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6-(4-Benzylpiperazin-1-yl)benzodioxanes as Selective Ligands at Cloned Primate Dopamine D_4 Receptors

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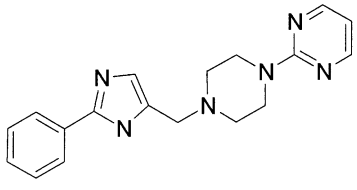
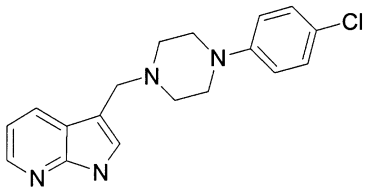
Abstract—A series of novel 6-(4-benzylpiperazin-1-yl)benzodioxanes were prepared and screened at selected dopamine receptor subtypes. 6-(4-[4-Chlorobenzyl]piperazin-1-yl)benzodioxane (**2d**) had high affinity and selectivity for the D_4 dopamine receptor subtype and was identified as a D_4 antagonist via its attenuation of dopamine-induced GTPγ^{35}S binding at the D_4 receptor.

Introduction

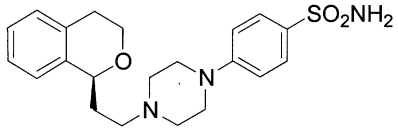
Schizophrenia is a debilitating disease affecting approximately 1% of the population. Affected individuals can experience delusions, hallucinations, disorganized thoughts and behavior resulting in an impairment of normal social function. The dopamine hypothesis postulates that schizophrenia is the result of hyperactivity of the dopaminergic system and that inhibition of dopaminergic processes by receptor blockade can be an effective means of treatment.[1] Classical antipsychotics such as haloperidol are effective in treating schizophrenic patients, however, they can cause severe side effects such as extrapyramidal symptoms and tardive dyskinesia.[2] These side effects have been attributed to a blockade of dopamine receptors in the striatum. Through cloning studies, it has been shown that the dopamine D_2 receptor family consists of D_2, D_3 and D_4 subtypes.[3] The distribution of the D_4 receptor throughout the brain differs from that of the D_2 receptor. The D_4 receptor is localized primarily in areas other than the striatum, such as the amygdala, thalamus, hypothalamus and cerebellum.[4] Subsequently, the D_4 receptor has been shown to be overexpressed in the postmortem brains of schizophrenics,[5] although this finding is the subject of some debate.[3] The atypical antipsychotic clozapine, which lacks the extrapyramidal side effects associated with most other antipsychotics, has a higher affinity for the D_4 receptor over the D_2 receptor.[6] These findings suggest that the D_4 receptor is a good target for the treatment of schizophrenia and a number of D_4 selective antagonists have been reported.[7] Several of these, including NGD 94-1,[8] L-745,870[9] and U-101387,[10] have been advanced to the clinic.



NGD 94-1



L-745,870



U-101387

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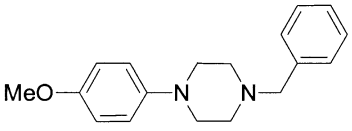
Results and Discussion

As part of a general program within the area of dopaminergic ligands, we have previously identified 1-phenyl-4-benzylpiperazines as selective agents for the D_4 receptor.[11] The 4-methoxyphenyl-piperazine **1** displayed modest potency at the D_4 receptor ($K_i = 93$ nM) but was inactive ($K_i > 5000$ nM) at the D_2 and D_3 receptors. The simplicity of the basic structure inferred that selective modifications could lead to higher affinity D_4 selective compounds. In the course of these studies, it was found that the alkoxy group could be incorporated into a benzodioxane ring system as shown for the parent compound **2a** (D_4 $K_i = 47$ nM). Compound **2a** displayed high selectivity for D_4 over the related D_2 (>100-fold) and D_3 (>100-fold) receptor subtypes and an exploration of the structure–activity relationship of this compound was carried out in order to improve the overall D_4 affinity. The structural variations of compound **2a** examined fell into three classes: (i) Substitutions upon the benzyl moiety by electron donating or electron withdrawing substituents. (ii) Incorporation of an alkyl group at the benzylic position. (iii) Variations in the size and oxygen content of the cyclic ether component of the original benzodioxane.



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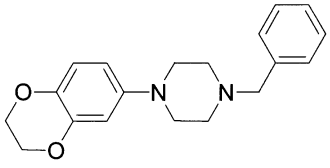
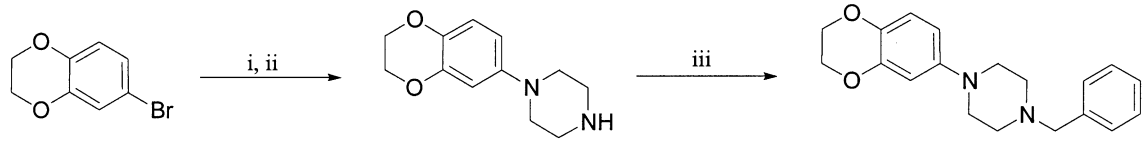


2a

As the number of dependent variables was high the benzylic portion of the molecule was optimized first. The required common intermediate, 1-(1,4-benzodioxane-6-yl)piperazine (**4**), was prepared via a two-step literature procedure from commercially available 6-bromo-1,4-benzodioxane (**3**).[12] Condensation with the appropriate benzyl halide furnished the target compounds **2a**–**2j** (Scheme 1).

To determine how the D_2, D_3 and D_4 affinities were affected by substituents on the benzyl group, a representative electron-withdrawing group (chlorine) and electron-donating group (methoxy) were introduced as single substituents at each of the available positions (**2b**–**g**, Table 1). Relative to the parent compound **2a**, both *ortho*-substituted compounds **2b** and **2e** were much weaker at D_4 and inactive at both D_2 and D_3. A significant improvement in D_4 affinity was achieved with the *meta*-substituted analogues **2c** and **2f** and weak affinity at both D_2 and D_3 receptors was observed. The highest D_4 potency was achieved with the *para*-substituted analogues **2d** ($K_i = 2$ nM) and **2g** ($K_i = 5$ nM). Although there was an increase in both D_2 and D_3 affinity, **2d** displayed high selectivity over D_2 (>250-fold) and D_3 (>500-fold). The finding that both electron-withdrawing and electron-donating substituents at the *para*-position gave compounds of similar potency indicates that a steric rather than electronic interaction may be responsible for these results. The *para*-fluoro **2h** and *para*-methyl **2i** analogues had similar binding profiles to **2d** and **2g**. The introduction of a methyl group at the benzylic position (**2j**) was detrimental to D_4 binding.

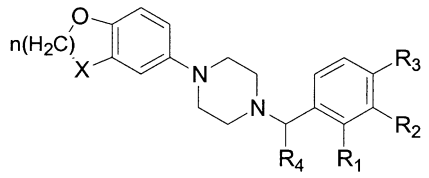
At this point, a further examination of the SAR was carried out by selected variations of the 1,4-benzodioxane portion of the molecule. The variations of ring size and oxygen content included the chromane **7**, the 1,3-dioxolane **8** and the benzofuran **9**. The high affinity 4-chlorobenzylpiperazine unit of compound **2d** was used with these examples to provide a point of reference. Each compound was prepared from the corresponding arylpiperazine in a manner similar to that shown for the preparation of compounds **2a**–**2j** (Scheme 1). 6-Piperazinylchromane (**6**) was prepared via the three-step procedure outlined in Scheme 2. 5-Piperazinyl-2H-benzo[*d*]1,3-dioxolane[13] and 5-piperazinyl-2,3-dihydrobenzo[*b*]furan[14] were prepared according to literature procedures. All three compounds (**7**, **8**, and **9**) were



3 **4** **2a-2j**

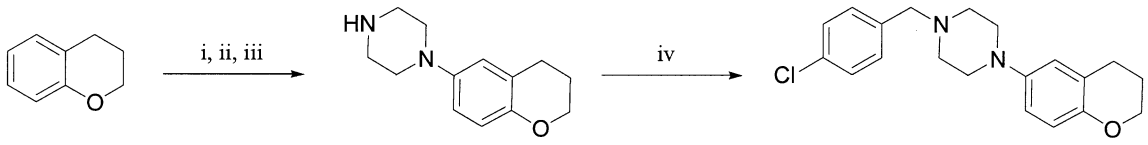
Scheme 1. Reaction conditions: (i) $Pd_2(dba)_3$, P(o-tolyl)$_3$, *tert*-butyl 1-piperazinecarboxylate, NaO^{t}Bu, PhMe, 100 °C, 55%; (ii) TFA, CH_2Cl_2, 20 °C, 82%; (iii) benzyl halide, K_2CO_3, CH_3CN, reflux, 48–89%.

Table 1. Dopamine receptor subtype binding



Compound	R_1	R_2	R_3	R_4	n	X	K_i (nM)[a] D_2	D_3	D_4
1							>5000	>5000	93±15
2a	H	H	H	H	2	O	>5000	>5000	47±8
2b	Cl	H	H	H	2	O	>5000	>5000	434±43
2c	H	Cl	H	H	2	O	2076±645	3770±1459	10±3
2d	H	H	Cl	H	2	O	526±96	1396±558	2±1
2e	OMe	H	H	H	2	O	>5000	>5000	160±23
2f	H	OMe	H	H	2	O	3297±1414	>5000	19±3
2g	H	H	OMe	H	2	O	1424±650	1293±910	5±2
2h	H	H	F	H	2	O	>5000	>5000	4±1
2i	H	H	Me	H	2	O	1212±148	2388±1318	2±1
2j	H	H	H	Me	2	O	>5000	>5000	1174±315
7	H	H	Cl	H	2	CH2	2152±1189	>5000	4±1
8	H	H	Cl	H	1	O	2657±233	>5000	9±5
9	H	H	Cl	H	1	CH2	>5000	>5000	10±4
Clozapine							113±9	Not determined	17±3
Haloperidol							2±1	14±2	5±1

[a]Binding data are the means of at least three independent experiments using standard displacement assays with [^{3}H]YM 09151 as the competitive ligand and primate dopamine receptor subtypes expressed in CHO cells.



5 **6** **7**

Scheme 2. Reaction conditions: (i) HNO_3, 0 °C, 79%; (ii) Raney nickel, H_2, 1 atm, 91%; (iii) bis-(2-chloroethyl)amine hydrochloride, K_2CO_3, chlorobenzene, reflux, 53%; (iv) 4-chlorobenzyl bromide, K_2CO_3, CH_3CN, reflux, 79%.

potent (K_i <10 nM) at the D_4 receptor with high selectivity over D_2 (>500 fold) and D_3 (>500 fold).

It next became important to determine the functional activity of these compounds at the D_4 receptor. Agonist-stimulated GTPγ^{35}S binding has been widely used for many G protein coupled receptors to distinguish agonists from antagonists and to determine their potency and efficacy for a given receptor. The GTPγ^{35}S binding functional assay can be used to demonstrate a dose-dependent agonist stimulation by the full D_4 receptor agonist, dopamine, in CHO cells producing stable expression of primate $D_{4.2}$ receptors. A typical assay exhibited a 3-fold stimulation over baseline, with an EC_{50} of 65 nM. When used alone compound **2d** gave no stimulation over baseline, suggesting that **2d** does not possess agonist properties at the human D_4 receptor. In combination with an EC_{74} level of dopamine (333 nM), **2d** reversed the agonist effect in a dose dependent manner (IC_{50} = 20 nM) leading to complete reversal. Thus, **2d** exerts functional antagonism within the D_4 receptor system.

Compound **2d** was also tested for affinity at the serotonergic and adrenergic receptors at which other D_4 antagonists have shown activity. Compound **2d** was inactive at 5HT-2 (>10 μM) and displayed weak activity at both alpha-1 (42 μM) and alpha-2 (5 μM).

Conclusion

A series of 6-(4-benzyl]piperazin-1-yl)benzodioxanes and related analogues were prepared and found to be high affinity, high selectivity D_4 receptor antagonists. Several compounds were more potent (K_i <10 nM) at D_4 and several orders of magnitude weaker at D_2 than clozapine. Reports on the antipsychotic effects in animal models and in clinical trials of selective D_4 receptor antagonists have so far proved disappointing. It appears that D_4 selective antagonists are no longer to be expected to reverse the symptoms of schizophrenia.[15] It is reasonable to assume, however, that compounds that include a component of D_4 antagonism in combination with other activities may lead to the desired therapeutic

effect with reduced side effects. Genomic studies have revealed that D_4 receptor gene polymorphism may be associated with other disorders and these may prove to be better targets for D_4 receptor antagonists. Indeed it was recently reported that the selective D_4 receptor antagonist, U-101387, prevents stress-induced cognitive deficits in monkeys.[16] The ever widening variety of structural classes with high affinity for the dopamine D_4 receptor holds promise for elaboration of the function of this receptor system in vivo.

Experimental

Chemistry

Melting points were determined using a Thomas–Hoover capillary melting apparatus and are uncorrected. Elemental analyses were obtained for all compounds tested for receptor binding. Elemental analysis were performed at Robertson Microlabs, Madison, NJ, USA and are within 0.4% of theoretical C, H, and N with the exception of **2c** where carbon analysis was 0.54% above the theoretical value. ^{1}H NMR spectra were recorded in $CDCl_3$ (unless otherwise noted) with tetramethylsilane (TMS) as the internal standard on a Varian Unity 400 MHz spectrometer. Electron ionization mass spectra (MS) were recorded on a Hewlett-Packard 5890 mass spectrometer. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was performed using silica gel and the flash technique. Representative procedures and physical properties for selected compounds are described.

1-(4-Methoxyphenyl)-4-benzylpiperazine (1). A solution of 1-(4-methoxyphenyl)piperazine (96 mg, 0.5 mmol) in acetonitrile (5 mL) was treated with potassium carbonate (0.4 g) and benzyl bromide (94 mg, 0.55 mmol). The mixture was heated to reflux for 4 h, cooled to room temperature and diluted with ethyl acetate (15 mL). The mixture was washed with water (20 mL), saturated NH_4Cl solution (15 mL), brine (20 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel eluting with hexane/ether (1:1) and gave the title compound as a white solid (130 mg, 92%): mp 78 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.60–2.63 (m, 4H), 3.08–3.11 (m, 4H), 3.57 (s, 2H), 3.76 (s, 3H), 6.83 (d, J = 9.0 Hz, 2H), 6.89 (d, J = 9.0 Hz, 1H), 7.26–7.36 (m, 5H); MS (LC-MS) m/e 283 (MH^+). Anal. ($C_{18}H_{22}N_2O$) C, H, N.

1-(1,4-Benzodioxane-6-yl)piperazine (4). Tri(*o*-tolyl)phosphine (0.3 g, 1.0 mmol) was added to a suspension of *tris*(dibenzylideneacetone)dipalladium(0) (0.3 g, 1.0 mmol) in toluene (150 mL) followed by the addition of sodium *tert*-butoxide (3.3 g, 34 mmol), *tert*-butyl 1-piperazinecarboxylate (5.2 g, 28.2 mmol) and 1,4-benzodioxan-6-bromide (5.16 g, 24 mmol). The mixture was heated to reflux for 18 h and then cooled. The reaction mixture was washed with water (200 mL), brine (200 mL), dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography on silica gel eluting with ether/hexane (1:1) gave *tert*-butyl 4-(2H,3H-benzo[*e*]1,4-dioxin-6-yl)piperazinecarboxylate (4.37 g, 57%) as a pale yellow solid: mp 53–55 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 2.97–2.99 (m, 4H), 3.53–3.56 (m, 4H), 4.19–4.24 (m, 4H), 6.45–6.48 (m, 2H), 6.78 (d, J = 9.0 Hz, 1H). Trifluoroacetic acid (10 mL) was added slowly at 0 °C to a solution of *tert*-butyl 4-(2H,3H-benzo[*e*]1,4-dioxin-6-yl)piperazinecarboxylate (3.2 g, 10 mmol) in dichloromethane (10 mL). The mixture was stirred for 1 h then poured onto ice water (50 mL). The aqueous solution was made basic by the addition of aqueous ammonia solution and extracted with dichloromethane (2×100 mL). The combined extracts were washed with brine (150 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel eluting with dichloromethane/methanol (4:1) and gave the title compound (1.65 g, 75%) as a white solid: mp 134–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.02 (brs, 8H), 4.17–4.23 (m, 4H), 6.44–6.47 (m, 2H), 6.76 (d, J = 9.5 Hz, 1H).

General procedure for the preparation of compounds 2a–2j

A solution of 1-(1,4-benzodioxane-6-yl)piperazine (**4**) (110 mg, 0.5 mmol) in acetonitrile (5 mL) was treated with potassium carbonate (0.4 g) and the appropriate benzyl halide (0.55 mmol). The mixture was heated to reflux for 4 h, or until complete by TLC, cooled to room temperature and diluted with dichloromethane (15 mL). The mixture was washed with saturated NH_4Cl solution (15 mL), brine (20 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel eluting with the appropriate hexane/ether mixture. The free base was finally converted to the salt form indicated in the text.

6-(4-Benzylpiperazin-1-yl)benzodioxane dihydrochloride (2a). 89% yield from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and benzyl bromide: mp 144–145 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.58–2.61 (m, 4H), 3.07–3.08 (m, 4H), 3.56 (s, 2H), 4.19–4.24 (m, 4H), 6.45–6.47 (m, 2H), 6.76 (d, J = 8.5 Hz, 1H), 7.26–7.34 (m, 5H); MS (LC-MS) m/e 311 (MH^+). Anal. ($C_{19}H_{22}N_2O_2$2HCl) C, H, N.

6-(4-[2-Chlorobenzyl]piperazin-1-yl)benzodioxane oxalate (2b). 71% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and 2-chlorobenzyl chloride: mp 175–176 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.66–2.68 (m, 4H), 3.09–3.11 (m, 4H), 3.68 (s, 2H), 4.19–4.25 (m, 4H), 6.46–6.48 (m, 2H), 6.77 (d, J = 8.5 Hz, 1H), 7.19–7.27 (m, 2H), 7.36 (d, J = 8 Hz, 1H), 7.50 (d, J = 8 Hz, 1H); MS (LC-MS) m/e 345 (MH^+). Anal. ($C_{19}H_{21}ClN_2O_2.HO_2CCO_2H$) C, H, N.

6-(4-[3-Chlorobenzyl]piperazin-1-yl)benzodioxane dihydrochloride (2c). 67% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and 3-chlorobenzyl chloride: mp 217–218 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.57–2.60 (m, 4H), 3.07–3.09 (m, 4H), 3.52 (s, 2H), 4.19–4.25 (m, 4H), 6.45–6.47 (m, 2H), 6.76 (d, J = 8 Hz, 1H), 7.23–7.26 (m, 3H), 7.36 (s, 1H); MS (LC-MS) m/e 345 (MH^+). Anal. ($C_{19}H_{21}ClN_2O_2$2HCl) C, H, N.

6-(4-[4-Chlorobenzyl]piperazin-1-yl)benzodioxane dihydrochloride (2d). 75% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and 4-chlorobenzyl chloride: mp 215–216 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.57–2.59 (m, 4H), 3.09–3.11 (m, 4H), 3.49 (s, 2H), 4.18–4.25 (m, 4H), 6.42–6.44 (m, 2H), 6.78 (d, J = 8.5 Hz, 1H), 7.22–7.25 (m, 4H); MS (LC-MS) m/e 345 (MH^+). Anal. ($C_{19}H_{21}ClN_2O_2.2HCl$) C, H, N.

6-(4-[4-Methoxybenzyl]piperazin-1-yl)benzodioxane dihydrochloride (2e). 48% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and 4-methoxybenzyl chloride: mp 195–196 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.56–2.59 (m, 4H), 3.06–3.08 (m, 4H), 3.49 (s, 2H), 3.81 (s, 3H), 4.20–4.22 (m, 4H), 6.43–6.46 (m, 2H), 6.76 (d, J = 8.5 Hz, 1H), 6.87 (d, J = 8.5 Hz, 2H), 7.25 (d, J = 8.5 Hz, 2H) ; MS (LC-MS) m/e 341 (MH^+). Anal. ($C_{20}H_{24}N_2O_3.2HCl$) C, H, N.

6-(4-[3-Methoxybenzyl]piperazin-1-yl)benzodioxane dihydrochloride (2f). 48% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and 3-methoxybenzyl chloride: mp 215–216 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.58–2.62 (m, 4H), 3.04–3.08 (m, 4H), 3.58 (s, 2H), 3.82 (s, 3H), 4.18–4.24 (m, 4H), 6.42–6.44 (m, 2H), 6.78–6.84 (M, 2H), 6.98 (M, *2*1H), 7.22 (t, J = 7.5 Hz, 1H); MS (LC-MS) m/e 341 (MH^+). Anal. ($C_{20}H_{24}N_2O_3$. 2HCl) C, H, N.

6-(4-[2-Methoxybenzyl]piperazin-1-yl)benzodioxane oxalate (2g). 66% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and 2-methoxybenzyl chloride: mp 143–144 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.62–2.64 (m, 4H), 3.07–3.10 (m, 4H), 3.61 (s, 2H), 3.82 (s, 3H), 4.19–4.25 (m, 4H), 6.42–6.44 (m, 2H), 6.78 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 7 Hz, 1H), 6.93 (t, J = 7.5 Hz, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.39 (d, J = 7 Hz, 1H); MS (LC-MS) m/e 341 (MH^+). Anal. ($C_{20}H_{24}N_2O_3HO_2CCO_2H$) C, H, N.

6-(4-[4-Fluorobenzyl]piperazin-1-yl)benzodioxane dihydrochloride (2h). 80% from 1-(1,4-benzodioxane-6-yl)-piperazine (**4**) and 4-fluorobenzyl chloride: mp 178–179 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.58–2.59 (m, 4H), 3.08–3.10 (m, 4H), 3.51 (s, 2H), 4.19–4.23 (m, 4H), 6.41–6.44 (m, 2H), 6.79 (d, J = 8.5 Hz, 1H), 6.99–7.03 (m, 2H), 7.25–7.31 (m, 2H); MS (LC-MS) m/e 329 (MH^+). Anal. ($C_{19}H_{21}FN_2O_2.2HCl$) C, H, N.

6-(4-[4-Methylbenzyl]piperazin-1-yl)benzodioxane dihydrochloride (2i). 56% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and 4-methylbenzyl chloride: mp 199–201 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.35 (s, 3H), 2.58–2.60 (m, 4H), 3.03–3.08 (m, 4H), 3.52 (s, 2H), 4.19–4.26 (m, 4H), 6.42–6.45 (m, 2H), 6.77 (d, J = 8.5 Hz, 1H), 7.16 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.5 Hz, 2H); MS (LC-MS) m/e 325 (MH^+). Anal. ($C_{20}H_{24}N_2O_22HCl$) C, H, N.

6-[4-(Phenylethyl)piperazinyl]-2H,3H-benzo[e]1,4-dioxin dihydrochloride (2j). 51% from 1-(1,4-benzodioxane-6-yl)piperazine (**4**) and (1-bromoethyl)benzene: mp 218–220 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 1.40 (d, J = 6.5 Hz, 3H), 2.49–2.56 (m, 2H), 2.61–2.68 (m, 2H), 3.04–3.07 (m, 4H), 3.40 (q, J = 6.5 Hz, 1H), 4.18–4.23 (m, 4H), 6.43–6.45 (m, 2H), 6.77 (d, J = 9.5 Hz, 1H), 7.25–7.34 (m, 5H); MS (LC-MS) m/e 325 (MH^+). Anal. ($C_{20}H_{24}N_2O_22HCl$) C, H, N.

Chromane (5). A solution of 4-chromanone (2.00 g, 13.50 mmol) in 20 mL of acetic acid was added to a suspension of zinc dust (20 g) in acetic acid (40 mL). The mixture was heated at 100 °C for 4 h. After cooling to room temperature, the mixture was filtered through Celite and the filtrate evaporated. The residue was partitioned between ethyl acetate (50 mL) and 1 N NaOH (50 mL) and extracted with further ethyl acetate (2×50 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel eluting with hexane/ethyl acetate (9:1) and gave the title compound 1.63 g (91%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99–2.06 (m, 2H), 2.80 (t, J = 6.5 Hz, 2H), 4.19 (t, J = 5.0 Hz, 2H), 6.79–6.86 (m, 2H), 7.03–7.11 (m, 2H).

6-Piperazinylchromane (6). Chromane (**5**) (1.34 g, 10 mmol) was added portionwise to 70% HNO_3 (6 mL) at 0 °C. The mixture was stirred at room temperature for a further 1 h. The solution was poured into ice water (15 mL) and extracted with ethyl acetate (3×25 mL), dried and concentrated. The residue was triturated with ethanol and gave 6-nitrochromane 1.41 g (79%) as pale yellow crystals: mp 99–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01–2.09 (m, 2H), 2.85 (t, J = 6.0 Hz, 2H), 4.28 (t, J = 5.5 Hz, 2H), 6.83 (d, J = 9.5 Hz, 1H), 7.96–7.98 (m, 2H). 6-Nitrochromane (1.25 g, 7 mmol) was dissolved in ethanol (50 mL), treated with Raney nickel (1.5 g) and hydrogenated for 3 h at atmospheric pressure. The resulting mixture was filtered and concentrated to afford chromane-6-ylamine 950 mg (91%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93–1.97 (m, 2H), 2.69 (t, J = 5.5 Hz, 2H), 3.25 (brs, 2H), 4.10 (t, J = 5.0 Hz, 2H), 6.39 (s, 1H), 6.45 (d, J = 8.5 Hz, 1H), 6.61 (d, J = 8.5 Hz, 2H). A solution of chromane-6-ylamine (0.745 g, 5 mmol), bis-(2-chloroethyl)amine hydrochloride (1.07 g, 6 mmol) and potassium carbonate (1.66 g, 12 mmol) in 25 mL chlorobenzene was heated to reflux for 24 h. The dark brown reaction mixture was partitioned between 3N NaOH (25 mL) and dichloromethane (50 mL). The organic layer was separated, dried and concentrated. The residue was purified by flash chromatography on silica gel eluting with dichloromethane/methanol (19:1) and gave the title compound 0.58 g (53%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97–2.00 (m, 2H), 2.75 (t, J = 6.0 Hz, 2H), 3.18 (brs, 8H), 4.13 (t, J = 5.0 Hz, 2H), 6.36 (s, 1H), 6.72–6.73 (m, 2H).

6-[4-(4-Chlorobenzyl)piperazinyl]chromane (7). A solution of 6-piperazinylchromane (**6**) (0.11 g, 0.5 mmol) in acetonitrile (5 mL) was treated with potassium carbonate (0.4 g) and 4-chlorobenzyl chloride (89 mg, 0.55 mmol). The mixture was heated to reflux for 4 h, cooled to room temperature and diluted with dichloromethane (15 mL). The mixture was washed with saturated NH_4Cl solution (15 mL), brine (20 mL), dried ($MgSO_4$)

and concentrated. The residue was purified by flash chromatography on silica gel eluting with hexane/ether (1:1) and gave the title compound 135 mg (79%) as a white solid: mp 94–95 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 1.95–1.99 (m, 2H), 2.58 (brs, 4H), 2.75 (t, J = 6.5 Hz, 2H), 3.06 (brs, 4H), 3.52 (s, 2H), 4.12 (t, J = 5.0 Hz, 2H), 6.62 (s, 1H), 6.72 (s, 2H), 7.28–7.29 (m, 4H); MS (LC-MS) m/e 343 (MH^+). Anal. ($C_{20}H_{23}ClN_2O{\cdot}C_2H_2O_4$) C, H, N.

5-{4-[(4-Chlorophenyl)methyl]piperazinyl}-2H-benzo[*d*]1,3-dioxolane dihydrochloride (8). 53% from 5-piperazinyl-2H-benzo[*d*]1,3-dioxolane and 4-chlorobenzyl chloride according to the procedure described for compound **7**: ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.59–2.60 (m, 4H), 3.07–3.09 (m, 4H), 3.62 (s, 2H), 5.90 (s, 2H), 5.99–6.04 (m, 2H), 6.49 (d, J = 8 Hz, 1H), 7.01 (d, J = 7 Hz, 2H), 7.15 (d, J = 7 Hz, 2H); MS (LC-MS) m/e 331 (MH^+). Anal. ($C_{18}H_{19}ClN_2O_2$2HCl) C, H, N.

5-{4-[(4-Chlorophenyl)methyl]piperazinyl}-2,3-dihydrobenzo[*b*]furan dihydrochloride (9). 55% from 5-piperazinyl-2,3-dihydrobenzo[*b*]furan and 4-chlorobenzyl chloride according to the procedure described for compound **7**: mp 218–219 °C; ^{1}H NMR (free base, 400 MHz, $CDCl_3$) δ 2.58–2.60 (m, 4H), 3.05–3.08 (m, 4H), 3.18 (t, J = 7 Hz, 2H), 3.52 (s, 2H), 4.53 (t, J = 7 Hz, 2H), 6.64–6.66 (m, 2H), 6.85 (s, 1H), 7.22–7.25 (m, 4H); MS (LC-MS) m/e 329 (MH^+). Anal. ($C_{19}H_{21}ClN_2O$.2HCl) C, H, N.

Biological methods

Expression of recombinant dopamine receptors. The recombinant primate $D_{4.2}$ receptor was prepared from the $D_{4.2}$ minigene expression construct by replacement of the NotI-KasI fragment containing two introns with a synthetic DNA fragment encoding the intron-deleted sequence (Genbank #HSD4DOP). Stable clones expressing each receptor were isolated under G418 selection after calcium phosphate transfection of CHO-K1 cells (the primate $D_{4.2}$ plasmid was cotransfected with pSV2Neo, Clontech). The membranes prepared from cell pellets were stored at −80 °C.

Membrane preparation. Pellets containing selected cloned dopamine receptor membrane were thawed on ice and resuspended in ice cooled 50 mM Tris buffer (pH 7.4 at 25 °C) containing 120 mM NaCl, 1 mM EDTA and 5 mM $MgCl_2$. All subsequent work was performed on ice. The membranes were homogenized on a Brinkmann Polytron (10 s, setting 5). The homogenate was centrifuged at 48,000g and 4 °C for 10 min (DuPont Sorvall RC5B). The pellet was resuspended in fresh buffer and centrifugation was repeated. The pellet was again resuspended in fresh buffer and centrifuged a final time at 48,000 g and 4 °C for 10 min. The pellet was resuspended to a final concentration of 40 mg protein/mL with 50 mM Tris buffer (pH 7.4 at 25 °C) containing 120 mM NaCl, just prior to addition to the assay tubes. The protein content was determined using the Bio-Rad assay (Hercules, CA), with bovine plasma gamma globulin as a standard.

Binding assay. For D_2, D_3, and D_4 binding, each sample was tested in triplicate in a final volume of 0.25 mL in Polypropylene microtube trips containing 0.1 nM [^{3}H]YM 09151 (81.4 Ci/mmol, NEN DuPont), and CHO cell homogenate (40 μg protein) in 50 mM Tris buffer (pH 7.4 at 25 °C) containing 120 mM NaCl. After a 2 h incubation at room temperature, the samples were rapidly filtered through 1% PEI treated GF/C filters using a Tomtec Harvester 96. The filters were rinsed with two washes of assay buffer. After air drying, bound radioactivity was then quantitated via the BetaPlate Scintillation Counter at an efficiency of 65%. Non-specific binding was defined with 10 μM spiperone for D_2 and D_4 and with 1 μM haloperidol for D_3.

Dopamine functional assays. CHO cells expressing primate $D_{4.2}$ receptor were grown to confluency in Ham's media supplemented with 10% fetal calf serum, harvested, and then stored as pellets at −80 °C. Thawed cells were homogenized using a Polytron (30 s, setting 5) in 50 mM Tris pH 7.4, 10 mM $MgCl_2$ and 2 mM EGTA. Membrane homogenates were centrifuged at 14,000g for 10 min and the pellet washed one time in cold PBS. The final pellet was resuspended in homoginization buffer and stored at −80 °C. On the day of the assay, thawed membrane homogenates were resuspended in assay buffer (50 mM Tris pH 7.4, 120 mM NaCl, 10 mM $MgCl_2$, 2mM EGTA, 0.1% BSA, 0.1 mM bacitracin, 100 KIU/mmL aprotinin, 5 μM GDP) and added to reaction tubes at a concentration of 25 μg/0.200 mL. Reactions were initiated by the addition of 100 pM GTPγ^{35}S, dopamine (0.01–10 μM) and individual compounds ranging in concentration from 0.1 nM to 10 μM. Following a 45 min incubation at 22 °C, the reaction was terminated by vacuum filtration over GF/C filters with ice-cold wash buffer (50 mM Tris pH 7.4, 120 mM NaCl). Bound GTPγ^{35}S was determined by liquid scintillation spectrometry. Non-specific binding was defined by 10 μM GTPγS and represented less than 10% of total binding.

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