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Laboratory note

Synthesis and structure–activity relationships of new arylpiperazines: *para* substitution with electron-withdrawing groups decrease binding to 5-HT_{1A} and D_{2A} receptors

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Abstract

Compounds in which *N*-phenylpiperazines were linked by a propyloxy chain to position 6 or 7 of a coumarin ring were designed and synthesised, and their affinities for 5-HT_{1A} and D_{2A} receptors were determined by radioligand binding assays. The influence of *para* substitution in the phenyl ring, substitution at position 4 of the coumarin system, and the coumarin position at which the piperazinylalkyl chain is linked was explored. Electron-withdrawing phenyl ring substituents *para* to the piperazine strongly reduced activity at both receptors. Binding at $5HT_{1A}$ was influenced by the bulk of substituents at position 4 of the coumarin system, and binding at D_{2A} by their electronic properties. Neither binding affinity was significantly affected by whether the piperazinylalkyl chain was inserted at position 6 or 7 of the coumarin system. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Disubstituted piperazines; Coumarins; 5-HT_{1A} receptors; D_{2A} receptors

1. Introduction

A number of psychiatric disorders, including anxiety, depression, schizophrenia and Parkinson's disease, are known to involve defects in the function of neural pathways sustained by the neurotransmitters dopamine and serotonin [1]. The blockade of dopamine receptors by agents such as haloperidol has antipsychotic effects [2], but the excessive affinity of these 'classical' antipsychotics for D_2 dopamine receptors causes extrapyramidal effects akin to those of Parkinson's disease [3]. The 'atypical' antipsychotic clozapine does not have severe extrapyramidal effects, but was withdrawn from the market because it causes agranulocytosis [4]. In recent years much effort has gone into the design of selective agonists of the presynaptic dopamine receptor D_{2A} with a view to depressing relevant dopamine pathways, without causing extrapyramidal effects, by inhibiting the synthesis and release of dopamine [5].

Dysfunction of serotonin-based pathways is involved in depression and anxiety [6], and numerous anxiolytics, such as buspirone, act at Type 1A serotonin receptors (5-HT_{1A}) [7]. Both buspirone and the compound 8-OH-DPAT not only act at 5-HT_{1A}, but also revert the catalepsy induced in rats by haloperidol [8,9]. This has led to a search for molecules with affinity for both D2 and 5-HT_{1A} as possible atypical antipsychotics [10]. Many of those that have been found possess an arylpiperazine moiety.

Compounds in which the *N*-arylpiperazine is the only ring system, such as mCCP and TFMPP (Fig. 1), are generally active at all varieties of 5-HT_1 receptor [11]. Linking a carbocyclic or heterocyclic system to the other piperazine nitrogen via a lipophilic chain sometimes results in selectivity for 5-HT_{1A}, as in the cases of

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PAPP [12] and compound **I** [13]; sometimes in mixed 5-HT_{1A}/D_{2A} activity, as in those of NAN-190 [14] and buspirone [7]; and sometimes in high selectivity for D_{2A}, as in those of OPC-4392 [15] and PD-119819 [16].

In previous work [17] on the synthesis of CNS-active compounds we prepared arylpiperazines linked to a coumarin system via a propyloxy chain, such as **II** (Fig. 1). These compounds have great affinity for both D_{2A} and 5-HT_{1A}, especially the 4-methyl compound **16**. In view of this, to investigate the possibility of modulating these affinities we have now prepared a series of **16** analogues with structures differing from that of **16** (a) in possessing an electron-donating group (OMe) or an electron—withdrawing group (NO_2) *para* to piperazine on the phenyl ring; (b) in the replacement of the 4-methyl group of **16** by substituents characteristic of each of the four quadrants of a Craig diagram for lipophilicity (π) versus electrophilicity (σ_p) (OSO_2Ph ($\pi+$, σ_p+), C_6H_{11} ($\pi+$, σ_p-), OMe ($\pi-$, σ_p-), and OSO_2Me ($\pi-$, σ_p+)); and (c) for half of the analogues, in having the arylpiperazinylpropyloxy moiety inserted at position 6 of the coumarin instead of position 7. Here we describe the synthesis and pharmacological evaluation of these compounds.

2. Chemistry

Compounds **4**, **5**, **14** and **15** were synthesized by direct condensation between 4-chloropropyl-phenylpiperazines with a nitro or methoxy group in the *para* position of the phenyl ring (compounds **3a** and **3b**) and 6(7)-hydroxy-4-methoxy- or 4-cyclohexyl-6(7)-hydroxy-coumarins (compounds **1** and **2** or **12** and **13**, respectively); see Fig. 2. The 4-chloropropyl-phenyl-piperazines **3** were prepared by alkylation of the corresponding phenylpiperazines with 1-bromo-3-chloropropane in acetone using NaOH as base; **3b** was

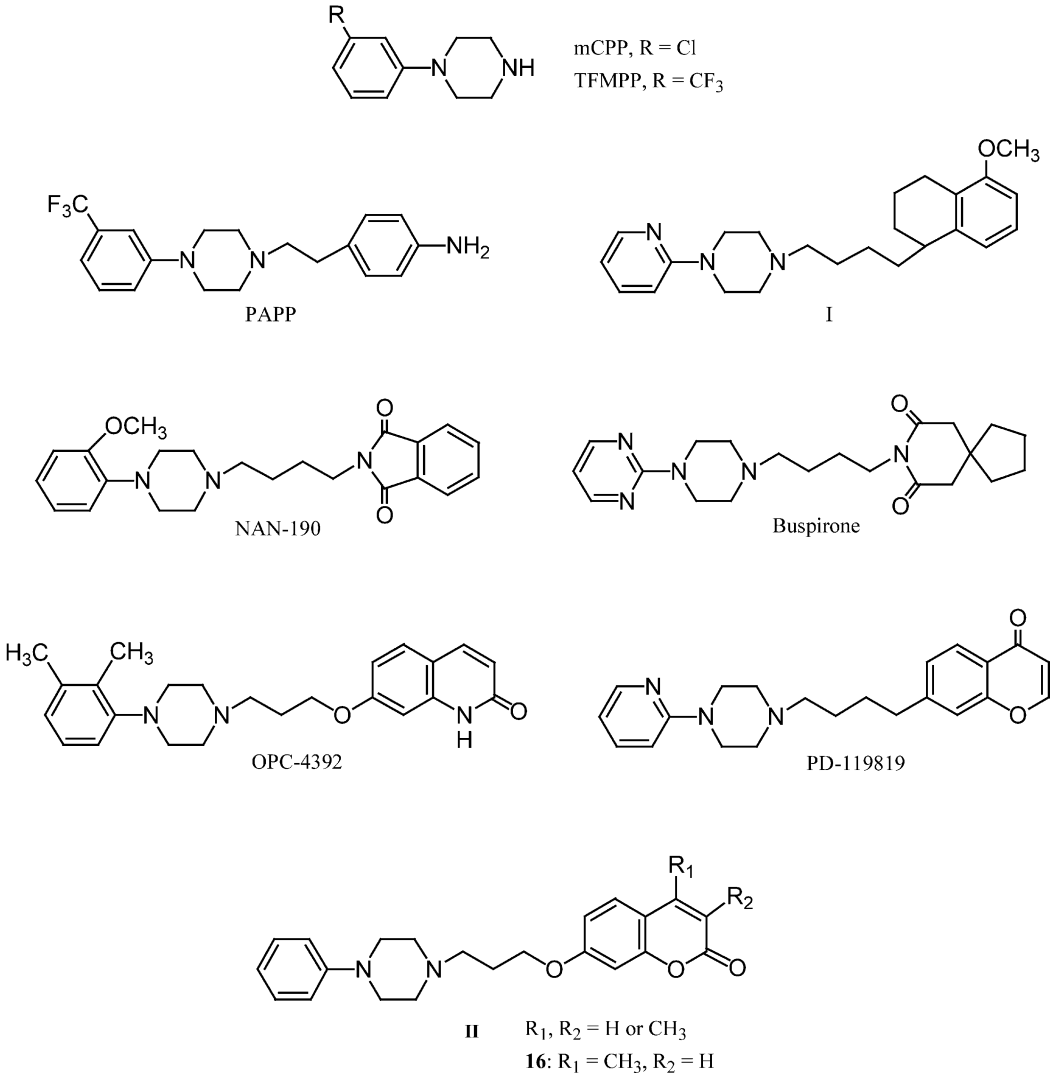


Fig. 1.

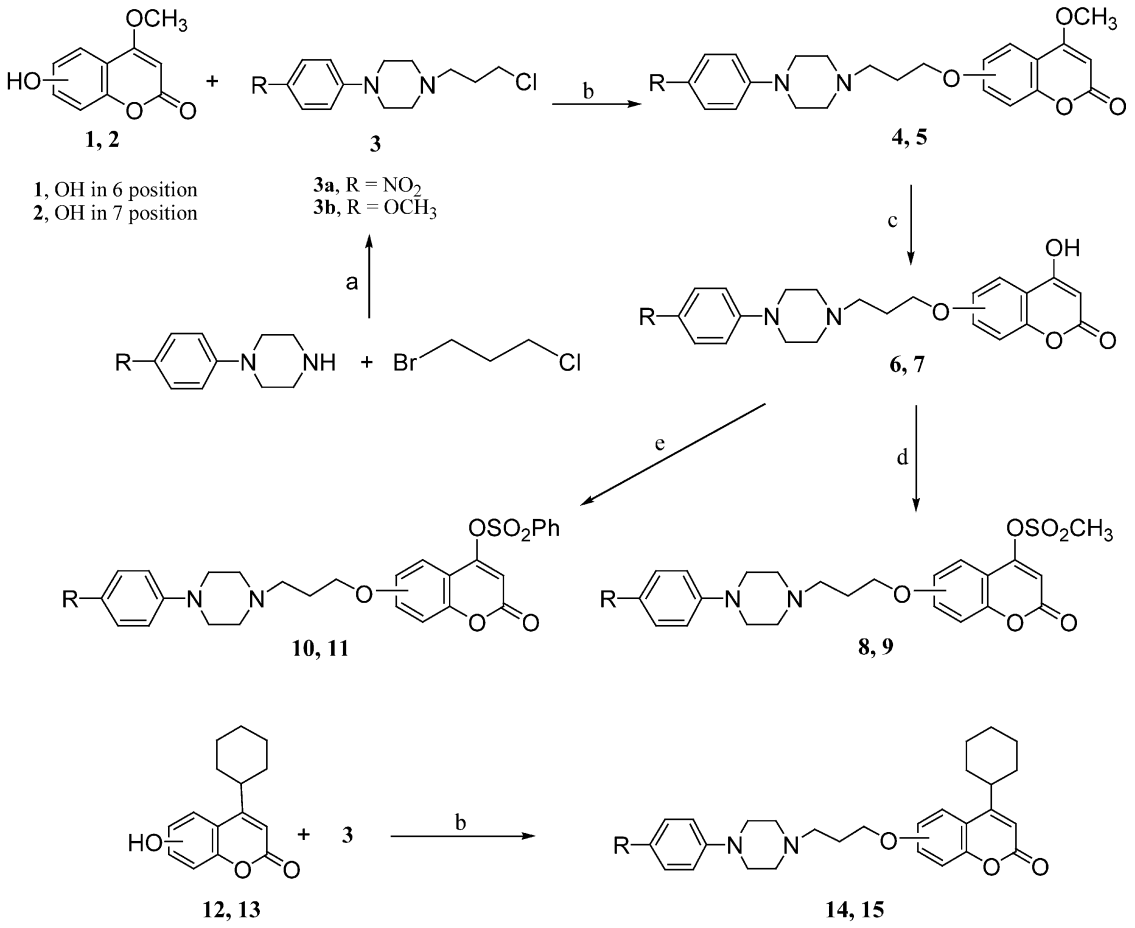


Fig. 2. Reagents: (a) NaOH/acetone; (b) (**1**) DMF/NaH, 100 °C, 1 h; (**2**) NaI, 100 °C, overnight; (c) HCl/AcOH, reflux, 45 min; (d) $CH_3SO_2Cl/CH_2Cl_2/Et_3N$; (e) $PhSO_2Cl/CH_2Cl_2/Et_3N$.

obtained in 77% yield [18] and the new compound **3a** in 82% yield. Coumarins **1** and **2** were obtained by selective methylation of the corresponding dihydroxycoumarins [19] by MeOH–HCl [20], and coumarins **12** and **13** as recently described elsewhere [21]. Reaction of **1**, **2**, **12** and **13** with compounds **3** in the presence of NaH and NaI in DMF afforded the desired compounds **4**, **5**, **14** and **15** in good yields.

Compounds **8**–**11** were obtained from **6** and **7** following preparation of the latter by hydrolysis of **4** and **5** with HCl in refluxing acetic acid. Treatment of **6** and **7** with methanesulphonylchloride and triethylamine in dichloromethane afforded **8** and **9** in, respectively, 54–60% yield (from **4**) and 60–63% yield (from **5**), and treatment of **6** and **7** with benzenesulphonylchloride and triethylamine in the same solvent gave **10** and **11** in, respectively, 46–50% yield (from **4**) and 53–55% yield (from **5**).

3. Pharmacology

For pharmacological assay, compounds **4**, **5**, **8**–**11**, **14** and **15** were converted to their water-soluble hydrochlorides. $5HT_{1A}$-binding assays were performed using rat hippocampus membranes with [^{3}H]-8-OH-DPAT as specific radioligand [22] (see Section 6). D_{2A}-binding assays were performed using membranes from mouse fibroblast (LTK-) cells transfected with human D_{2A}, with [^{3}H]-raclopride as radioligand [23].

4. Results and discussion

The results show that, for both 5-HT_{1A} and D_{2A} receptors, all the compounds studied in this work had very much less affinity than compound **16** (Table 1).

Previous studies have found that the steric hindrance in *para* position of the phenyl group is unfavourable for 5-HT_{1A} interaction specially for such electron-withdrawing substituents [24]. Nevertheless the substituents compared in these studies therefore differ not only in their electronic properties but also in other substituent parameters. In order to establish that the decrease in 5-HT_{1A} binding affinity is due directly to the electron-withdrawing effect, in this work we compare the nitro

and methoxy groups which quite exclusively differ in their σ_p parameters.

On the other hand, activity was not significantly or consistently affected by whether the phenylpiperazinylpropyloxy chain was inserted at position 6 or 7 of the coumarin. The effects of substituents at position 4 of the coumarin appear to depend on their bulk in the case of binding to 5-HT_{1A}: the most active compounds at this receptor were **4b** and **5b**, which have a 4-substitutent, MeO, that is similar in size to the Me substituent of compound **16** and much smaller than those of the other compounds assayed. Activity at D_{2A}, on the other hand, appears to depend more on the electronic properties of the 4-substituent: in this case, the most active compounds were **8b** and **10b**, which have 4-substituents that differ greatly in size and lipophilicity but have similar σ_p values.

5. Conclusions

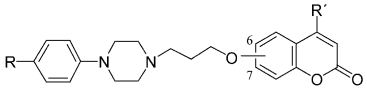
To sum up, our results show that the substitution in the *para* position of the phenyl ring is unfavourable for 5-HT_{1A} binding, especially for the electron-withdrawing groups and simultaneously decrease the D_{2A} receptor affinity. Binding at 5-HT_{1A} is reduced by increasing the bulk of the substituent in position 4 of the coumarin system, and binding at D_{2A} depends on its electronic properties. Neither binding affinity is significantly affected by whether the piperazinylalkyl chain is inserted at position 6 or 7 of the coumarin system.

6. Experimental protocols

6.1. Chemistry

Melting points were determined in a Reichert Kofler thermopan or in capillary tubes in a Büchi 510 apparatus, and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded in a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, J in Hz). Mass spectrometry was carried out in a Hewlett–Packard 5988A spectrometer. Elemental analyses were performed by a Perkin–Elmer 240B microanalyser (and were within $\pm 0.4\%$ of theoretical values for C, H and N). Flash chromatography (FC) was performed on silica gel (Merck 60, 230–400 mesh). Analytical TLC was performed on plates pre-coated with silica gel (Merck 60 F254, 0.25 mm).

Table 1
Affinities of compounds **4**, **5** **8**–**11** and **14**–**16** for 5-HT_{1A} and D_{2A} receptors labelled by [^{3}H]-8-OH-DPAT and [^{3}H]-raclopride, respectively



Compound	R	R′	Side-chain position	K_i (nM)	
				5-HT_{1A}	D_{2A}
4a	NO_2	OCH_3	6	>5000	>1000
4b	OCH_3	OCH_3	6	213	905
5a	NO_2	OCH_3	7	>500	>1000
5b	OCH_3	OCH_3	7	275	246
8a	NO_2	OSO_2CH_3	6	593	571
8b	OCH_3	OSO_2CH_3	6	1840	164
9a	NO_2	OSO_2CH_3	7	2910	>4000
9b	OCH_3	OSO_2CH_3	7	1070	696
10a	NO_2	OSO_2CH_3	6	5860	517
10b	OCH_3	OSO_2CH_3	6	695	159
11a	NO_2	OSO_2CH_3	7	>5000	4520
11b	OCH_3	OSO_2CH_3	7	4290	596
14a	NO_2	C_6H_{11}	6	4700	>1000
14b [a]	OCH_3	C_6H_{11}	6	448	1080
15a	NO_2	C_6H_{11}	7	>500	>1000
15b [a]	OCH_3	C_6H_{11}	7	>5000	>500
16 [b]	H	CH_3	7	5.54	13.7

[a] Synthesized as described in Ref. [21].
[b] Synthesized as described in Ref. [17].

*6.1.1. Preparation of 1-(3-chloropropyl)-4-arylpiperazines (**3**)*

*6.1.1.1. 1-(3-Chloropropyl)-4-nitrophenylpiperazine (**3a**).* To a stirred solution of *p*-nitrophenylpiperazine (2 g, 9.65 mmol) in 20 mL of acetone and 2 mL of 25% NaOH was added 1.6 g (10 mmol) of 1-bromo-3-chloropropane. After stirring for a further 48 h at room temperature (r.t.), the mixture was concentrated and the residue was taken into water. This solution was extracted with CH_2Cl_2, the organic phase was dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by FC using 19:1 CH_2Cl_2–MeOH as eluent to obtain 2.25 g (82%) of pure **3a**; m.p. 75–77 °C. ^{1}H-NMR ($CDCl_3$) δ 8.12 (d, $J = 9.42$, 2H, *o*-NO_2), 6.82 (d, $J = 9.42$, 2H, *m*-NO_2,), 3.63 (t, $J = 6.45$, 2H, CH_2-Cl), 3.44–3.40 (m, 2H, $N^4(CH_2)_2$), 2.63–2.52 (m, 6H, $CH_2N^1(CH_2)_2$), 1.98 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 154.91, 138.59, 125.97, 112.69, 55.14, 52.67, 47.03, 42.89, 29.73. MS: $m/z = 283$ $[M]^+$, 220, 177, 150, 120. Anal. Found: $C_{13}H_{18}ClN_3O_2$ (C, H, N).

*6.1.1.2. 1-(3-Chloropropyl)-4-methoxyphenylpiperazine (**3b**).* Prepared from *p*-methoxyphenyl piperazine in the same way as **3a.** Yield 77%; m.p. 54–56 °C. ^{1}H-NMR ($CDCl_3$) δ 6.89 (d, $J = 9.23$, 2H, ArH), 6.84 (d, $J = 9.23$, 2H, ArH), 3.76 (s, 3H, CH_3O), 3.62 (t, $J = 6.56$, 2H, CH_2Cl), 3.11–3.07 (m, 4H, $N^4(CH_2)_2$), 2.63–2.59 (m, 4H, $N^1(CH_2)_2$), 2.54 (t, $J = 6.85$, 2H, N^1CH_2), 1.99 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 153.90, 145.81, 118.20, 114.50, 55.54, 55.40, 53.36, 50.58, 43.15, 29.87. MS: $m/z = 268$ $[M]^+$, 205, 162, 135, 120. Anal. Found: $C_{14}H_{21}ClN_2O$ (C, H, N).

*6.1.2. General method for the preparation of arylpiperazinylpropyloxycoumarins **4**, **5**, **14** and **15***

For **4** and **5**, NaH (0.5 g, 20.8 mmol) was added to a solution of 4-methoxy-6(7)-hydroxycoumarin (**1** or **2**) (3.84 g, 20 mmol) in DMF (4 mL) and the mixture was stirred at 100 °C for 1 h. After addition of a solution of 1-(3-chloropropyl)-4-arylpiperazine **3** (20 mmol) in DMF (1 mL), followed by NaI (3 mg, 20 mmol), the mixture was stirred overnight at 100 °C. The DMF was evaporated and the residue purified by FC using 1:4 hexane–ethyl acetate as eluent to afford compounds **4** or **5**. Compounds **14** and **15** were synthesised analogously from 4-cyclohexyl-6(7)-hydroxycoumarins **12** and **13**, respectively.

*6.1.2.1. 4-Methoxy-6-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**4a**).* Yield 82%; m.p. (2·HCl) 230–232 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 8.11 (d, $J = 9.40$, 2H, *o*-OCH_3), 7.24 (d, $J = 8.73$, 1H, H-8), 7.22 (d, $J = 2.45$, 1H, H-5), 7.13 (dd, $J = 8.73$, 2.45, 1H, H-7), 6.87 (d, $J = 9.40$, 2H, *m*-OCH_3), 5.70 (s, 1H, H-3), 4.08 (t, $J = 6.14$, 2H, CH_2O), 3.98 (s, 3H, CH_3O), 3.44 (m, 4H, $N^4(CH_2)_2$), 2.62 (m, 6H, $CH_2N^1(CH_2)_2$), 2.03 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 166.50, 163.48, 159.96, 153.54, 147.54, 141.64, 126.38, 121.71, 118.51, 116.73, 113.16, 105.70, 90.51, 65.23, 57.19, 55.20, 52.51, 45.61, 26.86. MS: $m/z = 439$ $[M]^+$, 220, 177, 120. Anal.: $C_{23}H_{27}$ $Cl_2N_3O_6$ (C, H, N).

*6.1.2.2. 4-Methoxy-6-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**4b**).* Yield 86%; m.p. (2·HCl) 212–214 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 7.25 (d, $J = 8.70$, 1H, H-8), 7.24 (d, $J = 2.50$, 1H, H-5), 7.15 (dd, $J = 8.70$, 2.50, 1H, H-7), 6.88 (d, $J = 9.73$, 2H, *o*-OCH_3), 6.80 (d, $J = 9.73$, 2H, *m*-OCH_3), 5.70 (s, 1H, H-3), 4.10 (t, $J = 6.23$, 2H, CH_2O), 3.97 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 3.12 (m, 4H, $N^4(CH_2)_2$), 2.65 (m, 6H, $CH_2N^1(CH_2)_2$), 2.05 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 166.58, 163.50, 155.65, 154.14, 148.05, 146.05, 121.36, 118.49, 118.20, 116.27, 114.79, 105.95, 90.68, 67.27, 56.76, 55.93, 55.45, 53.80, 53.45, 50.98, 27.09. MS: $m/z = 424$ $[M]^+$, 205, 162, 135. Anal.: $C_{24}H_{30}$ $Cl_2N_2O_5$ (C, H, N).

*6.1.2.3. 4-Methoxy-7-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**5a**).* Yield 88%; m.p. (2·HCl) 243–245 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 8.12 (d, $J = 9.37$, 2H, *o*-NO_2), 7.69 (d, $J = 8.64$, 1H, H-5), 6.80 (d, $J = 8.64$, 1H, H-6), 6.81 (d, $J = 9.37$, 2H, *m*-NO_2), 6.76 (s, 1H, H-8), 5.57 (s, 1H, H-3), 4.12 (t, $J = 6.19$, 2H, CH_2O), 3.97 (s, 3H, CH_3O), 3.44 (m, 4H, $N^4(CH_2)_2$), 2.62 (m, 6H, $CH_2N^1(CH_2)_2$), 2.06 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 167.27, 163.88, 162.97, 155.43, 155.22, 138.70, 126.33, 124.48, 112.99, 112.87, 109.23, 101.37, 87.95, 66.93, 56.68, 55.12, 53.09, 47.35, 26.81. MS: $m/z = 439$ $[M]^+$, 422, 220, 192, 120. Anal.: $C_{23}H_{27}Cl_2N_3O_6$ (C, H, N).

*6.1.2.4. 4-Methoxy-7-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**5b**).* Yield 97%; m.p. (2·HCl) 233–234 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 7.68 (d, $J = 8.74$, 1H, H-5), 6.91 (d, $J = 8.74$, 1H, H-6), 6.88 (d, $J = 9.73$, 2H, *o*-OCH_3), 6.82 (d, $J = 9.73$, 2H, *m*-OCH_3), 6.79 (s, 1H, H-8), 5.55 (s, 1H, H-3), 4.09 (t, $J = 6.23$, 2H, CH_2O), 3.96 (s, 3H, CH_3O), 3.76 (s, 3H, CH_3O), 3.11 (m, 4H, $N^4(CH_2)_2$), 2.62 (m, 6H, $CH_2N^1(CH_2)_2$), 2.05 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 167.22, 163.80, 163.04, 155.45, 154.15, 146.07, 124.41, 118.53, 114.79, 112.85, 109.16, 101.40, 87.93, 67.16, 56.62, 55.93, 55.31, 53.78, 50.99, 26.91. MS: $m/z = 424$ $[M]^+$, 422, 205, 135. Anal.: ($C_{24}H_{30}$ $Cl_2N_2O_5$) C, H, N).

*6.1.2.5. 4-Cyclohexyl-6-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**14a**).* Yield 79%; m.p. (2·HCl) 243–244 °C. ^{1}H-NMR ($CDCl_3$) δ (free base)

8.11 (d, $J = 9.40$, 2H, o-NO_2), 7.28 (d, $J = 8.19$, 1H, H-8), 7.11 (m, 2H, H-5, H-7), 6.82 (d, $J = 9.40$, 2H, m-NO_2), 6.30 (s, 1H, H-3), 4.09 (t, $J = 6.20$, 2H, CH_2O), 3.44 (m, 4H, $N^4(CH_2)_2$), 2.82 (m, 1H, CH), 2.62 (m, 6H, $CH_2N^1(CH_2)_2$), 2.04 (m, 2H, $CH_2CH_2CH_2$), 1.99–1.81 (m, 5H), 1.70–1.28 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ 162.09, 160.77, 155.64, 155.22, 148.61, 138.86, 126.35, 119.76, 118.69, 118.58, 113.04, 112.55, 108.96, 67.14, 55.30, 53.15, 47.43, 39.37, 32.81, 27.15, 26.94, 26.43. MS: $m/z = 491$ $[M]^+$, 248, 220, 170, 120. Anal.: $C_{28}H_{35}Cl_2N_3O_5$ (C, H, N).

*6.1.2.6. 4-Cyclohexyl-7-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**15a**).* Yield 84%; m.p. (2·HCl) 246–248 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 8.10 (d, $J = 9.40$, 2H, o-NO_2), 7.54 (d, $J = 8.60$, 1H, H-5), 6.83 (m, 4H, H-6, H-8, m-NO_2), 6.13 (s, 1H, H-3), 4.10 (t, $J = 6.13$, 2H, CH_2O), 3.43 (m, 4H, $N^4(CH_2)_2$), 2.87 (m, 1H, CH), 2.60 (m, 6H, $CH_2N^1(CH_2)_2$), 2.08 (m, 2H, $CH_2CH_2CH_2$), 1.78–2.03 (m, 5H), 1.22–1.48 (m, 5H). ^{13}C-NMR ($CDCl_3$) δ 162.38, 162.10, 161.52, 155.98, 139.01, 126.38, 125.30, 112.57, 112.93, 112.48, 108.80, 102.15, 67.13, 55.40, 53.42, 50.30, 39.30, 32.84, 27.20, 26.53, 26.40. MS: $m/z = 491$ $[M]^+$, 248, 220, 170, 120. Anal.: $C_{28}H_{35}Cl_2N_3O_5$ (C, H, N).

*6.1.3. General procedure for the preparation of arylpiperazinylpropyloxycoumarins **6** and **7***

A solution of coumarin **4** or **5** (0.236 mmol) in acetic acid (20 mL) and HCl (5 mL) was refluxed for 1 h. The mixture was then concentrated under vacuum to obtain the crude enol, which was used for the next reaction without further purification.

*6.1.3.1. 4-Hydroxy-6-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**6a**).* ^{1}H-NMR (DMSO-d_6) δ 8.07 (d, $J = 9.45$, 2H, o-NO_2), 7.30 (d, $J = 8.90$, 1H, H-8), 7.23 (m, 2H, H-5, H-7), 7.09 (d, $J = 9.45$, 2H, m-NO_2), 5.74 (s, 1H, H-3), 4.12 (t, $J = 5.80$, 2H, CH_2O), 3.33 (m, 4H, $N^4(CH_2)_2$), 3.09 (m, 6H, $CH_2N^1(CH_2)_2$), 2.13 (m, 2H, $CH_2CH_2CH_2$). MS: $m/z =$ 425 $[M]^+$, 220, 165, 120.

*6.1.3.2. 4-Hydroxy-6-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**6b**).* ^{1}H-NMR (DMSO-d_6) δ 7.28 (d, $J = 2.87$, 1H, H-5), 7.23 (d, $J = 8.94$, 1H, H-8), 7.14 (dd, $J = 8.94$, 2.87, 1H, H-7), 6.89 (d, $J = 9.20$, 2H, o-OCH_3), 6.79 (d, $J = 9.20$, 2H, m-OCH_3), 5.32 (s, 1H, H-3), 4.07 (t, $J = 6.08$, 2H, CH_2O), 3.66 (s, 3H, CH_3O), 3.06 (m, 4H, $N^4(CH_2)_2$), 2.75 (m, 6H, $CH_2N^1(CH_2)_2$), 1.98 (m, 2H, $CH_2CH_2CH_2$). MS: $m/z = 410$ $[M]^+$, 205, 162, 135.

*6.1.3.3. 4-Hydroxy-7-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**7a**).* ^{1}H-NMR (DMSO-d_6) δ 8.03 (d, $J = 9.45$, 2H, o-NO_2), 7.70 (d, $J = 9.37$, 1H), 7.02 (d, $J = 9.45$, 2H, m-NO_2), 6.90 (m, 2H, H-6, H-8), 5.54 (s, 1H, H-3), 4.12 (t, $J = 6.18$, 2H, CH_2O), 3.50 (m, 4H, $N^4(CH_2)_2$), 2.62 (m, 6H, $CH_2N^1(CH_2)_2$), 2.10 (m, 2H, $CH_2CH_2CH_2$). MS: $m/z = 425$ $[M]^+$, 220, 165, 120.

*6.1.3.4. 4-Hydroxy-7-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**7b**).* ^{1}H-NMR (DMSO-d_6) δ 7.70 (d, $J = 9.11$, 1H, H-5), 6.92 (m, 4H, H-6, H-8, o-OCH_3), 6.82 (d, $J = 9.20$, 2H, m-OCH_3), 5.42 (s, 1H, H-3), 4.15 (t, $J = 5.95$, 2H, CH_2O), 3.68 (s, 3H, CH_3O), 3.20 (m, 10H, $N(CH_2)_5$), 2.08 (m, 2H, $CH_2CH_2CH_2$). MS: $m/z = 410$ $[M]^+$, 205, 162, 135.

*6.1.4. General procedure for the preparation of 4-methane/benzenesulphonyl-6(7)-aryl piperazinylpropyloxycoumarins **8**–**11***

To a solution of hydroxycoumarin **6** or **7** (2.36 mmol) in dichloromethane (10 mL) and triethylamine (0.49 mL, 3.54 mmol) at −5 °C was added the corresponding acid chloride (2.59 mmol). The mixture was stirred at −5 °C for 30 min and then transferred to a separating funnel containing dichloromethane (5 mL). The organic phase was washed with ice-water, 10% HCl, saturated $NaHCO_3$ solution and brine, and dried over Na_2SO_4. After filtration and evaporation of the solvent the residue was chromatographed, affording the pure compounds **8**–**11**.

*6.1.4.1. 4-Methanesulphonyl-6-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**8a**).* Yield 54% (from **4a**); m.p. (2·HCl) 168–170 °C. ^{1}H-NMR (DMSO-d_6) δ (free base) 8.10 (d, $J = 9.37$, 2H, o-NO_2), 7.24 (d, $J = 9.07$, 1H, H-8), 7.34 (dd, $J = 9.07$, 2.80, 1H, H-7), 7.24 (d, $J = 2.80$, 1H, H-5), 7.12 (d, $J = 9.37$, 2H, m-NO_2), 6.58 (s, 1H, H-3), 4.19 (t, $J = 6.07$, 2H, CH_2O), 3.65 (m, 4H, $N^4(CH_2)_2$), 3.37 (s, 3H, SCH_3), 3.35 (m, 6H, $CH_2N^1(CH_2)_2$), 2.30 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR (DMSO-d_6) δ 163.49, 160.73, 157.40, 155.48, 148.72, 139.10, 126.60, 119.83, 118.79, 116.62, 112.83, 107.93, 100.12, 67.15, 55.22, 53.10, 47.50, 39.70, 26.60. MS: $m/z = 503$ $[M]^+$, 220, 165, 136. Anal.: $C_{23}H_{27}Cl_2N_3O_8S$ (C, H, N).

*6.1.4.2. 4-Methanesulphonyl-6-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**8b**).* Yield 60% (from **4b**); m.p. (2·HCl) 166–168 °C. ^{1}H-NMR (DMSO-d_6) δ (free base) 7.48 (d, $J = 9.08$, 1H, H-8), 7.34 (m, 1H, H-7), 7.25 (s, 1H, H-5), 6.96 (d, $J = 8.80$, 2H, o-OCH_3), 6.85 (d, $J = 8.80$, 2H, m-OCH_3), 5.68 (s, 1H, H-3), 4.15 (t, $J = 5.98$, 2H, CH_2O), 3.68 (s, 3H, CH_3O), 3.43 (s, 3H, CH_3S), 3.39 (m, 4H, $N^4(CH_2)_2$), 3.20 (m, 6H, $CH_2N^1(CH_2)_2$), 2.30 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR (DMSO-d_6) δ 163.22, 160.57, 157.21, 155.28, 147.91, 143.27, 121.78, 118.76, 118.49, 115.65, 114.79, 106.75, 101.05, 67.52, 55.90, 55.58,

53.13, 50.55, 39.23, 26.50. MS: $m/z = 488$ $[M]^+$, 410, 205, 162, 135. Anal.: $C_{24}H_{30}Cl_2N_2O_7S$ (C, H, N).

*6.1.4.3. 4-Methanesulphonyl-7-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**9a**).* Yield 60% (from **5a**); m.p. (2·HCl) 194–98 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 8.12 (d, $J = 9.40$, 2H, o-NO_2), 7.62 (d, $J = 8.80$, H, H-5), 6.91 (d, $J = 2.40$, 1H, H-8), 6.86 (dd, $J = 8.80$, 2.40, 1H, H-6), 6.81 (d, $J = 9.40$, 2H, o-NO_2), 6.35 (s, 1H, H-3), 4.14 (t, $J = 6.24$, 2H, CH_2O), 3.44 (m, 4H, $N^4(CH_2)_2$), 3.37 (s, 3H, SCH_3), 2.62 (m, 6H, $CH_2N^1(CH_2)_2$), 2.05 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 162.71, 160.24, 157.97, 155.69, 153.55, 141.36, 126.36, 124.92, 113.29, 113.23, 112.08, 102.17, 101.08, 65.39, 55.59, 52.43, 45.58, 39.51, 25.12. MS: $m/z = 503$ $[M]^+$, 220, 165, 120. Anal.: $C_{23}H_{25}N_3O_8S$ (C, H, N).

*6.1.4.4. 4-Methanesulphonyl-7-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**9b**).* Yield 63% (from **5b**); m.p. (2·HCl) 177–179 °C. ^{1}H-NMR (DMSO-d_6) δ (free base) 7.70 (d, $J = 9.38$, 1H, H-5), 6.88 (d, $J = 9.60$, 4H), 6.82 (m, 6H, H-6, H-8, o, m-OCH_3), 5.31 (s, 1H, H-3), 4.12 (t, $J = 9.60$, 2H, CH_2O), 3.67 (s, 3H, CH_3O), 3.15 (s, 3H, CH_3S), 3.07 (m, 4H, $N^4(CH_2)_2$), 2.72 (m, 6H, $CH_2N^1(CH_2)_2$), 1.98 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR (DMSO-d_6) δ 163.50, 161.83, 157.45, 154.22, 154.14, 145.23, 126.49, 120.38, 114.83, 114.02, 112.22, 102.28, 101.72, 67.10, 55.43, 55.54, 53.13, 49.84, 38.43, 26.83. MS: $m/z = 488$ $[M]^+$, 205, 162, 135. Anal.: $C_{24}H_{30}Cl_2N_2O_7S$ (C, H, N).

*6.1.4.5. 4-Benzenesulphonyl-6-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**10a**).* Yield 46% (from **4a**); m.p. (2·HCl) 236–238 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 8.13 (d, $J = 9.40$, 2H, o-NO_2), 8.02 (d, $J = 7.25$, 2H, o-SO_2), 7.75 (m, 1H, p-SO_2), 7.64 (m, 2H, o-SO_2), 7.24 (d, $J = 9.01$, 1H, H-8), 7.15 (dd, $J = 9.01$, 2.86, 1H, H-7), 7.07 (d, $J = 2.86$, 1H, H-5), 6.88 (d, $J = 9.40$, 2H, m-NO_2), 6.21 (s, 1H, H-3), 4.05 (t, $J =$ 6.20, 2H, CH_2O), 3.45 (m, 4H, $N^4(CH_2)_2$), 2.63 (m, 6H, $CH_2N^1(CH_2)_2$), 2.05 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 163.33, 163.09, 160.85, 155.60, 150.81, 149.07, 138.72, 134.73, 129.12, 126.90, 126.48, 119.48, 118.64, 117.50, 112.92, 112.80, 101.03, 67.20, 54.60, 53.72, 49.60, 29.38. MS: $m/z = 565$ $[M]^+$, 318, 220, 141. Anal.: $C_{28}H_{29}Cl_2N_3O_8S$ (C, H, N).

*6.1.4.6. 4-Benzenesulphonyl-6-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**10b**).* Yield 50% (from **4b**); m.p. (2·HCl) 177–178 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 8.02 (d, $J = 7.64$, 2H, o-SO_2), 7.75 (m, 1H, p-SO_2), 7.63 (m, 2H, m-SO_2), 7.24 (d, $J = 9.06$, 1H, H-8), 7.14 (dd, $J = 9.06$, 2.85, 1H, H-7), 7.01 (d, $J = 2.85$, 1H, H-5), 6.78 (m, 4H, o, m-OCH_3), 6.27 (s, 1H, H-3), 4.02 (t, $J = 6.15$, 2H, CH_2O), 3.77 (m, 3H, CH_3O), 3.15 (m, 4H, $N^4(CH_2)_2$), 2.73 (m, 6H, $CH_2N^1(CH_2)_2$), 2.04 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 163.22, 162.10, 160.55, 154.18, 150.72, 148.60, 146.20, 136.36, 130.10, 129.32, 120.60, 119.46, 118.43, 114.77, 113.03, 108.90, 100.45, 67.02, 55.95, 53.70, 53.45, 51.12, 29.12. MS: $m/z = 550$ $[M]^+$, 410, 205, 162, 135. Anal.: $C_{29}H_{32}Cl_2N_2O_7S$ (C, H, N).

*6.1.4.7. 4-Benzenesulphonyl-7-{3-[4-(p-nitrophenyl)-1-piperazinyl]propyloxy}coumarin (**11a**).* Yield 53% (from **5a**); m.p. (2·HCl) 222–224 °C. ^{1}H-NMR ($CDCl_3$) δ (free base) 8.13 (d, $J = 9.38$, 2H, o-NO_2), 8.02 (d, $J = 7.85$, 2H, o-SO_2), 7.83 (m, 1H, p-SO_2), 7.75 (m, 2H, m-SO_2), 7.44 (d, $J = 8.68$, 1H, H-5), 7.18 (d, $J = 9.38$, 2H, m-NO_2), 7.05 (s, 1H, H-8), 6.90 (d, $J = 8.68$, 1H, H-6), 6.10 (s, 1H, H-3), 4.15 (t, 2H, CH_2O, $J = 6.20$), 3.45 (m, 4H, $N^4(CH_2)_2$), 2.63 (m, 6H, $CH_2N^1(CH_2)_2$), 2.10 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR ($CDCl_3$) δ 163.02, 162.71, 161.30, 160.43, 155.50, 150.70, 140.04, 136.24, 130.48, 129.33, 127.30, 124.67, 113.82, 112.83, 107.63, 105.48, 101.28, 66.49, 54.12, 53.90, 48.36 26.92. MS: $m/z = 565$ $[M]^+$, 425, 220, 165, 120. Anal.: $C_{28}H_{29}Cl_2N_3O_8S$ (C, H, N).

*6.1.4.8. 4-Benzenesulphonyl-7-{3-[4-(p-methoxyphenyl)-1-piperazinyl]propyloxy}coumarin (**11b**).* Yield 55% (from **5b**); m.p. (2·HCl) 221–223 °C. ^{1}H-NMR (DMSO-d_6) δ (free base) 8.11 (d, $J = 7.88$, 2H, o-SO_2), 7.86 (m, 1H, p-SO_2), 7.71 (m, 2H, m-SO_2), 7.46 (d, $J = 8.77$, 1H, H-5), 7.07 (s, 1H, H-8), 6.91 (m, 5H, H-6, o-and m-OCH_3), 6.11 (s, 1H, H-3), 4.18 (s 2H, CH_2O), 3.68 (s, 3H, CH_3O), 3.38 (m, 4H, $N^4(CH_2)_2$), 2.77 (m, 6H, $CH_2N^1(CH_2)_2$), 1.97 (m, 2H, $CH_2CH_2CH_2$). ^{13}C-NMR (DMSO-d_6) δ 163.38, 162.89, 161.49, 159.48, 154.23, 151.03, 146.12, 136.40, 130.02, 128.31, 125.80, 119.12, 114.64, 113.02, 108.64, 103.21, 101.44, 67.02, 56.03, 54.80, 53.67, 51.03, 28.09. MS: $m/z = 550$ $[M]^+$, 205, 162, 135. Anal.: $C_{29}H_{32}Cl_2N_2O_7S$ (C, H, N).

6.2. Pharmacology

6.2.1. 5-HT_{1A} receptor binding assay

Male Sprague–Dawley rats weighing about 200 g were decapitated and the cortex and hippocampus were dissected out and immediately homogenised (ultraTurrax homogeniser, 10 s) in 15 mL of ice-cold 50 mM Tris–HCl buffer containing 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$ and 1.0 mM $MnCl_2$ (pH 7.4). After centrifugation for 12.5 min at 17 000 rpm, the pellets were resuspended in the same buffer and homogenisation and centrifugation were repeated. The tissue homogenate was diluted to 5 mg mL^{-1} with the buffer, incubated for 10 min at 37 °C, treated with 10 nM pargyline, and reincubated for 10 min. Incubation mixtures (2 mL) contained various concentrations of test compound (diluted in 50 mM Tris–HCl buffer, pH 7.4), 1nM [^{3}H]-8-

OH-DPAT·HBr, and 5 mg mL^{-1} tissue homogenate in 50 mM pH 7.4 buffer. Nonspecific binding was measured by addition of 100 μM 5-HT.HCl to the reaction mixture. Binding reactions were started by the addition of tissue homogenate, incubated at 37 °C for 45 min, and terminated by rapid filtration through Whatman GF/B glass fibre using a Brandel cell harvester. The filters were washed with ice-cold buffer (50 mM Tris–HCl, pH 7.4), and the radioactivity was determined in a Packard 2200CA liquid scintillation analyser. The binding data were analysed by nonlinear regression using the LIGAND program [25].

6.2.2. D_{2A} receptor binding assay

D_{2A} binding was assayed essentially as described previously [22]. Mouse fibroblast (LTK-) cells expressing human D_{2A} receptors were obtained from Dr. O. Civelli (Vollum Institute, Portland). The membranes were prepared as described previously [22], resuspended in binding buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$ and 1 mM EDTA) and stored in aliquots at −70 °C. The frozen cell membranes were thawed, homogenised with a Branson 450 sonifier, and suspended in binding buffer. The binding assays were performed in a total volume of 0.5 mL with a receptor concentration of about 100 pM. The membranes were incubated with 1.20 nM [^{3}H]-raclopride and nonlabelled ligand for 1 h at 25 °C. Binding in the presence of (+)-butaclamol (1 μM) was defined as nonspecific. The incubations were terminated, the radioactivity determined and the data analysed as described for the 5-HT_{1A} binding assay.

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