Eur. J. Med. Chem. 34 (1999) 791–798

Original article

Heterocyclic congeners of PD 128,907 with a partially hydrogenated benzomorpholine moiety as potential dopamine D_3-receptor ligands

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(Received 19 November 1998; revised 14 April 1999; accepted 26 April 1999)

Abstract – With a straightforward seven-step synthesis, racemic perhydro[1,4]benzoxazin-6-on was synthesized in overall good yields via regioselective epoxid ring-opening to the corresponding β-aminoalcohol. The oxazine derivative was the key intermediate for the preparation of heteroaromatic analogues of the dopamine D_3-receptor preferring agonist PD 128,907. The morpholine moiety of PD 128,907 was incorporated in diazole and diazine compounds obtained by different ring closure reactions. The target compounds obtained were structurally related to non-ergot heteroaromatic dopamine agonists which display preferential activity at the D_3 receptor, e.g., quinpirole, quinerolane, or pramipexole. The five membered aminothiazole, aminoselenazole, and pyrazole derivatives showed at least one order of magnitude higher binding at the human D_3 receptor than that at the D_{2L} receptor. Although the novel compounds displayed K_i values only in the micromolar concentration range, the most active ones showed full agonist activity in a functional assay on mitogenesis. © 1999 Éditions scientifiques et médicales Elsevier SAS

dopamine / D_3-receptor / D_2-receptor / PD 128,907 / agonist / mitogenesis / pramipexole / quinpirole / quinerolane

1. Introduction

Dopamine is among the most widely studied neurotransmitters of the mammalian central nervous system (CNS). Recent advances in the molecular biology of dopamine receptors have resulted in the classification into D_1-like (D_1 and D_5) and D_2-like (D_2–D_4) receptor subtypes [1, 2]. Each of the five characterized dopamine receptor subtypes belongs to the superfamiliy of G protein-coupled receptors and contains approximately 400 amino acids arranged in similar tertiary structures of seven putative transmembrane domains. Although the receptors were well characterized by molecular biology and localized in different brain areas, their specific functions are poorly understood at present. The D_3 receptor has restricted expression in limbic brain areas, associated with cognitive functions and motivated behaviour [3, 4]. Due to the distinct neuroanatomical distribution of different D_2-like subreceptors, as well as pharmacological and behavioural studies, it is suggested that D_3 receptors seem to be important targets for the development of drugs for the treatment of Parkinson's disease [5], schizophrenia [6], and drug abuse, e.g., ligands with agonist activity at D_3 receptors reduced the self-administered cocaine intake in rats [7]. The development of more structurally diverse and selective D_3-receptor agonists is required to provide a better understanding of pharmacology and pathophysiology of dopamine receptor subtypes. Dopamine itself had 20 times higher affinity at the D_3 receptor than that at the D_2 receptor [3]. Different non-ergot agonists were described possessing varying degrees of preference for the D_3 receptor. Tetralin derivatives like 7-OH-DPAT (7-hydroxy-*N*,*N*-dipropyl-2-aminotetralin) [4] or PD 128,907 ((+)-4a*R*,10b*R*-3,4,4a,10b-tetrahydro-4-propyl-2*H*,5*H*-[1]benzopyrano [4,3-b]1,4-oxazin-9-ol) [8, 9] showed higher affinity at the D_3 receptor than that at other D_2-like receptors. The same is true for some heterocyclic analogues like pramipexole, quinerolane and quinpirole [10, 11] containing an aminothiazole, an aminopyrimidine and a pyrazole ring,

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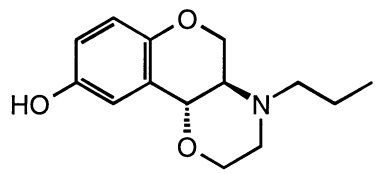

Figure 1. Structure of PD 128,907.

respectively. The reference agonist showing high activity in combination, with one of the highest degrees of D_3 receptor preferring selectivity, is PD 128,907 *(figure 1)*. This compound contains a morpholine moiety as one structural element which may be responsible for the increased selectivity compared to the structurally related 7-OH-DPAT. The afore mentioned heterocyclic agonists also displayed a preferential higher affinity at the D_3 receptor compared to that at other D_2-like receptors. The aim of this study was the synthesis and pharmacological investigation of compounds which have unified the structural morpholine element of PD 128,907 and the heterocyclic moiety of other D_3-receptor preferring agonists or related structures, e.g., pramipexole, quinerolane, and quinpirole.

2. Chemistry

The racemic *N*-substituted perhydro[1,4]benzoxazin-6-on ((±)-**7**) as key intermediate was synthesized by the straightforward seven-step route outlined in *figure 2*.

The synthesis of *trans*-configurated (±)-**3** relied on a modification of chemistry originally described by Cheng and co-workers [12] for the preparation of ligands for the κ opioid receptor. Cheng et al. obtained the epoxid **2** via a four-step route starting from 1,4-cyclohexanedione monoethylene ketal, but this can be done in a simple two-step procedure previously described [13, 14]. Thus, the enol ether of 1-methoxy-1,4-cyclohexadiene [15] was cleaved by a catalytic amount of *p*-toluyl sulfonic acid (*p*-TsOH) and the resulting carbonyl group was subsequently protected as ethylene ketal. Epoxidation of **1** with *m*-chloroperoxybenzoic acid (*m*-CPBA) provided **2** in

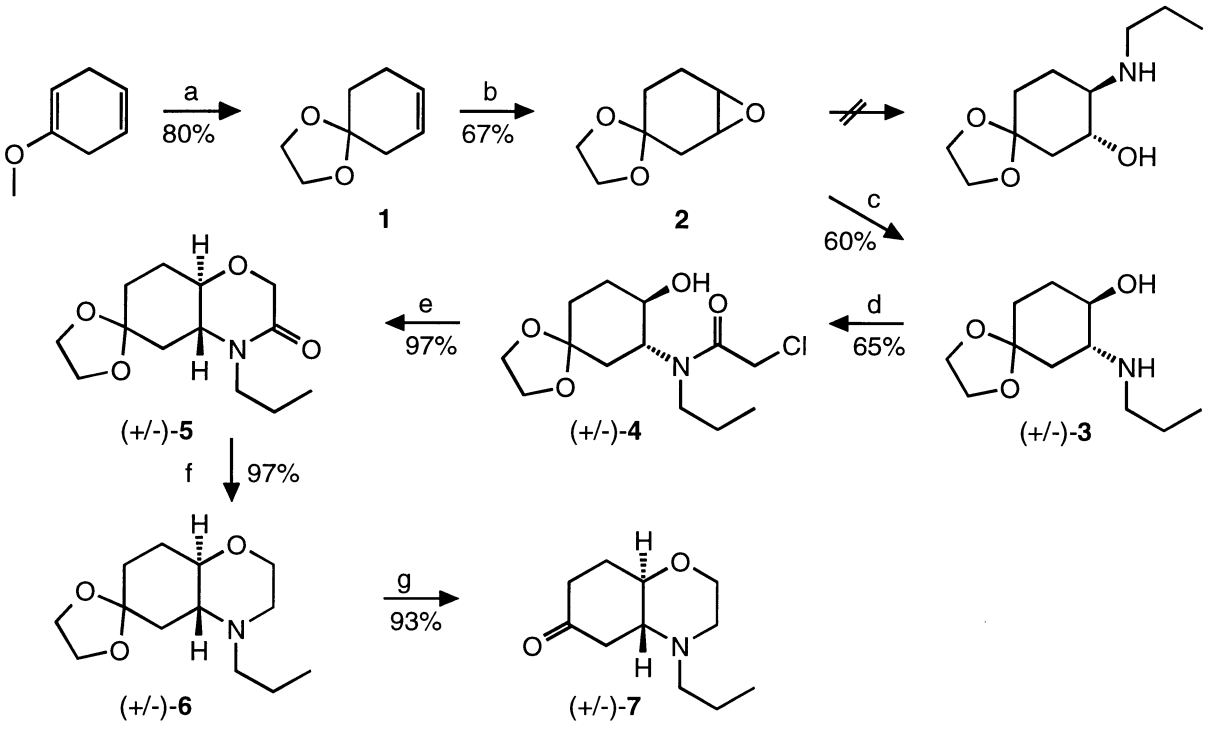

Figure 2. Synthesis of precursor (±)-**7**. (a) ethylene glycol, *p*-TsOH, toluene, ΔT; (b) *m*-chloroperoxybenzoic acid, CH_2Cl_2; (c) propylamine, water, ambient temperature; chloroacetyl chloride, Et_3N, CH_2Cl_2; (e) *n*-tetrabutylammonium hydrogensulphate (cat.), NaOH; (f) $LiAlH_4$, THF, ΔT; (g) 2 N HCl, ΔT.

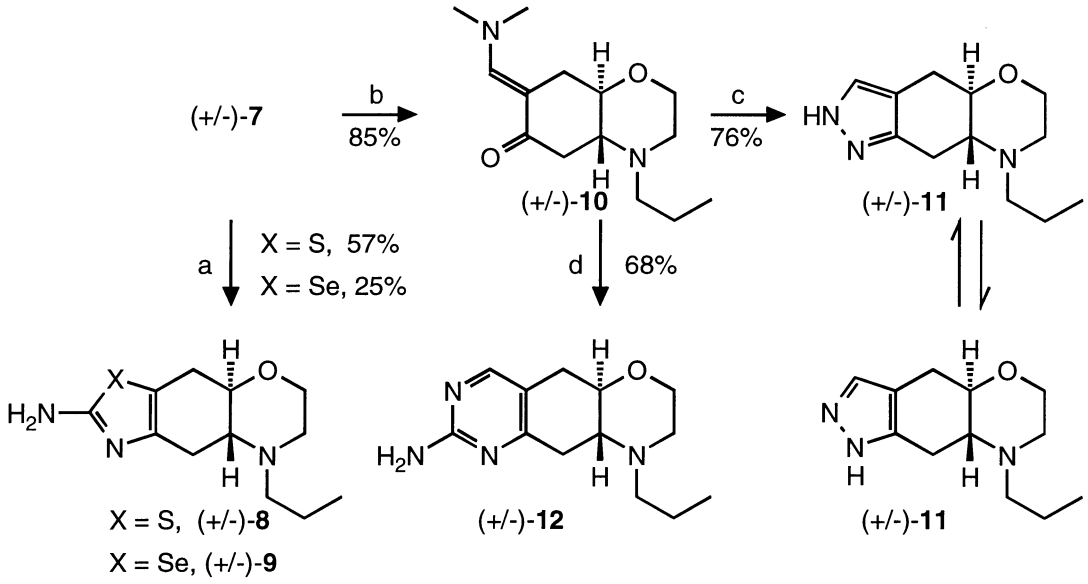

Figure 3. Synthesis of heterocyclic dopamine receptor ligands. (a) i: Br_2, HBr, 0 °C, ii: H_2N-CX-NH_2, ΔT; (b) $HC(NMe_2)_3$, DMF, 65 °C; (c) hydrazine, MeOH, ambient temperature; (d) guanidine x HCl, K_2CO_3, EtOH, ΔT.

good overall yield. Epoxid ring-opening of **2** with propyl amine resulted exclusively in the formation of the desired *trans*-configurated β-aminoalcohol (±)-**3**. The isomeric aminoalcohol could not be isolated, as depicted in *figure 2*. The selectivity and rate of conversion of this reaction is remarkable, since such nucleophilic epoxide ring openings with amines normally require heterogeneous catalysis [16] or amine activation with aluminia-organic reagents [17]. This observation of selectivity is congruent to the experiments of Chen et al. [12], who isolated, in the reaction of this epoxid with pyrrolidine, the two regioisomeric aminoalcohols in a ratio of 13:1 (89% yield).

Reaction of **3** with chloroacetyl chloride [18] led to the amide **4**, which is cyclized under phase-transfer conditions to the 1,4-benzoxazine **5**. In the ^{1}H NMR spectra of **4** both rotameres are present in equal amounts. This amide was reduced with $LiAlH_4$ in THF to give the amine **6**. Initial attemps to deprotect **6** with PPTS/wet acetone [19] failed, but this could be achieved by 2 N HCl [20] and afforded the desired racemic aminoketone **7**.

The heterocyclic substituted 1,4-benzoxazines were synthesized as shown in *figure 3*. The thiazolo- and selenazolo substituted compounds **8** and **9** were prepared in a well known manner [21] by treatment of **7** with bromine in acidic medium, and subsequent reaction of the α-haloketone intermediate either with thiourea or selenourea. Bromination of **7** could have taken place on either side of the carbonyl group to produce either the desired linear compounds **8** and **9** or the undesired angular products. However, bromination of position 5 is sterically unfavoured and the evidence of substitution could be done by an ^{1}H NMR off resonance decoupling experiment of compound **8**: decoupling of the 9a-proton resulted in a double doublet pattern of the protons in the 9-position, which would be impossible if the molecule was angularly annelated. Analogous observations were described by Kornfeld et al. [22] in the course of the preparation of rigid 3-(2-aminoethyl)pyrroles as dopamine agonists.

The precursor **10** as a masked 1,3-dicarbonyl compound was synthesized by the treatment of the free base of **7** with tris(dimethylamino)methane [23] in good yield. Treatment of this enaminone **10** either with guanidine hydrochloride/K_2CO_3 [24] or hydrazine [20, 22] resulted in the formation of the 2-aminopyrimidine derivative **12** and the pyrrole derivative **11** in its corresponding tautomeric forms, respectively. All compounds which were selected for pharmacological testing were converted with anhydrous HCl in their water soluble salts (see Experimental section). Due to the moderate pharmacological affinity the compounds were not separated into their enantiomers.

Table I. Binding and functional studies at Dopamine D_2 and D_3 receptors.

Compound	Inhibition of binding at			Stimulation of D_3 receptor	
	D_2 receptor K_i [μM]	D_3 receptor K_i [μM]	K_i (D_2)/ K_i (D_3)	EC_{50} [μM]	i.a.[a]
(±)-3	600	68 ± 3	13	nd[b]	nd
(±)-6	> 600	172 ± 28	> 3.4	nd	nd
(±)-8	99 ± 2	6.2 ± 0.5	16	7.1 ± 3.0	106
(±)-9	49 ± 3	2.9 ± 0.4	17	1.3 ± 0.08	101
(±)-11	99 ± 5	2.3 ± 0.3	43	0.54 ± 0.09	100
(±)-12	> 600	46 ± 5	> 12	nd	nd
Quinpirole[c,d]	1.4	0.039	36	0.00086	100
Quinerolane[c,d]	0.34	0.004	95	0.00015	96
Pramipexole[c]	0.79	0.004	193	0.00023	98
PD 128,907[c]	0.389	0.002	216	0.00064	96

(a) i.a. = intrinsic activity (i.a. (quinpirole) = 100); (b) nd = not determined; (c) ref. [32]; (d) ref. [33].

3. Pharmacology

Displacement assays were performed with chinese hamster ovary (CHO) cell lines stably transfected with human D_{2L} or D_3 receptor cDNA using [^{125}I]iodosulpiride at a concentration of 0.1 nM [25]. The nonspecific binding was determined in the presence of enomapride. Binding data were analysed by computerized nonlinear regression for a one-site model. The K_i values were derived from IC_{50} values according to the Cheng-Prussoff equation [26].

Additional functional experiments were performed to clarify the mode of action for the most active compounds of this series. NG 108-15 cells expressing the human dopamine D_3 receptor were incubated with forskoline and the drug in increasing concentrations [25]. Then, [^{3}H]thymidine was added, and increase in mitogenesis, as a measurement of agonist activity, could be measured by counting the radioactivity incorporated into the cells after 2 h. A decrease in, or inhibition of, mitogenesis in relation to the effect of the reference compound quinpirole would show a partial agonist or antagonist effect, respectively.

4. Pharmacological results and discussion

The target compounds (±)-**8–12** displayed at least 10 times higher binding affinities at the D_3 receptor than that at the D_2 receptor *(table I)*. Their affinity constants at the D_3 receptor were found to be in the micromolar concentration range. Although the compounds (±)-**8–11** were found to possess higher D_3-receptor affinity than the intermediate products of their chemical synthesis, the difference between these heteroaromatic products and their chemical educts were not important due to their overall moderate affinity constants. The aminopyrimidine derivative (±)-**12** showed equipotency to the secondary amine intermediate (±)-**3** and even lower D_3-receptor affinity than the non-aromatic morpholine precursor molecule (±)-**6**. Compounds possessing a five-membered heteroaromatic moiety ((±)-**8**–**11**) were found in this series to be active in a lower concentration range than the other tested compounds. Interestingly, the seleno analogue (±)-**9** of the pramipexole related derivative (±)-**8** showed about two times higher affinity than that of compound (±)-**8**. For dopamine agonists used in therapy for Parkinson's disease, an anti-oxidative or radical scavenger effect seemed to be beneficial. These properties may be enhanced in seleno heterocycles compared to sulfur heterocycles. Therefore, further investigations to study the anti-oxidative effects of aminothiazole and aminoselenazole derivatives are currently in progress.

Compared to PD 128,907, pramipexole, quinerolane, and quinpirole, the novel compounds possess the basic amino functionality on another side of the molecule (reversed orientation), i.e., the positions of the oxygen and the nitrogen in the morpholine moiety have to be changed to be able to superimpose with the reference agonists. Concerning the pharmacological activity, this seemed to be the major drawback for the compounds prepared. The position of the basic amino functionality in relation to the aromatic hydrogen-bond area were described in different molecular modelling and structure-activity relationship studies to be essential for high affinity D_2-like receptor binding [27]. These findings could be emphasized by the results of the present study for binding at D_3-receptors. Compounds with an opposite orientation of oxygen and nitrogen atom in the morpholine moiety possessing a stronger relationship to PD

128,907 compared to the ones described in this paper have already been reported as potent dopamine receptor agonists [28, 29].

Nevertheless, when the three most active compounds (±)-**8**–**11** were tested for their mode of action in the mitogenesis test it was found that all compounds were able to induce a full mitogenesis rate indicating a full agonist behaviour. Although the compounds displayed only moderate affinity they were still able to induce a change in the receptor structure that is required for signal transduction indicated by complete receptor activation.

5. Experimental protocols

Melting points were uncorrected. ^{1}H NMR spectra were determined with a Bruker AC 300 (300 MHz) or a Bruker Avance DPX 400 (400 MHz) instrument as indicated by spectrometer frequency. Chemical shifts were reported in δ values (ppm) relative to an internal standard of tetramethylsilane. Abbreviations used in NMR analysis were as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet; and dd, doublet of doublets. IR spectra were determined with a Perkin-Elmer 297 spectrophotometer and Mass spectra were obtained on a CH-7A-Varian MAT (70 eV) instrument. Microanalyses were performed with a Perkin-Elmer 240 B and C analyzer. Analyses indicated by the symbols of the elements or functions were within ± 0.4% of theoretical values. Thin layer chromatography was performed on Merck precoated TLC plates with silica gel 60-F254 and visualized with UV light (254 nm), after treatment with iodine or after treatment with Fast Blue B salt. Column (flash) chromatography [30] was performed with Merck silica gel 60 (230–400 mesh). Solvents and reagents were used as purchased, except as noted. Ether, THF and toluene were distilled from sodium metal/benzophenone ketyl. [^{125}I]Iodosulpiride (2 000 Ci/mmol) and [^{3}H]thymidine (110 Ci/mmol) were obtained from Amersham International, Ltd. (Buckinghamshire, UK) and emonapride (YM 09152) from the Yamanouchi Pharmaceutical Co. (Tokyo, Japan).

5.1. Chemistry

5.1.1. 1,4-Dioxaspiro[4,5]dec-7-ene (**1**)

Compound **1** was prepared according to the procedure of Lambert et al. [13] from 1-Methoxy-1,4-cyclohexadiene [15] in 80% yield: b.p. 70–71 °C (13 mbar) (lit. [13] b.p. 62–64 °C, 7 Torr); IR ($CHCl_3$ solution) 3 027, 2 934, 2 879, 1 687 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.74–1.81 (m, 2H), 2.27–2.28 (m, 4H), 3.99 (s, 4H), 5.60–5.74 (m, 2H); MS *m/e* 140 (M, 35), 125 (8), 112 (8), 99 (62), 86 (100).

5.1.2. (±)-Spiro[1,3-dioxolane-2,3′-[7]oxabicyclo[4,1,0] heptane] (**2**)

Compound **2** was prepared by the procedure of Cheng et al. [12] from **1** in 67% yield: b.p. 99 °C (9 mbar) (lit. [14] b.p. 100 °C, 16 Torr). IR ($CHCl_3$ solution) 2 942, 2 882 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.41–1.70 (m, 2H), 2.00–2.20 (m, 4H), 3.15–3.18 (m, 2H), 3.87–3.98 (m, 4H); MS *m/e* 156 (M, 2), 140 (22), 112 (12), 99 (85), 86 (100).

5.1.3. (±)-trans-7-Propylamino-1,4-dioxaspiro[4,5]decan-8-ol ((±)-**3**)

To a stirred mixture of **2** (6.25 g, 40.0 mmol) and propylamine (6.58 mL, 80.0 mmol) was added 4 mL H_2O dropwise at 0 °C. The reaction mixture was stirred at ambient temperature under N_2. After 40 h the mixture was evaporated in vacuo to remove the water and the excess of propylamine to give a red oil. This oil was purified by flash chromatography (10% MeOH in CH_2Cl_2 with 1% concentrated NH_4OH) on silica gel to give (±)-**3** (5.148 g, 60%) as a light red oil which slowly crystallized on standing: m.p. 65–66 °C; IR (KBr) 3 390, 3 300, 3 166, 2 956, 2 876, 2 824, 1 635 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.00 (m, 3H), 1.39–2.55 (m, 10H), 2.79 (m, 1H), 3.29 (m, 1H), 4.02 (m, 4H) (no OH/NH signals were detected); MS *m/e* 215 (M, 5), 186 (37), 156 (64), 129 (6), 101 (10), 86 (100). Anal. $C_{11}H_{21}NO_3$ (C, H, N).

5.1.4. (±)-trans-2-Chloro-N-(8-hydroxy-1,4-dioxaspiro [4,5]decan-7-yl)-N-propyl-acetamid ((±)-**4**)

To a stirred solution of (±)-**3** (5.08 g, 23.6 mmol) and triethylamine (3.29 mL, 23.6 mmol) in 100 mL CH_2Cl_2 was added chloroacetyl chloride (1.88 mL, 23.6 mmol) dropwise over a period of 10 min at 0 °C. After 30 min the mixture was allowed to warm to ambient temperature and stirred for a further 2 h. The organic layer was separated, washed with 20 mL 2 N HCl, 20 mL H_2O, 20 mL brine, dried ($MgSO_4$), and concentrated in vacuo whereupon red crystals formed. Recrystallization from CH_2Cl_2/n-hexane gave pure (±)-**4** (4.50 g, 65%) as colourless crystals: m.p. 172 °C; IR (KBr) 3 408, 2 961, 2 880, 1 647 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90–0.94 (m, 6H), 1.49–2.14 (m, 21H), 2.22 (s, 1H, collapse after D_2O-exchange), 2.56 (1H, collapse after D_2O-exchange), 2.94 (m, 1H), 3.23–3.34 (m, 2H), 3.65–3.76 (m, 2H), 3.97–4.13 (m, 9H), 4.33–4.35 (m, 1H); MS *m/e* 291 (M, 1), 256 (14), 232 (23), 156 (49), 86 (100). Anal. $C_{13}H_{22}ClNO_4$ (C, H, N).

5.1.5. (±)-trans-4-Propyl-2,3,4,4a,5,7,8,8a-octahydrospiro[6H-1,4-benzoxazine-6,2′-[1,3]dioxolane]-3-on ((±)-**5**)

Compound (±)-**4** (4.50 g, 15.4 mmol) and tetra-*n*-butylammonium hydrogen sulphate (525 mg, 1.54 mmol) were dissolved in 300 mL CH_2Cl_2 under an atmosphere of nitrogen. 61.7 mL of 0.5 N NaOH was added via syringe, and the biphasic system was stirred vigerously at ambient temperature for 20 h. The organic layer was separated, washed with water (2 × 40 mL), brine (40 mL), dried ($MgSO_4$), and concentrated in vacuo to give a yellow oil. This oil was purified by flash chromatography (10% MeOH in CH_2Cl_2) on silica gel to give (±)-**5** (3.804 g, 97%) as a light yellow oil which slowly crystallized on standing: m.p. 67 °C; IR (KBr) 3 448, 2 964, 2 880, 1 655 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (m, 3H), 1.43–2.13 (m, 8H), 3.04–3.74 (m, 4H), 3.98 (s, 4H), 4.26 (dd, *J* = 16.0/21.2 Hz, 2H); MS *m/e* 255 (M, 92), 226 (31), 198 (53), 155 (68), 141 (47), 99 (100). Anal. $C_{13}H_{21}NO_4$ (C, H, N).

5.1.6. (±)-trans-4-Propyl-2,3,4,4a,5,7,8,8a-octahydrospiro[6H-1,4-benzoxazine-6,2′-[1,3]dioxolane] hydrochloride ((±)-**6**)

$LiAlH_4$ (1.61 g, 42.3 mmol) was suspended in 20 mL THF under an atmosphere of nitrogen. The solution of (±)-**5** (3.60 g, 14.1 mmol) in 30 mL THF was added dropwise via syringe at 0 °C over a period of 20 min. The reaction mixture was allowed to warm to ambient temperature and then heated at reflux for 2 h. After cooling, excess hydride was destroyed by the careful addition of 10 mL water. The mixture was filtered and the solid was washed with ether (5 × 20 mL). The filtrate was washed with brine (2 × 30 mL), dried (K_2CO_3) and concentrated in vacuo. The resulting oil was purified by flash chromatography (10% MeOH in CH_2Cl_2 with 1% concentrated NH_4OH) on silica gel to give the free base of (±)-**6** as a light yellow oil. This oil was dissolved in dry ether and a stream of dry gasous HCl was bubbled through the solution at 0 °C to produce the hydrochloride (±)-**6** (3.793 g, 97%) as colourless crystals: m.p. 180–181 °C; IR (KBr) 3 431, 2 965, 2 881, 2 585, 2 475, 2 325 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.90 (m, 3H), 1.44–1.86 (m, 7H), 2.29 (m, 1H), 2.85–3.38 (m, 5H), 3.73 (m, 1H), 3.92 (m, 6H), 11.41 (br s, 1H); MS *m/e* 241 (M, 12), 212 (100), 139 (29). Anal. $C_{13}H_{23}NO_3 \cdot HCl$ (C, H, N).

5.1.7. (±)-trans-4-Propyl-3,4,4a,5,6,7,8,8a-octahydro-2H-1,4-benzoxazin-6-on hydrochloride ((±)-**7**)

Compound (±)-**6** (3.793 g, 13.7 mmol) was dissolved in 50 mL 2 N HCl and heated to reflux for 2 h, cooled, neutralized with 10% NaOH, saturated with NaCl and extracted with CH_2Cl_2 (5 × 20 mL). The organic extracts were dried over K_2CO_3 and concentrated in vacuo to provide a yellow oil. This oil was purified by flash chromatography (10% MeOH in CH_2Cl_2 with 1% concentrated NH_4OH) on silica gel to give the free base of (±)-**7** as a light yellow oil, which was converted in the hydrochloric salt as described before to give (±)-**7** (2.954 g, 93%) as colourless crystals: m.p. 197 °C; IR (KBr) 3 419, 2 969, 2 880, 2 581, 2 479, 2 419, 1 719 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.91 (t, *J* = 7.3 Hz, 3H), 1.63–1.69 (m, 3H), 2.06–3.47 (m, 10H), 4.02–4.03 (m, 2H), 4.13–4.14 (m, 1H), 11.83 (br s, 1H); MS *m/e* 197 (M, 34), 168 (100), 140 (42). Anal. $C_{11}H_{19}NO_2 \cdot HCl$ (C, H, N).

5.1.8. (±)-trans-8-Propyl-4a,6,7,8,8a,9-hexahydro-4H-thiazolo[4,5-g][1,4]benzoxazin-2-amine dihydrochloride ((±)-**8**)

To a stirred solution of (±)-**7** (233.7 mg, 1 mmol) in 10 mL 47% HBr was added Br_2 (1.76 mL of a 10% solution in 47% HBr, 1.1 mmol) dropwise at 0 °C. After 2 h, thiourea (83.7 mg, 1.1 mmol) was added as a solid, and the mixture was heated to reflux for further 2 h. After evaporation of the HBr in vacuo, the residue was treated with 10 mL 10% NaOH with cooling and extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo to provide a crude base of (±)-**8** as a brown solid. This residue was purified by flash chromatography (10% MeOH in CH_2Cl_2 with 1% concentrated NH_4OH) on silica gel to give the free base of (±)-**8** as a foam. This foam was dissolved in dry ethanol (15 mL), treated with saturated HCl in ethanol (2 mL) and evaporated in vacuo again. Recrystallization from dry 2-propanol/ether provided the title compound (185.7 mg, 57%) as a grey solid: m.p. 240–241 °C; IR (KBr) 3 375, 3 247, 3 064, 2 936, 2 720, 2 621, 2 510, 1 627, 1 577 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.97 (t, *J* = 7.1 Hz, 3H), 1.73–1.87 (m, 2H), 2.51–3.47 (m, 9H), 4.30–4.44 (m, 3H), 9.30 (br s, 3H), 12.67 (br s, 1H); MS *m/e* 253 (M, 28), 224 (29), 151 (8), 127 (100). Anal. $C_{12}H_{19}N_3OS \cdot 2\ HCl \cdot H_2O$ (C, H, N).

5.1.9. (±)-trans-8-Propyl-4a,6,7,8,8a,9-hexahydro-4H-selenazolo[4,5-g][1,4]benzoxazin-2-amine dihydrochloride ((±)-**9**)

This compound was prepared and purified in the same manner as described in the synthesis of (±)-**8** in a 1.5 mmol scale to provide (±)-**9** (137.8 mg, 25%) as a light red solid: m.p. 220–221 °C; IR (KBr) 3 397, 3 260, 3 073, 2 933, 2 722, 2 624, 1 623, 1 579 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.94 (t, *J* = 7.3 Hz, 3H),

1.73–1.77 (m, 2H), 2.89–3.52 (m, 9H), 4.03–4.19 (m, 3H), 9.58 (br s, 3H), 12.12 (br s, 1H); MS *m/e* 301 (M, Se main isotope, 12), 272 (13), 127 (100). Anal. $C_{12}H_{19}N_3OSe{\cdot}2\ HCl{\cdot}H_2O$ (C, H, N).

5.1.10. (±)-trans-(E)-7-Dimethylaminomethylidene-4-propyl-2,3,4,4a,5,6,8,8a-octahydro-1,4-benzoxazin-6-on ((±)-**10**)

To a solution of (±)-**7** (986.4 mg, 5 mmol, free base) in 5 mL of dry DMF was added tris(dimethylamino)methane (2.33 mL, 15 mmol). The solution was stirred for 16 h at 65 °C under a nitrogen atmosphere and then concentrated in vacuo to provide a brown oil. This oil was purified by flash chromatography (10% MeOH in CH_2Cl_2 with 1% concentrated NH_4OH) on silica gel to give (±)-**10** (1.066 g, 85%) as a yellow oil which very slowly crystallized on standing. A portion of this material was recrystallized from cyclohexane/n-hexane to give (±)-**10** as yellow crystals: m.p. 65 °C; IR (KBr) 3 451, 2 959, 2 859, 2 803, 1 648, 1 559 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.83 (t, *J* = 7.2 Hz, 3H), 1.29–1.46 (m, 2H), 1.89 (dd, *J* = 5.8/11.6 Hz, 1H), 2.03–2.62 (m, 6H), 2.73 (d, *J* = 11.4 Hz, 1H), 2.96 (dd, *J* = 4.2/6.7 Hz, 1H), 3.05 (s, 6H), 3.23–3.27 (m, 1H), 3.56 (t, *J* = 11.1 Hz, 1H), 3.77 (d, *J* = 11.1 Hz, 1H), 7.31 (s, 1H); MS *m/e* 252 (M, 29), 235 (7), 193 (25), 84 (84), 58 (100). Anal. $C_{14}H_{24}N_2O_2$ (C, H, N).

5.1.11. (±)-trans-8-Propyl-1,4,4a,6,7,8,8a,9-octahydropyrazolo[3,4-g][1,4]benzoxazine dihydrochloride ((±)-**11**) *(cf. [28, 29])*

To a solution of (±)-**10** (309.5 mg, 1.23 mmol) in 10 mL dry methanol was added hydrazine (0.39 mL, 12.3 mmol) and the mixture was stirred for 19 h. The solvent was evaporated and the crude product was purified by flash chromatography (10% MeOH in CH_2Cl_2 with 1% concentrated NH_4OH) on silica gel to give the base of (±)-**11** (206.8 mg, 76%) as a yellow oil. The HCl salt was formed in ethanol and crystallized from dry 2-propanol/ether to provide (±)-**11** as a hygroscopic foam. IR (KBr) 3 395, 2 932, 2 877, 2 688, 2 615, 2 487, 2 360, 1 676, 1 645, 1 570 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.96 (m, 3H), 1.75 (m, 2H), 2.51–3.48 (m, 9H), 4.03–4.11 (m, 3H), 7.59 (s, 1H), 7.75 (br s, 2H), 11.97 (br s, 1H); MS *m/e* 221 (M, 48), 192 (100), 127 (39), 98 (22). Anal. $C_{12}H_{19}N_3O{\cdot}2\ HCl$ (C, H, N).

5.1.12. (±)-trans-9-Propyl-5a,7,8,9,9a,10-hexahydro-5H-pyrimidino[4,5-g][1,4]benzoxazin-2-amine dihydrochloride ((±)-**12**)

To a solution of (±)-**10** (293.1 mg, 1.16 mmol) in 15 mL dry ethanol was added dry guanidine·HCl (1.11 g, 11.6 mmol) and K_2CO_3 (1.61 g, 11.6 mmol). The suspension was refluxed for 2 h and then evaporated to dryness in vacuo. The residue was treated with water (30 mL) and extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were dried (K_2CO_3) and concentrated in vacuo. The solid residue was converted into its hydrochloric salt and recrystallized from dry ethanol/ether to provide (±)-**12** (252.8 mg, 68%) as a yellow solid: m.p. 196 °C. IR (KBr) 3 229, 3 137, 2 969, 2 935, 2 880, 2 689, 2 542, 2 484, 2 349, 1 664 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 0.96 (t, *J* = 7.3 Hz, 3H), 1.72–1.77 (m, 2H), 2.54–3.53 (m, 9H), 4.05–4.16 (m, 3H), 7.99 (br s, 3H), 8.33 (s, 1H), 12.24 (br s, 1H); MS *m/e* 248 (M, 21), 219 (100). Anal. $C_{13}H_{20}N_4O{\cdot}2\ HCl$ (C, H, N).

5.2. Pharmacological testing

5.2.1. Displacement studies [25]

Transfected cell lines consisting of CHO cell lines stably transfected with human dopamine D_{2L} or D_3 receptor DNA [11] were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% foetal calf serum in a 5% CO_2 humidified atmosphere. They were harvested from culture dishes in the presence of 0.2% trypsin, centrifuged at 2 000 *g* for 5 min and homogenized in 10 mM Tris-HCl, pH 7.4 containing 5 mM $MgCl_2$ using a Polytron. The homogenate was centrifuged at 20 000 *g* for 15 min at 4 °C, and the pellet was resuspended by sonification in 50 mM Tris-HCl, pH 7.4, containing 120 mM: NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 8 mM $MgCl_2$ (incubation buffer). Membranes were either used immediately or after storage at –70 °C. Binding assays were started by the addition of 200 μL of membranes (1–20 μg of protein) from transfected cells diluted in incubation buffer 1 supplemented with 0.2% bovine serum albumin to polystyrene tubes containing, in 100 μL, 0.1 nM [^{125}I]iodosulpiride and drug diluted in 100 μL of incubation buffer. Nonspecific binding (1–20% of total binding) was determined in the presence of 1 μM enomapride. Incubations were run at 30 °C for 30 min. All reactions were stopped by vacuum filtration through Whatman GF/B glass-fibre filters coated in 0.3% polyethylenimine with an automated cell harvester (Brandel-Beckmann, Gauthersburg, MD) and were rinsed 3 times with 5 mL of ice-cold incubation buffer. Filters were counted by gamma spectrometry in 5 mL of ACS II (Amersham).

EC_{50} values (± SE) and maximal responses were calculated from concentration-response curves. Intrinsic activity was calculated relative to quinpirole, a full agonist [31].

5.2.2. Mitogenesis [25]

NG 108-15 cells expressing the human dopamine D_3-receptor [31] were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% foetal calf serum in a 5% CO_2 humidified atmosphere and plated in collagen-coated 96-well plates. After a 24 h culture, cells were washed twice with culture medium without foetal calf serum and incubated for 16 h with 1 μM forskolin and the drug in increasing concentrations or quinpirole as control. Then, [^{3}H]thymidine (1 μCi/well) was added for 2 h and cells were harvested by vacuum filtration through Whatman GF/C glass-fibre filters by using an automated cell harvester and were rinsed 15 times with 200 μL of phosphate-buffered saline. Radioactivity was counted by liquid scintigraphy in 5 mL of ACS (Amersham). K_i values were derived from IC_{50} values according to the Cheng-Prussoff equation [26], taking into account the K_d of [^{125}I]iodosulpiride for respective receptors. Data were means of K_i values from data obtained in at least three separate experiments, and the statistical error was expressed as ± SEM.

Acknowledgements

This work was supported by the Biomedical & Health Research programme (BIOMED) of the European Union and by the National Institute on Drug Abuse (NIDA) (DAII534-01), USA.

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