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Short communication

3-[2-[4-(4-Fluorobenzoyl)piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-4(3*H*)-quinazolinones: serotonin 5-HT_{2A} receptor antagonists endowed with potent central action

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Summary — A series of 5,6,7,8-tetrahydro-4(3*H*)-quinazolinones substituted at the 3-position with 4-benzoyl-1-ethylpiperidine, 4-(4-fluorobenzoyl)-1-ethylpiperidine, 4-[bis-(4-fluorophenyl)methylene]-1-ethylpiperidine, or 4-(4-fluorophenyl)-1-propylpiperazine have been prepared and evaluated in binding assays to determine their affinity at serotonin 5-HT_{2A} receptors as well as in a functional test, ie, wet dog shakes (WDS) induced by L-5-hydroxytryptophan (L-5-HTP), a behavioural response which is mediated by stimulation of 5-HT_{2A} receptors. Among the compounds prepared, 3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-4(3*H*)-quinazolinone (**10a**) and 2-methyl-3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-4(3*H*)-quinazolinone (**10b**) proved to be the most potent 5-HT_{2A} receptor antagonists. In binding assays, the two compounds displayed similar affinity for 5-HT_{2A} receptors in the nanomolar range to ketanserin and ritanserin. In the WDS test, they were even more potent than ketanserin and ritanserin. Compound **10b**, which was found to possess the highest potency and duration of action in the WDS test, was chosen for a preliminary evaluation of its ability to inhibit ethanol intake in rats, a response linked to blockade of the central 5-HT_{2A} receptors. This compound significantly reduced ethanol intake in rats from the first day of treatment. The results of the present study indicate that **10b** is a potent centrally acting antagonist at 5-HT_{2A} receptors.

tetrahydro-4(3*H*)-quinazolinone / synthesis / 5-HT_{2A} receptor affinity / 5-HT_{2A} antagonist

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been implicated in a variety of physiological and pathophysiological processes (cardiovascular regulation, memory, thermoregulation, sleep, feeding, depression, anxiety, drug abuse, migraine). At present, 5-HT receptors are subdivided into seven main classes (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, 5-HT_7 receptors). The 5-HT_2 receptors are further subdivided into 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} (formerly 5-HT_{1C}) [1].

Interest in the 5-HT_2 receptor family is related to involvement of these receptors in cardiovascular regulation [2], and in various mental disorders such as schizophrenia, hallucinations, depression, dysthymic disorders and anxiety [3, 4]. The first potent 5-HT_2 receptor antagonist discovered was 3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4(1*H*,3*H*)-quinazolinedione [ketanserin (**1**)] [5, 6] (fig 1). This compound displays high affinity for 5-HT_{2A} receptors, but also high affinity for α_1 adrenoreceptors. Blockade of α_1 adrenoreceptors appears to be mainly responsible for its antihypertensive effect [7]. Ketanserin displays lower but appreciable affinity for 5-HT_{2C}, dopaminergic and histaminergic binding sites [5]. Ketanserin does not readily cross the blood–brain barrier, thus reaching low concentrations in the brain of experimental animals [8].

Later, 6-[2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl]-7-methyl-5*H*-thiazole[3,2-*a*]pyrimidin-5-one [ritanserin (**2**)] was shown to be a potent and long-lasting 5-HT_2 receptor antagonist [9] which easily crosses the blood–brain barrier. The drug was found to reduce anxiety [10], depression and dysthymia [11]. Ritanserin does not discriminate between the three 5-HT_2 receptor subtypes.

More recently, other antagonists have been discovered, such as 2-[3-[4-(4-fluoro-phenyl)-1-piperazinyl]propyl]-2*H*-naphth[1,8-*cd*]isothiazole-1,1-dioxide [RP 62203, (**3**)] [12], which shows higher affinity for 5-HT_{2A} than 5-HT_{2C} receptors.

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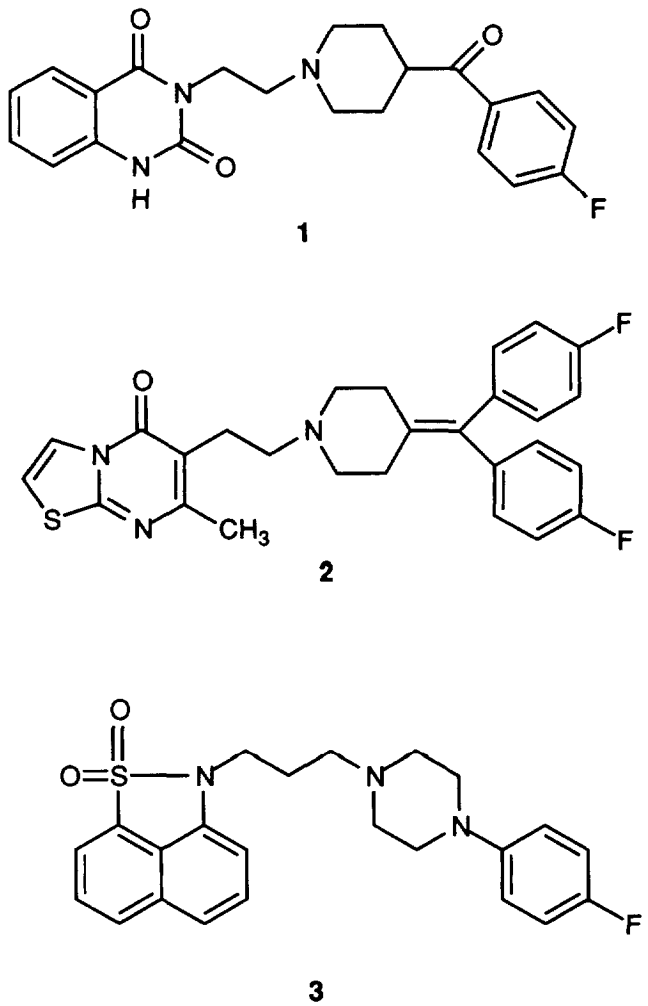


Fig 1. Structure of compounds **1–3**.

In the last few years, several studies have reported that the 5-HT_2 receptor antagonist ritanserin reduces ethanol intake in genetically heterogeneous rats [13–15]. Attenuation of ethanol intake has also been reported following risperidone treatment at doses at which the drug behaves as a selective 5-HT_2 receptor antagonist [16]. Both antagonists reduce ethanol intake by up to 50% after 3–5 days of subchronic treatment [16]. Recent experiments by one of our group suggest that reduction of ethanol intake might be due to antagonism at 5-HT_{2A} receptors (Ciccocioppo; pers commun).

Other studies, however, have reported no reduction in alcohol intake following ritanserin administration. In genetically heterogeneous rats the effect of the drug was not observed following acute or 3 days' treatment [17, 18]. Moreover, ritanserin did not reduce alcohol intake in genetically selected alcohol-preferring rats [19].

These findings indicate that 5-HT_2 receptor antagonists might be interesting pharmacological tools in the treatment of alcoholism and stimulate the investigation of new 5-HT_{2A} antagonists endowed with more prompt, efficacious and consistent inhibitory action on alcohol consumption.

The compounds **1–3** consist of two structural elements. One is the 4-(4-fluorobenzoyl)-1-ethylpiperidine, the 4-[bis(4-fluorophenyl)methylene]-1-ethylpiperidine or 4-(4-fluorophenyl)-1-propylpiperazine, which appears to be crucial for interaction with 5-HT_2 receptors. The other is the 2,4(1*H*,3*H*)-quinazolinedione, the 5*H*-thiazole[3,2-*a*]pyrimidin-5-one or the 2*H*-naphth[1,8-*cd*]isothiazole-1,1-dioxide, which appears to contribute to the affinity for 5-HT_2 receptors. Studies on ketanserin analogues showed that the 4-(4-fluorobenzoyl)-1-piperidine confers high 5-HT_2 receptor affinity, and also the 2,4(1*H*,3*H*)-quinazolinedione moiety contributes to the binding affinity [20].

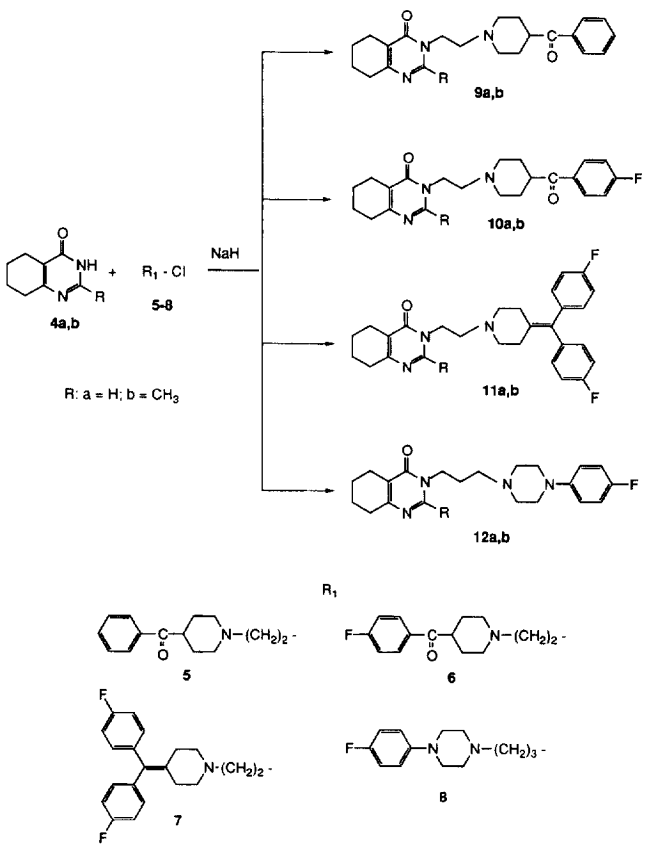
The aim of the present investigation was to find new centrally acting antagonists at 5-HT_{2A} receptors, which could be useful for the treatment of alcohol abuse and mental disorders. For this purpose some analogues of **1–3**, in which the 5,6,7,8-tetrahydro-4(3*H*)-quinazolinone nucleus replaces the quinazolinedione, the thiazolepyrimidinone or the naphthoisothiazoledioxide moieties, were synthesized. The 5,6,7,8-tetrahydro-4(3*H*)-quinazolinone nucleus was selected in order to obtain lipophilic compounds, which might therefore readily cross the blood–brain barrier.

Chemistry

The target compounds **9a,b–12a,b** were synthesized as outlined in scheme 1 by alkylation of 5,6,7,8-tetrahydro-4(3*H*)-quinazolinones **4a** [21] and **4b** [22] with the 2-chloroethylpiperidines **5–7** or 3-chloropropylpiperazine **8** in presence of sodium hydride in dimethylformamide. Compound **4b** is described in the literature [22] as 2-methyl-5,6,7,8-tetrahydroquinazolin-4-ol, but the IR spectrum indicates that it exists as 2-methyl-5,6,7,8-tetrahydro-4(3*H*)-quinazolinone. Condensation of 4-benzoylpiperidine with 2-iodoethanol gave the intermediate 4-benzoyl-1-(2-hydroxyethyl)piperidine, which was converted to the 4-benzoyl-1-(2-chloroethyl)piperidine **5** by treatment with thionyl chloride. The chloroalkylamines **6–8** were prepared in the same manner.

Pharmacology

The compounds **9a,b–12a,b** were first evaluated in in vitro binding tests for their affinity at 5-HT_{2A} receptors



Scheme 1.

using homogenized rat cerebral cortex and [^{3}H]ketanserin as radioligand. This is a valuable radioligand for 5-HT_{2A} receptors since ketanserin displays a pA_2 value for 5-HT_{2A} in the nanomolar range, but a pA_2 value for 5-HT_{2C} receptors in the micromolar range; in addition, ketanserin shows very low affinity for 5-HT_{2B} receptors [23]. In the present study prazosin was added to the incubation medium to minimize binding of [^{3}H]ketanserin to α_1-adrenoreceptors.

The new compounds were also evaluated in an in vivo functional test, wet dog shakes (WDS) induced by L-5-hydroxytryptophan (L-5-HTP). Given by subcutaneous administration together with the peripheral decarboxylase inhibitor carbidopa, L-5-HTP generates 5-HT in the central nervous system, thus inducing a strong WDS response. A large body of evidence indicates that WDS and head shakes are induced by stimulation of central 5-HT_2 receptors [24, 25] and several findings suggest that the 5-HT_{2A} receptor subtype may mediate them. In fact, in addition to L-5-HTP, WDS and head shakes are also evoked by (±)-DOI, a 5-$HT_{2A/2C}$ receptor agonist; however, rather selective 5-HT_{2C} receptor agonists, such as 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and 1-(3-chlorophenyl)piperazine (mCPP), do not induce WDS or head shakes in rats [26, 27], but hypolocomotion. Accordingly, a recent paper showed that the ability of several compounds to suppress head shakes induced by (±)-DOI is related to their affinity for 5-HT_{2A}, but not for 5-HT_{2C} receptors [28]. Moreover, WDS induced by L-5-HTP are abolished by the rather selective 5-HT_{2A} receptor antagonist ketanserin and by ritanserin, which although non-selective among 5-HT_2 receptor subtypes shows high affinity for 5-HT_{2A} receptors [24, 29]. So far no evidence has been provided for the role of 5-HT_{2B} receptors in 5-HT_2 receptor-mediated behaviour.

Compound **10b**, which showed the highest pK_i value as well as the highest potency and duration of action in the WDS test, was chosen for a preliminary evaluation of its ability to inhibit ethanol intake in genetically heterogeneous rats.

Results and discussion

The receptor binding affinities of the tested compounds are reported in table I. The affinity at 5-HT_{2A} receptors decreased in the order **10b** > **10a** > **11b**; **12b**; **11a** > **9a**; **9b**; **12a**. Compounds **10a** and **10b** showed pK_i values similar to that of ketanserin and ritanserin. For all compounds, inhibition of tracer labelling was completed over a concentration range of two order of magnitude. The Hill coefficient was

Table I. 5-HT_{2A} receptor binding affinity.

Compound	pK_i
Ritanserin	8.83 ± 0.06
Ketanserin	8.43 ± 0.17
9a	7.50 ± 0.22
9b	7.22 ± 0.37
10a	8.12 ± 0.15
10b	8.58 ± 0.29
11a	7.65 ± 0.28
11b	7.72 ± 0.35
12a	7.16 ± 0.15
12b	7.66 ± 0.24

Data represent the mean ± SEM of three independent determinations using [^{3}H]ketanserin as radioligand. pK_i is the –log of the K_i calculated according to the formula: $K_i = IC_{50}/1 + [L]/K_d$.

0.81 ± 0.09 for **10a** and 1.0 ± 0.14 for **10b**, thus indicating an independent binding. The other compounds showed lower but still evident affinity for 5-HT_{2A} receptors.

The results of the WDS test are reported in table II. Ketanserin and ritanserin given 1 h before the 10-min observation period reduced the number of WDS at doses of 0.1 and 1 mg/kg. Among our compounds, the analysis of variance revealed no significant treatment effect for **9a**, **9b**, and **12a** at doses up to 1 mg/kg (data not shown). On the other hand, **11a**, **11b**, and **12b** significantly reduced the number of WDS, but only at the dose of 1 mg/kg. Finally, **10a** and **10b** potently inhibited WDS induced by L-5-HTP. The analysis of variance for **10b** revealed a highly significant treatment effect [F (4,29) = 9.38; $P < 0.001$]. Pairwise comparisons showed a significant effect even at the dose of 0.01 mg/kg. Also for **10a** the analysis of variance revealed a highly significant treatment effect [F (4,34) = 12.55; $P < 0.001$], which was statistically significant at 0.03 mg/kg or higher doses. The antagonist activity decreased in the order **10b** > **10a** > **11a**; **11b**; **12b** > **9a**; **9b**; **12a**. Compounds **10a** and **10b** proved to be the most potent antagonists, being even more potent than ritanserin and ketanserin in the WDS test.

Table II. Inhibition of wet dog shake (WDS) responses induced by L-5-HTP.

Compound	*Dose (mg/kg)*	*No WDS*	*P*
Ketanserin	0	29.4 ± 3.8	
	0.01	22.8 ± 6.4	ns
	0.03	20.1 ± 5.9	ns
	0.1	4.8 ± 1.8	< 0.01
	1	3.7 ± 1.5	< 0.01
Ritanserin	0	35.1 ± 10.6	
	0.01	24.5 ± 11.0	ns
	0.03	22.8 ± 10.6	ns
	0.1	14.0 ± 7.3	< 0.05
	1	0.5 ± 0.3	< 0.01
10a	0	36.1 ± 7.5	
	0.01	24.7 ± 6.9	ns
	0.03	15.4 ± 3.9	< 0.01
	0.1	8.0 ± 2.5	< 0.01
	1	0.1 ± 0.1	< 0.01
10b	0	33.6 ± 7.1	
	0.01	14.3 ± 4.6	< 0.01
	0.03	12.0 ± 3.5	< 0.01
	0.1	6.3 ± 1.1	< 0.01
	1	0.1 ± 0.1	< 0.01
11a	0	36.0 ± 6.7	
	0.01	27.4 ± 3.8	ns
	0.03	28.2 ± 6.7	ns
	0.1	23.6 ± 3.9	ns
	1	9.2 ± 3.0	< 0.05
11b	0	29.2 ± 2.8	
	0.01	24.6 ± 1.9	ns
	0.03	20.2 ± 3.5	ns
	0.1	18.8 ± 3.6	ns
	1	7.0 ± 2.4	< 0.01
12b	0	28.0 ± 3.1	
	0.01	25.6 ± 3.9	ns
	0.03	23.2 ± 8.4	ns
	0.1	18.8 ± 4.9	ns
	1	8.2 ± 1.2	< 0.05

Mean number of wet dog shakes (WDS) in 10-min observation period. Each value is the mean ± SEM of 5–9 data.

To evaluate the time course of their effect on WDS, ritanserin, ketanserin, **10a** and **10b** were administered sc at a dose of 1 mg/kg, 1, 2, 3, 4, 5 or 6 h before the 10-min observation period. Ritanserin (fig 2) produced a very pronounced and statistically significant suppression of WDS at the different administration times (from 1 to 6 h before the observation period). These findings are in keeping with other reports that have shown a long-lasting effect of this compound [30]. Ketanserin (fig 2) and **10b** (fig 3) on the other hand, evoked a significant effect when administered up to 5 h before the observation period. The effect of **10a** was statistically significant up to 4 h before the observation period (fig 3).

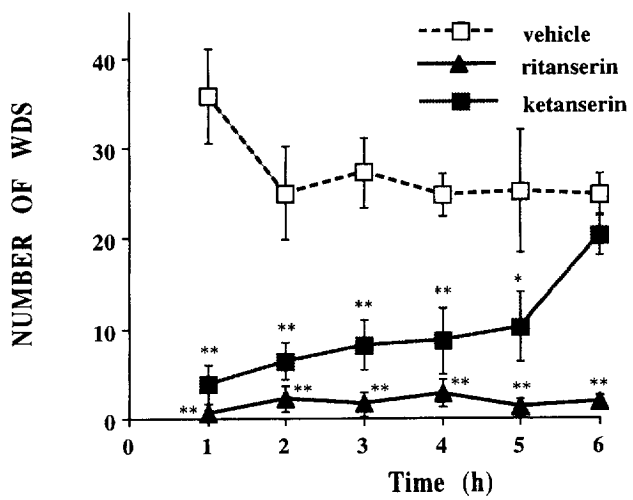


Fig 2. Time course of the effect of ritanserin and ketanserin on wet dog shake (WDS) responses elicited in rats by L-5-HTP plus carbidopa. Values are means ± SEM of 5–6 data. Difference from controls: $^{**}P < 0.01$; $^{*}P < 0.05$. Where not indicated, difference from controls was not statistically significant. For details, see *Experimental protocols*.

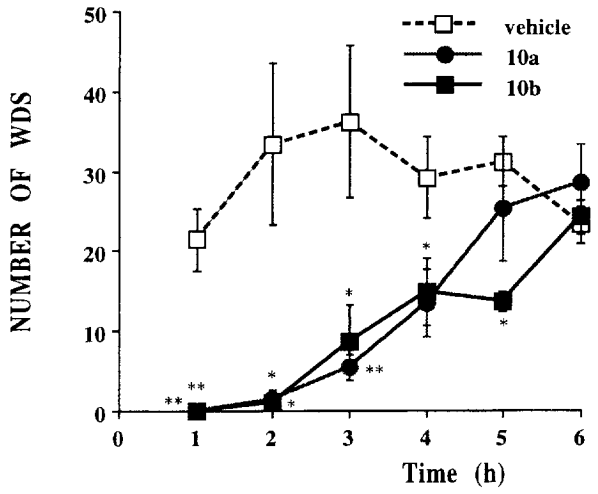


Fig 3. Time course of the effect of **10a** and **10b** on wet dog shake (WDS) responses elicited in rats by L-5-HTP plus carbidopa. Values are means ± SEM of 5–6 data. Difference from controls: $^{**}P < 0.01$; $^{*}P < 0.05$. Where not indicated, difference from controls was not statistically significant. For details, see *Experimental protocols*.

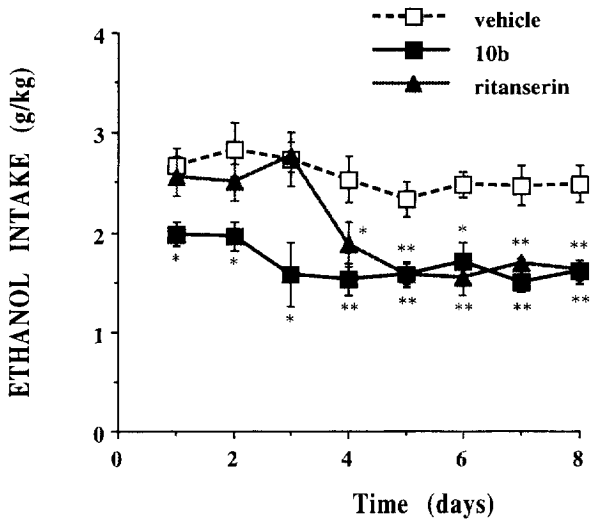


Fig 4. Cumulative 12-h ethanol intake in rats treated for 8 days with two subcutaneous injections/day of ritanserin or **10b**, 1 mg/kg. Values are means ± SEM of 5–6 data. Difference from controls: $^{**}P < 0.01$; $^{*}P < 0.05$. Where not indicated, difference from controls was not statistically significant. For details, see *Experimental protocols*.

The results concerning ethanol intake are reported in figure 4. The analysis of variance for ritanserin data showed a significant treatment effect [F (1,10) = 8.07; $P < 0.05$], and a time effect [F (7,70) = 25.43; $P < 0.001$]. Pairwise comparisons showed that the effect of ritanserin became statistically significant from the 4th day of treatment. The analysis of variance revealed also a significant treatment effect for **10b** [F (1,10) = 17.35; $P < 0.01$], as well as a significant time effect [F (7,70) = 3.12; $P < 0.01$]. Pairwise comparisons showed that the effect of **10b** was statistically significant from the 1st day of treatment. When data obtained following **10b** and ritanserin treatment were compared, the analysis revealed a significant difference among treatments [F (1,10) = 11.91; $P < 0.01$]. Pairwise comparisons showed a significant difference in the first three days of treatment. Both drugs did not modify either total fluid intake or food intake.

These results suggest some remarks concerning the structure–activity relationships. All compounds tested share the 5,6,7,8-tetrahydro-4(3*H*)-quinazolinone fragment, but differ in the piperidine or piperazine moiety. Derivatives **10a** and **10b** bearing the 4-(4-fluorobenzoyl)-1-ethylpiperidine moiety of ketanserin bind at 5-HT_{2A} receptors with the highest affinity. Other compounds, bearing the 4-benzoyl-1-ethylpiperidine, the 4-[bis(4-fluorophenyl)methylene]-1-ethylpiperidine or the 4-(4-fluorophenyl)-1-propylpiperazine have slightly less affinity. Thus it is evident that the 5-HT_{2A} receptor affinity is greatly determined by the piperidine or piperazine fragment. Andersen et al [31] developed a topographic model of the 5-HT_{2A} receptor on the basis of conformational analysis of some 5-HT_{2A} antagonists. The model may be visualized by the ritanserin pharmacophore 4-[bis(4-fluorophenyl)methylene]piperidine, and is described by the distance between two phenyl rings and by the distances of the phenyl rings from a point that interacts with piperidine nitrogen by a hydrogen bond. This model could also accommodate the 4-(4-fluorobenzoyl)piperidine moiety of ketanserin, but Ismaiel et al [32] suggested that 4-(4-fluorobenzoyl)piperidine binds with a different auxiliary binding site interacting with the aromatic ring. If so, it is possible that also the phenyl ring of 4-(4-fluorophenyl)piperazine binds at the same aromatic auxiliary site, and this may explain the 5-HT_{2A} affinity of compounds **12a** and **12b**. This is in agreement with the hypothesis that there are different modes of binding for the 5-HT_{2A} antagonists [32].

In a study on ketanserin analogues, Glennon et al [3] suggested that on the 5-HT_{2A} receptor there is a binding site that accommodates the fused phenyl ring of qiunazolinedione. Comparing ketanserin with **10a,b** it appears that the replacement of quinazolinedione with tetrahydroquinazolinone has little effect on

5-HT_{2A} receptor affinity. This observation indicates that the phenyl ring is not critical for binding and this is in agreement with the results obtained later by the same research group [32]. On the other hand, comparison of ritanserin with **11a** and **11b** shows that the replacement of thiazolepyrimidinone with tetrahydroquinazolinone decreases the affinity by about 10-fold. In this case, the structure of the bicyclic system seems to affect 5-HT_{2A} receptor affinity, as also observed in other ritanserin and ketanserin analogues [33].

In **12a** and **12b** the tetrahydroquinazolinone replaces the naphthoisothiazoledioxide moiety of RP 62203, and these compounds bind with an affinity similar to that of derivatives **11a** and **11b**. Likely a lipophilic hollow on the receptor with no stringent requirements can accommodate the 2,4-quinazolinedione, the thiazolepyrimidin-5-one, the naphthisothiazole-1,1-dioxide moieties of compounds **1–3** or the 5,6,7,8-tetrahydro-4-quinazolinone nucleus of our compounds.

Comparing the affinities of **9a** and **9b** with those of their fluorine derivatives **10a** and **10b**, it appears that the presence of a fluorine on the benzoyl moiety increases the binding affinity and that this effect is prominent in **10b**. The introduction of a methyl group at position 2 of the quinazolinone moiety has non-parallel consequences on the affinity that remains unchanged or is increased in the 4-fluorophenyl derivatives, whereas it is decreased in the nonfluorinated derivatives.

On the other hand, in the in vivo assays (WDS test) compounds **10a** and **10b** containing the same 4-(4-fluorobenzoyl)-1-ethylpiperidine moiety as ketanserin show higher antagonist activity at central 5-HT_{2A} receptors than ketanserin. Since binding tests have shown a similar affinity of **10a** and **10b** and ketanserin for the 5-HT_{2A} receptor, this finding from in vivo tests suggests that replacement of the 2,4-quinazolinedione of ketanserin with 5,6,7,8-tetrahydro-4-quinazolinone may increase the ability to cross the blood–brain barrier, and therefore the ability to influence the central serotonergic mechanism.

Moreover, the in vivo results indicate that in our derivatives the 4-(4-fluorobenzoyl)-1-ethylpiperidine moiety induces higher 5-HT_{2A} receptor antagonist activity than the 4-[bis(4-fluorophenyl)methylene]-1-ethylpiperidine moiety of ritanserin, or the 4-(4-fluorophenyl)-1-propylpiperazine portion of RP 62203.

A comparison between **10a**, **10b** and **9a**, **9b** suggests that the fluorine on the benzoyl moiety is a determining factor in high central 5-HT_{2A} antagonist activity.

On the other hand, the data in table I show that the 4-[bis(4-fluorophenyl)methylene]-1-ethylpiperidine derivatives **11a** and **11b** have a lower affinity than ritanserin for 5-HT_{2A} receptors. Moreover, table II shows that the same compounds significantly reduce the number of WDS only at a dose of 1 mg/kg, while ritanserin is active at 0.1 mg/kg. These findings suggest that 7-methyl-5*H*-thiazole[3,2-*a*]pyrimidin-5-one induces higher 5-HT_{2A} receptor antagonist activity than 2-methyl-5,6,7,8-tetrahydro-4-quinazolinone in the presence of the same piperidine moiety.

In conclusion, by substituting the 2,4-quinazolinedione moiety of ketanserin with the 5,6,7,8-tetrahydro-4-quinazolinone moiety the derivatives **10a** and **10b** were obtained, which show high affinity at 5-HT_{2A} receptors in binding tests and prove to be potent centrally acting 5-HT_{2A} receptor antagonists in in vivo tests. Moreover, compound **10b** shows an interesting inhibitory effect on alcohol consumption in rats.

Experimental protocols

Chemistry

Melting points were determined on a Büchi 510 apparatus and are uncorrected. Mycroanalyses 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 IR spectra were run on a Perkin–Elmer Model 297 spectrometer as nujol mulls or liquid films. The identity of all new compounds was confirmed by both elemental analysis and NMR data; homogeneity was confirmed by TLC on silica gel, Merck 60 F_{254}. Solutions were routinely dried over anhydrous Na_2SO_4 prior to evaporation. Chromatographic purification was carried out on Merck-60 silica gel columns, 70–230 mesh ASTM with a reported solvent.

5,6,7,8-Tetrahydro-4(3H)-quinazolinone ***4a***
This compound was prepared as reported in the literature [21]. IR (nujol) 1630, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 13.25 (bs, 1H, NH), 8.04 (s, 1H, H2), 2.69 (m, 2H, H5), 2.53 (m, 2H, H8), 1.8 (m, 4H, H6,7).

2-Methyl-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***4b***
This compound was prepared as reported in the literature [22]. IR ν cm^{-1}: 1645, 1600. ^{1}H-NMR ($CDCl_3$) δ 12.90 (bs, 1H, NH), 2.65 (m, 2H, H5), 2.52 (m, 5H, H8, CH_3), 1.78 (m, 4H, H6,7).

4-Benzoyl-1-(2-chloroethyl)piperidine ***5***
A mixture of 4-benzoylpiperidine (1.89 g, 10 mmol), potassium carbonate anhydrous (1.66 g, 12 mmol) and 2-iodoethanol (0.94 mL, 12 mmol) in acetonitrile (30 mL) was refluxed for 16 h. The reaction mixture was then evaporated and water added. The aqueous mixture was extracted with $CHCl_3$. The extracts were dried and evaporated to afford an oily residue which after trituration with petroleum ether, gave 4-benzoyl-1-(2-hydroxyethyl)-piperidine as a solid (yield 92%, mp = 47–49 °C). ^{1}H-NMR ($CDCl_3$) δ 7.93 (m, 2H, H_{arom}), 7.51 (m, 3H, H_{arom}), 3.66 (t, 2H, CH_2O), 3.30 (m, 2H, OH, H_{pip}-4), 3.04 (m, 2H, $H_{eq\text{-}pip}$-2,6), 2.62 (t, 2H, CH_2N), 2.37 (m, 2H, $H_{ax\text{-}pip}$-2,6), 1.91 (m, 4H, H_{pip}-3,5). This compound (8.97 g, 38 mmol) was dissolved in $CHCl_3$ (90 mL) and thionyl chlo-

ride (6.4 mL, 87 mmol) was added. The mixture was stirred at room temperature for 16 h. After evaporation of the solvent, water was added. Then the solution was extracted with Et_2O and basified with 2 N NaOH; the precipitate formed was collected by filtration and recrystallized from *n*-hexane; yield 89%, mp = 67–69 °C. ^{1}H-NMR ($CDCl_3$) δ 7.92 (m, 2H, H_{arom}), 7.50 (m, 3H, H_{arom}), 3.60 (t, 2H, CH_2Cl), 3.25 (m, 1H, H_{pip}-4), 3.0 (m, 2H, $H_{eq\text{-}pip}$-2,6), 2.75 (t, 2H, CH_2N), 2.28 (m, 2H, $H_{ax\text{-}pip}$-2,6), 1.85 (m, 4H, H_{pip}-3,5). Anal ($C_{14}H_{18}ClNO$) C, H, N.

General procedure for the preparation of the 5,6,7,8-tetrahydro-4(3H)-quinazolinones ***9a,b–12a,b***

To a solution of the quinazolinones **4a** or **4b** (0.01 mol) in anhydrous DMF (20 mL) sodium hydride (0.64 g, 0.016 mol) was added. The suspension was stirred at room temperature for 3 h. Then a solution of the chloroethylpiperidines **5–7** or chloropropylpiperazine **8** (0.01 mol) in DMF (10 mL) was added, and the mixture stirred at room temperarure for 24 h (compounds **9a,b** and **10a,b**) or at 90 °C for 6 h (compounds **11a,b** and **12a,b**). Then the solvent was evaporated in vacuo and water was added. The aqueous mixture was extracted with $CHCl_3$. The extracts were dried and evaporated to afford an oily residue, which was treated as described for each compound. The maleate salts were prepared by adding a saturated ethereal solution of maleic acid to the base dissolved in absolute EtOH. The salts were filtered and recrystallized.

3-[2-(4-Benzoylpiperidin-1-yl)ethyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***9a***. The oily residue was recrystallized from EtOAc/cyclohexane, mp = 121–123 °C; yield 56%. IR ν cm^{-1}: 1662, 1640. ^{1}H-NMR ($CDCl_3$) δ 7.91 (m, 3H, H2, H_{arom}), 7.50 (m, 3H, H_{arom}), 3.98 (t, 2H, CH_2NCO), 3.24 (m, 1H, H_{pip}-4), 2.97 (m, 2H, $H_{eq\text{-}pip}$-2,6), 2.68 (t, 2H, CH_2N_{pip}), 2.56 (m, 4H, H5,8), 2.23 (m, 2H, $H_{ax\text{-}pip}$-2,6), 1.82 (m, 8H, H_{pip}-3,5, H6,7). Maleate: mp = 183–185 °C (from EtOH). Anal ($C_{26}H_{31}N_3O_6$) C, H, N.

2-Methyl-3-[2-(4-benzoylpiperidin-1-yl)ethyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***9b***. The residue was purified by flash chromatography over a silica gel column eluted with EtOAc/MeOH, 95:5. The resulting material was recrystallized from isopropylacetate, mp = 144–146 °C, yield 52%. IR ν cm^{-1}: 1670, 1630. ^{1}H-NMR ($CDCl_3$) δ 7.92 (m, 2H, H_{arom}), 7.51 (m, 3H, H_{arom}), 4.12 (t, 2H, CH_2NCO), 3.25 (m, 1H, H_{pip}-4), 3.07 (m, 2H, $H_{eq\text{-}pip}$-2,6), 2.68 (t, 2H, CH_2N_{pip}), 2.57 (s, 3H, CH_3), 2.50 (m, 4H, H5,8), 2.25 (m, 2H, $H_{ax\text{-}pip}$-2,6), 1.80 (m, 8H, H_{pip}-3,5, H6,7). Maleate: mp = 202–203 °C (from abs EtOH). Anal ($C_{27}H_{33}N_3O_6$) C, H, N.

3-[2-[4-(4-Fluorobenzoyl)piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***10a***. The residue was dissolved in a mixture of EtOAc/MeOH, 9.5:0.5 and the solution filtered through silica gel. The filtrate was evaporated and the oily residue was dissolved in EtOH (10 mL) and concentrated HCl (4 mL) was added. The solution was evaporated and the residue was crystallized from EtOH; hydrochloride, mp = 265–267 °C, yield 52%. The hydrochloride was dissolved in 2 N NaOH and the solution extracted with $CHCl_3$. The organic extracts were dried and evaporated to afford a residue which was recrystallized from isopropylacetate, mp = 134–135 °C. IR ν cm^{-1}: 1671, 1654. ^{1}H-NMR ($CDCl_3$) δ 7.93 (m, 3H, H2, H_{arom}), 7.14 (m, 2H, H_{arom}), 3.98 (t, 2H, CH_2NCO), 3.21 (m, 1H, H_{pip}-4), 2.98 (m, 2H, $H_{eq\text{-}pip}$-2,6), 2.68 (t, 2H, CH_2N_{pip}), 2.57 (m, 4H, H5,8), 2.24 (m, 2H, $H_{ax\text{-}pip}$-2,6), 1.79 (m, 8H, H_{pip}-3,5, H6,7). Maleate: mp = 204–205 °C (from abs EtOH). Anal ($C_{26}H_{30}FN_3O_6$) C, H, N.

2-Methyl-3-[2-[4-(4-fluorobenzoyl)piperidin-1-yl]ethyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***10b***. Isopropylacetate (15 mL) was added to the residue and the mixture refluxed for 15 min. After cooling, the solid was filtered and recrystallized from EtOAc, mp = 180–181 °C, yield 56%. IR ν cm^{-1}: 1665, 1635. ^{1}H-NMR ($CDCl_3$) δ 7.98 (m, 2H, H_{arom}), 7.12 (m, 2H, H_{arom}), 4.11 (t, 2H, CH_2NCO), 3.20 (m, 1H, H_{pip}-4), 3.03 (m, 2H, $H_{eq\text{-}pip}$-2,6), 2.66 (t, 2H, CH_2N_{pip}), 2.58 (s, 3H, CH_3), 2.50 (m, 4H, H5,8), 2.24 (m, 2H, $H_{ax\text{-}pip}$-2,6), 1.75 (m, 8H, H_{pip}-3,5, H6,7). Maleate: mp = 215–216 °C (from abs EtOH). Anal ($C_{27}H_{32}FN_3O_6$) C, H, N.

3-[2-[4-[Bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***11a***. The residue was purified by flash chromatography over a silica gel column eluted with EtOAc/MeOH, 95:5. The resulting material was uncrystallizable, yield 63%. IR ν cm^{-1}: 1640. ^{1}H-NMR ($CDCl_3$) δ 7.92 (s, 1H, H2), 6.91 (m, 8H, H_{arom}), 3.95 (t, 2H, CH_2NCO), 2.63 (t, 2H, CH_2N_{pip}), 2.48 (m, 8H, $H_{eq\text{-}pip}$, H5,8), 2.27 (m, 4H, $H_{ax\text{-}pip}$), 1.78 (m, 4H, H6,7). Maleate: mp = 198–200 °C (from EtOH). Anal ($C_{32}H_{33}F_2N_3O_5$) C, H, N.

2-Methyl-3-[2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]ethyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***11b***. The residue was purified by flash chromatography over a silica gel column eluted with EtOAc/MeOH, 95:5. The resulting material was crystallizzed from isopropylacetate, mp = 146–148 °C, yield 68%. IR ν cm^{-1}: 1635. ^{1}H-NMR ($CDCl_3$) δ 7.02 (m, 8H, H_{arom}), 4.11 (t, 2H, CH_2NCO), 2.65 (t, 2H, CH_2N_{pip}), 2.50 (m, 10H, CH_3, $H_{eq\text{-}pip}$, H5,8), 2.35 (m, 4H, $H_{ax\text{-}pip}$), 1.73 (m, 4H, H6,7). Maleate: mp = 196–197 °C (from abs EtOH). Anal ($C_{33}H_{35}F_2N_3O_5$) C, H, N.

3-[3-[4-(4-Fluorophenyl)piperazin-1-yl]propyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***12a***. The residue was purified by flash chromatography over a silica gel column eluted with EtOAc/MeOH, 9:1. The resulting material was crystallized from isopropylacetate, mp = 111–112 °C, yield 53%. IR ν cm^{-1}: 1656. ^{1}H-NMR ($CDCl_3$) δ 8.01 (s, 1H, H2), 6.89 (m, 4H, H_{arom}), 3.98 (t, 2H, CH_2NCO), 3.07 (m, 4H, $H_{eq\text{-}pip}$), 2.56 (m, 8H, $H_{ax\text{-}pip}$, H5,8), 2.40 (t, 2H, CH_2N_{pip}), 1.98 (m, 2H, CH_2CN), 1.74 (m, 4H, H6,7). Maleate: mp = 145–147 °C (from abs EtOH). Anal ($C_{25}H_{31}FN_4O_5$) C, H, N.

2-Methyl-3-[3-[4-(4-fluorophenyl)piperazin-1-yl]propyl]-5,6,7,8-tetrahydro-4(3H)-quinazolinone ***12b***. The residue was purified by flash chromatography over a silica gel column eluted with EtOAc/MeOH, 9:1. The resulting material was crystallized from isopropylacetate, mp = 124–125 °C, yield 45%. IR ν cm^{-1}: 1653. ^{1}H-NMR ($CDCl_3$) δ 6.90 (m, 4H, H_{arom}), 4.07 (m, 2H, CH_2NCO), 3.12 (m, 4H, $H_{eq\text{-}pip}$), 2.61 (m, 4H, $H_{ax\text{-}pip}$), 2.53 (s, 3H, CH_3), 2.50 (m, 6H, CH_2N_{pip}, H5,8), 1.94 (m, 2H, CH_2CN), 1.73 (m, 4H, H6,7). Maleate: mp = 181–182 °C (from abs EtOH). Anal. ($C_{26}H_{33}FN_4O_5$) C, H, N.

Pharmacology

Animals

Male Wistar rats (Charles River, Calco, CO, Italy) weighing 280–310 g at the beginning of the experiments were used. They were kept in individual cages on a 12:12 h light/dark cycle (lights on at 8:00 pm). They had free access to food pellets (diet No 4RF18, Mucedola, Settimo Milanese, MI, Italy) and tap water.

Materials
Ketanserin and ritanserin were generously donated by Janssen Pharmaceutica, Beerse, Belgium, Carbidopa by DuPont Merck, Wilmington, DE, USA, L-5-hydroxytryptophan (L-5-HTP) by Sigma Tau Res Lab, Pomezia, Rome, Italy. All antagonists tested were dissolved in a vehicle containing 20% propylene glycol and a few drops of lactic acid. The pH of the solution was adjusted to 5 by adding 2 N NaOH. The drugs were subcutaneously injected in a volume of 1 mL/kg body weight. L-5-HTP and carbidopa were separately dissolved in distilled water containing a few drops of 5 N HCl. The pH of solution was adjusted to 6 by adding 2 N NaOH. Carbidopa was injected ip, while L-5-HTP was given sc. The two drugs were administered in a volume of 4 mL/kg body weight. Compounds **9a,b–12a,b** were employed as maleates.

Receptor binding tests
The compounds were evaluated for their in vitro binding affinity at 5-HT_2 receptors using [^{3}H]ketanserin (spec act 63.7 Ci/mmol) at conc 0.4 nM. The experiment was carried out according to Leysen et al [34]. Male Wistar rats (200–300 g) were killed by cervical dislocation and the cerebral cortex was rapidly dissected and placed in approximately 20 vol ice-cold Tris–HCl buffer (pH 7.7). The compounds tested in binding assays were dissolved in a minimum quantity of distilled water and diluted Tris–HCl buffer. To reduce [^{3}H]ketanserin binding to α_1 adrenoceptor, prazosin (final conc 0.1 mM) was added to the radioligand solution. Prazosin was dissolved in a minimum quantity of ethanol and diluted in Tris-buffer. Ritanserin was dissolved in a minimum quantity of glacial acetic acid and diluted in Tris–HCl buffer. [^{3}H]Ketanserin was supplied in ethanol and diluted in Tris–HCl buffer. Competition data were analyzed by computer-assisted iterative curve fitting according to the equation:

$$b = B_{max}[L]^n/[L]^n + K^n$$

(b = maximum binding at equilibrium; K = molar concentration of competing compound to reduce the specific binding by 50%; L = molar concentration of competing compound; n = Hill coefficient). The affinities of competing compounds (K_i) were calculated using the Cheng–Prusoff equation [35].

L-5-HTP-induced wet dog shakes (WDS) in rats
Carbidopa (12.5 mg/kg) was injected ip 30 min before sc injection of L-5-HTP (100 mg/kg). Since the WDS response reaches the maximum 2 h after administration of L-5-HTP [36], the number of WDS in rats was recorded for 10 min beginning 2 h after L-5-HTP injection. The newly synthesized compounds as well as the standards ritanserin and ketanserin were administered sc at doses up to 1 mg/kg 1 h after L-5-HTP injection, ie, 1 h before the 10-min observation period. Immediately after antagonist injection, each animal was placed in a perspex box where the number of characteristic shakes of neck, head and trunk (WDS) was recorded for 10 min. Also the number of shakes of head alone (HS) was recorded during the 10-min observation period; since the occurrence of HS was far lower than that of WDS, only data concerning WDS are reported. The experiment was carried out according to a within-subject design in which each group of animals received each dose of a single antagonist as well the injection of vehicle (controls).

To evaluate the time course of their effect, ritanserin, ketanserin, **10a** and **10b** were administered sc at a dose of 1 mg/kg. Their administration took place 1, 2, 3, 4, 5 or 6 h before the 10-min observation period. In relation to the large number of treatments, the experiment was carried out according to a between-subject design in which different groups of rats received either injection of vehicle or the antagonist tested.

*Effect on ethanol intake of ritanserin and **10b***
Preference for 3% (v/v) ethanol was induced by a classical acclimatation procedure in which rats had 3% ethanol as the only fluid available for one week [13]. The following week they only had access to water; afterwards during the experiments they were given free choice between 3% ethanol and water. Food was available ad libitum. The WDS test showed that the effect of **10b** was statistically significant for at least 5 h after administration. Therefore **10b** was administered in two sc injections at 6-h intervals. The first administration took place at 8:00 pm, ie, just before the beginning of the dark phase; the second administration took place at 2:00 am. Also ritanserin-treated rats received drug injection at 8:00 pm and 2:00 am. Both drugs were given at a dose of 1 mg/kg/injection. Intake of 3% ethanol, water and food was determined at 8:00 am.

Statistical analysis
Statistical analysis of WDS data (means ± SEM) was performed by one-way analysis of variance (repeated measures for dose–response relationship and randomized design for time course). Statistical analysis of ethanol intake experiments was carried out by split-plot analysis of variance (ANOVA) with between-group comparisons for drug treatment and within-group comparisons for time (treatment day). Pairwise comparisons were carried out by means of the Mann–Whitney test. Statistical significance was set at $p < 0.05$.

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