Eur J Med Chem (1996) 31, 957–964

Synthesis and binding affinities of a series of 1,2-benzisoxazole-3-carboxamides to dopamine and serotonin receptors

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(Received 26 February 1996; accepted 12 June 1996)

Summary — A series of 1,2-benzisoxazole-3-carboxamides derived from tertiary cycloalkylamines was synthesized and evaluated for affinity for serotonergic (5-HT_3 and 5-HT_4) and dopaminergic (D_2) receptors using radioligand binding assays. The majority of compounds displayed a very weak affinity for the studied neurotransmitter receptors. Only amides containing a conformationally rigid system retained a relative 5-HT_3 receptor affinity. The presence of a quinuclidine group affected receptor interaction more favorably than the tropane framework.

5-HT_3 receptor / 5-HT_4 receptor / dopamine D_2 receptor / 1,2-benzisoxazole-3-carboxamide

Introduction

Over the last decade, a wide expansion has occurred in the search for 5-HT_3/5-HT_4 receptor ligands, because of their numerous potential applications in therapy. 5-HT_3 receptor antagonists are useful in preventing the emesis associated with anticancer chemotherapy [1] and are attractive for the possible treatment of anxiety [2] or migraine [3]. On the other hand, the 5-HT_4 class of serotonin receptors is of clinical interest due to its role in the regulation of gastrointestinal motility [4] and possible involvement in various affective disorders [5]. The potential therapeutic utility of 5-HT_4 receptor antagonists has recently been reviewed [6].

Benzamides (fig 1) represent a starting point for the design of ligands for the 5-HT_3 receptor, but the modest selectivity observed with some compounds has limited their usefulness. Metoclopramide, zacopride, renzapride and cisapride also act as agonists at the 5-HT_4 receptor [7, 8] explaining the gastroprokinetic activity of these derivatives. The benzamide framework often has dopamine D_2 receptor antagonistic activity [9, 10], which is responsible for unfavorable side effects such as extrapyramidal symptoms and central nervous system depression. With the aim of increasing 5-HT_3 receptor selectivity, extensive research has led to various structural classes such as imidazoles [11, 12], indoles [13] and quinolines [14].

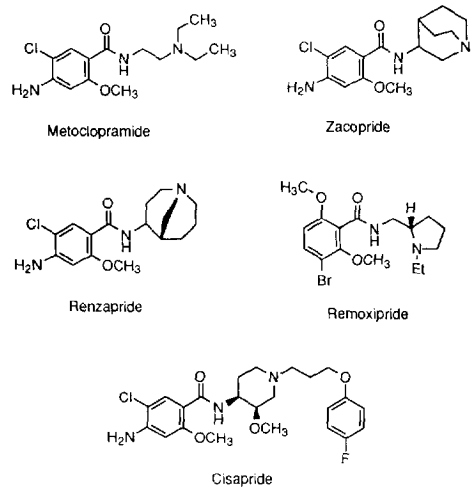

Fig 1. Some benzamides with ligand properties to dopamine and/or serotonin receptors.

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Recent papers [15, 16] have demonstrated the value of designing conformationally constrained benzamide derivatives to gain selectivity. It is well established that the *ortho*-methoxybenzamides are characterized by an intramolecular hydrogen bond between the amidic N-H and the *o*-methoxy group, thus forming a planar pseudo-ring system [17]. In order to study the effect of further conformational restriction about the aromatic nucleus, we decided to investigate 1,2-benzisoxazole-3-amide derivatives containing an azacycloalkyl group connected via an aliphatic linkage and corresponding to the general formula shown in figure 2. In this paper, we report the synthesis of these compounds and their affinities for the serotonin ($5\text{-}HT_3$ and $5\text{-}HT_4$) and dopamine (D_2) receptors according to radioligand binding techniques.

Chemistry

The general synthetic procedures are outlined in figure 3. Commercially available 2,4-dinitrophenyl acetic acid **1** was converted into methyl ester **2**. The key intermediate (methyl 6-nitro-1,2-benzisoxazole-3-carboxylate) **3** was prepared by reaction of **2** with an alkyl nitrite, according to a modified procedure of that initially described by Borsche [18]. Indeed, even when commercial isoamyl nitrite was distilled prior to use, it gave only poor yields in this cyclization reaction. The utilization of freshly prepared pentyl nitrite [19] was preferred on account of the better results obtained.

The target benzisoxazole carboxamides were obtained by a one-step procedure [20] involving reaction of the appropriate amine with **3** in refluxing methanol. The synthesis of the 3-quinuclidinylbenzisoxazole carboxamide **13** was accomplished from the commercially available (±) 3-aminoquinuclidine and gave a mixture of (+) and (–) epimers which were not separated. The tropane amides **14a** and **15a** were generated from 3β- and 3α-aminotropane, respectively. The 3β-aminotropane was obtained by reduction of tropinone oxime with sodium in 1-pentanol whereas the α-isomer was synthesized via the benzyl intermediate

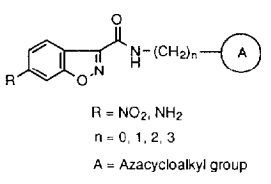


Fig 2. General formula of 1,2-benzisoxazole-3-amide derivatives.

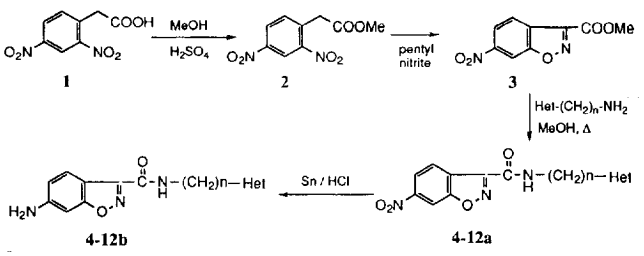


Fig 3. Synthesis of compounds **4**–**12**. The structure of Het-$(CH_2)_n$-NH_2 is shown in table I.

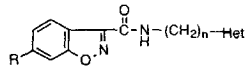
which was subjected to catalytic hydrogenation over palladium on carbon [21]. The chemical characteristics of the products obtained are listed in table I.

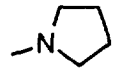
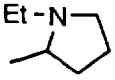
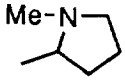
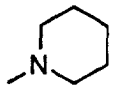
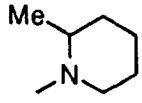
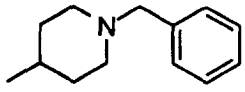
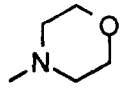
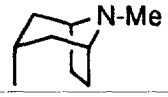
The stereochemistry of the tropane amides **14a** and **15a** was determined by ^1H and ^{13}C NMR spectroscopy, including two-dimensional ^1H,^1H-COSY spectra [22] and heteronuclear correlation experiments HMQC [23] and HMBC [24]. The ^1H and ^{13}C data and selected HMBC correlations for the two isomers are given in table II.

The preferred conformation of the tropane system can be deduced from the ^{13}C NMR chemical shifts. In both compounds, the values for the corresponding carbons are practically the same. The δ value of the C12(12') signal at ca 34–35 ppm is in good agreement with previously reported data for a tropane system with a flattened chair conformation of the piperidine ring and an axial position of the *N*-methyl with respect to this ring [27].

Assignments of proton resonances were made taking into account literature data for other tropane amides [25–27]. Two remarkable differences in ^1H chemical shifts were observed between the two isomers. The protons H15(15')n in the **15a** isomer appeared at δ 1.87 ppm, whereas the corresponding proton in the **14a** isomer resonated at δ 1.58 ppm. This fact can be attributed to the change of the stereochemistry at C11 and the deshielding effect exerted by the amido group on H15(15')n protons [25] in **15a**. In addition, protons H12(12')β appeared at 2.08 ppm for the **15a** isomer, and at 1.60 ppm for the derivative **14a**, respectively. The decisive information on the stereochemistry at C11 is given by the HMBC experiment. Analysis of the data for **15a** revealed a key correlation from C13(13') to H11. This is consistent with the β position for proton H11, thus defining the amide group in the α position. Since this correlation does not show up in compound **14a**, it is concluded that dihedral angle between proton H11 and carbon C13(13') is close to 90° and therefore the amide substituent in **14a** isomer is β.

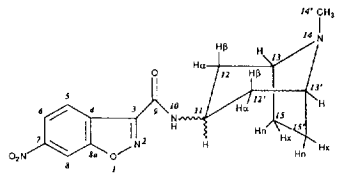
Table I. Chemical data for 1,2-benzisoxazole-3-carboxamides **4–15.**



Compound	*Het*	*n*	*R*	*Yield (%)*	*Mp (°C)*	*Formula (MW)*
4a		2	NO_2	81	146	$C_{14}H_{16}N_4O_4$ (304.31)
4b		2	NH_2	50	152	$C_{14}H_{18}N_4O_2$ (274.32)
5a		1	NO_2	65	80–81	$C_{15}H_{18}N_4O_4$ (318.33)
6a		2	NO_2	72	94	$C_{15}H_{18}N_4O_4$ (318.33)
6b		2	NH_2	31	114	$C_{15}H_{20}N_4O_2$ (288.35)
7a		3	NO_2	78	182	$C_{15}H_{16}N_4O_5$ (332.32)
7b		3	NH_2	22	147	$C_{15}H_{18}N_4O_3$ (302.33)
8a		2	NO_2	87	100	$C_{15}H_{18}N_4O_4$ (318.33)
8b		2	NH_2	48	167	$C_{15}H_{20}N_4O_2$ (288.35)
9a		3	NO_2	80	112	$C_{17}H_{22}N_4O_4$ (346.39)
9b		3	NH_2	54	150	$C_{17}H_{24}N_4O_2$ (316.41)
10a		0	NO_2	61	173	$C_{20}H_{20}N_4O_4$ (380.41)
10b		0	NH_2	53	181	$C_{20}H_{22}N_4O_2$ (350.42)
11a		2	NO_2	89	171	$C_{14}H_{16}N_4O_5$ (320.31)
11b		2	NH_2	65	147	$C_{14}H_{18}N_4O_3$ (290.32)
12a		3	NO_2	83	144[a]	$C_{15}H_{18}N_4O_5$ (334.33)
12b		3	NH_2	54	115	$C_{15}H_{20}N_4O_3$ (304.35)
12c		3	NH-Ac	73	168	$C_{17}H_{22}N_4O_4$ (346.39)
12d		3	Cl	20	95	$C_{15}H_{18}ClN_3O_3$ (323.78)
13a		0	NO_2	41	172–173	$C_{15}H_{16}N_4O_4$ (316.32)
13b		0	NH_2	50	202–204	$C_{15}H_{18}N_4O_2$ (286.34)
14a		0	NO_2	32		$C_{16}H_{18}N_4O_4$ (330.34)
14b		0	NH_2	40	245	$C_{16}H_{20}N_4O_2$ (300.36)
15a		0	NO_2	22	198	$C_{16}H_{18}N_4O_4$ (330.34)
15b		0	NH_2	34	216	$C_{16}H_{20}N_4O_2$ (300.36)

[a]Melting point unexpressed in reference [20].

Table II. ^{1}H (500 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) NMR data[a] and selected HMBC correlations for **14a** and **15a**.



Position	***14a***			***15a***		
	^{1}H NMR	*^{13}C NMR*	*HMBC*	*^{1}H NMR*	*^{13}C NMR*	*HMBC*
3	–	151.9	C3, H5	–	152.2	C3, H5
4	–	123.7	C4, H5 C4, H6 C4, H8	–	123.5	C4, H5 C4, H6 C4, H8
5	8.29 (dd, 8.8, 1.6)	119.4	C5, H8	8.31 (dd, 8.8, 1.6)	119.5	C5, H8
6	8.26 (d, 8.8)	124.0	–	8.26 (d, 8.8)	123.8	–
7	–	148.5	C7, H6 C7, H8	–	148.6	C7, H5 C7, H8
8	8.76 (br s)	105.9		8.82 (br s)	106.0	C8, H6
8a	–	161.5	C8a, H8	–	161.5	C8a, H5 C8a, H8
9	–	156.3	C9, H10 C9, H11	–	156.9	C9, H10 C9, H11
10	9.04 (d, 8.3)	–		8.60 (d, 8.3)	–	
11	4.20 (m)	40.6	C11, H10 C11, H13(13') C11, H12(12')α C11, H12(12')β	4.04 (m)	41.3	C11, H10 C11, H13(13') C11, H12(12')α C11, H12(12')β
12 (12')	Hα: 1.78 (m) Hβ: 1.60 (m)[b]	34.8		Hα: 1.78 (d, 14.1) Hβ: 2.08 (m)	34.2	
13 (13')	3.09 (m)	59.2		3.03 (m)	58.5	C13(13'), H11
14'	2.21 (s)	37.9		2.15 (s)	39.2	
15 (15')	Hx: 1.95 (m) Hn: 1.58 (m)[b]	25.7		Hx: 1.98 (m) Hn: 1.87 (m)	24.9	

[a]Chemical shifts, ppm (multiplicity, *J* in Hz); [b]overlapping protons were assigned by COSY experiments.

Pharmacology

The binding affinities of the prepared compounds for three neurotransmitter receptors were evaluated via radioligand assays on different tissue homogenates. The affinity for the serotonin 5-HT_3 receptor was examined by studying the ability of the derivatives to inhibit specific binding of [^{3}H]BRL 43694 to NG 108-15 cells. Binding at the 5-HT_4 receptor was studied in pig hippocampus tissue labeled with [^{3}H]GR 113808. Bovine striatum labeled with [^{3}H]raclopride was used to define binding to D_2 dopamine receptor. In these experiments, ICS 205930, 5-hydroxytryptamine and spiperone were chosen to assess non-specific binding. Structures of the radioligand and reference products used in the assays are indicated in figure 4.

Biological results and discussion

The pharmacological behavior of some selected derivatives at 5-HT_3, 5-HT_4 and D_2 receptors is indicated in table III. As seen from the data, the K_i values of the most interesting compounds were in the micromolar range and therefore demonstrated a modest activity.

None of the nitro compounds (**4a**, **5a**, **6a** and **13a**) at concentrations of 15–100 μM could displace the specific radioligands to the studied receptor sites. In contrast, amino compounds **4b** and **6b** showed weak but measurable affinity and revealed the beneficial effect of the reduction of the nitro group. Replacement of the NH_2 substituent by a chlorine atom (**12d**) led only to undesirable losses of affinity.

Although the interaction of compounds **10b** and **12b** with the 5-HT_4 receptor site (K_i values ranged from 2.0 to 2.6 μM) was not very significant, it might be related to the length of the hydrocarbon linkage between the amidic NH and the tertiary nitrogen atom. The common structural feature for these compounds was a three carbon bridge separating the two heteroatoms (propyl in **12b** or part of a 4-piperidine ring in **10b**).

The most interesting responses were obtained with compounds bearing conformationaly restrained bicyclic amine systems. Thus, the quinuclidinyl compound **13b** presented a relative affinity and a fairly good selectivity for serotonin 5-HT_3 receptors (K_i = 0.25 μM) and did not bind to 5-HT_4 and D_2 receptors (K_i > 10 μM). This result contrasts with zacopride which is a potent 5-HT_3 antagonist but also interacts with 5-HT_4 receptors [7].

Introduction of a tropane group led to compounds with 5-HT_3 receptor binding properties (**14b** and **15b**). As commonly described in the tropanyl-derived 5-HT_3 receptor antagonists, biological activity proved to be extremely sensitive to the stereochemistry of the molecule [17, 28]. Thus, the *exo* isomer **14b** modestly interacted with both serotonergic receptors with a very low selectivity for $5HT_4$ receptor (K_i = 2.34 μM; 5-HT_3/$5HT_4$ = 0.46). In contrast, the *endo* isomer **15b** had an affinity and a selectivity for serotonin 5-HT_3 receptors (K_i = 0.65 μM; 5-HT_3/5-HT_4 = 4.6) and did not bind to D_2 receptors (K_i > 10 μM). The lack of D_2 activity could be explained by the absence of a methoxy group in the *ortho* position with respect to the amide linkage, since it was demonstrated that such a characteristic is essential for D_2 activity in the benzamide family [29].

In conclusion, our results are in agreement with the commonly recognized chemical features implicated in the 5-HT_3 receptor affinity for potential antagonists.

Table III . Binding profile to serotonergic and dopaminergic receptors.

Compounds	*Inhibition constant, K_i (μM)*		
	Dopamine	*Serotonin*	
	D_2	*5-HT_3*	*5-HT_4*
4a	29	> 100	> 100
4b	29	6.2	5.9
5a	15	> 100	> 100
6a	> 100	> 100	> 100
6b	17	18	16
10b	6.4	31	2.6
12b	4.6	> 100	2.0
12d	39	> 100	29
13a	> 100	15	17
13b[a]	> 10	0.25	> 10
14b	> 10	5.08	2.34
15b	> 10	0.65	3.41
Pimozide	0.51×10^{-3}		
ICS 205930		0.9×10^{-3}	
Cisapride			7.4×10^{-3}

[a]This compound was tested as racemate.

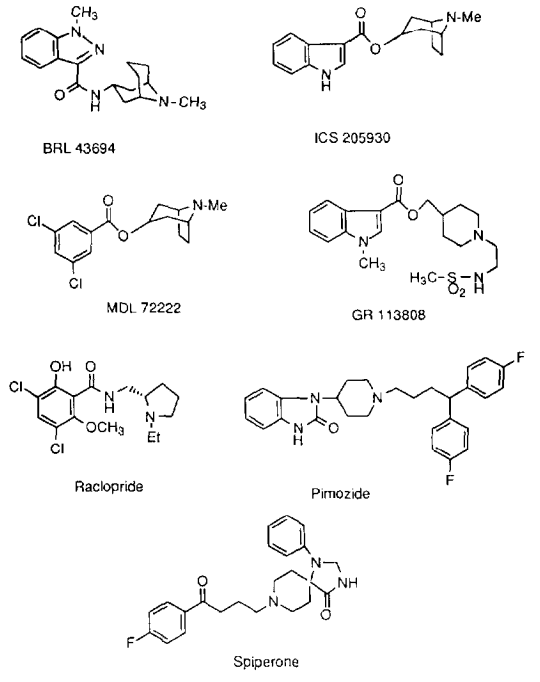


Fig 4. Structures of radioligand and reference products used in the pharmacological assays.

The favorable role [30] of azabicycloalkyl substituents such as tropane or quinuclidine was again highlighted. However, the incorporation of a carboxamide linkage led to a very limited activity. It has been demonstrated in other serotonergic ligands (ICS 205930, MDL 72222) that the presence of an ester spacer group resulted in a relative enhancement of the receptor affinity in comparison with the amide derivatives. Such a structural modification is in preparation in our laboratory.

Experimental protocols

Chemistry

The structures of all the compounds were supported by the IR spectra (KBr pellets, Shimadzu IR 470 spectrometer) and ^{1}H NMR data (60 MHz, Varian EM 360 L spectrometer; tetramethylsilane internal reference). High-resolution spectra (^{1}H, ^{13}C, HMQC, HMBC, ^{1}H, ^{1}H-COSY) were obtained on a Bruker AMX 500 spectrometer. Melting points were determined with an Electrothermal digital capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Service central d'analyse du CNRS (Vernaison, France) and were within ± 0.4% of the calculated values.

General procedure for the preparation of 6-nitro-1,2-benzisoxazole-3-carboxamides ***4a–15a***

To a solution of methyl 6-nitro-1,2-benzisoxazole-3-carboxylate [18] (3.33 g, 15 mmol) in warm methanol (50 mL) was added the appropriate amine (18 mmol). The reaction mixture was refluxed for 5 h under vigorous stirring and then kept at room temperature for 12 h. The resulting precipitate was collected by suction filtration, washed with the minimum volume of diethyl ether, then recrystallized from heptane/acetone (90:10). According to this method, the following compounds were prepared.

6-Nitro-N-[(1-pyrrolidinyl)ethyl]-1,2-benzisoxazole-3-carboxamide ***4a***. Compound **4a** was prepared from 1-(2-aminoethyl)pyrrolidine. IR (KBr, cm^{-1}): 3200 (νNH); 1665 (νCO); 1350, 1525 (νNO$_2$). ^{1}H NMR ($CDCl_3$) δ 1.70–2.05 (m, 4H, 2 × CH_2); 2.45–2.95 (m, 6H, 3 × CH_2); 3.50–3.85 (m, 2H, CH_2NH); 7.65 (br, s, 1H, NH); 8.25–8.65 (m, 3H, ArH). Anal $C_{14}H_{16}N_4O_4$ (C, H, N).

6-Nitro-N-[(1-ethyl-2-pyrrolidinylmethyl]-1,2-benzisoxazole-3-carboxamide ***5a***. Compound **5a** was prepared from 2-aminomethyl-1-ethyl pyrrolidine. IR (KBr, cm^{-1}): 3400 (νNH); 1680 (νCO); 1340, 1530 (νNO$_2$). ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3H, CH_3); 1.60–3.73 (m, 10H, 5 × CH_2); 3.83–4.05 (m, 1H, CHN-Pyr); 7.65 (br, s, 1H, NH); 8.23–8.66 (m, 3H, ArH). Anal $C_{15}H_{18}N_4O_4$ (C, H, N).

6-Nitro-N-[(1-methyl-2-pyrrolidinylethyl]-1,2-benzisoxazole-3-carboxamide ***6a***. Compound **6a** was pepared from 2-(2-aminoethyl)-1-methyl pyrrolidine. IR (KBr, cm^{-1}): 3400 (νNH); 1675 (νCO); 1345, 1535 (νNO$_2$). ^{1}H NMR (CCl_4) δ 1.60–2.63 (m, 6H, 3 × CH_2); 2.40 (*s*, 3H, CH_3); 3.00–4.06 (m, 5H, 2 × CH_2 + CHN-Pyr); 8.10–8.55 (m, 3H, ArH); 8.90 (br, s, 1H, NH). Anal $C_{15}H_{18}N_4O_4$ (C, H, N).

6-Nitro-N-[(2-oxo-1-pyrrolidinyl)propyl-1,2-benzisoxazole-3-carboxamide ***7a***. Compound **7a** was prepared from 1-(3-aminopropyl)-2-pyrrolidinone. IR (KBr, cm^{-1}): 3290 (νNH); 1665, 1680 (νCO); 1350, 1520 (νNO$_2$). ^{1}H NMR (DMSO-d_6) δ 1.60–2.30 (m, 6H, 3 × CH_2 Pyr); 3.15–3.60 (m, 6H, 3 × CH_2); 8.20–8.40 (m, 2H, ArH); 8.75–8.85 (m, 1H, ArH); 9.15 (t, 1H, NH). Anal $C_{15}H_{16}N_4O_5$ (C, H, N).

6-Nitro-N-[(1-piperidinyl) ethyl]-1,2-benzisoxazole-3-carboxamide ***8a***. Compound **8a** was prepared from 1-(2-aminoethyl) piperidine. IR (KBr, cm^{-1}): 3200 (νNH); 1665 (νCO); 1350, 1515 (νNO$_2$). ^{1}H NMR ($CDCl_3$) δ 1.45–1.85 (m, 6H, 3 × CH_2 Pip); 2.35–2.75 (m, 6H, 3 × CH_2); 3.45–3.85 (m, 2H, CH_2-NH); 7.75 (br, s, 1H, NH); 8.15–8.65 (m, 3H, ArH). Anal $C_{15}H_{18}N_4O_4$ (C, H, N).

6-Nitro-N-[(2-methyl-1-piperidinyl)propyl]-1,2-benzisoxazole-3-carboxamide ***9a***. Compound **9a** was preapred from 1-(3-aminopropyl)-2-methyl piperidine. IR (KBr, cm^{-1}): 3250 (νNH); 1680 (νCO); 1345, 1530 (νNO$_2$). ^{1}H NMR (CCl_4) δ 1.15 (d, 3H, CH_3); 1.45–2.00 (m, 8H, 4 × CH_2); 2.05–2.55 (m, 3H, CH_2 + CHN Pip); 2.65–3.25 (m, 2H, CH_2); 3.35–3.85 (m, 2H, CH_2); 8.10–8.55 (m, 3H, ArH); 9.35 (br, s, 1H, NH). Anal $C_{17}H_{22}N_4O_4$ (C, H, N).

6-Nitro-N-[(1-benzyl-4-piperidinyl)-1,2-benzisoxazole-3-carboxamide ***10a***. Compound **10a** was prepared from 4-amino-1-benzyl piperidine. IR (KBr, cm^{-1}): 3400 (νNH); 1675 (νCO); 1325, 1535 (νNO$_2$). ^{1}H NMR ($CDCl_3$) δ 1.45–3.10 (m, 8H, 4 × CH_2); 3.55 (*s*, 2H, CH_2); 3.85–4.35 (m, 1H, C*H*-NH); 6.95 (d, 1H, NH); 7.35 (*s*, 5H, ArH); 8.15–8.60 (m, 3H, ArH). Anal $C_{20}H_{20}N_4O_4$ (C, H, N).

6-Nitro-N-[(1-morpholinyl)ethyl]-1,2-benzisoxazole-3-carboxamide ***11a***. Compound **11a** was prepared from 1-(2-aminoethyl) morpholine. IR (KBr, cm^{-1}): 3360 (νNH); 1675 (νCO); 1350, 1530 (νNO$_2$). ^{1}H NMR ($CDCl_3$) δ 2.45–2.85 (m, 6H, 3 × CH_2); 3.55–3.95 (m, 6H, 3 × CH_2); 7.55 (br, s, 1H, NH); 8.15–8.65 (m, 3H, ArH). Anal $C_{14}H_{16}N_4O_5$ (C, H, N).

6-Nitro-N-[(1-morpholinyl)propyl]-1,2-benzisoxazole-3-carboxamide ***12a***. Compound **12a** was prepared from 1-(3-aminopropyl) morpholine. IR (KBr, cm^{-1}): 3320 (νNH); 1660 (νCO); 1350, 1535 (νNO$_2$). ^{1}H NMR ($CDCl_3$) δ 1.60–2.10 (m, 2H, CH_2); 2.45–2.80 (m, 6H, 3 × CH_2); 3.45–3.95 (m, 6H, 3 × CH_2); 8.05–8.55 (m, 3H, ArH); 9.10 (br, s, 1H, NH). Anal $C_{15}H_{18}N_4O_5$ (C, H, N).

(±) *6-Nitro-N-(1-azabicyclo[2.2.2]octan-3-yl)-1,2-benzisoxazole-3-carboxamide* ***13a***. Compound **13a** was prepared from (±) 3-aminoquinuclidine. IR (KBr, cm^{-1}): 3400 (νNH); 1660, 1680 (νCO); 1350, 1530 (νNO$_2$). ^{1}H NMR (DMSO-d_6) δ 1.30–2.05 (m, 4H, 2 × CH_2); 2.45–3.25 (m, 6H, 3 × CH_2); 3.30–3.50 (m, 1H, CH); 3.80–4.30 (m, 1H, CH-N); 8.25–8.45 (m, 2H, ArH); 8.80–8.95 (m, 1H, ArH); 9.30 (d, 1H, NH). Anal $C_{15}H_{16}N_4O_4$ (C, H, N).

6-Nitro-N-(8-methyl-8-azabicyclo[3.2.1]octan-3-yl)-1,2-benzisoxazole-3-carboxamide ***14a***. Compound **14a** was prepared from 3β-aminotropane [21]. IR (KBr, cm^{-1}): 3400 (νNH); 1675 (νCO); 1345, 1535 (νNO$_2$). ^{1}H and ^{13}C NMR: see table II. Anal $C_{16}H_{18}N_4O_4$ (C, H, N).

6-Nitro-N-[(8-methyl-8-azabicyclo[3.2.1]octan-3-α-yl)-1,2-benzisoxazole-3-carboxamide ***15a***. Compound **15a** was prepared from 3α-aminotropane [21]. IR (KBr, cm^{-1}): 3420 (νNH); 1680 (νCO); 1345, 1530 (νNO$_2$). ^{1}H and ^{13}C NMR: see table II. Anal $C_{16}H_{18}N_4O_4$ (C, H, N).

General procedure for the preparation of 6-amino-1,2-benzisoxazole-3-carboxamides ***4b–15b***

The appropriate 6-nitro-1,2-benzisoxazole-3-carboxamide **4a–15a** (10 mmol) and powdered tin (3.56 g, 30 mmol) were thoroughly ground together and then concentrated hydrochloric acid (10 mL) was added slowly with vigorous stirring. After all the acid had been added, the reaction mixture was boiled for 30 min, and then allowed to cool to room temperature. The solution was diluted with water (50 mL), cooled in an ice bath and made alkaline with a 20% solution of sodium hydroxide added over 5–10 min. The resulting precipitate was filtered, washed with 2 M NaOH, and then with water. For purification, the material was dissolved in hot ethanol (50 mL) and filtered hot. After the filtrate was concentrated, the obtained solid was recrystallized from heptane/ethyl acetate (50:10). According to this method, the following compounds were prepared.

6-Amino-N-[(1-pyrrolidinyl)ethyl]-1,2-benzisoxazole-3-carboxamide ***4b***. Compound **4b** was prepared from **4a**. IR (KBr, cm^{-1}): 3300, 3450 (νNH_2); 3200 (νNH); 1650 (νCO). ^1H NMR (DMSO-d_6) δ 1.55–1.90 (m, 4H, 2 × CH_2); 2.35–2.85 (m, 6H, 3 × CH_2); 3.50 (q, 2H, CH_2NH); 6.10 (*s*, 2H, NH_2); 6.73–6.95 (m, 2H, ArH); 7.80 (d, 1H, ArH); 8.65 (t, 1H, NH). Anal $C_{14}H_{18}N_4O_2$ (C, H, N).

6-Amino-N-[(1-methyl-2-pyrrolidinyl)ethyl]-1,2-benzisoxazole-3-carboxamide ***6b***. Compound **6b** was prepared from **6a**. IR (KBr, cm^{-1}): 3400, 3500 (νNH_2); 3350 (νNH); 1660 (νCO). ^1H NMR ($CDCl_3$) δ 1.45–2.55 (m, 8H, 4 × CH_2); 2.35 (s, 3H, CH_3); 2.90–3.30 (*m*, 1H, CH); 3.35–3.85 (m, 2H, CH_2NH); 4.45 (s, 2H, NH_2); 6.65–6.90 (m, 2H, ArH); 8.00 (d, 1H, ArH); 8.25 (br, s, 1H, NH). Anal $C_{15}H_{20}N_4O_2$ (C, H, N).

6-Amino-N-[(2-oxo-1-pyrrolidinyl)propyl]-1,2-benzisoxazole-3-carboxamide ***7b***. Compound **7b** was prepared from **7a**. IR (KBr, cm^{-1}): 3350, 3420 (νNH_2); 3240 (νNH); 1650 (νCO). ^1H NMR (DMSO-d_6) δ 1.60–2.30 (m, 6H, 3 × CH_2 Pyr); 3.25–3.55 (m, 6H, 3 × CH_2); 6.10 (s, 2H, NH_2); 6.65–6.90 (m, 2H, ArH); 7.75 (d, 1H, ArH); 8.80 (t, 1H, NH). Anal $C_{15}H_{18}N_4O_3$ (C, H, N).

6-Amino-N-[(1-piperidinyl)ethyl]-1,2-benzisoxazole-3-carboxamide ***8b***. Compound **8b** was prepared from **8a**. IR (KBr, cm^{-1}): 3320, 3450 (νNH_2); 3200 (νNH); 1655 (νCO). ^1H NMR (DMSO-d_6) δ 1.30–1.65 (m, 6H, 3 × CH_2 Pip); 2.25–2.65 (m, 6H, 3 × CH_2); 3.25–3.65 (m, 2H, CH_2NH); 6.05 (s, 2H, NH_2); 6.65–6.95 (m, 2H, ArH); 7.80 (d, 1H, ArH); 8.60 (t, 1H, NH). Anal $C_{15}H_{20}N_4O_2$ (C, H, N).

6-Amino-N-[(2-methyl)-1-piperidinyl)propyl]-1,2-benzisoxazole-3-carboxamide ***9b***. Compound **9b** was prepared from **9a**. IR (KBr, cm^{-1}): 3360, 3450 (νNH_2); 3250 (νNH); 1655 (νCO). ^1H NMR (DMSO-d_6) δ 1.00 (d, 3H, CH_3); 1.15–3.00 (m, 15H, 7 × CH_2 + CHN-Pip); 3.15–3.60 (q, 2H, CH_2NH); 6.05 (s, 2H, NH_2); 6.60–6.90 (m, 2H, ArH); 7.75 (d, 1H, ArH); 9.05 (t, 1H, NH). Anal $C_{17}H_{24}N_4O_2$ (C, H, N).

6-Amino-N-(1-benzyl-4-piperidinyl)-1,2-benzisoxazole-3-carboxamide ***10b***. Compound **10b** was prepared from **10a**. IR (KBr, cm^{-1}): 3400, 3500 (νNH_2); 3300 (νNH); 1645 (νCO). ^1H NMR (DMSO-d_6) δ 1.55–3.00 (m, 8H, 4 × CH_2); 3.45 (*s*, 2H, CH_2); 3.65–4.05 (m, 1H, CHNH); 6.10 (*s*, 2H, NH_2); 6.75–6.95 (m, 2H, ArH); 7.35 (s, 5H, ArH); 7.75 (d, 1H, ArH); 8.75 (d, 1H, NH). Anal $C_{20}H_{22}N_4O_2$ (C, H, N).

6-Amino-N-[(1-morpholinyl)ethyl]-1,2-benzisoxazole-3-carboxamide ***11b***. Compound **11b** was prepared from **11a**. IR (KBr, cm^{-1}): 3400, 3500 (νNH_2); 3300 (νNH); 1655 (νCO). ^1H NMR ($CDCl_3$) δ 2.35–2.75 (m, 6H, 3 × CH_2); 3.40–3.85 (m, 6H, 3 × CH_2); 4.45 (s, 2H, -NH_2); 6.60–6.85 (m, 2H, ArH); 7.50 (br, s, 1H, NH); 8.00 (d, 1H, ArH). Anal $C_{14}H_{18}N_4O_3$ (C, H, N).

6-Amino-N-[(1-morpholinyl)propyl]-1,2-benzisoxazole-3-carboxamide ***12b***. Compound **12b** was prepared from **12a**. IR (KBr, cm^{-1}): 3360, 3430 (νNH_2); 3350 (νNH); 1655 (νCO). ^1H NMR ($CDCl_3$) δ 1.50–2.05 (m, 2H, CH_2); 2.25–2.75 (m, 6H, 3 × CH_2); 3.45–3.95 (m, 6H, 3 × CH_2); 4.35 (*s*, 2H, NH_2); 6.55–6.85 (m, 2H, ArH); 7.95 (d, 1H, ArH); 8.65 (br, s, 1H, NH). Anal $C_{15}H_{20}N_4O_3$ (C, H, N).

6-Amino-N-(8-methyl-8-azabicyclo[3.2.1]octan-3-β-yl)-1,2-benzisoxazole-3-carboxamide ***14b***. Compound **14b** was prepared from **14a**. IR (KBr, cm^{-1}): 3380, 3500 (νNH_2); 3320 (νNH); 1655 (νCO). ^1H NMR (DMSO-d_6) δ 1.35–2.15 (m, 8H); 2.25 (s, 3H, N-CH_3); 2.95–3.30 (m, 2H, CHN); 3.80–4.45 (m, 1H, CHNCO); 6.00 (s, 2H, NH_2); 6.55–6.85 (m, 2H, ArH) ; 7.65 (d, 1H, ArH); 8.60 (d, 1H, NH). Anal $C_{16}H_{20}N_4O_2$ (C, H, N).

6-Amino-N-(8-methyl-8-azabicyclo[3.2.1]octan-3-α-yl)1,2-benzisoxazole-3-carboxamide ***15b***. Compound **15b** was prepared from **15a**. IR (KBr, cm^{-1}): 3320, 3450 (νNH_2); 3150 (νNH); 1670 (νCO). ^1H NMR (DMSO-d_6) δ 1.45–2.05 (m, 8H); 2.15 (s, 3H, NCH_3); 2.90–3.25 (m, 2H, CHN); 3.85–4.25 (m, 1H, CHNCO); 6.10 (s, 2H, NH_2); 6.65–6.95 (m, 2H, ArH); 7.75 (d, 1H, ArH); 8.25 (d, 1H, NH). Anal $C_{16}H_{20}N_4O_2$ (C, H, N).

(±)-6-Amino-N-(1-azabicyclo[2.2.2]octan-3-yl)-1,2-benzisoxazole-3-carboxamide ***13b***

A solution of tin(II) chloride (9.48 g, 50 mmol) in concentrated hydrochloric acid (10 mL) was added to a suspension of **13a** (1.58 g, 5 mmol) in hydrochloric acid (10 mL) and stirred at room temperature for a week. The reaction mixture was concentrated to dryness. The residue was taken up into 2 M sodium hydroxide (200 mL) and extracted with ethyl acetate (3 × 50 mL). Evaporation of the dried (Na_2SO_4) extracts and recrystallization of the residue from heptane/ethyl acetate (75:25) afforded 0.71 g (50%) of **13b**, mp 202–204 °C. IR (KBr, cm^{-1}): 3300, 3450 (νNH_2); 3125 (νNH); 1655 (νCO). ^1H NMR (DMSO-d_6) δ 1.30–2.05 (m, 4H, 2 × CH_2); 2.50–3.35 (m, 6H, 3 × CH_2); 3.80–4.30 (m, 1H, CHN); 6.10 (s, 2H, NH_2); 6.70–6.95 (m, 2H, ArH); 7.70 (d, 1H, ArH); 8.85 (d, 1H, NH). Anal $C_{15}H_{18}N_4O_2$ (C, H, N).

6-Chloro-N-[(morpholinyl)propyl]-1,2-benzisoxazole-3-carboxamide ***12d***

To a well-stirred mixture of **12b** (3.04 g, 10 mmol) in concentrated hydrochloric acid (5 mL) and water (5 mL), cooled to 0–5 °C, was slowly added a solution of sodium nitrite (0.76 g, 11 mmol) in water (5 mL) until the reaction mixture showed a positive starch-iodide test for nitrous acid.

A solution of copper(I) chloride was prepared as follows. To a solution of copper(II) sulfate pentahydrate (3.74 g, 15 mmol) and sodium chloride (0.99 g, 17 mmol) in hot water (15 mL), was added a solution of sodium metabisulfite (0.76 g, 4 mmol) in water (10 mL), with constant stirring. The precipitate obtained was collected by filtration, washed thoroughly with water and dissolved in concentrated hydrochloric acid (7.5 mL).

To 5 mL of this solution was added the cold diazonium solution under vigorous stirring. The mixture was allowed to warm to room temperature and then heated at 90 °C until complete evolution of nitrogen. After cooling, the mixture was basified with 2 M sodium hydroxide solution and then extracted with ethyl acetate. The dried extract (Na_2SO_4) was concentrated in vacuo and chromatographed on a silica-gel column eluting with

diethyl ether/tetrahydrofuran (50:50). The first fraction (20 mL) was removed; the second fraction (60 mL) was evaporated and recrystallized from hexane to give 0.65 g (20%) of **12d**, mp 95 °C. IR (KBr, cm^{-1}): 3210 (νNH); 1670 (νCO). ^1H NMR (CCl_4) δ 1.50–2.05 (m, 2H, CH_2); 2.25–2.65 (m, 6H, 3 × CH_2); 3.35–3.95 (m, 6H, 3 × CH_2); 7.20–8.35 (AB system, J = 9 Hz, 2H, ArH); 7.60 (*s*, 1H, ArH); 8.70 (s, 1H, NH). Anal $C_{15}H_{18}ClN_3O_3$ (C, H, Cl, N).

6-Acetylamino-N-[(1-morpholinyl)propyl]-1,2-benzisoxazole-3-carboxamide ***12c***

A stirred solution of **12b** (1.52 g, 5 mmol) in acetic anhydride (10 mL) was heated at 80 °C for 15 min. After cooling, the reaction mixture was poured into ice water (100 mL). Following 1 h hydrolysis, the resulting mixture was extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to afford 1.26 g (73%) of **12c**, mp 168 °C after recrystallization from tetrahydrofuran. IR (KBr, cm^{-1}): 3300 (νNH); 1670 (νCO). ^1H NMR (DMSO-d_6) δ 1.60–1.95 (m, 2H, CH_2); 2.15 (s, 3H, CH_3); 2.05–2.55 (m, 6H, 3 × CH_2); 3.20–3.80 (m, 6H, 3 × CH_2); 7.40–8.15 (AB system, J = 9 Hz, 2H, ArH); 8.40 (s, 1H, ArH); 9.15 (t, 1H, N*H*CH_2); 10.20 (s, 1H, N*H*$COCH_3$).

Pharmacology. Radioligand binding assays

Membranes were prepared according to the methods of Peroutka and Snyder [31, 32]. In brief, brain tissue was homogenized in 20 vol of 5 mM Tris-HCl, 0.65 M sucrose, pH 7.5 at 4 °C, using a Potter Teflon/glass 1000 *g* AR. The homogenate was centrifuged at 500 *g* for 10 min at 4 °C and the pellet discarded. The supernatant was centrifuged in a Kontron Centrikon T-2070 at 48 000 g for 10 min at 4 °C. Pellet was homogenized in 20 vol of 50 mM Tris-HCl, 2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride pH 7.5 and then incubated for 10 min at 37 °C and centrifuged at 48 000 *g* for 10 min. Pellet was washed twice in the same buffer, centrifuged and finally homogenized in binding buffer.

The 5-HT_3 serotonin receptor in NG 108-15 cells (European Collection of Animal Cell Culture) was labeled with [^3H]BRL 43694 (83.5 Ci/mmol). Cells were homogenized in 50 mM Tris-HCl buffer pH 7.4 (10^6 cells/mL) using polytron (plot 10, two times 10 s burst).

The experiments were performed by incubating the homogenate in the presence of 1 nM [^3H]BRL 43694 and different concentrations of the tested compounds (11 concentrations, 10^{-4} to 10^{-11} M) dissolved in the assay buffer, at 25 °C for 60 min. Non-specific binding was determined in the presence of 10^{-5} M ICS 205930. MDL 72222 was used as reference product.

The 5-HT_4 serotonin receptor in pig hippocampus (Cellubio, France) was labeled with [^3H]GR 113808 (85 Ci/mmol). Tissue (P2) was homogenized in 50 mM Tris-HCl buffer pH 7.4 containing 10 μM pargyline and 0.1 mM phenylmethylsulfonyl fluoride, and diluted to a final protein concentration of about 0.75 mg/mL. The experiments were performed by incubating the homogenate in the presence of 0.1 nM [^3H]GR 113808 and different concentrations of the tested compounds (11 concentration 10^{-4} to 10^{-11} M) dissolved in the assay buffer at 37 °C for 30 min. Non-specific binding was determined in the presence of 10^{-5} M 5-hydroxytryptamine. Cisapride was used as reference product.

The D_2 dopamine receptor in bovine striatum (Cellubio, France) was labeled with [^3H]raclopride (82.4 Ci/mmol). Tissue (P2) was homogenized in 50 mM Tris-HCl buffer pH 7.4 containing 120 mM NaCl, and diluted to a final protein concentration of about 0.7 mg/mL.

The experiments were performed by incubating the homogenate in the presence of 1.5 nM [^3H]raclopride and different concentrations of the tested compounds (11 concentrations, 10^{-4} to 10^{-11} M) dissolved in the assay buffer at 25 °C for 60 min. Non-specific binding was determined in the presence of 10^{-5} M spiperone. Pimozide was used as reference product.

Competition experiments were analysed using the iterative non-linear least-squares curve fitting the computer programme Inplot 4, Graphpad software (San Diego, CA). K_i were calculated according to the method of Cheng and Prusoff [33].

Acknowledgments

The authors are grateful to the Société ADIR & Cie (Groupe Servier) and LDK France for their financial support. They also thank A Lagardère, S Larrouture and N Pinaud for their able technical assistance. The secretarial help of A Carrère is also gratefully acknowledged.

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