ArH), 7.08 (1H, s, ArH), 7.23 (2H, d, J = 8.5 Hz, ArH), 7.58 (2H, d, J = 8.5 Hz, ArH). Anal. $C_{17}H_{19}IO_5S$ (C, H).

5.1.3. 4-(4-Fluorobenzoyl)-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine hydrochloride **7**

A mixture of **6** (3 g, 6.5 mmol), 4-fluorobenzoylpiperidine (1.35 g, 6.5 mmol) and K_2CO_3 (1.28 g, 9.3 mmol) in acetone (30 mL) was warmed to reflux for 20 h. The solvent was removed by evaporation, and the residue was partitioned between H_2O and $CHCl_3$. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was purified by chromatography eluting with $CHCl_3$:acetone:MeOH (6.7:3:0.3, v/v) and crystallized from isopropyl ether; m.p.: 98–100 °C; yield 84%. ^{1}H-NMR ($CDCl_3$) δ 1.88 (4H, m, $H_{3,5pip}$), 2.21 (2H, m, $H_{2,6ax\text{-}pip}$), 2.58 (2H, m, $ArCH_2$), 2.80 (2H, m, NCH_2), 3.08 (2H, m, $H_{2,6eq\text{-}pip}$), 3.21 (1H, m, H_{4pip}), 3.78 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 6.70 (1H, s, ArH), 7.14 (2H, m, ArH), 7.20 (1H, s, ArH), 7.98 (2H, m, ArH). Hydrochloride: crystallized from absolute EtOH, m.p.: 232–234 °C. Anal. $C_{22}H_{25}FINO_3$.HCl (C, H, N).

5.1.4. (4-Fluorophenyl)-{1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidin-4-yl}methanone oxime hydrochloride **8**

A mixture of **7** (1 g, 2 mmol), hydroxylamine hydrochloride (0.35 g, 5 mmol), and pyridine (2 mL) in absolute EtOH (5 mL) was warmed to reflux for 3 h. After evaporation of the solvent, water was added. The precipitate was filtered and crystallized from absolute EtOH; m.p.: 239–241 °C; yield 92%. ^{1}H-NMR (DMSO-d_6) δ 1.88 (4H, m, $H_{3,5pip}$), 2.23 (1H, m, H_{4pip}), 2.96 (4H, m, $ArCH_2$, $H_{2,6ax\text{-}pip}$), 3.11 (2H, m, NCH_2), 3.55 (2H, m, $H_{2,6eq\text{-}pip}$), 3.75 (6H, s, OCH_3), 6.93 (1H, s, ArH), 7.38 (5H, m, ArH), 10.24 (1H, bs, NH^+), 10.90 e 11.45 (1H, 2s, OH). Anal. $C_{22}H_{26}FIN_2O_3$.HCl (C, H, N).

5.1.5. (4-Fluorophenyl)-{1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidin-4-yl}methanone O-acetyloxime hydrochloride **9**

A mixture of **8** (0.8 g, 1.6 mmol) and acetic anhydride (2 mL) was stirred at 60 °C for 1.5 h. The mixture was cooled and partitioned between 5% aqueous Na_2CO_3 and Et_2O. The organic extracts were washed with H_2O, dried (Na_2SO_4) and evaporated. The residue was crystallized from isopropyl ether; m.p.: 78–80 °C, yield 77%. The residue was dissolved in Et_2O and treated with ethereal HCl. The solid obtained was crystallized from acetone;

m.p.: 168–170 °C. NMR (DMSO-d_6) δ 2.0 (7H, m, $H_{3,5pip}$, CH_3), 3.11 (7H, m, $ArCH_2$, $H_{4pip,}$ $H_{2,6ax\text{-}pip}$), 3.61 (2H, m, $H_{2,6eq\text{-}pip}$), 3.78 (6H, s, OCH_3), 6.96 (1H, m, ArH), 7.42 (5H, m, ArH), 10.35 (1H, bs, NH^+). Anal. $C_{24}H_{28}FIN_2O_4$.HCl (C, H, N).

5.1.6. (4-Fluorophenyl)-{1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidin-4-yl}methanone-O-ethyloxime hydrochloride **10**

This was made in the same way as **8** from **7** and O-ethylhydroxylamine hydrochloride. The precipitate obtained after addition of water was filtered and treated with a saturated solution of Na_2CO_3. The mixture was extracted with Et_2O. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was dissolved in absolute EtOH, and EtOH saturated with HCl gas was added. The solvent was evaporated and the residue was crystallized from 2-propanol; m.p.: 203–205 °C, yield 75%. ^{1}H-NMR ($CDCl_3$) δ 1.1 (3H, t, J = 6.7 Hz, CH_3), 2.0 (2H, m, $H_{3,5ax\text{-}pip}$), 2.39 (2H, m, $H_{3,5eq\text{-}pip}$), 2.68 (3H, m, $H_{2,6ax\text{-}pip}$, H_{4pip}), 3.19 (4H, m, $ArCH_2$, NCH_2), 3.66 (2H, m, $H_{2,6eq\text{-}pip}$), 3.78 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 4.08 (2H, q, J = 6.7 Hz, OCH_2), 6.82 (1H, s, ArH), 7.10 (2H, m, ArH), 7.25 (3H, m, ArH), 12.42 (1H, bs, NH^+). Anal. $C_{24}H_{30}FIN_2O_3$.HCl (C, H, N).

5.1.7. (4-Fluorophenyl)-{1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidin-4-yl}methanone O-benzyloxime hydrochloride **11**

This was made in the same way as **8** from **7** and O-benzylhydroxylamine hydrochloride. The precipitate obtained after addition of water was filtered and treated with a saturated solution of Na_2CO_3. The mixture was extracted with Et_2O. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was dissolved in absolute EtOH, and EtOH saturated with HCl gas was added. The solvent was evaporated and the residue was crystallized from 2-propanol; m.p.: 145–147 °C, yield 71%. ^{1}H-NMR (DMSO-d_6) δ 1.82 (3H, m, $H_{3,5ax\text{-}pip}$, H_{4pip}), 2.94 (6H, m, $ArCH_2$, $H_{2,6ax\text{-}pip}$, $H_{3,5eq\text{-}pip}$), 3.12 (2H, m, NCH_2), 3.45 (2H, m, $H_{2,6eq\text{-}pip}$), 3.76 (6H, s, OCH_3), 5.02 (2H, s, OCH_2), 6,92 (1H, s, ArH), 7.30 (10H, m, ArH), 9.97 (1H, bs, NH^+). Anal. $C_{29}H_{32}FIN_2O_3$.HCl (C, H, N).

5.1.8. (4-Fluorophenyl)-{1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidin-4-yl}methanol fumarate **12**

To a solution of the ketone **7** (0.5 g, 1 mmol) in CH_3OH (12 mL), stirred at room temperature, $NaBH_4$ (0.038 g, 1 mmol) was added. The mixture was stirred for 12 h. The solvent was evaporated and the residue was partitioned between H_2O and CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was purified by chromatography eluting with $CHCl_3$:acetone:MeOH (6.7:3:0.3, v/v) to give an uncrystallizable oil; yield 84%. ^{1}H-NMR ($CDCl_3$) δ 1.43 (4H, m, $H_{3,5pip}$), 2.0 (4H, m, OH, H_{4pip}, $H_{2,6ax\text{-}pip}$), 2.49 (2H, m, $ArCH_2$), 2.74 (2H, m, NCH_2), 3.02 (2H, m, $H_{2,6eq\text{-}pip}$), 3.70 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 4.35 (1H, m, HCO), 6.65 (1H, s, ArH), 7.01 (2H, m, ArH), 7.18 (1H, s, ArH), 7.25 (2H, m, ArH). The oil was dissolved in absolute EtOH and treated with a solution of fumaric acid in absolute EtOH. The fumarate was filtered and crystallized from absolute EtOH, m.p.: 189–191 °C. Anal. $C_{22}H_{27}FINO_3.C_4H_4O_4$ (C, H, N).

5.1.9. 4-[2-(4-Fluorophenyl)-[1,3]dioxolan-2-yl]-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine hydrochloride **13**

A mixture of ketone **7** (0.5 g, 1 mmol) and ethylene glycol (3 mL) was saturated with HCl gas and heated at 90 °C for 1 h. The resulting solid was collected and crystallized from absolute EtOH; m.p.: 263–265 °C, yield 93%. ^{1}H-NMR (DMSO-d_6) δ 1.64 (4H, m, $H_{3,5pip}$), 2.09 (1H, m, H_{4pip}), 2.88 (4H, m, $ArCH_2$, $H_{2,6ax\text{-}pip}$), 3.11 (2H, m, NCH_2), 3.50 (2H, m, $H_{2,6eq\text{-}pip}$), 3.70 (8H, m, OCH_3, OCH_2), 3.98 (2H, m, OCH_2), 6.92 (1H, s, ArH), 7.21 (2H, m, ArH), 7.38 (3H, m, ArH), 9.93 (1H, bs, NH^+). Anal. $C_{24}H_{29}FINO_4$.HCl (C, H, N).

5.1.10. (4-Fluorophenyl)-{1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidin-4-yl}methylacetate fumarate **14**

A mixture of **12** (0.5 g, 1 mmol), dry pyridine (5 mL) and acetic anhydride (0.47 mL, 5 mmol) was stirred at room temperature for 12 h, and then poured into ice. EtOH (2 × 30 mL) was added and the mixture was partially evaporated. The aqueous concentrate was made basic with 2 N NaOH, and the mixture was extracted with Et_2O. The organic extracts were dried (Na_2SO_4) and evaporated to give an uncrystallizable oil; yield 76%. ^{1}H-NMR ($CDCl_3$) δ 1.40 (3H, m, $H_{3,5ax\text{-}pip}$, H_{4pip}), 1.85 (4H, m, $H_{2,6ax\text{-}pip}$, $H_{3,5eq\text{-}pip}$), 2.07 (3H, s, CH_3), 2.52 (2H, m, $ArCH_2$), 2.77 (2H, m, NCH_2), 3.01 (2H, m, $H_{2,6eq\text{-}pip}$), 3.75 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 5.48 (1H, d, J = 8.8 Hz, OCH), 6.68 (1H, s, ArH), 7.0 (2H, m, ArH), 7.18 (1H, s, ArH), 7.25 (2H, m, ArH). The oil was dissolved in absolute EtOH and treated with a solution of fumaric acid in absolute EtOH. The fumarate was filtered and crystallized from absolute EtOH, m.p.: 188–190 °C. Anal. $C_{24}H_{29}FINO_4.C_4H_4O_4$ (C, H, N).

5.1.11. (4-Fluorophenyl)-{1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidin-4-yl}methylbenzoate fumarate **15**

A mixture of **12** (0.5 g, 1 mmol), dry pyridine (5 mL) and acetic anhydride (0.52 mL, 4.5 mmol) was stirred at room temperature for 12 h, and then poured into ice.

EtOH (2 × 30 mL) was added and the mixture was partially evaporated. The aqueous concentrate was made basic with 2 N NaOH and the mixture was extracted with Et_2O. The organic extracts were dried (Na_2SO_4) and evaporated to give an uncrystallizable oil; yield 74%. ^{1}H-NMR ($CDCl_3$) δ 1.51 (3H, m, $H_{3,5ax\text{-}pip}$, H_{4pip}), 2.0 (4H, m, $H_{2,6ax\text{-}pip}$, $H_{3,5eq\text{-}pip}$), 2.57 (2H, m, $ArCH_2$), 2.79 (2H, m, NCH_2), 3.07 (2H, m, $H_{2,6eq\text{-}pip}$), 3.75 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 5.78 (1H, d, *J* = 8.1 Hz, OCH), 6.70 (1H, s, ArH), 7.04 (2H, m, ArH), 7.19 (1H, s, ArH), 7.42 (5H, m, ArH), 8.08 (2H, m, ArH). The oil was dissolved in absolute EtOH and treated with a solution of fumaric acid in absolute EtOH. The fumarate was filtered and crystallized from absolute EtOH, m.p.: 181–183 °C. Anal. $C_{29}H_{31}FINO_4.C_4H_4O_4$ (C, H, N).

5.1.12. 4-(4-Fluorobenzyl)-1-[2-(4-iodo-2,5-dimethoxyphenyl)ethyl]piperidine hydrochloride **16**

A mixture of **6** (0.46 g, 1 mmol), 4-fluorobenzoylpiperidine (0.19 g, 1 mmol) and K_2CO_3 (0.2 g, 1.5 mmol) in acetone (10 mL) was warmed to reflux for 20 h. The precipitate was filtered and the solution was evaporated. The residue was partitioned between H_2O and $CHCl_3$. The organic extracts were dried (Na_2SO_4) and evaporated. The residue was purified by chromatography eluting with $CHCl_3$:acetone:MeOH (6.7:3:0.3, v/v) and crystallized from acetone; m.p.: 96–98 °C; yield 66%. ^{1}H-NMR ($CDCl_3$) δ 1.47 (5H, m, $H_{3,5pip,}$ H_{4pip}), 1.97 (2H, m, $H_{2,6ax\text{-}pip}$), 2.50 (4H, m, $H_{2,6eq\text{-}pip,}$ $ArCH_2$), 2.75 (2H, m, $ArCH_2$), 2.98 (2H, m, NCH_2), 3.77 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 6.70 (1H, s, ArH), 6.98 (2H, m, ArH), 7.10 (2H, m, ArH), 7.20 (1H, s, ArH). The purified residue was dissolved in Et_2O and treated with ethereal HCl. The solid obtained was crystallized from absolute EtOH; m.p.: 204–205 °C. Anal. $C_{22}H_{27}FINO_2$.HCl (C, H, N).

5.2. Pharmacology

5.2.1. Materials

Ketanserin tartrate was purchased from Research Biochemicals International (Natick, MA, USA). [^{3}H]Ketanserin (64.1 Ci/mmol) was purchased from New England Nuclear, Boston, Mass., 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 was obtained from male Wistar rats (250–300 g body weight) purchased 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 homogenate was centrifuged at 40 000 *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 [23] 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 fibre 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 [25] $K_i = IC_{50}/1 + (ligand/K_d)$, where K_d = 0.8 nmol/L for [^{3}H]ketanserin and K_d = 1.9 nmol/L for [^{3}H]mesulergine [26].

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

Experiments were performed as described by Nozulak et al. [27]. 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 (composition 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 1 h. 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 deter-

mined 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 [24]. Caudate nucleus slices (400 μm thick) were incubated with 0.1 μmol [^{3}H]choline (81 Ci/mmol) 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; $NaHCO_3$ 25, glucose, 11; hemicholinium-3, 0.01), bubbled with 95% O_2 and 5% CO_2. 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. The net increase of [^{3}H]choline efflux, induced by 5-HT 30 μM, added to the Krebs solution from the 45th min of superfusion, was 0.89 ± 0.11% (n = 11).

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