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Original article

New 3-benzisothiazolyl and 3-benzisoxazolylpiperazine derivatives with atypical antipsychotic binding profile☆

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Abstract

New 3-benzisothiazolyl and 3-benzisoxazolylpiperazine derivatives were synthesised and their 5-HT_{1A}, 5-HT_{2A} and D_2 receptor binding affinities evaluated. The compounds displayed high affinity for the 5-HT_{2A} receptor combined with moderate to low 5-HT_{1A} and D_2 affinities. Two of them, **18** and **25**, have been selected for further pharmacological studies to be evaluated as potential atypical antipsychotics. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Serotonin; Dopamine; 5-HT receptors; D_2 receptor; Antipsychotic

1. Introduction

Clozapine (Fig. 1), an atypical antipsychotic agent, is effective in treating positive and negative symptoms of schizophrenia without inducing dose-limiting extrapyramidal side effects (EPS) [1–4], but it has been associated with agranulocytosis [5,6], a potentially fatal blood disorder. In the past, many attempts have been made to obtain compounds with a clozapine-like antipsychotic profile without its toxicological problem.

One of the theories to explain clozapine's properties is the combination of potent serotonin 5-HT_{2A} antagonism with moderate dopamine D_2 antagonism (mixed dopamine D_2–serotonin 5-HT_{2A} receptor antagonism hypothesis) [7] as found in risperidone [8] and ziprasidone [9,10] (Fig. 1) which are known atypical antipsychotic drugs. It has been postulated that the additional 5-HT_{1A} agonist activity shown by several atypical antipsychotic agents could reduce EPS and alleviate the anxiety that often precipitates psychotic episodes in schizophrenic patients [11,12].

Norman et al. [11,12] reported that a series of cyclic and noncyclic benzamides connected through a link with the 3-benzisothiazolylpiperazine moiety exhibited in vitro and in vivo activities which suggested atypical antipsychotic activity. This pharmacological profile may result from the combination of dopamine antagonism of the cyclic and noncyclic benzamides with the known serotonin antagonism of the benzisothiazolylpiperazine moiety [13,14]. Additionally, the reported derivatives presented serotonin 5-HT_{1A} receptor agonism.

Recently, we have reported [15] that a series of naphthylpiperazines linked to different phtalazinones exhibited affinities for the serotonin and dopamine D_2 receptors. Several of these derivatives possessed in vivo activities that suggested they would be useful in the treatment of schizophrenia and are currently under development.

As a continuation of that work, we have now prepared novel compounds **13**–**28** (Fig. 2) attempting to combine the known serotonin activity of 3-benzisothiazolyl and 3-benzisoxazolylpiperazine moieties with the potential dopamine activity of different heterocycles **1**–**10** (Fig. 3). In all cases, both fragments are linked through an ethylene chain. The preparation and preliminary pharmacology of these new compounds are described herein.

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2. Chemistry

The derivatives **13**–**28** were prepared as outlined in Fig. 2 through the *N*-alkylation of heterocycles **1**–**10** (Fig. 3) with the corresponding chloride **11** (Z = O) or **12** (Z = S).

The required chlorides **11** (Z = O) and **12** (Z = S) were synthesised by alkylation of 3-(1-piperazinyl)-1,2-benzisothiazole and 3-(1-piperazinyl)-1,2-benzisoxazole with chloroethanol and transformation of the corresponding alcohols to chlorides **11** and **12** [13].

The heterocycles **1**–**3** were available from commercial suppliers, but heterocycles **4**–**10** required the use of several synthetic approaches. Quinazolinone **5** was obtained from *o*-nitrobenzylchloride following the procedure reported by Reilly et al. [16]. Dimethylated isoquinolinone **8** was prepared from dimethyl phenylacetamide by the method described by Ben-Ishai et al.

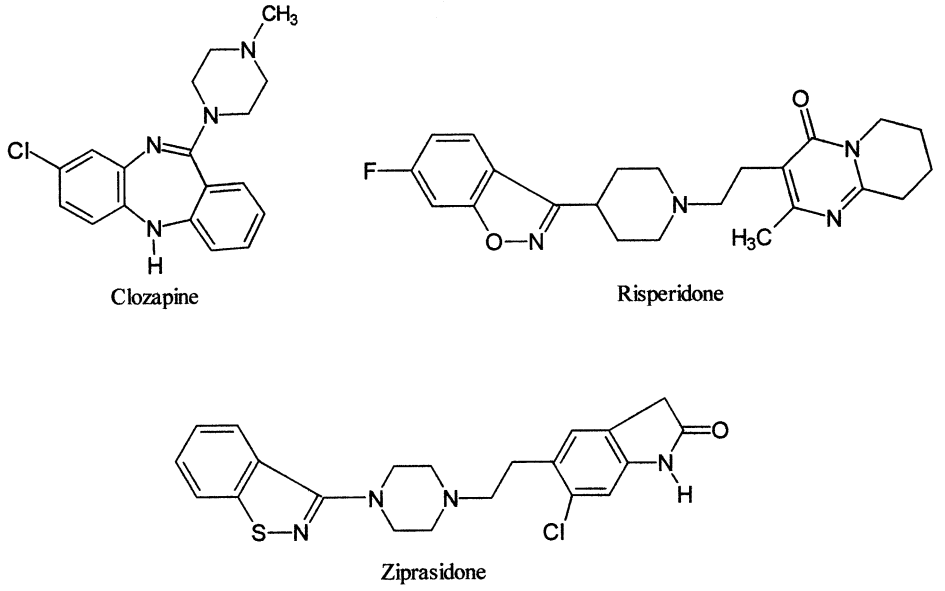


Fig. 1. Chemical structures of clozapine, risperidone and ziprasidone.

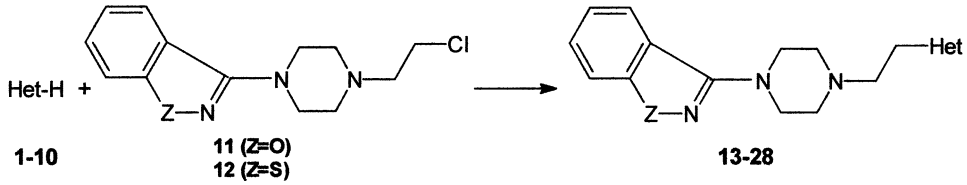
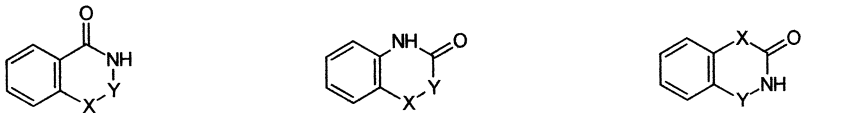


Fig. 2. Synthetic procedure to compounds **13**–**28**.



1 X-Y = CH=CH

2 X-Y = CH=N

3 X-Y = N=CH

4 X = C=O, Y = N-CH_3

5 X = CH_2, Y = N-CH_3

6 X = CH_2, Y = CH_2

7 X = C=O, Y = $C(CH_3)_2$

8 X = $C(CH_3)_2$, Y = CH_2

9 X = N-CH_3, Y = CH_2

10 X= $C(CH_3)_2$, Y = C=O

Fig. 3. Heterocycles **1**–**10**.

[17]. Quinazolinone **9** was obtained from *N*-methylisatoic anhydride by the procedure reported by Coyne et al. [18]. Isoquinolinedione **10** was synthesised from homophthalimide by refluxing an aqueous alcohol solution of methyl iodide, sodium hydroxide and homophthalimide [19]. An alternative for the preparation of isoquinolinedione **10** was to reflux a mixture of the appropriate dicarboxylic acid and urea in xylene removing the water azeotropically using a Dean–Stark apparatus [20]. Quinazolinedione **4** was prepared from isatoic anhydride using the procedure described by Kappe et al. [21]. Quinolinone **6** was obtained from 2-nitrobenzaldehyde by the method of Mali et al. [22]. Quinolinedione **7** was synthesised from the acid chloride of dimethylmalonic acid and aniline following the procedure reported by Taylor et al. [23].

The alkylation of the heterocycles **1**–**10** with the appropriate chlorides I was carried out either using classical reaction conditions (NaH in *N*,*N*-dimethylformamide) or phase transfer catalysis (tetrabutylammonium bromide, KOH, toluene) in a two-phase solid/liquid system. Only *N*-alkylated products **13**–**28** were obtained, according to their ^{13}C-NMR (CH_2–N–C=O shift) and IR (C=O st) spectra. These products were isolated as free bases or salts.

3. Pharmacology

All compounds were initially evaluated for in vitro dopamine D_2 receptor affinity by radioligand binding assay. For each compound, the ability to displace the specific ligand [^{3}H]raclopride from D_2 receptors of rat striatum was determined [24]. Concentrations required to inhibit 50% of radioligand specific binding (IC_{50}) and K_i values were calculated from only one competition experiment with samples in triplicate, using 10–12 different concentrations of displacer. For each compound, the ability to displace the specific ligands [^{3}H]8-OH-DPAT and [^{3}H]ketanserin from 5-HT_{1A} sites of rat cerebral cortex or 5-HT_{2A} sites of rat prefrontal cortex, respectively, was also determined [25,26]. The pharmacological results are reported in Table 1.

4. Results and discussion

The compounds displayed lower affinities (K_i 25.2 to $IC_{50} > 1000$ nM) than risperidone (K_i 5.65 nM) and ziprasidone (K_i 4.6 nM) for the D_2 receptor while **18** and **25**–**27** displayed higher affinities than clozapine (K_i 55.04 nM) for that receptor. As clozapine compounds **13**, **16**, **18**, **20**, **22** and **28** showed very low affinity ($IC_{50} > 1000$ nM) for the 5-HT_{1A} receptor while the rest of compounds at least equalled that shown by risperidone (K_i 217.54 nM) and one of them, **25** (K_i 6.05 nM), was even better than ziprasidone (K_i 12 nM). Most of the derivatives exhibited a very high affinity for the 5-HT_{2A} receptor, reaching values at the level of risperidone (K_i 0.22 nM) and ziprasidone (K_i 1.4 nM), and superior to that of clozapine (K_i 13.69 nM). Compounds with the 3-benzisothiazolyl moiety showed affinities for the D_2 receptor that were higher than those displayed by their 3-benzisoxazolylpiperazine analogues. The exception was compound **28** with 5-fold lower affinity than its 3-benzisoxazolylpiperazine analogue **18**. Similarly, affinities for the 5-HT_{1A} receptor were higher for 3-benzisothiazolylpiperazine derivatives, particularly when substituted with heterocycles **1**, **5** and **9**; the highest affinity for this receptor was found in compound **25**. The same pattern was observed with the affinities for the 5-HT_{2A} receptor. Thus, the 3-benzisothiazolylpiperazine derivatives had affinities that were higher than those of the 3-benzisoxazolylpiperazine analogues. All compounds were potent ligands for that receptor, particularly **24**, **26**–**28**, which were ten times more potent than ziprasidone.

From these results some observations are noteworthy: (i) compound **18** has an affinity profile very close to those of risperidone and clozapine, and (ii) as ziprasidone compound **25** displays high affinities for all three receptors. Therefore, these two compounds have been selected for further pharmacological tests to be evaluated as potential atypical antipsychotic drugs.

5. Experimental protocols

5.1. Chemistry

Flash column chromatography was performed with silica gel 60, 230–400 mesh. The salts were prepared by addition of an ethanolic or ethereal solution of hydrogen chloride to a solution of the free base in ethanol or ether. Melting points were determined in open capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. Elemental analyses indicated by the symbols of the elements were within $\pm 0.4\%$ of the theoretical values. Infrared (IR) spectra were recorded (in KBr) on a Perkin–Elmer Spectrum 1310 spectrophotometer and only noteworthy absorption values (cm^{-1}) are listed. ^{1}H-NMR (200 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded using a Brucker AC 200 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Spectral data are consistent with assigned structures.

5.1.1. Preparation of intermediates **11** and **12**

*5.1.1.1. 3-[4-(2-Chloroethyl)-1-piperazinyl]-1,2-benzisoxazole (**11**, Z = O).* A suspension of 3-(1-piper-

Table 1
5-HT_{2A}, 5-HT_{1A} and D_2 receptor binding affinities

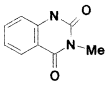
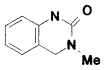
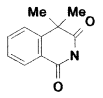
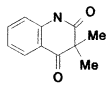
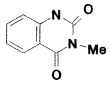
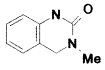
Compound	Z	Heterocycle (Het)	5-HT_{2A}[a] (K_i nM)	5-HT_{1A}[b] (K_i nM)	D_2[c] (K_i nM)
13	O	 **4**	11.99	>1000[d]	169.96
14	O	 **5**	0.79	178.6	206.10
15	O	 **1**	13.62	66.2	165.81
16	O	 **8**	0.41	>1000[d]	110.26
17	O	 **9**	0.57	183	89.61
18	O	 **10**	0.73	>1000[d]	25.25
19	O	 **6**	2.44	224.04	784.7
20	O	 **7**	3.63	>1000[d]	321.98
21	O	 **2**	4.15	187.03	459.5

Table 1 (*Continued*)

Compound	Z	Heterocycle (Het)	5-HT$_{2A}$[a] (K_i nM)	5-HT$_{1A}$[b] (K_i nM)	D_2[c] (K_i nM)
22	O	 **3**	5.61	>1000[d]	>1000[d]
23	S	 **4**	0.80	55.44	95.91
24	S	 **5**	0.18	31.15	82.23
25	S	 **1**	0.86	6.05	45.68
26	S	 **8**	0.12	84.9	27.71
27	S	 **9**	0.11	33.7	46.04
28	S	 **10**	0.13	>1000[d]	133.83
Risperidone			0.22	217.54	5.65
Ziprasidone[e]			1.4	12	4.6
Clozapine			13.69	≈1000[d]	55.04

[a] 5-HT$_{2A}$:[^{3}H]ketanserin binding.
[b] 5-HT$_{1A}$:[^{3}H]8-OH-DPAT binding.
[c] D_2:[^{3}H]raclopride binding.
[d] IC_{50} (nM).
[e] Schotte et al. [28].

azinyl)-1,2-benzisoxazole [13] (29 g, 0.14 mol) in dioxane (570 mL) was treated with anhydrous K_2CO_3 (69 g, 0.5 mol) and KI (2 g, 12 mmol). After addition of 2-chloroethanol (55 mL) the mixture was refluxed for 20 h. The solvent was distilled and the residue was treated with water and extracted with $CHCl_3$. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated to give 26 g of 3-[4-(2-hydroxyethyl)-1-piperazinyl]-1,2-benzisoxazole (74%).

3-[4-(2-Hydroxyethyl)-1-piperazinyl]-1,2-benzisoxazole (13.6 g, 54.4 mmol) in $CHCl_3$ (220 mL) was treated dropwise with a solution of $SOCl_2$ (9.5 mL, 131 mmol) in $CHCl_3$ (30 mL) and the mixture was heated at reflux for 4 h. The reaction was allowed to cool to room temperature, and water (300 mL) and solid KOH until pH > 10 were added. The layers were separated and the aqueous phase extracted with $CHCl_3$. The mixture of organic layers was dried over anhydrous Na_2SO_4, filtered and concentrated to give a solid which was washed with hexane to give 11.6 g (80%) of the desired product, m.p. 96–99 °C. ^{1}H-NMR ($CDCl_3$) δ 2.73 (t, 4H, $J = 5$ Hz), 2.81 (t, 2H, $J = 6.9$ Hz), 3.60 (t, 4H, $J = 5$ Hz), 3.64 (t, 2H, $J = 6.9$ Hz), 7.18–7.25 (m, 1H), 7.36–7.49 (m, 2H), 7.68 (d, 1H, $J = 8$ Hz). ^{13}C-NMR (DMSO-d_6) δ 41.3 (CH_2), 47.6 (CH_2), 51.8 (CH_2), 59.1 (CH_2), 110.0 (CH), 115.5 (C), 122.6 (CH), 122.8 (CH), 129.9 (CH), 160.7 (C), 163.2 (C).

*5.1.1.2. 3-[4-(2-Chloroethyl)-1-piperazinyl]-1,2-benzisothiazole (**12**, Z = S).* It was synthesised analogously to **11** (Z = O). Yield: 80%, m.p. 102–104 °C. ^{1}H-NMR ($CDCl_3$) δ 2.76 (t, 4H, $J = 5.7$ Hz), 2.84 (t, 2H, $J = 7.2$ Hz), 3.58 (t, 4H, $J = 5.7$ Hz), 3.65 (t, 2H, $J = 7.2$ Hz), 7.35 (t, 1H, $J = 6.9$ Hz), 7.47 (t, 1H, $J = 6.9$ Hz), 7.88 (m, 2H).

^{13}C-NMR ($CDCl_3$) δ 40.7 (CH_2), 49.7 (CH_2), 52.6 (CH_2), 59.6 (CH_2), 120.3 (CH), 123.6 (CH), 123.7 (CH), 127.3 (CH), 127.7 (C), 152.4 (C), 163.5 (C).

*5.1.2. General procedure for the preparation of compounds **14**–**17**, **25**–**28***

*5.1.2.1. As an example, preparation of 2-[2-[4-(1,2-benzisoxazol-3-yl)-1-piperazinyl]ethyl]-1(2H)-isoquinolinone (**15**).* A mixture of 3-[4-(2-chloroethyl)-1-piperazinyl]-1,2-benzisoxazole (2.4 g, 9 mmol), isocarbostyril (1 g, 6.9 mmol), tetrabutylammonium bromide (0.75 g, 2.3 mmol) and KOH (powder, 0.6 g, 10.3 mmol) in toluene (140 mL) was heated under reflux for 5 h. The reaction mixture was allowed to cool to room temperature, transferred to a separatory funnel and washed with saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4 and concentrated to give an oil that solidified upon standing. The solid was triturated with ether and filtered to give 2.2 g (85%) of **15** as a white solid, m.p. 115–120 °C. ^{1}H-NMR ($CDCl_3$) δ 2.71–2.86 (m, 6H), 3.54–3.64 (m, 4H), 4.17 (t, 2H, $J = 6.5$ Hz), 6.50 (d, 1H, $J = 7.3$ Hz), 7.11–7.25 (m, 2H), 7.42–7.54 (m, 4H), 7.60–7.70 (m, 2H), 8.43 (d, 1H, $J = 7.6$ Hz). ^{13}C-NMR ($CDCl_3$) δ 46.5 (CH_2), 48.2 (CH_2), 52.6 (CH_2), 56.7 (CH_2), 105.7 (CH), 110.3 (CH), 116.0 (C), 122.0 (CH), 122.2 (CH), 125.8 (CH), 126.0 (C), 126.6 (CH), 127.6 (CH), 129.4 (CH), 132.0 (CH), 132.1 (CH), 137.0 (C), 161.2 (C), 162.0 (C), 163.8 (C). IR (KBr) 1610, 1640. Anal. $C_{22}H_{22}N_4O_2$ (C, H, N).

*5.1.2.2. 1-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-3,4-dihydro-3-methyl-2(1H)-quinazolinone (**14**).* It was purified by flash column chromatography using EtOAc–hexane 9/1 as eluent (34% yield, m.p. 150–152 °C). ^{1}H-NMR ($CDCl_3$) δ 2.70 (t, 4H, $J = 4.6$ Hz), 2.78 (t, 4H, $J = 4.6$ Hz), 3.03 (s, 3H), 3.60 (t, 4H, $J = 4.6$ Hz), 4.07 (t, 2H, $J = 7.4$ Hz), 4.38 (s, 2H), 6.92–7.07 (m, 3H), 7.18–7.3 (m, 2H), 7.42–7.53 (m, 2H), 7.69 (d, 1H, $J = 8$ Hz). ^{13}C-NMR ($CDCl_3$) δ 35.4 (CH_3), 40.4 (CH_2), 48.1 (CH_2), 50.2 (CH_2), 52.7 (CH_2), 55.1 (CH_2), 110.3 (CH), 112.7 (CH), 116.0 (C), 119.6 (C), 121.6 (CH), 122.0 (CH), 122.1 (CH), 125.6 (CH), 128.1 (CH), 129.3 (CH), 138.3 (C), 154.6 (C), 161.1 (C), 163.8 (C). IR (KBr) 1600, 1640. Anal. $C_{22}H_{25}N_5O_2$ (C, H, N).

*5.1.2.3. 2-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-1,4-dihydro-4,4-dimethyl-3(2H)-isoquinolinone (**16**).* It was purified by flash column chromatography using Et_2O–CH_2Cl_2 4/6 as eluent (86% yield). Hydrochloride: m.p. > 240 °C.

^{1}H-NMR (DMSO-d_6) δ 1.40 (s, 6H), 3.34–4.07 (m, 12H), 4.69 (s, 2H), 7.19–7.40 (m, 5H), 7.60 (d, 2H, $J = 3.7$ Hz), 8.03 (d, 1H, $J = 8.0$ Hz). ^{13}C-NMR (DMSO-d_6 + CD_3OD-d_4) δ 26.1 (CH_3), 41.9 (CH_2), 45.3 (C), 49.6 (CH_2), 50.7 (CH_2), 52.7 (CH_2), 110.3 (CH), 115.4 (C), 122.9 (CH), 123.2 (CH), 125.0 (CH), 125.7 (CH), 126.5 (CH), 127.9 (CH), 130.5 (CH), 130.8 (C), 141.2 (C), 160.3 (C), 163.7 (C), 174.2 (C). IR (KBr) 1600, 1630. Anal. $C_{24}H_{28}N_4O_2 \cdot HCl$ (C, H, N, Cl).

*5.1.2.4. 3-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-3,4-dihydro-1-methyl-2(1H)-quinazolinone (**17**).* It was purified by flash column chromatography using Et_2O–CH_2Cl_2 1/1 as eluent (59% yield). Hydrochloride: m.p. > 240 °C.

^{1}H-NMR ($CDCl_3$) δ 2.65–2.75 (m, 6H), 3.30 (s, 3H), 3.54–3.64 (m, 6H), 4.48 (s, 2H), 6.83 (d, 1H, $J = 8.1$ Hz), 6.93–7.07 (m, 2H), 7.17–7.29 (m, 2H), 7.41–7.51 (m, 2H), 7.68 (d, 1H, $J = 8.0$ Hz). ^{13}C-NMR (DMSO-d_6) δ 30.2 (CH_3), 42.2 (CH_2), 44.8 (CH_2), 47.6 (CH_2), 50.5 (CH_2), 52.9 (CH_2), 110.3 (CH), 113.0 (CH), 115.2 (C), 120.0 (C), 122.0 (CH), 122.8 (CH), 123.2 (CH), 125.6 (CH), 128.3 (CH), 130.5 (CH), 138.9 (C), 154.8 (C), 160.1 (C), 163.4 (C). IR (KBr) 1600, 1640. Anal. $C_{22}H_{25}N_5O_2 \cdot HCl$ (C, H, N, Cl).

*5.1.2.5. 2-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-ethyl]-1(2H)-isoquinolinone (**25**).* It was purified by flash column chromatography using Et_2O–CH_2Cl_2 1/1 as eluent (74% yield). Hydrochloride: m.p. 236–246 °C (dec.).

^{1}H-NMR ($CDCl_3$) δ 2.75–2.87 (m, 6H), 3.51–3.56 (m, 4H), 4.17 (t, 2H, $J = 6.5$ Hz), 6.49 (d, 1H, $J = 7.4$ Hz), 7.14 (d, 1H, $J = 7.3$ Hz), 7.31–7.68 (m, 5H), 7.78–7.92 (m, 2H), 8.43 (d, 1H, $J = 7.7$ Hz). ^{13}C-NMR ($CDCl_3$) δ 46.6 (CH_2), 50.1 (CH_2), 53.1 (CH_2), 56.7 (CH_2), 105.6 (CH), 120.5 (CH), 123.8 (CH), 123.9 (CH), 125.8 (CH), 126.1 (C), 126.6 (CH), 127.5 (CH), 127.7 (CH), 127.9 (C), 132.0 (CH), 132.3 (CH), 137.1 (C), 152.6 (C), 162.1 (C), 163.9 (C). IR (KBr) 1620, 1640. Anal. $C_{22}H_{22}N_4OS$·HCl (C, H, N, Cl).

*5.1.2.6. 2-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-ethyl]-1,4-dihydro-4,4-dimethyl-3(2H)-isoquinolinone (**26**).* It was purified by flash column chromatography using Et_2O–CH_2Cl_2 1/1 as eluent (54% yield). Hydrochloride: m.p. 236–238 °C.

^{1}H-NMR (DMSO-d_6) δ 1.42 (s, 6H), 3.38–4.11 (m, 12H), 4.72 (s, 2H), 7.17–7.62 (m, 5H), 8.06–8.11 (m, 3H). ^{13}C-NMR (DMSO-d_6) δ 26.1 (CH_3), 41.6 (CH_2), 41.8 (C), 46.6 (CH_2), 49.5 (CH_2), 50.9 (CH_2), 52.2 (CH_2), 121.3 (CH), 124.1 (CH), 124.8 (CH), 124.9 (CH), 125.6 (CH), 126.4 (CH), 127.0 (C), 127.7 (CH), 128.3 (CH), 130.7 (C), 141.0 (C), 152.2 (C), 162.3 (C), 173.8 (C). IR (KBr) 1595, 1635. Anal. $C_{24}H_{28}N_4OS$·HCl (C, H, N, Cl).

*5.1.2.7. 3-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-ethyl]-3,4-dihydro-1-methyl-2(1H)-quinazolinone (**27**).* It was purified by flash column chromatography using Et_2O–CH_2Cl_2 1/1 as eluent (41% yield). Hydrochloride: m.p. 223–228 °C.

^{1}H-NMR (DMSO-d_6) δ 3.18 (s, 3H), 3.30–3.84 (m, 10H), 4.03–4.09 (m, 2H), 4.51 (s, 2H), 6.91–7.02 (m, 2H), 7.12–7.29 (m, 2H), 7.40–7.61 (m, 2H), 8.06–8.15 (m, 2H). ^{13}C-NMR (DMSO-d_6) δ 30.1 (CH_3), 42.1 (CH_2), 46.3 (CH_2), 47.5 (CH_2), 50.8 (CH_2), 52.4 (CH_2), 112.9 (CH), 120.0 (C), 121.2 (CH), 121.7 (CH), 124.1 (CH), 124.6 (CH), 125.5 (CH), 126.9 (C), 128.1 (CH), 138.9 (C), 152.1 (C), 154.4 (C), 162.2 (C). IR (KBr) 1600, 1650. Anal. $C_{22}H_{25}N_5OS$·HCl (C, H, N, Cl).

*5.1.2.8. 2-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-ethyl]-3,4-dihydro-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione (**28**).* It was purified by flash column chromatography using Et_2O–CH_2Cl_2 1/9 as eluent (45% yield). Hydrochloride: m.p. 200–203 °C.

^{1}H-NMR ($CDCl_3$) δ 1.69 (s, 6H), 3.20–3.39 (m, 4H), 3.78–3.84 (m, 2H), 4.05–4.21 (m, 4H), 4.47–4.53 (m, 2H), 7.37–7.56 (m, 4H), 7.62–7.70 (m, 1H), 7.82–7.87 (m, 2H), 8.21 (d, 1H, $J = 7.8$ Hz). ^{13}C-NMR ($CDCl_3$) δ 29.4 (CH_3), 34.4 (CH_2), 43.8 (C), 46.4 (CH_2), 50.8 (CH_2), 54.8 (CH_2), 120.7 (CH), 123.2 (CH), 123.3 (C), 124.5 (CH), 125.3 (CH), 127.3 (CH), 127.4 (C), 128.0 (CH), 129.1 (CH), 134.5 (CH), 145.1 (C), 152.9 (C), 161.5 (C), 164.4 (C), 177.5 (C). IR (KBr) 1600, 1660, 1705. Anal. $C_{24}H_{26}N_4O_2S$·HCl (C, H, N, Cl).

*5.1.3. General procedure for the preparation of compounds **13**, **18**–**24***

*5.1.3.1. As an example, preparation of 3-[2-[4-(1,2-benzisoxazol-3-yl)-1-piperazinyl]ethyl]-4(3H)-quinazolinone (**22**).* Sodium hydride as a 50% oil dispersion (0.5 g, 10.4 mmol) was placed in a 100 mL, flame dried, round-bottomed flask equipped with a magnetic stirring bar and an argon gas inlet. The sodium hydride was washed three times with hexane and the waste hexane removed each time with a pipet. Anhydrous DMF (8 mL) was added followed by a solution of 4(3*H*)-quinazolinone (1 g, 6.8 mmol) in anhydrous DMF (16 mL). The mixture was heated to 60 °C for 1 h 30 min. A solution of 3-[4-(2-chloroethyl)-1-piperazinyl]-1,2-benzisoxazole (2 g, 7.5 mmol) in anhydrous DMF (15 mL) was added dropwise and the reaction mixture was heated to 120 °C for 5 h. The reaction was allowed to cool to room temperature, stirred for 16 h and poured onto water. The mixture was stirred and the resulting precipitate was filtered. The white solid was washed with water and dried to afford 1.6 g (64%) of **22**, m.p. 158–161 °C. ^{1}H-NMR ($CDCl_3$) δ 2.71–2.84 (m, 6H), 3.54–3.59 (m, 4H), 4.15 (t, 2H, $J = 5.9$ Hz), 7.17–7.25 (m, 2H), 7.42–7.82 (2m, 5H), 8.09 (s, 1H), 8.32 (d, 1H, $J = 7.7$ Hz). ^{13}C-NMR ($CDCl_3$) δ 43.6 (CH_2), 48.1 (CH_2), 52.5 (CH_2), 56.5 (CH_2), 110.4 (CH), 116.0 (C), 121.9 (C), 122.0 (CH), 122.2 (CH), 126.5 (CH), 127.1 (CH), 127.3 (CH), 129.4 (CH), 134.1 (CH), 146.9 (CH), 148.0 (C), 161.0 (C), 163.8 (C). IR (KBr) 1600, 1650. Anal. $C_{21}H_{21}N_5O_2$ (C, H, N).

*5.1.3.2. 1-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-3-methyl-2,4(1H,3H)-quinazolinedione (**13**).* Yield: 52%, m.p. 158–161 °C. ^{1}H-NMR ($CDCl_3$) δ 2.66–2.82 (m, 6H), 3.50 (s, 3H), 3.59 (t, 4H, $J = 4.8$ Hz), 4.34 (t, 2H, $J = 7.1$ Hz), 7.18–7.30 (m, 3H), 7.46–7.52 (m, 2H), 7.65–7.74 (m, 2H), 8.25 (m, 1H). ^{13}C-NMR (DMSO-d_6) δ 28.1 (CH_3), 40.8 (CH_2), 47.7 (CH_2), 52.3 (CH_2), 54.6 (CH_2), 110.0 (CH), 114.5 (CH), 114.8 (C), 115.5 (C), 122.6 (CH), 122.9 (CH), 127.9 (CH), 129.9 (CH), 135.2 (CH), 139.5 (C), 150.4 (C), 160.8 (C), 161.2 (C), 163.2 (C). IR (KBr) 1600, 1640, 1690. Anal. $C_{22}H_{23}N_5O_3$ (C, H, N).

*5.1.3.3. 2-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-3,4-dihydro-4,4-dimethyl-1,3(2H,4H)-isoquinolinedione (**18**).* It was purified by flash column chromatography using Et_2O–CH_2Cl_2 0.5/9.5 as eluent (42% yield). Hydrochloride: m.p. 230–234 °C (dec.).

^{1}H-NMR ($CDCl_3$) δ 1.64 (s, 6H), 2.66–2.76 (m, 6H), 3.48–3.53 (m, 4H), 4.21 (t, 2H, $J = 6.4$ Hz), 7.16–7.24 (m, 1H), 7.39–7.50 (m, 4H), 7.59–7.70 (m, 2H), 8.22 (d, 1H, $J = 7.8$ Hz). ^{13}C-NMR ($CDCl_3$) δ 29.3 (CH_3), 37.1 (CH_2), 43.5 (C), 48.2 (CH_2), 52.5 (CH_2), 55.6 (CH_2), 110.3 (CH), 116.1 (C), 122.1 (CH), 123.8 (C), 125.0 (CH), 127.2 (CH), 128.8 (CH), 129.3 (CH), 133.9 (CH), 144.9 (C), 161.2 (C), 163.8 (C), 164.0 (C), 177.0 (C). IR (KBr) 1600, 1650, 1690. Anal. $C_{24}H_{26}N_4O_3 \cdot HCl$ (C, H, N, Cl).

*5.1.3.4. 1-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-3,4-dihydro-2(1H)-quinolinone (**19**).* It was purified by flash column chromatography using EtOAc–hexane 7/3 as eluent (53% yield). Hydrochloride: m.p. 244–247 °C (dec.).

^{1}H-NMR ($CDCl_3$) δ 2.61–2.94 (m, 10H), 3.58 (t, 4H, $J = 4.9$ Hz), 4.14 (t, 2H, $J = 7.2$ Hz), 6.98–7.24 (m, 5H), 7.30–7.48 (m, 2H), 7.68 (d, 1H, $J = 8$ Hz). ^{13}C-NMR ($CDCl_3$) δ 25.2 (CH_2), 31.6 (CH_2), 39.6 (CH_2), 47.9 (CH_2), 52.4 (CH_2), 54.7 (CH_2), 110.0 (CH), 114.5 (CH), 115.8 (C), 121.9 (CH), 122.0 (CH), 122.6 (CH), 126.3 (C), 127.1 (CH), 127.7 (CH), 129.1 (CH), 139.2 (C), 160.9 (C), 163.6 (C), 169.8 (C). IR (KBr) 1590, 1655. Anal. $C_{22}H_{24}N_4O_2 \cdot HCl$ (C, H, N, Cl).

*5.1.3.5. 1-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-3,3-dimethyl-2,4(1H,3H)-quinolinedione (**20**).* It was purified by flash column chromatography using EtOAc–hexane 7/3 as eluent (37% yield, m.p. 114–116 °C). ^{1}H-NMR ($CDCl_3$) δ 1.49 (s, 6H), 2.70 (t, 2H, $J = 6.9$ Hz), 2.77 (t, 4H, $J = 4.6$ Hz), 3.57 (t, 4H, $J = 4.6$ Hz), 4.24 (t, 2H, $J = 6.9$ Hz), 7.14–7.23 (m, 2H), 7.43–7.54 (m, 2H), 7.59–7.7 (m, 3H), 8.02 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ 23.7 (CH_3), 39.7 (CH_2), 48.1 (CH_2), 52.7 (CH_2), 53.0 (C), 54.7 (CH_2), 110.3 (CH), 114.4 (CH), 116.0 (C), 120.0 (C), 122.0 (CH), 122.2 (CH), 122.9 (CH), 128.4 (CH), 129.4 (CH), 135.7 (CH), 142.0 (C), 161.1 (C), 163.8 (C), 174.2 (C), 197.4 (C). IR (KBr) 1600, 1650, 1690. Anal. $C_{24}H_{26}N_4O_3$ (C, H, N).

*5.1.3.6. 2-[2-[4-(1,2-Benzisoxazol-3-yl)-1-piperazinyl]-ethyl]-1(2H)-phthalazinone (**21**).* It was purified by flash column chromatography using EtOAc as eluent (36% yield, m.p. 115–118 °C). ^{1}H-NMR ($CDCl_3$) δ 2.78 (t, 4H, $J = 5$ Hz), 2.92 (t, 2H, $J = 6.8$ Hz), 3.57 (t, 4H, $J = 5$ Hz), 4.44 (t, 2H, $J = 6.8$ Hz), 7.17–7.25 (m, 1H), 7.41–7.51 (m, 2H), 7.67–7.85 (m, 4H). 8.18 (s, 1H), 8.41–8.46 (m, 1H). ^{13}C-NMR ($CDCl_3$) δ 48.0 (CH_2), 48.2 (CH_2), 52.4 (CH_2), 56.1 (CH_2), 110.3 (CH), 116.1 (C), 122.1 (CH), 125.9 (CH), 126.6 (CH), 127.8 (C), 129.4 (CH), 129.5 (C), 131.5 (CH), 133.0 (CH), 137.7 (CH), 159.4 (C), 161.2 (C), 163.8 (C). IR (KBr) 1600, 1630, 1700. Anal. $C_{21}H_{21}N_5O_2$ (C, H, N).

*5.1.3.7. 1-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-ethyl]-3-methyl-2,4(1H,3H)-quinazolinedione (**23**).* Yield: 68%, m.p. 158–160 °C. ^{1}H-NMR ($CDCl_3$) δ 2.78 (t, 2H, $J = 6.9$ Hz), 2.83 (t, 4H, $J = 4.8$ Hz), 3.50 (s, 3H), 3.57 (t, 4H, $J = 4.8$ Hz), 4.35 (t, 2H, $J = 6.9$ Hz), 7.23–7.92 (m, 7H), 8.25 (d, 1H, $J = 7.9$ Hz). ^{13}C-NMR ($CDCl_3$) δ 28.0 (CH_3), 40.9 (CH_2), 49.6 (CH_2), 52.9 (CH_2), 54.5 (CH_2), 113.2 (CH), 115.1 (C), 120.1 (CH), 122.5 (CH), 123.5 (CH), 123.6 (CH), 127.2 (CH), 127.5 (CH), 128.5 (CH), 134.7 (CH), 139.2 (C), 150.5 (C), 152.2 (C), 160.7 (C), 163.4 (C). IR (KBr) 1600, 1650, 1700. Anal. $C_{22}H_{23}N_5O_2S$ (C, H, N).

*5.1.3.8. 1-[2-[4-(1,2-Benzisothiazol-3-yl)-1-piperazinyl]-ethyl]-3,4-dihydro-3-methyl-2(1H)-quinazolinone (**24**).* It was purified by flash column chromatography using EtOAc as eluent (65% yield, m.p. 133–136 °C). ^{1}H-NMR ($CDCl_3$) δ 2.73 (t, 2H, $J = 7.4$ Hz), 2.82 (t, 4H, $J = 4.8$ Hz), 3.03 (s, 3H), 3.58 (t, 4H, $J = 4.8$ Hz), 4.08 (t, 2H, $J = 7.4$ Hz), 4.38 (s, 2H), 6.94–7.07 (m, 3H), 7.22–7.51 (m, 3H), 7.81 (d, 1H, $J = 7.9$ Hz), 7.91 (d, 1H, $J = 7.9$ Hz). ^{13}C-NMR ($CDCl_3$) δ 35.4 (CH_3), 40.4 (CH_2), 49.9 (CH_2), 50.2 (CH_2), 53.1 (CH_2), 55.1 (CH_2), 112.7 (CH), 119.5 (C), 120.3 (CH), 121.5 (CH), 123.7 (CH), 123.8 (CH), 125.5 (CH), 127.3 (CH), 127.8 (C), 128.1 (CH), 138.3 (C), 152.5 (C), 154.6 (C), 163.7 (C). IR (KBr) 1600, 1645. Anal. $C_{22}H_{25}N_5OS$ (C, H, N).

5.2. Pharmacology

5.2.1. 5-HT_{2A} receptor binding assay [24]

Adult male Wistar rats weighing 220–280 g were used. Animals were killed by decapitation, the whole brain with the exception of the brain stem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. Prefrontal cortex used for the binding experiments were homogenised with an Ultra-Turrax (setting 5 for 20 s) in 10 volumes of ice-cold 0.25 M sucrose buffer and centrifuged at $1080 \times g$ for 10 min (4 °C), and the supernatant was recentrifuged at $35000 \times g$ for 10 min (4 °C). The resulting pellet was resuspended in 40 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.7) and then centrifuged once more at $35000 \times g$ for 10 min (4 °C). The final pellet was resuspended in 10 volumes of ice-cold 50 mM Tris–HCl buffer and was stored at −70 °C until use. At the time of the experiment, the membranes were diluted in the same ice-cold buffer (final dilution 1:60, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration in Tris–HCl buffer with 10% ethanol (0.1 mL of Tris–ethanol buffer if no competing drug was added) and 0.1 mL of [^{3}H]ketanserin (NEN, 60–90 Ci/mmol) in Tris–ethanol buffer (final concentration 0.8 nM). Nonspecific bind-

ing was determined using 1 μM cold methysergide. Binding experiment was initiated by addition of 0.8 mL of membrane suspension (400–500 μg of protein). After incubation for 15 min at 37 °C the reaction was stopped for vacuum filtration through Whatman GF/B glass pretreated filters (1% polyethylenimine in 50 mM Tris–HCl buffer), using a Brandel Cell Harvester, followed by two washes with 5 mL of ice-cold 50 mM Tris–HCl (pH 7.7) buffer. Filters were placed in scintillating polyethylene vials (with 5 mL scintillation cocktail) and equilibrated. Filter-retained radioactivity was measured in a liquid scintillation counter (Kontron Beta V). IC_{50} and K_i values were calculated using the computer program EBDA (McPherson) [27].

5.2.2. 5-HT_{1A} receptor binding assay [25]

Adult male Wistar rats weighing 220–280 g were used. Animals were killed by decapitation, the whole brain with the exception of the brain stem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. The cerebral cortex used for the binding experiments was homogenised with an Ultra-Turrax (setting 5 for 20 s) in 10 volumes of ice-cold 0.32 M sucrose buffer, centrifuged at 900 × *g* for 10 min (4 °C), and the supernatant was recentrifuged at 48000 × *g* for 25 min (4 °C). The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.5), incubated at 37 °C for 15 min, and then centrifuged once more at 48000 × *g* for 25 min (4 °C). The final pellet was resuspended in 4 volumes of ice-cold 50 mM Tris–HCl buffer containing 4 mM $CaCl_2$ and 0.1% ascorbic acid and was stored at −70 °C until use. At the time of the experiment, the membranes were diluted in the same ice-cold buffer with 10 μM pargyline (final dilution 1:28, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration and 0.1 mL of [^{3}H]8-OH-DPAT (NEN, 148–163 Ci/mmol) in Tris–HCl buffer (final concentration 1.5 nM). Nonspecific binding was determined using 10 μM cold 5-HT. The binding experiment was initiated by addition of 0.8 mL of membrane suspension (700–800 μg of protein). After incubation for 30 min at 37 °C, the reaction was stopped for vacuum filtration through Whatman GF/B glass filters, using a Brandel cell harvester, followed by two washes with 5 mL of ice-cold 50 mM Tris–HCl (pH 7.5) buffer. Filters were placed in scintillating polyethylene vials (with 5 mL scintillation cocktail) and equilibrated. Filter-retained radioactivity was measured in a liquid scintillation counter (Kontron Beta V). IC_{50} and K_i values were calculated using the computer program EBDA (McPherson) [27].

5.2.3. D_2 receptor binding assay [26]

Adult male Wistar rats weighing 220–280 g were used. Animals were killed by decapitation, the whole brain with the exception of the brain stem and cerebellum was quickly removed, and the various areas were dissected, weighed, and immediately frozen at −70 °C. Striatum used for the binding experiments was homogenised with an Ultra-Turrax (setting 5 for 20 s) in 50 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.7) and centrifuged at 48000 × *g* for 10 min (4 °C). The resulting pellet was resuspended in 50 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.7), incubated at 37 °C for 10 min, and then centrifuged once more at 48000 × *g* for 10 min (4 °C). The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) and was stored at −70 °C until use. At the time of experiment, the membranes were diluted in the same ice-cold buffer with 10 μM pargyline (final dilution 1:150, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration and 0.1 mL of [^{3}H]raclopride (NEN, 60–87 Ci/mmol) in Tris HCl buffer (final concentration 1 nM). Nonspecific binding was determined using 1 μM cold (+)-butaclamol. The binding experiment was initiated by addition of 0.8 mL of membrane suspension (300–400 μg of protein). After incubation for 60 min at 25 °C, the reaction was stopped for vacuum filtration through Whatman GF/B glass filters, using a Brandel cell harvester, followed by two washes with 5 mL of ice-cold 50 mM Tris–HCl (pH 7.7) buffer. Filters were placed in scintillating polyethylene vials (with 5 mL scintillation cocktail) and equilibrated. Filter-retained radioactivity was measured in a liquid scintillation counter (Kontron Beta V). IC_{50} and K_i values were calculated using the computer program EBDA (McPherson) [27].

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