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Synthesis and circular dichroism of optically active 1,3-disubstituted isochromans of dopamine D_4 antagonist activity

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Dedicated to Prof. András Lipták on the occasion of his 75th birthday

ABSTRACT

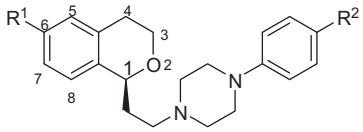
The C-1 epimers of the 3-methyl-6,7-dimethoxy analogues of the D_4 antagonist sonepiprazole **1b** and 5-HT_{1D} agonist PNU-109291 **1a** containing both the isochroman and *p*-methoxyphenylpiperazine chromophores were prepared in order to study the applicability of circular dichroism for the assignment of the configuration of 1,3-disubstituted isochromans and test their dopamine D_4 antagonist activity.

1. Introduction

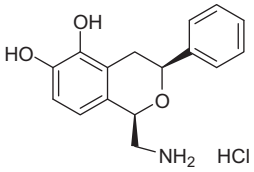
There are several synthetic optically active isochroman derivatives reported with remarkable pharmacological activities, such as selective 5-HT_{1D} agonist,[1] D_1 agonist[2] and D_4 antagonist[3], which are promising for the treatment of migraines, Parkinson's disease and schizophrenia, respectively. Moreover, it has been shown that the absolute configurations of these isochroman derivatives play a decisive role in their pharmacological activities; the (*S*)-enantiomer of the 5-HT_{1D} agonist **1a** (PNU-109291)[1a] and the selective D_4 antagonist sonepiprazole **1b** (U-101387)[3] possessed a superior affinity for the binding site compared to the (*R*)-enantiomer, while the (1*R*,3*S*)-enantiomer of **2** (A68930) is almost exclusively responsible for the observed selective D_1 agonist activity of the racemate (Chart 1).[2a]

In spite of the apparent importance of chirality in these derivatives, there is no direct and general method for the assignment of the configuration of the isochroman skeleton available which can be used on μg quantity of a non-crystalline derivative. Thus, so far X-ray diffraction[2c,3] and analogous techniques[1a,2a] have been applied to determine the absolute configurations of optically active isochromans. Herein, we outline a general method for the configurational assignment of 1,3-disubstituted isochromans that exploits the combination of heteronuclear couplings constants or NOE effect and CD data.

In a simple sequence, both C-1 epimers of the 3-methyl-6,7-dimethoxy analogues of the 5-HT_{1D} agonist PNU-109291 **1a** containing both isochroman and *p*-methoxyphenylpiperazine chromophores were prepared and the relative and absolute configurations of the epimeric isochroman derivatives were studied by the measurements of their heteronuclear coupling constants and CD spectra. The activities of the prepared isochromans were tested on dopamine D_4 receptors in radio ligand binding assays.



1	R^1	R^2
a	CONHMe	OMe
b	H	SO_2NH_2



(1*R*,3*S*)-**2** (A68930)

Chart 1. Structures of pharmacologically active synthetic isochromans.

2. Results and discussion

In order to study the chiroptical properties of pharmacologically active 1,3-disubstituted isochromans of potential pharmacological

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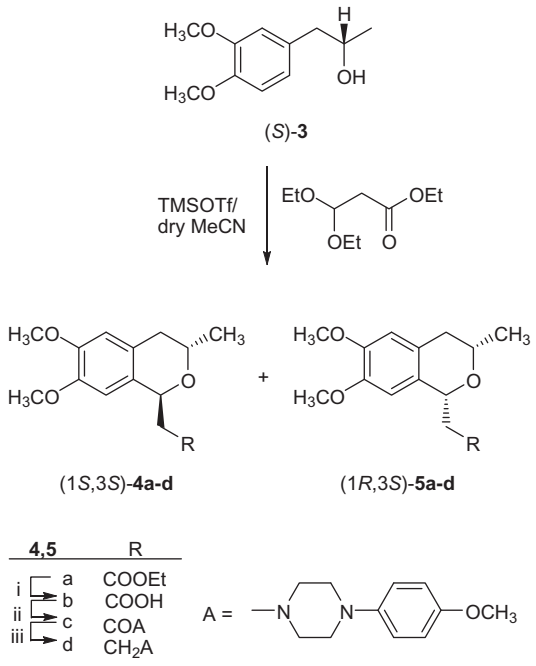
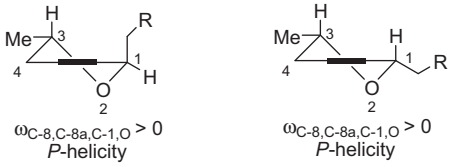


Scheme 1. Reagents: (i) LiOH, THF; (ii) 4-methoxyphenylpiperazine, EDC, dry CH_2Cl_2; (iii) BH_3, THF.

activity, (1*S*,3*S*)-**4d** and (1*R*,3*S*)-**5d** analogues of the 5-HT_{1D} agonist **1a** were synthesized (Scheme 1).

This was achieved by an oxa-Pictet–Spengler cyclization[4] of the optically active secondary alcohol (+)-(*S*)-**3**, readily available from the bioreduction of the corresponding ketone,[5] with ethyl-3,3-diethoxypropionate in the presence of trimethylsilyl triflate (TMSOTf), which resulted in a 1:2 mixture of epimers (1*S*,3*S*)-**4a** and (1*R*,3*S*)-**5a**, which were subsequently separated by column chromatography. The inherent (*S*)-C-3 stereogenic centre allowed the assignment of the configuration at C-1 by means of the heteronuclear coupling constants and an NOE effect, since it was not effected during the ring closure. The three-bond carbon–proton coupling constants of (1*S*,3*S*)-**4a** ($^3J_{C3,1H}$ = 6.0 Hz; $^3J_{C1,3H}$ = 1–2 Hz) and the absence of an NOE effect[6] between its 1-H and 3-H protons proved the *trans* relative configuration and hence the (1*S*,3*S*)-**4a** absolute configuration. The three-bond carbon–proton coupling constants also confirmed that the C-1 ethoxycarbonyl substituent of (1*S*,3*S*)-**4a** has an axial orientation and the half-chair conformation of the hetero ring is not distorted to relieve the 1,3-diaxial interaction. In contrast, the small $^3J_{C3,1H}$ value (<2 Hz) of (1*R*,3*S*)-**5a** and the NOE effect between the *axial* 1-H and 3-H protons allowed its assignment as *cis*, that is, (1*R*,3*S*). The absolute configurations of the isochroman derivatives have recently been studied by circular dichroism and a correlation was established between the helicity of the hetero ring and the ^1L_b band Cotton effect (CE),[7] which was used to determine the absolute configurations of 3-substituted isochroman natural products, pseudoanguillosporins A and B.[8]



Scheme 2. Preferred *P*-helicity conformations of the fused heteroring in (1*S*,3*S*)-**4a**–**d** and (1*R*,3*S*)-**5a**–**d** with axial and equatorial C-1 substituents, respectively.

According to this correlation, the *P*-helicity (Scheme 2) of the hetero ring results in a positive ^1L_b band CE regardless of the nature(s) and position(s) of the substituent(s) on the aromatic ring. In both (1*S*,3*S*)-**4a** and (1*R*,3*S*)-**5a**, the hetero rings adopt a *P*-helicity with equatorial 3-methyl group, as indicated in Scheme 2 and in agreement with the rule, their ^1L_b band CEs are positive at about 280 nm (Table 1). In contrast, below 250 nm, the CD spectra of the epimers were markedly different reflecting their different C-1 absolute configurations.

Since the absolute configuration of C-3 was known in **4a** and **5a**, the determination of the relative configuration gave the absolute configuration of C-1, making the CD analysis redundant. However, for the 1,3-disubstituted isochromans of unknown absolute configuration, the sign of the ^1L_b CE allows us to determine the helicity of the hetero ring which in combination with the relative configuration deduced from heteronuclear coupling constants and/or NOE effects, gives the absolute configuration. For 1-monosubstituted isochromans, only the helicity of the heteroring can be determined from the sign of the measured ^1L_b CE, which also gives the absolute configuration of C-1.

The isochroman epimers (1*S*,3*S*)-**4a** and (1*R*,3*S*)-**5a** served as starting materials for a three-step synthesis of isochroman derivatives (1*S*,3*S*)-**4d** and (1*R*,3*S*)-**5d** possessing potential dopaminergic activity (Scheme 1).

Esters (1*S*,3*S*)-**4a** and (1*R*,3*S*)-**5a** were hydrolysed to the corresponding carboxylic acids (1*S*,3*S*)-**4b** and (1*R*,3*S*)-**5b** by lithium hydroxide in THF in high yield (93–95%). Similar to the ester precursors, the carboxylic acid derivatives (1*S*,3*S*)-**4b** and (1*R*,3*S*)-**5b** also had positive ^1L_b CE around 280 nm (Fig. 1) in accordance with the *P*-helicity of their hetero ring. Coupling with 4-methoxyphenylpiperazine provided the amides (1*S*,3*S*)-**4c** and (1*R*,3*S*)-**5c**, which in contrast to the ester derivatives, had near mirror image CD spectra in the region 350–180 nm (Fig. 2). It is evident that with the introduction of the methoxyphenylpiperazine moiety, the relative orientation of the two aryl chromophores, that is, the absolute configuration of C-1, determines the CD spectra, which overrides the contribution from the helicity of the isochroman ring.

The reduction of the amides with borane in THF resulted in (1*S*,3*S*)-**4d** and (1*R*,3*S*)-**5d** with 75% and 85% overall yields, respectively. For (1*S*,3*S*)-**4d** and (1*R*,3*S*)-**5d**, similar three-bond heteronuclear NMR coupling constants were measured as for (1*S*,3*S*)-**4a** and

Table 1
CD data of isochromans (1*S*,3*S*)-**4a**–**d** and (1*R*,3*S*)-**5a**–**d** in acetonitrile

Compound	CD: λ_{max} (nm) ($\Delta\varepsilon$)
(1*S*,3*S*)-**4a**	290sh (+0.50), 281 (+0.62), 274sh (+0.47), 228 (+4.00), 223sh (+3.94), 210 (+4.35)
(1*R*,3*S*)-**5a**	288sh (+0.22), 283 (+0.30), 241 (−0.91), 229 (+0.40), 220 (−0.23), 205 (+15.95)
(1*S*,3*S*)-**4b**	290sh (+0.67), 286sh (+0.83), 281 (+0.86), 227 (+5.96), 211sh (+5.61), 196 (−7.28)
(1*R*,3*S*)-**5b**	289 (+0.20), 284sh (+0.18), 277sh (+0.13), 241 (−1.03), 229 (+0.09), 220 (−0.78), 201 (+19.31), 193 (−6.09)
(1*S*,3*S*)-**4c**	313sh (+0.04), 304 (+0.06), 289sh (−0.19), 285 (−0.26), 230 (+5.53), 206 (−11.70), positive below 198 nm
(1*R*,3*S*)-**5c**	315sh (−0.03), 309 (−0.04), 292sh (+0.78), 282 (+1.08), 242 (−0.67), 227sh (+1.19), 207 (+25.68), 195 (−15.77)
(1*S*,3*S*)-**4d**	288 (+0.82), 282 (+0.89), 232 (+7.28), 213 (−2.88), 199 (+6.35)
(1*R*,3*S*)-**5d**	288 (+0.07), 240 (−2.72), 206.5 (+9.25), 196 (−4.80)

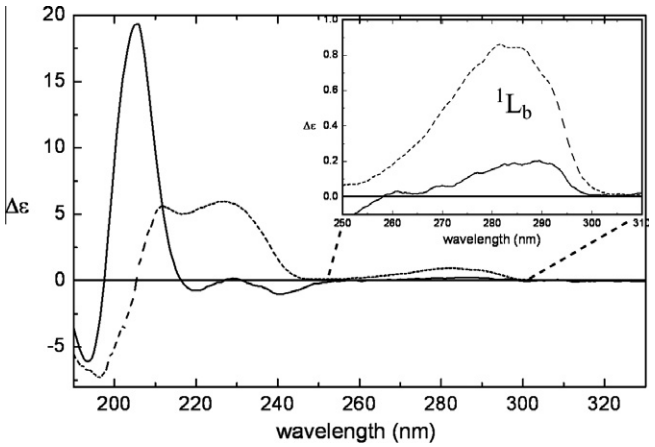


Figure 1. CD spectra of (1*S*,3*S*)-**4b** (dashed line) and (1*R*,3*S*)-**5b** (solid line) in acetonitrile with the ^1L_b region enlarged.

(1*R*,3*S*)-**5a**, respectively, which confirmed that the large axial C-1 substituent of (1*S*,3*S*)-**4d** did not distort the half-chair conformation of the hetero ring and thus the compounds in both series **4** and **5** have *P*-helicity (Scheme 1, Scheme 2, Fig. 2).

With the amide carbonyl group reduced as in (1*S*,3*S*)-**4d** and (1*R*,3*S*)-**5d**, the C-1 side-chain became more flexible leading to multiple conformations and hence significantly different ^1L_b CE in (1*S*,3*S*)-**4d** and (1*R*,3*S*)-**5d** as a result of the interaction of the isochroman chromophore and the *p*-methoxyphenyl group. Although both of them had positive CE in the ^1L_b region, which apparently follows the isochroman helicity rule, it cannot be applied due to the aforementioned consideration. A CD calculation of the epimers (1*S*,3*S*)-**4d** and (1*R*,3*S*)-**5d** would be highly demanding considering the large conformational freedom of the C-1 substituent.

3. Pharmacological study

The activity of the prepared compounds were tested on dopamine $D_{4,2}$, $D_{4,4}$, $D_{4,7}$ receptors with radio ligand binding assays using human recombinant CHO-K1 cells and spiperone and haloperidol as reference compounds (Scheme 3).[9] Compound (1*S*,3*S*)-**4d** showed 66% inhibition with a dopamine $D_{4,2}$ receptor at 10 μM concentration and smaller inhibition activities with dopamine $D_{4,4}$, $D_{4,7}$ receptors (Table 2). Unfortunately, the (1*R*,3*S*)-**5d** could not be tested, since it partially decomposed while being stored when awaiting the assay. According to the literature data, the (1*R*)-sonepiprazole **1b** has an inferior D_4 antagonist activity compared to its (1*S*)-enantiomer. The conformationally more rigid amides (1*S*,3*S*)-**4c** had practically no activity on the tested dopamine D_4 receptors and the **4a** and **5a** precursors did not show either activity (Scheme 3).

Table 2
Results of the biochemical assays of the compounds tested

Compound	Concent ration (μM)	Inhibition (%)		
		Dopamine $D_{4,2}$	Dopamine $D_{4,4}$	Dopamine $D_{4,7}$
4a	10	10	−1	6
5a	10	0	1	3
4c	10	−2	3	−1
5c	10	6	1	4
4d	10	66	46	34

4. Conclusion

Both C-1 epimers of the 3-methyl-6,7-dimethoxy analogues of the 5-HT_{1D} agonist PNU-109291 were prepared in order to study the applicability of the isochroman helicity rule in the 1,3-disubstituted isochromans and in the presence of a remote aryl chromophore. Their absolute configurations and the conformations of their hetero ring were determined by means of their three-bond heteronuclear coupling constants. Although the *p*-methoxyphenyl group is separated by several single bonds from the C-1 stereogenic centre, their ^1L_b band CE does not follow the isochroman helicity rule and the absolute configuration of the C-1 stereogenic centre determines the CD spectra. However, the near mirror image CEs below 250 nm are indicative of the C-1 absolute configuration of the epimers and can be used for the configurational assignment of C-1 in analogous compounds. Pharmacological studies revealed that (1*S*,3*S*)-**4d** showed moderate non-selective antagonist activity on dopamines $D_{4,2}$, $D_{4,4}$, $D_{4,7}$. It was also demonstrated that the isochroman helicity rule can be safely used for the configurational assignment of 1,3-disubstituted isochromans such as (1*S*,3*S*)-**4a,b** and (1*R*,3*S*)-**5a,b**; i.e. in the absence of an interacting C-1 aromatic substituent.

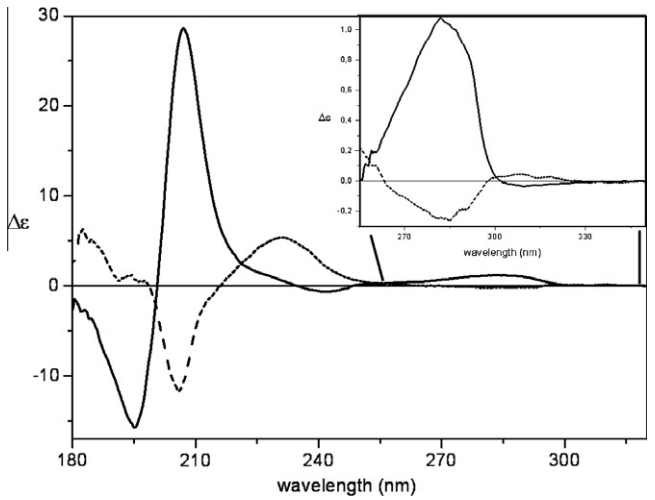
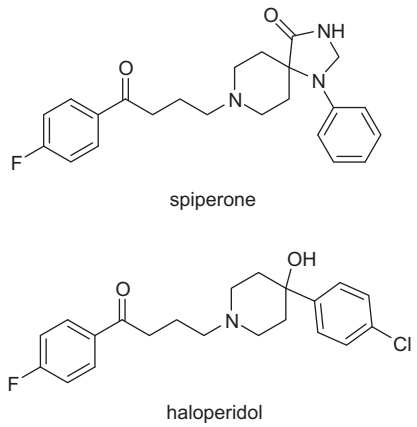


Figure 2. CD spectra of (1*S*,3*S*)-**4c** (dashed line) and (1*R*,3*S*)-**5c** (solid line) in acetonitrile with the 250–350 nm region enlarged.



Scheme 3. Structure of the reference compounds spiperone and haloperidol.

5. Experimental

5.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Elemental analysis was measured with a Carlo-Elba analyser Tpy 1106. The NMR spectra were recorded on a Bruker-AMX 500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) Bruker WP 200 SY (^{1}H: 200 MHz) spectrometer using TMS as an internal standard. Chemical shifts were reported in ppm. Optical rotation was determined with a Perkin–Elmer 241 polarimeter. CD spectra were recorded on a J-810 spectropolarimeter. The CD spectra were measured in millidegrees and normalized into $\Delta\varepsilon_{max}$ (l mol^{-1} cm^{-1})/λ (nm) units. In the UV spectra, the red shift of the tweezer Soret band indicated that complexation took place. IR spectra were recorded on a Perkin Elmer 16 PC FTIR spectrometer and absorption bands presented in cm^{-1}. Precoated silica gel plates (Kieselgel 60 F_{254}, 0.25 mm, Merck) were used for analytical and preparative TLC. High resolution FAB mass spectra were measured on a JEOL JMS-DX303 HF mass spectrometer using a glycerol matrix and Xe ionizing gas.

5.1.1. (+)-Ethyl-[(1*S*,3*S*)-6,7-dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]acetate 4a and (+)-ethyl-[(1*R*,3*S*)-6,7-dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]acetate 5a

Trimethylsilyl triflate (50 μl) was added to a mixture of **3** (300 mg, 1.53 mmol) and ethyl-(3,3-diethoxy)propionate (0.44 ml, 2.28 mmol) in acetonitrile (10 ml). The reaction mixture was heated at a gentle reflux for 30 min. The mixture was washed with $NaHCO_3$ solution (15 ml) and extracted with CH_2Cl_2 (3 × 15 ml), dried (Na_2SO_4), filtered and concentrated in vacuo to give 568 mg of a colourless oil, which contained two components (**5a**:**4a**) in a ratio of 5:3, separated by column chromatography (hexane/ethyl acetate 4:1). Eluted first was **5a** (225.3 mg, 50%), which was then crystallized from hexane: mp 92–94 °C; $[\alpha]_D^{20} = +108.2$ (*c* 1.27, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.29 (t, *J* = 7.5 Hz, 3H, CH_2–*CH*$_3$), 1.30 (d, *J* = 6.0 Hz, 3H, CH–*CH*$_3$), 2.56 (dd, *J* = 13.9, 2.0 Hz, 1H, 4-Hb), 2.67–2.70 (m, 2H, H-4a, 1'-Hb), 2.89 (dd, *J* = 15.0, 4.0 Hz, 1H, 1'-Ha), 3.76–3.83 (m, 1H, 3-H), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.21 (q, *J* = 7.5 Hz, 2H, *CH*$_2$–CH_3), 5.17 (m, 1H, 1-H), 6.55 (s, 1H, Ar-H), 6.57(s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.20 (CH_2–*CH*$_3$), 21.55 (CH–*CH*$_3$), 35.96 (C-1'), 42.18 (C-4), 55.84 (OCH_3), 55.96 (OCH_3), 60.51 (*CH*$_2$–CH_3), 70.62 (C-3), 73.54 (C-1), 107.18 [CH,(Ar)], 111.45 [CH,(Ar)], 126.57 (C-5a), 128.65 (C-8a), 147.20 (C-6), 147.78 (C-7), 171.50 (CO). $^3J_{C3,1H}$ = 2.0 Hz, $^3J_{C1,3H}$ = 1–2 Hz; IR (KBr): 2990, 2968, 2832, 1734, 1248, 1234 cm^{-1}. Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.43; H, 7.50.

Eluted second was **4a** (140.7 mg, 31%), which was crystallized from hexane: mp 98–101 °C; $[\alpha]_D^{20} = +30.2$ (*c* 1.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26–1.33 (m, 6H, CH–*CH*$_3$, CH_2–*CH*$_3$), 2.58–2.62 (m, 2H, 4-H), 2.69 [dd, *J* = 14.5, 4.0 Hz, 1H, CH_2a(CO)], 2.90 [dd, *J* = 15.0, 10.5 Hz, 1H, CH_2b(CO)], 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.05 (m, 1H, 3-H), 4.23 (m, 2H, *CH*$_2$–CH_3), 5.30 (m, 1H, 1-H), 6.53 (s, 1H, Ar-H), 6.57 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.27 (CH_2–*CH*$_3$), 21.24 (CH–*CH*$_3$), 35.26 [CH_2(CO)], 41.79 (C-4), 55.86 (OCH_3), 55.96 (OCH_3), 60.63 (*CH*$_2$–CH_3), 64.33 (C-3), 71.98 (C-1), 107.86 [CH,(Ar)], 111.42 [CH,(Ar)], 125.50 (C-5a), 128.17 (C-8a), 146.50 (C-7), 147.20 (C-6), 171.12 (CO). $^3J_{C3,1H}$ = 6.0 Hz, $^3J_{C1,3H}$ = 1–2 Hz; IR (KBr): 2972, 2932, 2836, 1732, 1278, 1252, 1230, 1106 cm^{-1}. Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.26; H, 7.52.

5.2. General procedure for the preparation of 5b, 4b

5.2.1. (+)-[(1*R*,3*S*)-6,7-Dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]acetic acid 5b

To a solution of **5a** (50 mg, 0.17 mmol) in THF (5 ml), an aqueous solution of lithium hydroxide (0.3 M, 2.2 ml) was added and the mixture was stirred at room temperature for 18 h. The volatiles were then removed in vacuo and the residue was acidified with 1 M HCl. The resulting cloudy mixture was extracted with CH_2Cl_2 (3 × 15 ml) and the combined organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue was purified by preparative TLC (toluene/acetic acid 9:1) to give **5b** (43 mg, 95%). The product was crystallized (from CH_2Cl_2/hexane 1:10): mp 119–121 °C; $[\alpha]_D^{20} = +117.5$ (*c* 1.28, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (d, *J* = 6.2 Hz, 3H, CH_3), 2.59 [dd, *J* = 15.7, 2.2 Hz, 1H, CH_2a(CO)],), 2.70 (dd, *J* = 15.7, 11.4 Hz, 4-CH_2a), 2.76 [dd, *J* = 15.7, 8.5 Hz, 1H, CH_2b(CO)], 3.00 (dd, *J* = 15.7, 3.6 Hz, 4-CH_2b), 3.81–3.88 (m, 7H, 3-H, 2 × OCH_3), 5.16 (dd, *J* = 8.5, 3.3 Hz, 1H, 1-H), 6,55 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.62 (CH_3), [36.23 (CH_2(CO)], 42.28 (C-4), 56.49 (OCH_3), 71.60 (C-3), 73.59 (C-1), 107.62 (C-Ar), 112.10 (C-Ar), 126.71, 127.87 (C-8a, C-5a), 143.97 (C-7), 148.13 (C-6), 175.69 (CO), $^3J_{C3,1H}$ = 2.0 Hz, $^3J_{C1,3H}$ = 1–2 Hz; IR (KBr): 2972, 2934, 2836, 1740, 1316, 1252, 1104 cm^{-1}. Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.03; H, 6.83.

5.2.2. (+)-[(1*S*,3*S*)-6,7-Dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]acetic acid 4b

Solid (93%). The product was crystallized from hexane: mp 135–136 °C; $[\alpha]_D^{20} = +21.1$ (*c* 0.88, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (d, *J* = 6.2 Hz, 3H, CH_3), 2.62 [m, 2H, CH_2(CO)], 2.77 (d, *J* = 14.5 Hz, 4-CH_2a), 2.76 (m, 1H, 4-CH_2b), 3.84 (s, 3H, OCH_3), 3.85 (s, 1H, OCH_3), 4.08 (m, 1H, 3-H), 5.32 (d, *J* = 8.5, Hz, 1H, 1-H), 6,53 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.29 (CH_3), 35.40 [CH_2(CO)], 38.22 (C-4), 56.49 (OCH_3), 65.28 (C-3), 72.10 (C-1), 108.11 (C-Ar), 111.77 (C-Ar), 125.88, 128.20 (C-8a, C-5a), 148.08, 148.53 (C-6, C-7), 170.05 (CO), $^3J_{C3,1H}$ = 6.0 Hz, $^3J_{C1,3H}$ = 1–2 Hz; IR (KBr): 3160, 2966, 2926, 2832, 1714, 1294, 1250, 1108 cm^{-1}. Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.08; H, 6.80.

5.3. General procedure for the preparation of 4c, 5c

5.3.1. (+)-1-{[(1*S*,3*S*)-6,7-Dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]acetyl}-4-(4-methoxyphenyl) piperazine 4c

To a cooled (0 °C) solution of **4b** (22 mg, 0.08 mmol) and *N*-(*p*-methoxyphenyl)piperazine (20.6 mg, 0.11 mmol) in dichloromethane (5 ml), EDC (47.5 mg, 0.25 mmol) and *N*,*N*-dimethylaminopyridine (5 mg, 0.04 mmol) were added. The reaction mixture was allowed to warm to ambient temperature and it was stirred for further 2 h. The reaction mixture was concentrated in vacuo, suspended in water (5 ml) and then extracted with CH_2Cl_2 (3 × 15 ml). The combined organic layer was dried (Na_2SO_4), filtered and concentrated. The residue was purified by preparative TLC (toluene/acetic acid 8:1) to give **4c** (35 mg, 96%) as a reddish oil: $[\alpha]_D^{20} = +6.4$ (*c* 1.35, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (d, *J* = 6.0 Hz, 3H, CH_3), 2.72 [dt, *J* = 16.0, 3.5 Hz, 2H, CH_2(CO)], 3.11 [overlapped, 4H, 2 × N–CH_2(CO)], 3.12 [overlapped, 2H, N–CH_2(Ar)], 3.65–3.67 [m, 2H, N–CH_2(Ar)], 3.74 (s, 3H, OMe), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.87–3.92 (m, 2H, 4-H), 3.97–4.02 (m, 1H, 3-H), 5.39–5.40 (m, 1H, 1-H), 6.56 (s, 1H, Ar-H), 6.65 (s, 1H, Ar-H), 6.85 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.91 (d, *J* = 9.0 Hz, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.00 (CH_3), 35.15 [CH_2(CO)], 39.73 [N–CH_2(CO)], 41.91 (C-4), 46.10 [N–CH_2(CO)], 50.85 [N–CH_2(Ar)], 51.47 [N–CH_2(Ar)], 55.45 (OCH_3), 55.79 (OCH_3), 55.94 (OCH_3), 64.90 (C-3), 72.02 (C-1), 108.02 (C-5), 111.09 (C-8),

114.42 (C-Ar), 118.89 (C-Ar), 125.09 (C-5a), 128.45 (C-8a), 145.00 (C-6), 147.45 (C-7), 147.75 (C-q-Ar), 154.39 (C-q-Ar), 169.70 (CO); IR (KBr): 2930, 2856, 2834, 1634, 1250, 1076 cm^{-1}.

5.3.2. (+)-1-{[(1*R*,3*S*)-6,7-Dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]acetyl}-4-(4-methoxy-phenyl)piperazine 5c

Reddish oil (96%): $[\alpha]_D^{20} = +79.5$ (*c* 1.47, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.28 (d, *J* = 6.0 Hz, 3H, CH_3), 2.62 (m, 2H, 4-H), 2.91 [overlapped, 2H, N–CH_2(CO)], 3.08–3.13 [overlapped, 4H, 2 × N–CH_2(Ar)], 3.64–3.66 [overlapped, 2H, CH_2a(CO), 3-H], 3.67–3.68 [overlapped, 2H, N–CH_2(CO)], 3.76 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.08 [m, 1H, CH_2b(CO)], 5.20 (m, 1H, 1-H), 6.57 (s, 1H, Ar-H), 6.69 (s, 1H, Ar-H), 6.86 (d, *J* = 9.0 Hz, 2H, Ar-H), 6.92 (d, *J* = 9.0 Hz, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.77 (CH_3), 35.99 (C-4), 40.35 [N–CH_2(CO)], 41.92 [CH_2(CO)], 46.44 [N–CH_2(CO)], 50.90 [N–CH_2(Ar)], 51.30 [N–CH_2(Ar)], 55.42 (OCH_3), 55.78 (OCH_3), 55.94 (OCH_3), 70.43 (C-3), 74.83 (C-1), 107.33 (C-5), 111.19 (C-8), 114.39 (C-Ar), 118.87 (C-Ar), 126.12 (C-5a), 128.84 (C-8a), 145.07 (C-6), 147.41 (C, C-7), 147.64 (C-q-Ar), 154.30 (C-q-Ar), 170.38 (CO); IR (KBr): 2964, 2832, 1634, 1248, 1104 cm^{-1}.

5.4. General procedure for the preparation of 4d, 5d

5.4.1. (+)-1-{2-[(1*S*,3*S*)-6,7-Dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]ethyl}-4-(4-methoxyphenyl)piperazine 4d

A solution of **4c** (40 mg, 0.09 mmol) in THF (15 ml) was cooled to 0 °C and treated with a 1 M solution of borane in THF (0.27 ml, 0.27 mmol). The cooling bath was removed and the mixture heated at reflux for 4 h. Then a 1 M solution of borane in THF (0.1 ml, 0.1 mmol) was added to the mixture, which was then heated at reflux for an additional 3 h. The reaction was then cooled to 0 °C and slowly quenched with aqueous 1 M HCl (1.05 ml) and refluxed for an additional 1.5 h. The solution was cooled to room temperature and the volatiles were removed in vacuo. The resulting aqueous residue was diluted with brine and the pH was set to 14 with aqueous NaOH, followed by extraction with CH_2Cl_2 (3 × 15 ml). The combined organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by preparative TLC (toluene/acetic acid 2:1) to give **4d (**33.2 mg, 86%), which was crystallized from hexane: mp 123–124 °C; $[\alpha]_D^{20} = +14.9$ (*c* 0.11, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.34 (d, *J* = 6.2 Hz, 3H, CH_3), 1.90 (m, 1H, CH_2a), 2.14 (m, 1H, CH_2b), 2.60 (dd, *J* = 16.0, 9.7 Hz, 2H, 4-H), 2.67 [overlapped, 3H, CH_2(pip)(Ar), N–CH_2a], 2.68 (overlapped, 1H, N–CH_2b), 2.69 [overlapped, 2H, CH_2(pip)], 2.70 [overlapped, 2H, CH_2(pip)(Ar)], 3.13 [overlapped, 2H, CH_2(pip)], 3.70 (s, 3H, OCH_3), 3.78 (s, 6H, 2 × OCH_3), 4.04–4.07 (m, 1H, 3-H), 4.86 (dd, *J* = 10.7, 2.5 Hz, 1H, 1-H), 6.56 (s, 1H, Ar-H), 6.59 (s, 1H, Ar-H), 6.86 (d, *J* = 9.1 Hz, 2H, Ar-H), 6.93 (d, *J* = 9.1 Hz, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.38 (CH_3), 33.34 (CH_2), 35.46 (C-4), 50.62 [CH_2(pip)], 53.55 [CH_2(pip)(Ar)], 55.57 (OCH_3), 55.77 (N–CH_2), 55.89 (OCH_3), 56.02 (OCH_3), 63.71 (C-3), 73.44 (C-1), 108.24 (C-5), 111.41 (C-8), 114.46 (C-Ar), 118.18 (C-Ar), 125.29 (C-5a), 129.81 (C-8a), 145.73 (C-7), 147.43 (C-6), 147.68 (N-C-q-Ar), 153.82 (C-q-Ar), $^3J_{C3,1H}$ = 4.9 Hz, $^3J_{C1,3H}$ = 1–2 Hz; IR (KBr): 2940, 2820, 1246, 1106 cm^{-1}. Anal. Calcd for $C_{25}H_{34}N_2O_4$: C, 70.39; H, 8.03; N, 6.57. Found: C, 70.32; H, 8.06; N, 6.58.

5.4.2. (+)-1-{2-[(1*R*,3*S*)-6,7-Dimethoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-yl]ethyl}-4-(4-methoxyphenyl)piperazine 5d

Oil (87%): $[\alpha]_D^{20} = +67.1$ (*c* 0.54, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.15 (d, *J* = 6.1 Hz, 3H, CH_3), 2.00 (m, 1H, CH_2a), 2.23 (overlapped, 1H, CH_2b), 2.56 (overlapped, 1H, 4-Ha), 2.59 [overlapped, 2H, CH_2(pip)(Ar)], 2.63 (overlapped, 1H, 4-Hb), 2.67 [overlapped, 2H, CH_2(pip)(Ar)], 2.70 [overlapped, 4H, 2 × CH_2(pip)], 3.15 (overlapped, 2H, N–CH_2), 3.39 (overlapped, 1H, 3-H), 3.62 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.70 (m, 1H, 1-H), 6.48 (s, 1H, Ar-H), 6.54 (s, 1H, Ar-H), 6.73–6.85 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.20 (CH_3), 33.60 (CH_2), 36.50 (C-4), 50.70 (N–CH_2), 53.70 [CH_2(pip)], 54.86 [CH_2(pip)(Ar)], 55.90 (OCH_3), 56.30 (2 × OCH_3), 70.90 (C-3), 75.50 (C-1), 107.70 (C-5), 111.71 (C-8), 114.70 (C-Ar), 118.50 (C-Ar), 127.16 (C-5a), 129.85 (C-8a), 145.40 (C-6), 147.05 (C-7), 147.81 (N–C-q-Ar), 153.42 (C-q-Ar); $^3J_{1H-C3}$ = 2.0 Hz, $^3J_{3H-C1}$ = 1–2 Hz; IR (KBr): 2930, 2830, 1248, 1104 cm^{-1}.

6. Pharmacological assays

6.1. Radio ligand binding assays at human recombinant CHO-K1 cells (dopamine D4,2 receptor)

The incubation buffer: 50 mM Tris–HCl, pH 7.4, 1.4 mM ascorbic acid, 0.001% BSA, 150 mM NaCl.

The samples were incubated for 2 h at 25 °C. Ligand: 0.5 nM [^3H] spiperone, vehicle: 1% DMSO, Non-specific ligand: 10 μM haloperidol, K_D: 0.32 nM, B_{max}: 0.55 pmol/mg protein, specifics binding: 90%.

6.2. Radio ligand binding assays at human recombinant CHO-K1 cells (dopamine D4,4 receptor)

The incubation buffer: 50 mM Tris–HCl, pH 7.4, 1.4 mM ascorbic acid, 0.001% BSA, 150 mM NaCl.

The samples were incubated for 2 h at 25 °C. Ligand: 1.2 nM [^3H] spiperone, vehicle: 1% DMSO, non-specific ligand: 10 μM haloperidol, K_D: 0.46 nM, B_{max}: 0.63 pmol/mg protein, specifics binding: 85%.

6.3. Radio ligand binding assays at human recombinant CHO-K1 cells (dopamine D4,7 receptor)

The incubation buffer: 50 mM Tris–HCl, pH 7.4, 1.4 mM ascorbic acid, 0.001% BSA, 150 mM NaCl.

The samples were incubated for 2 h at 25 °C. Ligand: 1.5 nM [^3H] spiperone, vehicle: 1% DMSO, non-specific ligand: 10 μM haloperidol, K_D: 0.48 nM, B_{max}: 0.77 pmol/mg protein, specifics binding: 85%.

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References

1. (a) Ennis, M. D.; Ghazal, N. B.; Hoffman, R. L.; Smith, M. W.; Schlachter, S. K.; Lawson, C. F.; Im, W. B.; Pregenzer, J. F.; Svensson, K. A.; Lewis, R. A.; Hall, E. D.; Sutter, D. M.; Harris, L. T.; McCall, R. B. *J. Med. Chem.* **1998**, *41*, 2180–2183; (b) Bouchelet, I.; Case, B.; Olivier, A.; Hamel, E. *Br. J. Pharm.* **2000**, *129*, 501–508; (c) Bhattacharya, A.; Schenck, K. W.; Xu, Y.-C.; Nisenbaum, L.; Galbreath, E.; Cohen, M. C. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 825–832.
2. (a) DeNinno, M. P.; Schoenleber, R.; Asin, K. E.; MacKenzie, R.; Kebabian, J. W. *J. Med. Chem.* **1990**, *33*, 2948–2950; (b) DeNinno, M. P.; Schoenleber, R.; Perner, R. J.; Lijewski, L.; Asin, K. E.; Britton, D. R.; MacKenzie, R.; Kebabian, J. W. *J. Med. Chem.* **1991**, *34*, 2561–2569; (c) DeNinno, M. P.; Perner, R. J.; Morton, H. E.; DiDomenico, S., Jr. *J. Org. Chem.* **1992**, *57*, 7115–7118; (d) Isacson, R.; Kull, B.; Wahlestedt, C.; Salmi, P. *Neuroscience* **2004**, *124*, 33–42.
3. TenBrink, R. E.; Bergh, C. L.; Duncan, J. N.; Harris, D. W.; Huff, R. M.; Lahti, R. A.; Lawson, C. F.; Lutzke, B. S.; Martin, I. J.; Rees, S. A.; Schlachter, S. K.; Sih, J. C.; Smith, M. W. *J. Med. Chem.* **1996**, *39*, 2435–2437.
4. (a) Hegedűs, A.; Hell, Z. *Org. Biomol. Chem.* **2006**, *4*, 1220–1222; (b) Larghi, L. E.; Kaufman, S. T. *Synthesis* **2006**, *2*, 187–220.
5. (a) Vicenzi, J. T.; Zmijewski, M. J.; Reinhard, M. R.; Landen, B. E.; Muth, W. L.; Marler, P. G. *Enzyme Microb. Technol.* **1997**, *20*, 494–499; (b) Erdélyi, B.; Szabó, A.; Birincsik, L.; Hoschke, Á. *J. Mol. Catal. B: Enzym.* **2004**, *29*, 195–199.