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Binding of 2,4-disubstituted morpholines at human D_4 dopamine receptors

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Abstract

The synthesis of a series of 2,4-disubstituted morpholines is described and their affinities at human dopamine receptors reported. The orally bioavailable 7-azaindole compound **11** has nanomolar affinity at the hD_4 receptor with >1000-fold selectivity over the hD_2 receptor.

Key words: Morpholine, ligands, dopamine, receptors, psychosis.

1. Introduction

Recent advances in the molecular biology of dopamine receptors have seen the cloning of a number of different subtypes [1]. These receptors, based on their pharmacology, can be classified into two classes, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4). Studies have suggested that D_4 receptors are preferentially located in brain areas associated with antipsychotic activity [2] and a report [3] has suggested that D_4 receptor density may be elevated in post-mortem schizophrenic brain, although a more recent study has not confirmed the findings [4]. Therefore, there is clearly a need to characterise selective D_4 receptor ligands in order to elucidate the role of human D_4 receptors (hD_4) in psychosis.

Recent literature reports [5] have described novel, selective, D_4 receptor antagonists (e.g., **1**, **2**, and **3**, Fig. 1). These agents will be valuable in determining the relevance of blocking the receptor within the central nervous system. Studies leading to the identification of **1** noted that the 4-hydroxypiperidine (**4**) is D_2 selective, whereas the piperazine (**5**) proves to be D_4 selective [5a]. The major interest within our laboratories has focused on the identification of selective ligands for the hD_4 receptor.

This report describes the evolution, synthesis and in vitro evaluation of an isosteric morpholine series. These analogues, related to **1**, provide high affinity, selective hD_4 receptor ligands with good oral bioavailability.

2. Chemistry

The synthesis of compounds within the indole and azaindole series (Scheme 1) is exemplified by the preparation of **11**. The formation of the morpholines was accomplished by reaction of the appropriate epoxide with 2-aminoethyl hydrogen sulphate under basic conditions. Opening of the epoxide by the amine occurred followed by ring cyclisation under more rigorous basic conditions. The morpholines were isolated by a simple acid-base extraction procedure then purified by chromatography or vacuum distillation and obtained pure in moderate yields (30–40%). Although not a high yielding process this simple procedure, employing a chemical isolation work-up to remove many of the unwanted byproducts, allowed ready access to 2-substituted morpholines on a multi-gram scale. The target compounds were readily prepared in good yields (65–90%) by the displacement of gramine (**6–8**) or 7-azagramine [6] (**9–13**) with the morpholine in refluxing toluene. The isolation of products in this series was achieved without the use of chromatography, allowing the procedure to be amenable to rapid analogue synthesis. Analogues derived from (3,4-epoxybutyl)-benzene (**19**) were pre-

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pared from hydrocinnamaldehyde (**20**) and the ylide available from trimethylsulphonium iodide.

The benzimidazole (**14**) and pyridyl analogues (**16**) were obtained (Scheme 1) from 2-(chloromethyl)-benzimidazole (**22**) and 3-picolyl chloride (**23**) respectively, by nucleophilic displacement with 2-(phenoxymethyl)-morpholine in ethanol at reflux (66–72%). The quinoline analogue (**15**) was synthesized in moderate yield (36%) from quinoline-3-carboxaldehyde (**24**) and the morpholine in refluxing formic acid under Leuckart–Wallach conditions [7].

3. Results and discussion

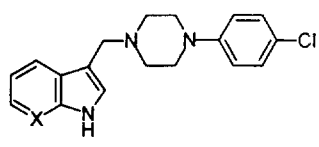
Receptor binding was determined by the displacement of [^{3}H]-spiperone from cloned human receptors; the D_2 being stably expressed in CHO cells and D_3 and D_4 in HEK293 cells [8]. Simple molecular modelling (using the Merck modelling facility and the molecular mechanics programme OPTIMOL [9]) suggested that a two atom unit, rather than a single atom substituent between the morpholine and an aromatic ring, more closely resembled the pharmacophore mapped out by analogues such as **1**. Indeed this proves to be the case (Table 1), the phenethyl analogue (**7**) has 12-fold greater affinity for the hD_4 receptor compared to the benzyl analogue (**6**). The azaindole analogues were prepared based on the observation in the piperazine series (cf **1**) that some enhancement of D_4 receptor selectivity could be obtained. The comparison of **9** with **7** indicates that this trend is the same in this morpholine series. For ease of synthesis the phenoxy compounds were prepared to compare with their phenethyl counterparts and have essentially identical affinity at the hD_4 receptor in vitro, although the selectivity over D_2 is reduced. This deficiency was overcome by the addition of lipophilicity to the morpholine 2-substituent. The inclusion of a chloro substituent at the 3 or 4-position of the aromatic ring attains nanomolar affinity and >1000-fold selectivity over hD_2 receptor affinity (**11** and **12**). Replacement of the 4-chloro substituent by a methoxy group (**13**) results in a tenfold reduction in hD_4 receptor affinity. The reduction in affinity of the benzimidazole (**14**) and quinoline (**15**) analogues may be partly explained by the reduction in pKa (Table 2) of the morpholine basic nitrogen. Removal of the fused benzo ring in **15**, to give the pyridyl analogue (**16**), results in total loss of affinity for dopamine receptors. This result cannot be ascribed to a pKa effect alone.

Compound **11** had a measured pKa of 6.65 and LogD (LogP at pH 7.4) of 3.18. This physicochemical data (Table 2) suggests that compounds from within this series would be highly brain penetrant and therefore suitable as ligands to interact with D_4 receptors within the central nervous system.

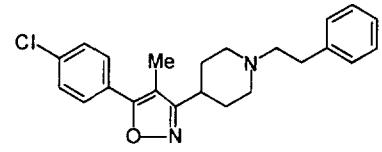
In view of its high receptor affinity and selectivity, **11** was investigated in further detail in vivo. In rats **11** is well absorbed following po administration at 20 mg/kg, with a plasma C_{max} of 1500 ng/ml and an oral bioavailability of 43%. Plasma concentrations reach their maximum at 30 min and remain at the 800 ng/ml level 2 h post dose.

4. Summary

The synthesis and dopamine receptor binding of a series of morpholines has been described. These ligands are related to highly selective D_4 receptor antagonists containing a phenyl-piperazine group (exemplified by **1**).



1 X = N
5 X = CH



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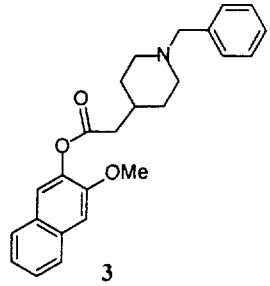

3

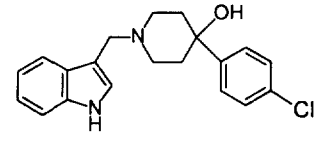

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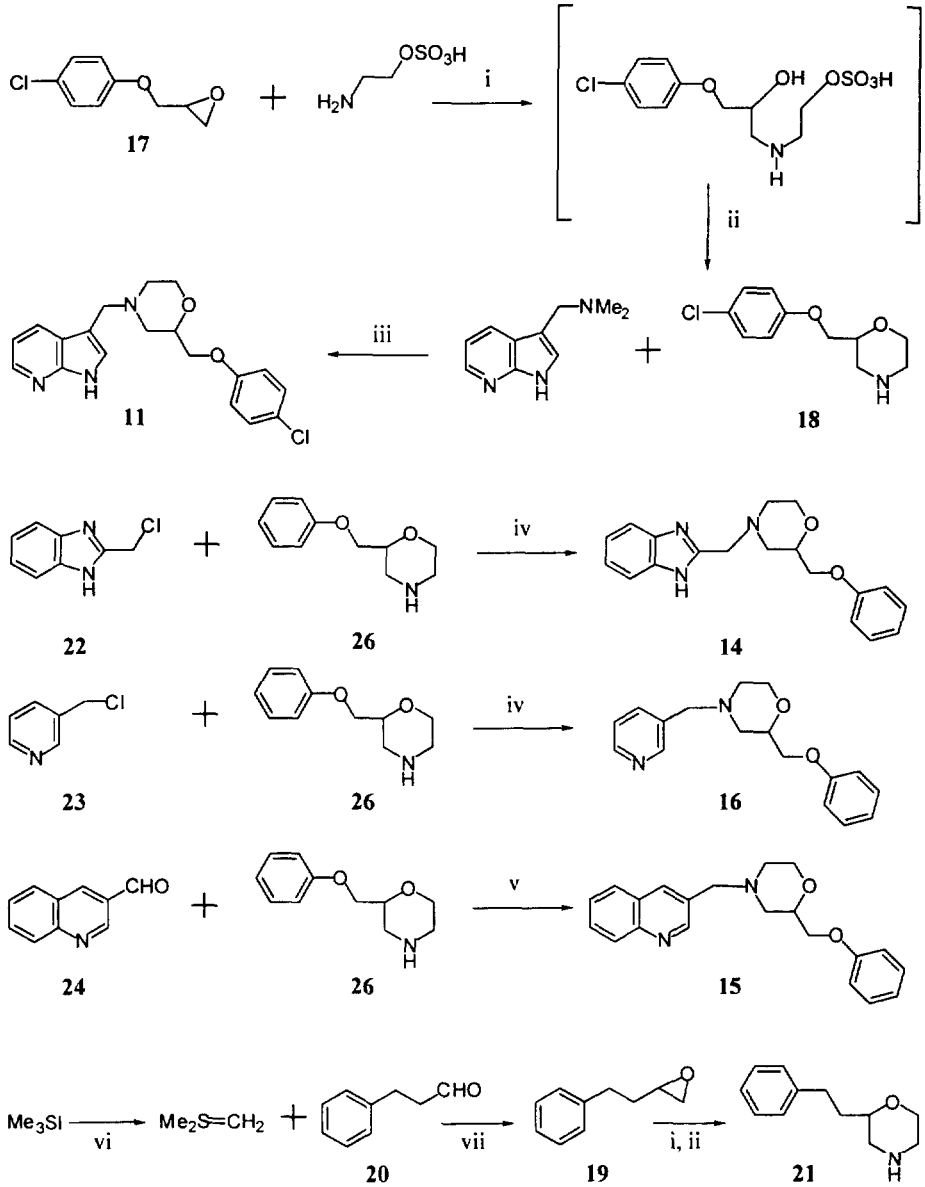
Fig. 1.

Structure–activity studies have led to the identification of the 2-(4-chlorophenoxymethyl)-morpholine analogue (**11**), which shows nanomolar affinity for the human D_4 receptor and > 1000-fold selectivity over the hD_2 receptor. In vivo studies in rats show **11** has good bioavailability with high plasma levels after oral administration. These data, along with the favourable physicochemical parameters within this series, suggest that **11** would provide a valuable tool, complementary to the piperazine series, in elucidating the function of D_4 receptors within the central nervous system.

5. Experimental

5.1 General conditions

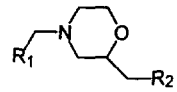
^{1}H NMR were recorded at 360 MHz on a Bruker AM360 spectrometer or at 250 MHz on a Bruker AC250 spectrometer. Mass spectra were recorded on a VG Quattro mass spectrometer. HPLC were run on a spherisorb ODS2 column using acetonitrile/0.2% triethylamine (pH to 3, phosphoric acid) mixtures, flow rate 1 ml/min at $\lambda = 230$ nm, on a Hewlett–Packard 1090

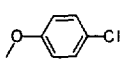
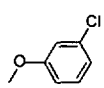
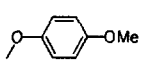
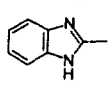
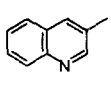


Scheme 1. Preparation of 2,4-disubstituted morpholines. Reagents and conditions: (i) NaOH, H_2O, MeOH, 40°C, 2 h; (ii) NaOH, H_2O, toluene, 65°C, 7 h; (iii) toluene, Δ, 18 h; (iv) K_2CO_3, Δ, 2–3 h; (v) HCO_2H, 120°C, 6 h; (vi) NaH, DMSO, THF, 5°C to rt, 2 h.

instrument. All preparative chromatography was performed using Merck Kieselgel 60 (0.035-0.070 mm). Melting points are uncorrected and were measured on a capillary Electrothermal melting point apparatus. Elemental analyses were carried out by Butterworths Laboratories, Twickenham, United Kingdom, and

Table 1
Binding affinities for, 2,4-disubstituted morpholines compared to ligands **1** and **5**



Compound[b]	R_1	R_2	K_i(nM)[a]		
			D_2	D_3	D_4
1			860 (1030, 715)	2300 (2670, 1920)	0.43 (0.61, 0.33)
5			71 (85, 60)	150 (170, 115)	1.6 (1.9, 1.2)
6			> 2000 (36%)[c]	> 3600 (3%)	74[d]
7			1300 (1540, 1110)	3210 (3770, 2740)	6.2 (7.5, 5.1)
8			400 (455, 353)	1480 (2270, 965)	5.6 (6.7, 4.7)
9			> 1800 (20%)	> 4700 (26%)	7.1 (7.3, 7.0)
10			1300 (1550, 1160)	> 4000 (35%)	6.5 (9.5, 4.5)
11			> 1700 (40%)	3200 (4070, 2540)	1.5 (1.6, 1.4)
12			> 1900 (25%)	4300 (4780, 3860)	2.1 (2.6, 1.7)
13			> 1900 (13%)	> 4900 (11%)	21[d]
14			> 1200 (12%)	> 3400 (8%)	39[d]
15			> 1800 (46%)	> 4600 (33%)	25 (27, 24)
16			> 950 (11%)	> 2900 (0%)	> 2000 (21%)

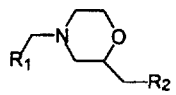
[a]Displacement of [^{3}H]spiperone. Data are the geometric means of two to five independent determinations. Statistical limits are given in parentheses.
[b]All morpholine compounds are racemic.
[c]Full K_i not obtained, percentage inhibition at the concentration shown given in parentheses.
[d]Single determination.

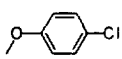
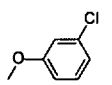
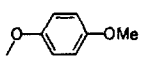
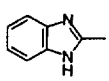
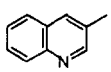
were within 0.4% of their theoretical values unless otherwise reported.

*5.1.1 2-[4-Chlorophenoxymethyl]-morpholine (**18**)*

2-Aminoethyl hydrogen sulphate (78.5 g, 0.556 mol) was added in portions to a stirred mixture of 4-chlorophenyl-2,3-epoxypropyl ether (**17**, 24.5 g, 0.133 mol) and NaOH (42.4 g, 1.06 mol) in H_2O (100 ml) and MeOH (40 ml). After addition the reaction mixture was stirred at 40°C for 2 h. After cooling the mixture was treated with NaOH (32.8 g, 0.82 mol) then toluene (150 ml) and stirred at 65°C for 7 h. The mixture was cooled, diluted

Table 2
Physical and physicochemical properties of 2,4-disubstituted morpholines **6** to **16**



Compound	R_1	R_2	mp(°C)	HPLC (%)[a]	CHN[b]	LogD[c]	pKa[d]
6			122–124	>99.5	$C_{20}H_{22}N_2O.C_2H_2O_4$		7.55
7			170–171	98.4	$C_{21}H_{24}N_2O.0.9C_2H_2O_4$		[e]
8			169–171	>99.5	$C_{20}H_{22}N_2O_20.7C_2H_2O_4$		
9			200–201	>99.5	$C_{20}H_{23}N_3O.C_2H_2O_4$		
10			188–189	96.1	$C_{19}H_{21}N_3O_2.C_2H_2O_4$	3.01	6.61
11			190–191	99.0	$C_{19}H_{20}ClN_3O_2.C_2H_2O_4$	3.18	6.65
12			177–178	98.2	$C_{19}H_{20}ClN_3O_2.C_2H_2O_40.25H_2O$		
13			183–184	>99.5	$C_{20}H_{23}N_3O_3.C_2H_2O_4$		
14			171–172	99.0	$C_{19}H_{21}N_3O_2$	3.17	5.07
15			164–166	>99.5	$C_{21}H_{22}N_2O_2.C_2H_2O_40.5H_2O$		5.69
16			160–161	97.4	$C_{17}H_{20}N_2O_2.C_2H_2O_4$		5.63

[a]Spherisorb ODS2 column, λ = 210 or 230 nm, 70% CH_3CN/30% of 0.2% NEt_3, pH to 3 with H_3PO_4, flow rate 1 ml/min.
[b]C, H, and N values within 0.4% of the theoretical values. All hydrogen oxalate salts recrystallised from EtOH/H_2O or EtOH/acetone mixtures. The free base of **14** was recrystallised from EtOAc:*n*-hexane (2:1).
[c]LogD = LogP (at pH 7.4). Octanol/pH 7.4 aqueous buffer shake flask method used.
[d]Recorded on a Sirius PCA-101 titrator.
[e]Accurate value of **7** could not be recorded due to poor aqueous solubility.

with toluene (60 ml) and H_2O (200 ml). The toluene layer was extracted with 2 M HCl (2×100 ml). The combined acid extracts were basified to pH 12 with 40% NaOH then extracted with toluene (3×100 ml). The combined organics were dried (Na_2SO_4) then evaporated to give a yellow gum (20 g), which was purified by column chromatography on silica using EtOAc to remove lipophilic impurities followed by EtOAc: MeOH:NH_3 (9:1:0.1) to elute the product as a pale-yellow gum (10.8 g, 36%) which solidified on standing, mp 51–52°C. ^{1}H NMR ($CDCl_3$) δ 2.71–3.04 (4H, m), 3.67 (1H, ddd, $J_1=3$, $J_2=J_3=11$ Hz), 3.82–3.98 (4H, m), 6.84 (2H, d, $J=8$ Hz), 7.21 (2H, d, $J=8$ Hz); MS, CI$^+$, m/z 228 $(M+H)^+$; calcd for $C_{11}H_{14}ClNO_2$:C, 58.03; H, 6.20; N, 6.15%. Found C, 57.92; H, 5.94; N, 5.92%. Using the same procedure the following were prepared:

*5.1.2 2-[2-Phenylethyl]-morpholine (**21**)*

In 38% yield. ^{1}H NMR ($CDCl_3$) δ 1.63–1.85 (2H, m), 2.53–2.91 (6H, m), 3.38–3.42 (1H, m), 3.58 (1H, ddd, $J_1=3$, $J_2=J_3=11$ Hz), 3.88 (1H, dd, $J_1=2$, $J_2=11$ Hz), 7.15–7.29 (5H, m); MS, CI$^+$, m/z 192 $(M+H)^+$; calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32%. Found C, 75.61; H, 8.62; N, 7.04%.

*5.1.3 2-[3-Chlorophenoxymethyl]-morpholine (**25**)*

In 32% yield. ^{1}H NMR ($CDCl_3$) δ 2.72–3.05 (4H, m,), 3.68 (1H, ddd, $J_1=3$, $J_2=J_3=11$ Hz), 3.82–4.00 (4H, m), 6.78–6.82 (1H, m), 6.90–6.95 (2H, m), 7.18 (1H, dd, $J_1=J_2=11$ Hz); MS, CI$^+$, m/z 228 $(M+H)^+$; calcd for $C_{11}H_{14}ClNO_2$: C, 58.03; H, 6.20; N, 6.15%. Found C, 57.53; H, 6.21; N, 5.75%.

*5.1.4 2-[Phenoxymethyl]-morpholine (**26**)*

In 44% yield. ^{1}H NMR ($CDCl_3$) δ 2.54–2.85 (2H, m), 2.92 (1H, ddd, $J_1=2$, $J_2=J_3=12$ Hz), 3.05 (1H, dd, $J_1=2$, $J_2=12$ Hz), 3.67 (1H, ddd, $J_1=2$, $J_2=J_3=12$ Hz), 3.83–4.03 (4H, m), 6.88–6.97 (3H, m), 7.23–7.30 (2H, m); MS, CI$^-$, m/z 194 $(M+H)^+$; calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25%. Found C, 68.75; H, 7.99; N, 6.80%.

*5.1.5 2-[4-Methoxyphenoxymethyl]-morpholine (**27**)*

In 35% yield. ^{1}H NMR ($CDCl_3$) δ 2.71–3.10 (4H, m), 3.67 (1H, ddd, $J_1=3$, $J_2=J_3=11$ Hz), 3.76 (3H, s), 3.80–4.00 (4H, m), 6.81 (2H, d, $J=7$ Hz), 6.85 (2H, d, $J=7$ Hz); MS, CI$^+$, m/z 224 $(M+H)^+$; calcd for $C_{12}H_{17}NO_3$. $0.5H_2O$: C, 62.05; H, 7.81; N, 6.03%. Found C, 62.24; H, 7.61; N, 6.23%.

*5.1.6 2-Benzylmorpholine (**28**)*

In 32% yield. ^{1}H NMR ($CDCl_3$) δ 2.57–2.90 (6H, m), 3.53–3.67 (2H, m), 3.86 (1H, ddd, $J_1=J_2=1$, $J_3=12$ Hz), 7.18–7.31 (5H, m); MS, CI$^+$, m/z 178 $(M+H)^+$; calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90%. Found C, 74.05; H, 8.21; N, 7.44%.

*5.1.7 3-[2-(4-Chloro-phenoxymethyl)-morpholin-4-yl-methyl]-1H-pyrrolo[2,3-b]pyridine hydrogen oxalate (**11**)*

7-Azagramine [6] (500 mg, 2.85 mmol) and **18** (650 mg, 2.85 mmol) were heated at reflux in toluene (20 ml), with stirring, for 18 h. The reaction mixture was cooled, treated with silica (200 mg) and activated charcoal (200 mg), stirred at ambient temperature for 15 min then filtered, evaporated to dryness to afford the product free base as a flaky, colourless solid (870 mg, 85%), mp 140–141°C.

The hydrogen oxalate salt had mp 190–191°C (EtOH:H_2O); ^{1}H NMR (DMSO-d_6) δ 2.50–2.68 (2H, m), 3.04 (1H, d, $J=12$ Hz), 3.18 (1H, d, $J=12$ Hz), 3.64 (1H, dd, $J_1=J_2=12$ Hz), 3.90–4.02 (4H, m), 4.11 (2H, s), 6.94 (2H, d, $J=9$ Hz), 7.11 (1H, dd, $J_1=4$, $J_2=8$ Hz), 7.31 (2H, d, $J=9$ Hz), 7.54 (1H, s), 8.12 (1H, dd, $J_1=1$, $J_2=8$ Hz), 8.24 (1H, dd, $J_1=1$, $J_2=4$ Hz), 11.76 (1H, s), MS, CI$^+$, m/z 358 $(M+H)^+$; calcd for $C_{19}H_{20}ClN_3O_2.C_2H_2O_4$: C, 56.32; H, 4.95; N, 9.38%. Found C, 56.57; H, 4.77; N, 9.18%.

Using the same methodology the following were prepared (see Table 2 for physical and physicochemical properties):

5.1.8 Compound 6

Yield 92%. The hydrogen oxalate salt had mp 122–124°C; ^{1}H NMR (D_2O) δ 2.84 (2H, d, $J=6$ Hz), 2.96 (1H, dd, $J_1=J_2=12$ Hz), 3.16 (1H, ddd, $J_1=4$, $J_2=J_3=12$ Hz), 3.40–3.47 (2H, m), 3.74 (1H, dd, $J_1=J_2=12$ Hz), 3.97–4.10 (2H, m), 4.51 (1H, d, $J=14$ Hz), 4.58 (1H, d, $J=14$ Hz), 7.20–7.36 (7H, m), 7.55–7.58 (2H, m), 7.71 (1H, d, $J=7$ Hz); MS, CI$^+$, m/z 307 $(M+H)^+$; calcd for $C_{20}H_{22}N_2O$. $C_2H_2O_4$: C, 66.65; H, 6.10; N, 7.07%. Found C, 66.31; H, 6.01; N, 7.01%.

5.1.9 Compound 7

Yield 95%. The hydrogen oxalate salt had mp 170–171°C; ^{1}H NMR (DMSO-d_6) δ 1.64–1.70 (2H, m), 2.44–2.69 (4H, m), 3.05–3.16 (2H, m), 3.53–3.60 (2H, m), 3.92 (1H, d, $J=12$ Hz), 4.14 (2H, s), 7.02–7.28 (7H, m), 7.39-7.42 (2H, m), 7.70 (1H, d, $J=8$ Hz), 11.29 (1H, s); MS, CI$^+$, m/z 321 $(M+H)^+$; calcd for $C_{21}H_{24}N_2O$. $0.9C_2H_2O_4$: C, 68.21; H, 6.48; N, 6.98%. Found C, 68.04; H, 6.44; N, 6.98%.

5.1.10 Compound 8

Yield 91%. The hydrogen oxalate salt had mp 169–171°C; ^{1}H NMR (DMSO-d_6) δ 2.40–2.60 (1H, m), 3.01 (1H, d, $J=12$ Hz), 3.17 (1H, d, $J=12$ Hz), 3.62 (1H, dd, $J=J_2=12$ Hz), 3.90–4.05 (7H, m), 6.88–7.41 (9H, m), 7.70 (1H, d, $J=8$ Hz), 11.19 (1H, br. s); MS, CI$^+$, m/z 323 $(M+H)^+$; calcd for $C_{20}H_{22}N_2O_2.0.7C_2H_2O_4$: C, 66.69; H, 6.12; N, 7.27%. Found C, 66.94; H, 6.15; N, 7.12%.

5.1.11 Compound 9

Yield 78%. The hydrogen oxalate salt had mp 200–201°C; ^{1}H NMR (DMSO-d_6) δ 1.64–1.71 (2H, m), 2.47–2.70 (4H, m), 3.02–3.14 (2H, m), 3.43–3.59 (2H, m), 3.93 (1H, d, J = 10 Hz), 4.13 (2H, s), 7.10–7.28 (6H, m), 7.55 (1H, s), 8.12 (1H, d, J = 7 Hz), 8.25 (1H, dd, J_1 = 1, J_2 = 5 Hz), 11.83 (1H, s); MS, CI$^+$, m/z 322 $(M+H)^+$; calcd for $C_{20}H_{23}N_3O \cdot C_2H_2O_4$: C, 64.22; H, 6.12; N, 10.21%. Found C, 64.07; H, 5.98; N, 10.02%.

5.1.12 Compound 10

Yield 65%. The hydrogen oxalate salt had mp 188–189°C; ^{1}H NMR (DMSO-d_6) δ 2.55–2.75 (2H, m), 3.14 and 3.30 (2H, each d, J = 12 Hz), 3.86–4.03 (4H, m), 4.08–4.20 (3H, m), 6.84–7.27 (6H, m), 7.46 (1H, s), 8.07 (1H, dd, J_1 = 1, J_2 = 8 Hz), 8.27 (1H, dd, J_1 = 1, J_2 = 5 Hz), 11.43 (1H, s); MS, CI$^+$, m/z 324 $(M+H)^+$; calcd for $C_{19}H_{21}N_3O_2.C_2H_2O_4$: C, 61.01; H, 5.61; N, 10.16%. Found C, 60.61; H, 5.43; N, 9.97%.

5.1.13 Compound 12

Yield 80%. The hydrogen oxalate salt had mp 177–178°C; ^{1}H NMR (DMSO-d_6) δ 2.50–2.68 (2H, m), 3.06 (1H, d, J = 12 Hz), 3.20 (1H, d, J = 12 Hz), 3.66 (1H, dd, $J_1 = J_2$ = 12 Hz), 3.92–4.07 (4H, m), 4.13 (2H, s), 6.88–6.92 (1H, m), 6.98–7.02 (2H, m), 7.11 (1H, dd, J_1 = 4, J_2 = 8 Hz), 7.29 (1H, dd, $J_1 = J_2$ = 8 Hz), 7.56 (1H, s), 8.13 (1H, d, J = 8 Hz), 8.25 (1H, dd, J_1 = 1, J_2 = 4 Hz), 11.80 (1H, s); m/z 358 $(M+H)^+$; calcd for $C_{19}H_{20}ClN_3O_2.C_2H_2O_4.0.25H_2O$: C, 55.76; H, 5.01; N, 9.29%. Found C, 55.58; H, 5.21; N, 8.92%.

5.1.14 Compound 13

Yield 79%. The hydrogen oxalate salt had mp 183–184°C; ^{1}H NMR (DMSO-d_6) δ 2.49–2.75 (2H, m), 3.05 (1H, d, J = 12 Hz), 3.21 (1H, d, J = 12 Hz), 3.64 (1H, dd, $J_1 = J_2$ = 12 Hz), 3.69 (3H, s), 3.85–4.00 (4H, m), 4.13 (2H, s), 6.84 (4H, s), 7.11 (1H, dd, J_1 = 5, J_2 = 8 Hz), 7.55 (1H, s), 8.13 (1H, dd, J_1 = 1, J_2 = 8 Hz), 8.24 (1H, dd, J_1 = 1, J_2 = 5 Hz), 11.79 (1H, s); MS, CI$^+$, m/z 354 $(M+H)^+$; calcd for $C_{20}H_{23}N_3O_3.C_2H_2O_4$: C, 59.59; H, 5.68; N, 9.47%. Found C, 59.42; H, 5.69; N, 9.23%.

*5.1.15 2-[2-(Phenoxymethyl)-morpholin-4-ylmethyl]-benzimidazole (**14**)*

Compound **22** (172 mg, 1.03 mmol), **26** (200 mg, 1.03 mmol) and K_2CO_3 (142 mg, 1.03 mmol) in EtOH (8 ml) were heated at 80°C for 2 h. The mixture was cooled, evaporated then the residue partitioned between H_2O (10 ml) and EtOAc (25 ml). The organic layer was separated and the aqueous re-extracted with EtOAc (25 ml). The combined organics were dried (Na_2SO_4) then evaporated to give a beige gum. The crude product was purified by column chromatography on silica (using EtOAc then EtOAc:MeOH (25:1)) to give the title compound as a pale-yellow solid (220 mg, 66%), mp 171–172°C (EtOAc:hexane, 2:1); ^{1}H NMR ($CDCl_3$) δ 2.40 (1H, dd, $J_1 = J_2$ = 10 Hz), 2.51 (1H, ddd, J_1 = 3, $J_2 = J_3$ = 10 Hz), 2.79 (1H, d, J = 10 Hz), 2.99 (1H, d, J = 10 Hz), 3.82 (1H, ddd, J_1 = 3, $J_2 = J_3$ = 10 Hz), 3.91–4.06 (6H, m), 6.88 (2H, dd, J_1 = 1, J_2 = 8 Hz), 6.95 (1H, ddd, J_1 = 1, $J_2 = J_3$ = 8 Hz), 7.24–7.29 (4H, m), 7.57–7.61 (2H, m); MS, CI$^+$, m/z 324 $(M+H)^+$; calcd for $C_{19}H_{21}N_3O_2$: C, 70.57; H, 6.55; N, 12.99%. Found C, 70.55; H, 6.51; N, 12.96%.

Using the same procedure compound **16** was obtained in 72% yield. The hydrogen oxalate salt had mp 160–161°C (EtOH); ^{1}H NMR (DMSO-d_6) δ 2.18 (1H, dd, $J_1 = J_2$ = 11 Hz), 2.28 (1H, ddd, J_1 = 3, $J_2 = J_3$ = 11 Hz), 2.72 (1H, d, J = 11 Hz), 2.91 (1H, d, J = 11 Hz), 3.58 (1H, ddd, J_1 = 2, $J_2 = J_3$ = 11 Hz), 3.68 (3H, s), 3.81–3.87 (2H, m), 3.96 (2H, d, J = 5 Hz), 6.88–6.95 (3H, m), 7.27 (2H, dd, $J_1 = J_2$ = 8 Hz), 7.39 (1H, dd, J_1 = 5, J_2 = 8 Hz), 7.76 (1H, d, J = 8 Hz), 8.50 (1H, d, J = 5 Hz), 8.54 (1H, d, J = 2 Hz); MS, CI$^+$, m/z 285 $(M+H)^+$; calcd for $C_{17}H_{20}N_2O_2.C_2H_2O_4$: C, 60.85; H, 5.92; N, 7.48%. Found C, 60.43; H, 5.53; N, 7.21%.

*5.1.16 3-[2-(Phenoxymethyl)-morpholin-4-ylmethyl]-quinoline hydrogen oxalate (**15**)*

Compound **24** (447 mg, 2.8 mmol), **26** (500 mg, 2.6 mmol), and anhydrous HCO_2H (0.1 ml, 2.6 mmol) were heated at 120°C, with stirring, for 6 h. The reaction mixture was cooled, treated with 2 M HCl (20 ml) then washed with EtOAc (20 ml). The aqueous was basified to pH 11 with 2 M NaOH then extracted with EtOAc (2×30 ml). The combined organics were dried (Na_2SO_4) then evaporated to give an orange gum which was purified by column chromatography on silica (using EtOAc then EtOAc:MeOH, 25:1) to afford the compound as a pale-yellow gum (340 mg, 36%). The hydrogen oxalate salt had mp 164–166°C (EtOH:H_2O), ^{1}H NMR (DMSO-d_6) δ 2.23 (1H, dd, $J_1 = J_2$ = 11 Hz), 2.35 (1H, ddd, J_1 = 3, $J_2 = J_3$ = 11 Hz), 2.80 (1H, d, J = 11 Hz), 2.97 (1H, d, J = 11 Hz), 3.61 (1H, ddd, J_1 = 2, $J_2 = J_3$ = 11 Hz), 3.80–3.92 (4H, m), 3.96 (2H, d, J = 5 Hz), 6.89–6.94 (3H, m), 7.23–7.28 (2H, m), 7.59–7.64 (1H, m), 7.73–7.78 (1H, m), 7.97–8.04 (2H, m), 8.29 (1H, d, J = 2 Hz), 8.90 (1H, d, J = 2 Hz); MS, CI$^+$, m/z 335 $(M+H)^+$; calcd for $C_{21}H_{22}N_2O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$: C, 63.73; H, 5.81; N, 6.46%. Found C, 63.38; H, 5.84; N, 6.48%.

5.2 Molecular modelling

Basic modelling studies were performed within the Merck Molecular Modelling facility using OPTIMOL as the molecular mechanics programme [9]. The phenyl-piperazine part structure was obtained from crystal structure data of compound **1** [10]. The 2-benzyl and 2-phenethyl-morpholines were constructed within the

modelling programme and low energy conformations identified using the OPTIMOL programme.

5.3 Pharmacokinetic methods

Compound **11** was administered at doses of 6 and 20 mg/kg iv and po respectively in rat (three animals per route of administration). The rats were serially sampled for plasma up to 6 h. The vehicle used for the iv formulation was a mixture of 1-methyl-2-pyrrolidine:water. The vehicle used for the po formulation was PEG400. Each plasma sample (200 μl) was spiked with 1000 ng of an internal standard. Each sample was treated with 0.1 M NaOH (250 μl) followed by EtOAc (5 ml). After vortex mixing and centrifugation (10 min at 2000 rpm) the supernatent was removed, evaporated to dryness at 70°C under nitrogen then reconstituted in 120 μl mobile phase. 70 μl injections were made onto the following HPLC system. Hichrom Excel KR100 C8 5 μm column (150 mm×4.6 mm id), 25% CH_3CN in 0.1% TFA mobile phase at a flow rate of 1 ml/min. UV detection at 220 nm. A calibration of standards of 0, 2, 5, 10, 25, 100, 250, and 1000 ng of **11** with 1000 ng of internal standard were prepared by spiking into 200 μl control rat plasma. These were extracted as above giving a linear response (r^2 0.998).

5.4 Biochemical methods: [^{3}H]-spiperone binding studies

Clonal cell lines expressing the human dopamine D_2, D_3, and D_4 receptor subtypes were harvested in PBS (phosphate buffered saline) and then lysed in 10 mM Tris-HCl pH 7.4 buffer containing 5 mM $MgSO_4$ for 20 min on ice. Membranes were centrifuged at 50,000 g for 15 min at 4°C and the resulting pellets resuspended in assay buffer (50 mM Tris-HCl) pH 7.4 containing 5 mM EDTA, 1.5 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM KCl, 120 mM NaCl, and 0.1% ascorbic acid at 20 mg wet wt/ml (human D_4 HEK cells), 10 mg wet wt/ml (human D_2 CHO cells and D_3 HEK cells). Incubations were carried out for 120 min at ambient temperature (22°C) in the presence of 0.2 nM [^{3}H]-spiperone for displacement studies and were initiated by the addition of 20–100 μg protein in a final assay volume of 0.5 ml. The incubation was terminated by rapid filtration over GF/B filters presoaked in 0.3% PEI (polyethylenimine) and washed with ice-cold 50 mM Tris-HCl, pH 7.4. Specific binding was determined by 10 μM apomorphine and radioactivity determined by counting in a LKB beta counter. Binding parameters were determined by non-linear least squares regression analysis, from which the inhibition constant K_i could be calculated for each test compound.

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