Design and Synthesis of trans-N-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoguinolin-2vl)ethvl]cvclohexvl]-4-quinolinecarboxamide (SB-277011): A Potent and Selective Dopamine D₃ Receptor Antagonist with High Oral Bioavailability and **CNS Penetration in the Rat**

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A selective dopamine D_3 receptor antagonist offers the potential for an effective antipsychotic therapy, free of the serious side effects of currently available drugs. Using clearance and brain penetration studies as a screen, a series of 1,2,3,4-tetrahydroisoquinolines, exemplified by 13, was identified with high D_3 affinity and selectivity against the D_2 receptor. Following examination of molecular models, the flexible butyl linker present in 13 was replaced by a more conformationally constrained cyclohexylethyl linker, leading to compounds with improved oral bioavailability and selectivity over other receptors. Subsequent optimization of this new series to improve the cytochrome P450 inhibitory profile and CNS penetration gave trans-N-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (24, SB-277011). This compound is a potent and selective dopamine D_3 receptor antagonist with high oral bioavailability and brain penetration in the rat and represents an excellent new chemical tool for the investigation of the role of the dopamine D_3 receptor in the CNS.

Introduction

Dopaminergic neurotransmission is mediated by five receptor subtypes (D_1-D_5) , which can be grouped into two receptor families. D_1 -like receptors include the D_1 and D₅ subtypes, whereas D₂-like receptors include the D_2 , D_3 , and D_4 subtypes.

Clinically effective antipsychotic agents share the property of dopamine D_2 and D_3 receptor antagonism. At the doses used in the clinic, these drugs occupy D_3 as well as D₂ receptors, and their antipyschotic effects could therefore be mediated via D₂ and/or D₃ receptors. Blockade of dopamine D₂ receptors in the striatum leads to serious extrapyramidal side effects, which result in poor patient compliance and consequently poor control of the disease. However, dopamine D_3 receptors are preferentially located in limbic brain regions, such as the nucleus accumbens, where dopamine receptor blockade has been associated with antipsychotic activity. A selective dopamine D₃ receptor antagonist therefore offers the potential for an effective antipsychotic therapy, free of the serious side effects of currently available drugs.^{1–4} The presence of the dopamine D_3 receptor in projection regions of the mesocorticolimbic system also suggests a potential therapeutic role in reinforcement processes and drug abuse.⁵

Pharmacological approaches to understanding the role of the dopamine D_3 receptor have been hindered

by a lack of compounds (either agonists or antagonists) with the required selectivity and pharmacokinetic profiles. The aim of our research was therefore to identify orally bioavailable, CNS penetrant antagonists with high affinity (p $K_i \ge 8$) for the dopamine D₃ receptor and high selectivity (\geq 100-fold) over other receptors and ion channels in order to test the above hypothesis and to further characterize the role of the dopamine D₃ receptor in the central nervous system.⁶

Several dopamine agonists have been identified with selectivity for the dopamine D_3 receptor over the D_2 receptor (Chart 1). Although 7-OH-DPAT (1), quinelorane (2), and PD-128907 (3), show high selectivity in radioligand binding assays, these agonists lack the





7-OH-DPAT 1

quinelorane 2





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Chart 2. Dopamine D₃ Receptor Antagonists



appropriate functional selectivity 7 to allow clear discrimination between D_3 and D_2 receptor function in vivo.

Antagonists with varying degrees of selectivity for the D₃ receptor compared to the D₂ receptor (Chart 2) have been reported. The compounds identified to date include U 99194 (4),⁸ nafadotride (5),⁹ aryl pyrrole 6,¹⁰ arylpiperazines 7,¹¹ 8,¹² and 9,¹³ aminotetralins 10^{14} and 11,¹⁵ benzopyranopyrrolidine 12,¹⁶ and tetrahydroisoquinoline **13**.¹⁷ Of these antagonists, U 99194, nafadotride, and arylpiperazine 8 are only 10-30-fold selective for the D_3 over the D_2 receptor, and GR 103691 (7) is only 10-fold selective over the 5-HT_{1A} receptor. The arylpyrrole derivative **6** and aminotetralin **11** are metabolically unstable. Neither functional activity nor metabolic stability data have been reported for aminotetralin derivative 10. NGB 2904 (9) and S 33084 (12) have been identified as potent and selective dopamine D₃ receptor antagonists. Some evidence for pharmacological activity in vivo has been described for S 33084;18 however, no details of the pharmacokinetic profile of either 9 or 12 have been disclosed.

Experiments using mice where the dopamine D_3 receptor has been genetically deleted have allowed an assessment of the selectivity of some compounds. As

such, PD-128907 and nafadotride have been shown to lack the necessary in vivo selectivity to define the role of the dopamine D_3 receptor¹⁹ and have highlighted the need for compounds with an improved selectivity and pharmacokinetic profile.

Recently, we described the design and synthesis of a novel series of 1,2,3,4-tetrahydroisoquinolines, exemplified by 3-indolyl-2-propenamide derivative 13, which had high affinity, pK_i 8.4 (K_i 4 nM), for the dopamine D_3 receptor and 150-fold selectivity against the D_2 receptor.¹⁷ Studies in the rat showed that compound 13 had a low blood clearance of 27 mL/min/kg, with a plasma half-life of 2.5 h. However, further studies demonstrated that 13 had oral bioavailability of only 7% in the rat and moderate selectivity against 5-HT_{1B} $(pK_i 7.0)$ and 5-HT_{1D} $(pK_i 7.2)$ receptors. Alternative substituents to the $7-CF_3SO_2$ group of 13 were then investigated, with the aim of reducing lipophilicity and deactivating the tetrahydroisoguinoline ring toward metabolism. From this approach, the 7-cyano derivative 14 (Table 1) was identified with an improved oral bioavailability of 11% in the rat, although D₃ affinity was reduced compared to 13 and selectivity over 5-HT_{1B} and 5-HT_{1D} receptors remained modest.



^{*a*} Analyses for the elements indicated were within $\pm 0.4\%$ of the theoretical values. ^{*b*} pK_i values at human cloned receptors represent the mean of at least three determinations.

Table 2. Receptor Binding Profiles of *trans*-1,4-Cyclohexylethyl Derivatives

compd	R ¹	R ²	mp °C (HCl salt)	formula	anal."	D ₃ ^b	$\mathbf{D}_2^{\ b}$	5-HT _{1B} ^b	5-HT _{1D} ^b
15	7-CN	Ph	288-291	C ₂₇ H ₃₁ N ₃ O.HCl	M^+	8.9	6.8	6.8	7.0
16	7-CN		261-263	$C_{29}H_{32}N_4O.HCl$	M^{+}	8.5	6.9	\mathbf{NT}^{c}	NT ^c
17	7-CN		278-280	C ₂₇ H ₃₀ N ₄ O.HCl	C,H,N	8.7	7.0	6.2	7.0
18	6-CN		253-255	C ₂₇ H ₃₀ N ₄ O.HCl	C,H,N	7.8	6.1	NT ^c	NT ^c
19	6-CN	Ph	282-285	C ₂₇ H ₃₁ N ₃ O.HCl	C,H,N	8.5	6.7	6.0	6.5
20	6-CN	Ph(3-SO ₂ Me)	268-269	C ₂₈ H ₃₃ N ₃ O ₃ S.HCl	C,H,N	8.3	6.5	5.2	5.4
21	6-CN	Ph(4-F)	263-264	C27H30FN3O.HCl	$H,N;C^d$	8.5	6.7	<5	6.2
22	6-CN	2-naphthyl	255-258	C ₂₉ H ₃₁ N ₃ O.HCl	C,H,N	8.0	5.8	<5	7.0
23	6-CN	l-naphthyl	191-194	C ₂₉ H ₃₁ N ₃ O.HCl	C,H,N	8.1	6.4	NT^{c}	NT^{c}
24	6-CN	4-quinolinyl	216-219	$C_{28}H_{30}N_4O.HCl.0.5H_2O$	C,H,N	8.0 ^e	6.0 ^e	<5.2	5.9

^{*a,b*} See footnotes *a*, *b* in Table 1. ^{*c*} NT = not tested. ^{*d*} Calcd: C, 69.23; found: C, 68.81. ^{*e*} pK_i values represent the mean of 15 determinations.

Inspection of molecular models of compounds **13** and **14** and comparison of these with known selective 5-HT_{1B} and 5-HT_{1D} ligands²⁰ suggested that the flexible butyl linker present in **13** and **14** could be partly responsible for the moderate selectivity against these receptors. Using parallel solution phase chemistry, a range of more conformationally constrained linkers was investigated. From this exercise, the *trans*-1,4-cyclohexylethyl derivative **15** (Table 2) was identified with high D₃ receptor affinity (p K_i 8.9) and 110-fold selectivity over the D₂ receptor. In agreement with our hypothesis, compound **15** also had improved selectivity over 5-HT_{1B} and 5-HT_{1D} receptors compared to **13** and **14**. Using compound **15** as a new lead for further parallel chemistry, we have now identified **24**, *trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (SB-277011), as a potent and selective dopamine D₃ receptor antagonist with high oral bioavailability and CNS penetration in the rat.

Chemistry

Compounds **15–24** (Table 2) were readily prepared as shown in Scheme 1. Esterification of *trans*-(4-amino)cyclohexylacetic acid **25**,²¹ followed by reaction with di*tert*-butyl dicarbonate, gave **26**. Reduction of **26** with DIBAL-H gave aldehyde **27**, which was reductively

Scheme 1



aminated with the appropriately substituted 1,2,3,4tetrahydroisoquinolines **28** ($\mathbb{R}^1 = 7$ -CN) and **29** ($\mathbb{R}^1 = 6$ -CN) to give the protected amines **30** ($\mathbb{R}^1 = 7$ -CN) and **31** ($\mathbb{R}^1 = 6$ -CN), respectively. Deprotection with TFA to the corresponding amines **32** ($\mathbb{R}^1 = 7$ -CN) and **33** ($\mathbb{R}^1 = 6$ -CN) followed by coupling to the appropriate acids gave **15**-**17** ($\mathbb{R}^1 = 7$ -CN) and **18**-**24** ($\mathbb{R}^1 = 6$ -CN).

Results and Discussion

Following the highly encouraging receptor binding profile obtained with the cinnamide derivative 15, this new series of compounds required further optimization to reduce the cytochrome P450 inhibitory potential and improve the pharmacokinetic profile. For comparison with the previous series of butyl-linked compounds, the 3-indolyl-2-propenamide derivative 16 was prepared, but this modification reduced D₃ receptor affinity and selectivity over the D₂ receptor compared to **15**. Investigation of the effect of conformational constraint of the cinnamide moiety of 15 resulted in the synthesis of 2-indolyl derivative 17, which retained affinity for the D₃ receptor. Interestingly, **17** had improved selectivity over the 5-HT_{1B} receptor compared to **15**, but selectivity over the D₂ receptor was reduced to 50-fold. However, it was particularly encouraging that 17 was found to have an excellent pharmacokinetic profile in the rat, with an oral bioavailability of 43%, blood clearance of 6 mL/min/kg, and half-life of 6.3 h. A CNS penetration study showed 17 to have a brain:blood ratio of 3:1. Unfortunately, the 7-cyanotetrahydroisoquinoline derivatives 15–17 were shown to be inhibitors of cytochrome P450 2D6, and 17 was the most potent with an IC₅₀ of 0.8 µM. However, the corresponding 6-cyanotetrahydroisoquinoline derivative 18 had greatly reduced liability to inhibit 2D6 (17% inhibition at 10 μ M), but D₃ receptor affinity was also reduced. Following this important observation, a range of 6-cyanotetrahydroisoquinolines was prepared with the initial aim of improving affinity for the dopamine D_3 receptor. Cinnamide **19** had an improved D_3 receptor affinity compared to **18** and also had 100-fold selectivity against 5-HT_{1B} and 5-HT_{1D} receptors. Substitution around the phenyl ring of the cinnamide moiety was allowed, as exemplified by compounds **20** and **21**. Although neither compound was 100-fold selective against the D_2 receptor, selectivity against the 5-HT_{1B} and 5-HT_{1D} receptors remained high.

Interestingly, the 3-methylsulfonylcinnamide 20 had an encouraging oral bioavailability of 45% in the rat, but in CNS penetration studies, this compound showed a negligible level (<2%) of brain penetration. However, the 4-fluorocinnamide 21 had a similar oral bioavailability to that of 20 in the rat and a brain: blood ratio of 1:1. Presumably the improved CNS penetration of 21 is a result of both increased lipophilicity and reduced hydrogen-bond acceptor potential compared to 3-methylsulfonylcinnamide 20. Additional analogues with physicochemical properties similar to those of 21 were therefore prepared. Although the 2-naphthyl derivative **22** achieved 100-fold selectivity against the D_2 and 5-HT_{1B} receptors, selectivity against 5-HT_{1D} receptors was only 10-fold. The 1-naphthyl analogue 23 retained D_3 receptor affinity, although selectivity against D_2 receptors was reduced compared to **22**. Replacement of the 1-naphthyl group of **23** by a 4-quinolinyl group, as in 24, maintained D_3 receptor affinity and restored \geq 100-fold selectivity against the D₂, 5-HT_{1B}, and 5-HT_{1D} receptors. Subsequent cross-screening showed 24 to be \geq 100-fold selective against a package of 63 other receptors and ion channels.

An in vitro functional assay using microphysiometry in CHO cells expressing human cloned dopamine D_3 and D_2 receptors⁷ demonstrated that **24** was devoid of agonist-like activity and was a potent and selective antagonist (D_3 , pK_b 8.4; D_2 , pK_b 6.5). Compound **24** (SB-277011) was shown to have an excellent pharmacokinetic profile in the rat (oral bio-availability 43%, half-life 2.0 h, plasma clearance 19 mL/min/kg) and to be highly brain-penetrant (brain:blood ratio of 3.6:1), with a clean P450 profile.

Using in vivo microdialysis in the rat, the dopamine agonist quinelorane has been shown to reduce dopamine levels in the nucleus accumbens and the striatum.²² Compound **24** dose dependently reversed the effects of quinelorane in the nucleus accumbens, with complete reversal at a dose of 3 mg/kg po. In contrast, the effects of quinelorane in the striatum were not reversed by **24** at a high dose of 93 mg/kg. The regional selectivity of the effect of **24** on the reversal of the quinelorane-induced reduction in dopamine efflux is in good agreement with the regional distribution of D₃ receptors in rat forebrain.²³

The low level of dopamine D_3 receptors in the dorsal striatum and the pituitary gland has led to the hypothesis that selective D_3 receptor antagonists would have reduced liability to induce extrapyramidal movement disorders and would not induce hyperprolactinaemia. In agreement with this hypothesis, **24** dosed up to 80 mg/kg po displayed no cataleptic activity in the rat and did not elevate prolactin levels. In contrast, the typical antipsychotic agent haloperidol produced a complete cataleptic response and significantly elevated serum prolactin levels at a dose of 3 mg/kg po.

Conclusion

The tetrahydroisoquinoline derivatives 13 and 14, designed as dopamine D₃ receptor antagonists with improved metabolic stability compared to the N-propyl-2-aminotetralin derivative 11, were the starting point for a chemical program aimed at further improving oral bioavailability and selectivity over other receptors within this class of compound. Molecular modeling studies suggested that conformational constraint of the flexible butyl linker present in 13 and 14 would improve selectivity, and this approach led to the identification of cinnamide derivative 15 containing a *trans*-cyclohexylethyl linker. Further optimization of 15 to improve the cytochrome P450 inhibitory potential and pharmacokinetic profile resulted in the identification of 24 (SB-277011). This compound is a potent and selective dopamine D₃ receptor antagonist with excellent oral bioavailability and brain penetration in the rat. Preliminary studies using in vivo microdialysis in the rat suggest that the regional selectivity of the effect of 24 on the reversal of the quinelorane-induced reduction in dopamine efflux is in good agreement with the regional distribution of D₃ receptors in rat forebrain. The lack of induction of catalepsy or elevation of serum prolactin with high doses of 24 provides further support for the hypothesis that a selective dopamine D_3 antagonist would have reduced liability to induce extrapyramidal movement disorders and would not induce hyperprolactinaemia. Further details of these encouraging preliminary findings, together with a more extensive evaluation of 24 in a range of pharmacological models designed to probe the biological relevance of the dopamine D₃ receptor, will be reported.

Experimental Section

Chemistry. Melting points were determined on a Buchi melting point apparatus and are uncorrected. ¹H NMR spectra were recorded on a Bruker AMX 400, a JEOL GX 270, or a Bruker AC 250. High-resolution mass spectra were recorded using a JEOL DX303 at 70 eV. Low-resolution mass spectra (API⁺) were recorded using a Fisons VG Platform. LC-mass spectra (LCMS) were recorded using a Hewlett-Packard HP 1100 System attached to a Waters Micomass ZMD electrospray ionization mass spectrometer. A SORBAX SB C_{18} 3.5 μ m (30 imes 2.1 mm) column was used with elution using the following conditions: eluant A, 0.1% TFA/H₂O v/v, eluant B, 0.1% TFA/ CH₃CN v/v; flow rate, 0.75 mL/min. The eluation gradient was 5-95% B increased over 1.5 min and then held for 0.5 min. A detection wavelength of 215 nm was used. Karl Fischer analysis was used to confirm the presence of water in analytical samples submitted for combustion analysis. Merck Kieselgel 60 was used for column chromatography. All solvent evaporation was performed under vacuum.

trans-2-(1-(4-(*N*-*tert*-Butyloxycarbonyl)amino)cyclohexyl)acetic Acid, Methyl Ester (26). A mixture of *trans*-(4-amino)cyclohexylacetic acid hydrogen sulfate²¹ (27.0 g, 106 mmol), concentrated H₂SO₄ (3 mL), and methanol (300 mL) was stirred at reflux for 5 h. The resulting solution was filtered and the filtrate evaporated in vacuo to give a brown oil (36 g). A mixture of this material, triethylamine (36 mL, 26.1 g, 259 mmol), dichloromethane (600 mL) and di-*tert*-butyl dicarbonate (25.5 g, 117 mmol) was stirred at 20 °C for 18 h. The resulting solution was partitioned between saturated aqueous NaHCO₃ (500 mL) and dichloromethane (3 × 200 mL), and the combined extracts were dried (Na₂SO₄) and evaporated in vacuo to give **26** (24.6 g, 86%) as a colorless solid. ¹H NMR (CDCl₃) δ : 1.08 (4H, m), 1.43 (9H, s), 1.76 (3H, m), 2.00 (2H, m), 2.20 (2H, d, J = 7 Hz), 3.37 (1H, m), 3.66 (3H, s), 4.39 (1H, br s).

trans-2-(1-(4-(N-tert-Butyloxycarbonyl)amino)cyclohexyl)acetaldehyde (27). To a stirred solution of trans-2-(1-(4-(N-tert-butyloxycarbonyl)amino)cyclohexyl) acetic acid, methyl ester (46.0 g, 170 mmol), in dry toluene (920 mL) at -78 °C under argon was added a solution of diisobutylaluminum hydride (1 M, 285 mL, 285 mmol), dropwise over 0.5 h. The resulting solution was stirred for a further 0.3 h and quenched with a mixture of methanol (28 mL) in toluene (50 mL) and then poured into saturated aqueous potassium sodium tartrate (1.2 L). The resultant mixture was extracted with ether (4 \times 1 L). The combined organic extracts were dried (Na₂SO₄) and evaporated in vacuo to give a waxy solid which was purified using silica gel, eluting with 10-50% ethyl acetate/hexane to give 27 ($\overline{2}1.77$ g, $53\overline{8}$) as a colorless solid. ¹H NMR (CDCl₃) δ: 1.12 (4H, m), 1.44 (9H, s), 1.78 (3H, m), 2.00 (2H, m), 2.33 (2H, dd, J = 7, 2 Hz), 3.37 (1H, m), 4.40 (1H, m), 9.75 (1H, m).

trans-2-(2-(1-(4-(N-tert-Butyloxycarbonyl)amino)cyclohexyl)ethyl)-6-cyano-1,2,3,4-tetrahydroisoquinoline (31). A mixture of trans-2-(1-(4-(N-tert-butyloxycarbonyl)amino)cyclohexyl) acetaldehyde (6.0 g, 24.9 mmol), 6-cyano-1,2,3,4tetrahydroisoquinoline (3.93 g, 24.9 mmol), and sodium triacetoxyborohydride (7.7 g, 36.3 mmol) in 1,2-dichloroethane (270 mL) was stirred at 20 $^\circ \rm C$ for 16 h. The resulting solution was partitioned between aqueous K_2CO_3 (200 mL) and dichloromethane (100 mL), and the combined extracts were washed with brine (200 mL), dried (Na₂SO₄), and evaporated in vacuo to a minimum volume and filtered through a pad of silica (100 g), washing with ethyl acetate. The filtrate was evaporated in vacuo to give 31 (8.33 g, 87%) as a yellow solid. ¹H NMR (CDCl₃) δ: 1.08 (4H, m), 1.28 (1H, m), 1.44 (9H, s), 1.48 (2H, m), 1.78 (2H, m), 1.99 (2H, m), 2.52 (2H, m), 2.72 (2H, t, J= 7 Hz), 2.91 (2H,,m), 3.37 (1H, m), 3.63 (2H, m), 4.40 (1H, m), 7.12 (1H, d, J = 8 Hz), 7.39 (2H, m).

trans•2-(2-(1-(4-(*N*-*tert*-Butyloxycarbonyl)amino)cyclohexyl)ethyl)-7-cyano-1,2,3,4-tetrahydroisoquinoline (30) was prepared in a manner similar to compound 31. ¹H NMR (CDCl₃) δ: 1.06 (4H, m), 1.28 (1H, m), 1.44 (9H, s), 1.47 (2H, m), 1.77 (2H, m), 1.99 (2H, m), 2.52 (2H, m), 2.72 (2H, t, *J* = 7 Hz), 2.94 (2H, m), 3.37 (1H, m), 3.60 (2H, s), 4.37 (1H, m), 7.18 (1H, d, J = 8 Hz), 7.32 (1H, s), 7.39 (1H, d, J = 8 Hz).

trans-2-(2-(1-(4-Amino)cyclohexyl)ethyl)-6-cyano-1,2,3,4tetrahydroisoquinoline (33). A mixture of *trans*-2-(2-(1-(4-(*N*-*tert*-butyloxycarbonyl)amino)cyclohexyl)ethyl)-6-cyano-1,2,3,4tetrahydroisoquinoline (8.3 g, 21.7 mmol), trifluoroacetic acid (15 mL), and dichloromethane (180 mL) was stirred at 20 °C for 2 h. The resulting solution was evaporated in vacuo and the residue partitioned between saturated aqueous K₂CO₃ (200 mL) and dichloromethane (2 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried (Na₂SO₄), and evaporated in vacuo to give **33** (4.99 g, 81%) as a brown oil. ¹H NMR (CDCl₃) δ : 0.91–1.16 (4H, m), 1.22–1.40 (3H, m), 1.47 (2H, m), 1.72–1.91 (4H, m), 2.52 (2H, m), 2.59 (1H, m), 2.72 (2H, t, *J* = 7 Hz), 2.92 (2H, m), 3.64 (2H, s), 7.11 (1H, d, *J* = 8 Hz), 7.39 (2H, m). Mass spectrum (API⁺): Found 284 (MH⁺). C₁₈H₂₅N₃ requires 283.

trans-2-(2-(1-(4-Amino)cyclohexyl)ethyl)-7-cyano-1,2,3,4tetrahydroisoquinoline (32) was prepared in a manner similar to compound 33. ¹H NMR (CDCl₃) δ : 0.91–1.16 (4H, m), 1.18–1.40 (3H, m), 1.47 (2H, m), 1.73–1.92 (4H, m), 2.53 (2H, m), 2.62 (1H, m), 2.72 (2H, t, J = 7 Hz), 2.94 (2H, m), 3.60 (2H, s), 7.19 (1H, d, J = 8 Hz), 7.32 (1H, s), 7.41 (1H, d, J = 8 Hz). Mass spectrum (API⁺): Found 284 (MH⁺). C₁₈H₂₅N₃ requires 283.

trans-N-[4-[2-(7-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-1H-indole-2-carboxamide (17). A mixture of trans-2-(2-(1-(4-amino)cyclohexyl)ethyl-7-cyano-1,2,3,4-tetrahydroisoquinoline (350 mg, 1.24 mmol), indole-2carboxylic acid (200 mg, 1.24 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (238 mg, 1.24 mmol), 1-hydroxybenzotriazole (catalytic amount), and dichloromethane (8 mL) was shaken for 16 h. Saturated sodium bicarbonate (4 mL) was then added and the mixture shaken for 0.25 h. Chromatography of the organic layer on silica with 50-100% ethyl acetate in hexane and 0-10% methanol in ethyl acetate gradient elution gave 17 as a yellow solid (90 mg, 17%). ¹H NMR (CDCl₃) δ : 1.08–1.36 (4H, m), 1.50–1.70 (4H, m), 1.86 (1H, m), 2.12 (2H, m), 2.55 (2H, m), 2.73 (2H, t, J = 7 Hz), 2.94 (2H, m), 3.60 (2H, s), 3.95 (1H, m), 5.97 (1H, d, J = 8 Hz), 6.81 (1H, m), 7.17 (2H, m), 7.34 (2H, m), 7.42 (2H, t, J = 8 Hz), 7.64 (1H, d, J = 8 Hz), 9.22 (1H, br s). Mass spectrum (API⁺): Found 427 (MH⁺). C₂₇H₃₀N₄O requires 426.

The following compounds were prepared in a manner similar to compound **17**.

trans- (*E*)-*N*-4-[2-(7-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-3-phenyl-2-propenamide (15). ¹H NMR (DMSO- d_6) δ : 1.00–1.60 (7H, m), 1.90 (4H, m), 2.54 (2H, m), 2.74 (2H, t, J = 6 Hz), 2.95 (2H, t, J = 6 Hz), 3.65 (2H, s), 3.67 (1H, m), 6.69 (1H, d, J = 16 Hz), 7.38 (1H, d, J =8 Hz), 7.46 (4H, m), 7.65 (4H, m), 8.07 (1H, d, J = 8 Hz). Mass spectrum (API⁺): Found 414 (MH⁺). C₂₇H₃₁N₃O requires 413. The purity was determined as >98% by LC-MS, retention time 1.15 min.

trans-(*E*)-*N*-[4-[2-(7-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-3-(3-1*H*-indole)-2-propenamide (16). ¹H NMR (DMSO- d_6) δ : 0.85–1.50 (7H, m), 1.80 (4H, m), 2.35 (2H, m), 2.61 (2H, m), 2.84 (2H, m), 3.52 (3H, m), 6.56 (1H, d, J = 16 Hz), 7.13 (2H, m), 7.27 (1H, d, J = 8 Hz), 7.41 (1H, d, J = 8 Hz), 7.52 (3H, m), 7.69 (2H, m), 7.84 (1H, m), 11.50 (1H, br s). Mass spectrum (API⁺): Found 453 (MH⁺). C₂₉H₃₂N₄O requires 452. The purity was determined as >98% by LC-MS, retention time 1.07 min.

trans-(*E*)-*N*-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-3-[3-(methylsulfonyl)phenyl]-2-propenamide (20). A mixture of *trans*-2-(2-(1-(4-amino)cyclohexyl)ethyl)-6-cyano-1,2,3,4-tetrahydroisoquinoline (0.10 g, 0.35 mmol), (*E*)-3-(3-methylsulfonyl)phenylpropenoic acid (0.079 g, 0.35 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.067 g, 0.35 mmol), 1-hydroxybenzotriazole (catalytic amount), and dichloromethane (5 mL) was treated in a manner to similar to compound 5 to give 20 (0.065 g, 38%) as an off-white solid. ¹H NMR (DMSO-*d*₆) δ : 0.97– 1.38 (5H, m), 1.48 (2H, m), 1.84 (4H, m), 2.52 (2H, m), 2.68 (2H, m), 2.87 (2H, m), 3.29 (3H, s), 3.63 (3H, m), 6.81 (1H, d, J = 16 Hz), 7.31 (1H, d, J = 8 Hz), 7.52 (1H, d, J = 16 Hz), 7.61 (2H, m), 7.72 (1H, t, J = 8 Hz), 7.93 (2H, m), 8.02 (2H, m). Mass spectrum (API⁺): Found 492 (MH⁺). C₂₈H₃₃N₃O₃S requires 491.

The following compounds were prepared in a manner similar to compound 20.

trans-N-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-1*H*-indole-2-carboxamide (18). ¹H NMR (DMSO- d_6) δ : 0.88 (2H, m), 1.03–1.32 (5H, m), 1.60 (4H, m), 2.29 (2H, m), 2.46 (2H, m), 2.65 (2H, m), 3.42 (2H, m), 3.56 (1H, m), 6.82 (1H, m), 6.95 (2H, m), 7.09 (1H, d, J = 8Hz), 7.23 (1H, d, J = 8 Hz), 7.38 (3H, m), 8.01 (1H, d, J = 8Hz), 11.34 (1H, s). Mass spectrum (API⁺): Found 427 (MH⁺). C₂₇H₃₀N₄O requires 426.

trans-(*E*)-*N*-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquino-lin-2-yl)ethyl]cyclohexyl]-3-phenyl-2-propenamide (19). ¹H NMR (DMSO- d_6) δ : 0.83–1.22 (5H, m), 1.34 (2H, m), 1.70 (4H, m), 2.38 (2H, m), 2.54 (2H, m), 2.73 (2H, m), 3.50 (3H, m), 6.49 (1H, d, J = 16 Hz), 7.17 (1H, d, J = 8 Hz), 7.29 (4H, m), 7.45 (4H, m), 7.88 (1H, d, J = 8 Hz). Mass spectrum (API⁺): Found 414 (MH⁺). $C_{27}H_{31}N_3O$ requires 413.

trans-(*E*)-*N*-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-3-(4-fluorophenyl)-2-propenamide (21). ¹H NMR (CDCl₃ + CD₃OD) δ : 1.03–1.40 (5H, m), 1.54 (2H, m), 1.84 (2H, m), 2.05 (2H, m), 2.55 (2H, m), 2.75 (2H, t, J = 7 Hz), 2.94 (2H, m), 3.66 (2H, s), 3.82 (1H, m), 5.90–6.15 (1H, m), 6.30 (1H, d, J = 16 Hz), 6.97–7.17 (3H, m), 7.35–7.61 (5H, m). Mass spectrum (API⁺): Found 432 (MH⁺). C₂₇H₃₀ FN₃O requires 431. Purity was determined as >98% by LC-MS, retention time 1.05 min.

trans-*N*-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-2-naphthalenecarboxamide (22). ¹H NMR (DMSO- d_6) δ : 1.10 (2H, m), 1.24–1.53 (5H, m), 1.88 (4H, m), 2.52 (2H, m), 2.67 (2H, m), 2.86 (2H, m), 3.63 (2H, m), 3.82 (1H, m), 7.30 (1H, d, J = 8 Hz), 7.61 (4H, m), 8.00 (4H, m), 8.42 (2H, m). Mass spectrum (API⁺): Found 438 (MH⁺). C₂₉H₃₁N₃O requires 437.

trans-N-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-1-naphthalenecarboxamide (23). ¹H NMR (DMSO- d_6) δ : 1.08 (2H, m), 1.30 (3H, m), 1.47 (2H, m), 1.83 (2H, m), 1.97 (2H, m), 2.52 (2H, m), 2.67 (2H, m), 2.86 (2H, m), 3.63 (2H, s), 3.81 (1H, m), 7.30 (1H, d, J = 8Hz), 7.54 (6H, m), 7.99 (2H, m), 8.17 (1H, m), 8.42 (1H, d, J =8 Hz). Mass spectrum (API⁺): Found 438 (MH⁺). C₂₉H₃₁N₃O requires 437.

trans-N-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (24). A mixture of trans-2-(2-(1-(4-amino)cyclohexyl)ethyl)-6-cyano-1,2,3,4-tetrahydroisoquinoline (4 g, 14.1 mmol), quinoline-4carboxylic acid (2.45 g, 14.1 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.71 g, 14.1 mmol), 1-hydroxybenzotriazole (0.251 g. 1.86 mmol), and dichloromethane (150 mL) was stirred at room temperature for 3 h. Further dichloromethane (50 mL) was added and stirring continued for 17 h. Dichloromethane (200 mL) was added and the mixture washed with saturated aqueous sodium hydrogen carbonate (500 mL). The aqueous phase was extracted with dichloromethane (2 \times 250 mL). Combined organic extracts were dried (Na₂SO₄) and evaporated in vacuo. The residue was purified by chromatography on silica gel (350 mL) using 10-100% ethyl acetate-hexane and then a 1-5% methanol-ethyl acetate gradient elution to give 24 (3.06 g, 49.4%) as a colorless solid. A sample recrystallized from ethyl acetate-dichloromethane, mp 207–210 °C. ¹H NMR (CDCl₃) δ : 1.17–1.45 (5H, m), 1.53 (2H, m), 1.89 (2H, m), 2.20 (2H, m), 2.55 (2H, m), 2.73 (2H, t, J = 6 Hz), 2.91 (2H, t, J = 6 Hz), 3.65 (2H, s), 4.07 (1H, m), 5.83 (1H, d, J = 8 Hz), 7.10 (1H, d, J = 8 Hz), 7.38 (3H, m), 7.60 (1H, m), 7.76 (1H, m), 8.12 (1H, m), 8.19 (1H, m), 8.90 (1H, d, J = 4 Hz). Mass spectrum (API⁺): Found 439 (MH⁺). C₂₈H₃₀N₄O requires 438.

Treatment of a solution of the free base of **24** (1.84 g, 4.3 mmol) in methanol (40 mL) and dichloromethane (20 mL) with hydrochloric acid (2 M, 2.15 mL), followed by evaporation in

vacuo, gave a solid. Recrystallization from ethanol (250 mL) gave the monohydrochloride salt (0.86 g) as an off-white solid, mp 216–219 °C. ¹H NMR (DMSO- d_6) δ : 1.03–1.25 (2H, m), 1.25–1.45 (3H, m), 1.67–1.90 (4H, m), 2.01 (2H, m), 3.09 (1H, m), 3.26 (4H, m), 3.72 (1H, m), 3.82 (1H, m), 4.37 (1H, m), 4.65 (1H, m), 7.45 (1H, d, J = 8 Hz), 7.51 (1H, d, J = 4 Hz), 7.64–7.87 (4H, m), 8.10 (2H, m), 8.70 (1H, d, J = 8 Hz), 8.97 (1H, d, J = 4 Hz), 11.01 (1H, br s).

Biological Assays. In vivo studies were conducted in compliance with the Home Office Guidance on the operation of the UK Animals (Scientific Procedures) Act 1986 and were approved by the SmithKline Beecham Procedures Review Panel.

Cloned Cell Lines Expressing D₂ and D₃ Receptors. Cloned human D₂ (long) receptors expressed in CHO cells were obtained from the Garvan Institute of Medical Research, Sydney, Australia. Human D₃ receptors expressed in CHO or NG108-15 cells were obtained from Unite de Neurobiologie et Pharmacologie (U.109) de l'INSERM, Paris, France.

Radioligand Binding Assays. Radioligand binding assays at hD₂ and hD₃ receptors were carried out using membranes from CHO cells. Membranes (5–15 μ g of protein) were incubated with [¹²⁵I]iodosulpride (0.1 nM) in buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, and 1 mM MgCl₂ (pH 7.4) for 30 min at 37 °C in the presence or absence of competing ligands. Nonspecific binding was defined with 0.1 mM iodosulpride. Binding to a wide variety of monoamine receptors was performed as described in ref 20. Radioligand binding assays were also performed on 55 receptors, ion channels, and enzymes by Cerep, Le Bois l'Eveque, B. P. 1, 86000 Celle L'Evescault, France.

Cell Culture. CHO cells expressing hD₂ receptors were grown in 50:50 Dulbecco's modified Eagles Medium (DMEM; without sodium pyruvate, with glucose):Ham's F-12 containing 10% (v/v) foetal bovine serum (FBS). For hD₃ CHO, clones the growth medium was DMEM (without sodium pyruvate, with glucose) containing 10% FBS, 100 nM methotrexate, 2 mM glutamine, 500 nM (–)-sulpiride, and 1% (v/v) essential amino acids. Cells were removed from confluent plates by scraping and were harvested by centrifugation (200*g*, 5 min, room temperature). Following resuspension in 10 mL of fresh culture medium, an aliquot was counted and the cells passaged at 12 500 or 25 000 cells cm². Cultures between passages 5 and 30 were used for functional studies.

Determination of Extracellular Acidification Rates in Microphysiometer. Cells were seeded into 12 mm transwell inserts (Costar) at 300 000 cells/cup in FBS-containing growth medium. The cells were incubated for 6 h at 37 °C in 95%O₂/ 5%CO₂, before changing to FBS and sulpiride-free medium. After a further 16-18h, cups were loaded into the sensor chambers of the Cytosensor microphysiometer (Molecular Devices, California). The chambers were perfused with running medium (bicarbonate-free Dulbecco's modified Eagles medium containing 2 mM glutamine and 44 mM NaCl) at a flow rate of 100 μ L/min and temperature of 37 °C. Each pump cycle lasted 90 s. The pump was on for the first 60 s and the acidification rate determined between 68 and 88 s. Cells were exposed (4.5 min for hD₂, 7.5 min for hD₃) to increasing concentrations (at half log unit intervals) of quinpirole at half hourly intervals. For antagonist studies, a control concentration-response curve to quinpirole was conducted, and the cells were then exposed to antagonist for at least 42 min prior to construction of a further quinpirole concentration-effect curve in the presence of antagonist. Each chamber therefore acted as its own control. Drug additions were performed using the Cytosensor autosampler (Molecular Devices) from deep well blocks.

Data Analysis and Statistics. Radioligand binding studies were analyzed using an iterative four-parameter logistic model to generate IC_{50} values and from these pK_i values were determined. Concentration–effect curves from microphysiometry experiments were constructed from the peak acidification response and analyzed using a four-parameter logistic equation. Antagonist data were analyzed as the concentration

required to shift the quinpirole concentration–effect curve. Antagonist affinity was expressed as pK_b (–log K_b).

In Vivo Microdialysis. Male Sprague Dawley rats (250-350 g) were anaesthetized with medetomidine HCl (0.4 mg/ kg, sc) and fentanyl (0.45 mg/kg, ip), and a guide cannula (BAS, Congleton, UK) was implanted in either the nucleus accumbens (AP + 2.7 mm from bregma, L + 1.6 mm from midline, V - 5.6 mm from dura), striatum (A/P + 0.0 mm, L + 2.8 mm, V-3.5 mm), or frontal cortex (A/P + 3.2 mm, L+2.0 mm, V- 1.2 mm) according to the atlas of Paxinos & Watson. Anaesthesia was reversed with atipamezole HCl (2.5 mg/kg, sc) and nalbuphine HCl (2 mg/kg, sc). Rats were housed singly, and at least 2 weeks were allowed for postoperative recovery. The rats were allowed food and water ad libitum up to approximately 400 g in weight, when their diet was restricted to 20 g/day. On the day of an experiment, rats were anaesthetized with isoflurane to facilitate insertion of the microdialysis probe (BAS Congleton, UK; 4 mm membrane for striatum, 2 mm membrane for nucleus accumbens and cortex) into the guide cannula and allowed to recover for 1 h. Probes were perfused with artificial cerebrospinal fluid (NaCl, 125 mM; KCl, 2.5 mM; MgCl₂, 1.18 mM; CaCl₂, 1.26 mM; pH 7.4) at a flow rate of 1 μ L/min. Perfusate from the first 2 h was discarded and subsequent samples were collected at 1 h intervals for 6 h. Each sample was collected into 10 μ L acetic acid (0.3% w/v) to prevent degradation of dopamine.

After the first hourly fraction had been collected, either SB-277011 (0.28–2.8 mg/kg, po; nucleus accumbens) or SB-277011 (93 mg/kg, po for striatum) or vehicle (1% methylcellulose, 2 mL/kg) was administered. Two hours later either quinelorane (30 μ g/kg, sc) or vehicle (saline, 1 mL/kg, sc) was administered. Samples were collected for a further 3 h.

Samples were analyzed for dopamine using HPLC-ECD. A Jasco (PU-980) HPLC pump (flow rate 0.3 mL/min) pumped mobile phase (0.07 M KH₂PO₄, 1 mM octane sulfonic acid, 0.1 mM EDTA, 10% methanol, pH 2.5) through a Symmetry C18 analytical column (3.5 μ m, 2.1 × 150 mm plus guard column). Eluates were detected using an ANTAC Decade detector set at a voltage of 0.8 V. The amount of dopamine in the first 1 h collection sample was used as the baseline, and all subsequent values were calculated as a percentage of this for each individual rat. A standard sample was included every six samples to enable quantification and check for reproducibility.

Catalepsy. Catalepsy was assessed by positioning rats with their hindpaws on the bench and their forelimbs rested on a 1 cm diameter horizontal bar, 10 cm above the bench. The length of time in this position was recorded to a maximum of 120 s. Vehicle (1% methylcellulose, 2 mL/kg po) or SB-277011 (2.5, 7.9, 25.2 or 78.8 mg/kg po) or haloperidol (2.8 mg/kg po) was injected (2 mL/kg). Catalepsy was assessed 180 and 210 min (for habituation purposes) and 240 min after drug administration. Rats were judged cataleptic and assigned a score of 1 if they maintained an immobile attitude for 30 s or more at the 240 min time point; otherwise, they were given a score of 0. A logistic regression analysis (SAS-RA, version 6.11; SAS Institute Inc.) was used to analyze the data at the 240 min time point. In a separate experiment, vehicle (1% methylcellulose, 2 mL/kg po) or SB-277011 (2.5, 7.9, 25.2 or 78.8 mg/kg po) was injected in a volume of 2 mL/kg, followed 150 min later by saline or haloperidol (1.13 mg/kg ip) in a volume of 1 mL/kg. Catalepsy was assessed 180, 210, and 240 min after SB-277011 administration.

Plasma Prolactin Levels. Animals were pretreated with either, haloperidol (3 mg/kg po), SB-277011 (93 mg/kg po) or vehicle (1% methylcellulose, 2 mL/kg po). After 2 h the animals were decapitated and the blood collected into glass vials. Samples were kept at 4 °C overnight, and then the serum was separated and stored at -70 °C until subsequent assay. Serum prolactin was assayed by radioimmunoassay (Amersham Life Sciences). Serum prolactin measures were transformed (log) prior to analysis by analysis of variance and Dunnett's *t*-test (Statistica Version 6.0).

Supporting Information Available: Table of analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

- (1) Sokoloff, P.; Giros, B.; Martres, M.-P., Bouthenet, M.-L.; Schwartz, J.-C. Molecular cloning and characterisation of a novel dopamine receptor (D₃) as a target for neuroleptic drugs. *Nature* **1990**, *347*, 146–151.
- (2) Schwartz, J.-C.; Levesque, D.; Martres, M.-P.; Sokoloff, P. Dopamine D_3 receptor: basic and clinical aspects. *Clin. Neuropharmacol.* **1993**, *16*, 295–314.
- (3) Shafer, R. A.; Levant, B. The D₃ dopamine receptor in cellular and organismal function. *Psychopharmacology* **1998**, *135*, 1–16.
 (4) Joyce, J. N.; Meador-Woodruff, J. H. Linking the family of D₂
- (4) Joyce, J. N.; Meador-Woodruff, J. H. Linking the family of D₂ receptors to neuronal circuits in human brain: insights into schizophrenia. *Neuropsychopharmacology* **1997**, *16*, 375–384.
- (5) Pilla, M.; Perachon, S.; Sautel, F.; Garrido, F.; Mann, A.; Wermuth, C. G.; Schwartz, J.-C.; Everitt, B. J.; Sokoloff, P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D₃ receptor agonist. *Nature* **1999**, *400*, 371–375.
- (6) Joyce, J. N.; Gurevich, E. V. Dopamine D₃ receptors: from anatomy to neuropsychiatry. *NeuroSci. News* **1999**, *2*, 11−21.
 (7) Coldwell, M. C.; Boyfield, I.; Brown, A. M.; Stemp, G.; Middle-
- (7) Coldwell, M. C.; Boyfield, I.; Brown, A. M.; Stemp, G.; Middlemiss, D. N. Pharmacological characterisation of extracellular acidification rate responses in human D_{2(long)}, D₃ and D_{4.4} receptors expressed in Chinese hamster ovary cells. Br. J. Pharmacol. **1999**, 127, 1135–1144.
- (8) Waters, N.; Svensson, K.; Haandsma-Svensson, S. R.; Smith, M. W.; Carlsson, A. The dopamine D₃ receptor: a postsynaptic receptor inhibitory on rat locomotor activity. *J. Neur. Trams.* **1993**, *94*, 11–19.
- (9) Sautel, F.; Griffon, N.; Sokoloff, P.; Schwartz, J.-P.; Launey, C.; Simon, P.; Costentin, J.; Schoenfelder, A.; Garrido, F.; Mann, A.; Wermuth, C. G. Nafadotride, a potent preferential dopamine D₃ receptor antagonist, activates locomotion in rodents. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 1239–1246.
- D₃ receptor antagonist, activates locomotion in rodents. J. Pharmacol. Exp. Ther. 1995, 275, 1239–1246.
 (10) Bolton, D.; Boyfield, I.; Coldwell, M. C.; Hadley, M. S.; Johns, A.; Johnson, C. N.; Markwell, R. E.; Nash, D. J.; Riley, G. J.; Scott, E. E.; Smith, S. A.; Stemp, G.; Wadsworth, H. J.; Watts, E. A. 2-[(Substituted)phenyl]-5-[1-(2-phenylaza-cycloheptyl)methyl]-1H-pyrroles with high affinity and selectivity for the dopamine D₃ receptor. *Bioorg. Med. Chem. Lett.* 1997, 7, 485–488.
- Murray, P. J.; Harrison, L. E.; Johnson, M. R.; Robertson, G. M.; Scopes, D. I. C.; Bull, D. R.; Graham, E. A.; Hayes, A. G.; Kilpatrick, G. J.; Den Dass, I.; Large, C.; Sheehan, M. J.; Stubbs, C. M.; Turpin, M. P. A novel series of arylpiperazines with high affinity and selectivity for the dopamine D₃ receptor. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 219–222.
 Belliotti, T. R.; Kesten, S. R.; Rubin, J. R.; Wustrow, D. J.; Georgic, L. M.; Zoski, K. T.; Akunne, H. C.; Wise, L. D. Novel
- Belliotti, T. R.; Kesten, S. R.; Rubin, J. R.; Wustrow, D. J.; Georgic, L. M.; Zoski, K. T.; Akunne, H. C.; Wise, L. D. Novel cyclohexyl amides as potent and selective D₃ dopamine receptor ligands. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2403–2408.
 Yuan, J.; Chen, X.; Brodbeck, R.; Primus, R.; Braun, J.; Wasley,
- (13) Yuan, J.; Chen, X.; Brodbeck, R.; Primus, R.; Braun, J.; Wasley, J. W. F.; Thurkauf, A. NGB 2904 and NGB 2849: two highly selective dopamine D₃ receptor antagonists. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2715–2718.

- (14) Murray, P. J.; Helden, R. M.; Johnson, M. R.; Robertson, G. M.; Scopes, D. I. C.; Stokes, M.; Wadman, S.; Whitehead, J. W. F.; Hayes, A. G.; Kilpatrick, G. J.; Large, C.; Stubbs, C. M.; Turpin, M. P. Novel 6-substituted 2-aminotetralins with potent and selective affinity for the dopamine D₃ receptor. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 403–8.
- (15) Boyfield, I.; Coldwell, M. C.; Hadley, M. S.; Johnson, C. N.; Riley, G. J.; Scott, E. E.; Stacey, R.; Stemp, G.; Thewlis, K. A novel series of 2-aminotetralins with with high affinity and selectivity for the dopamine D₃ receptor. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1995–1998.
- (16) Dubuffet, T.; Newman-Tancredi, A.; Cussac, D.; Audinot, V.; Loutz, A.; Millan, M. J.; Lavielle, G. Novel benzopyrano[3,4-c]pyrrole derivatives as potent and selective dopamine D₃ receptor antagonists. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2059–2064.
- (17) Austin, N. E.; Avenell, K. Y.; Boyfield, I.; Branch, C. L.; Coldwell, M. C.; Hadley, M. S.; Jeffrey, P.; Johns, A.; Johnson, C. N.; Nash, D. J.; Riley, G. J.; Smith, S. A.; Stacey, R. C.; Stemp, G.; Thewlis, K. M.; Vong, A. K. K. Novel 1,2,3,4-tetrahydroisoquinolines with high affinity and selectivity for the dopamine D₃ receptor. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 179–184.
- (18) Cussac, D.; Newman-Tancredi, A.; LeJeune, F.; Gobert, A.; Audinot, V.; Rivet. J. M.; Dubuffet, T.; Lavielle, G.; Millan, M. J. S 33084: A novel, potent and selective benzopyrano-pyrrolidine antagonist and radioligand at dopamine D₃ receptors. *Soc. Neurosci. Abstr.* **1999**, *25*, 1467.
- (19) Xu, M.; Koeltzow, T. E.; Cooper, D. C.; Tonegawa, S.; White, F. J. Dopamine D_3 receptor mutant mice exhibit identical responses to putative D_3 receptor selective agonists and antagonists. *Synapse* **1999**, *31*, 210–215.
- (20) Gaster, L. M.; Blaney, F. E.; Davies, S.; Duckworth, D. M.; Ham, P.; Jenkins, S.; Jennings, A. J.; Joiner, G. F.; King, F. D.; Mulholland, K. R.; Wyman, P. A.; Hagan, J. J.; Hatcher, J.; Jones, B. J.; Middlemiss, D. N.; Price, G. W.; Riley, G. J.; Roberts, C.; Routledge, C.; Selkirk, J.; Slade, P. D. The selective 5-HT_{1B} receptor inverse agonist 1'-methyl-5-[[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3, 4'-piperidine] (SB-224289) potently blocks terminal 5-HT autoreceptor function both in vitro and in vivo. *J. Med. Chem.* **1998**, *41*, 1218–1235.
- (21) Johnston, T. P.; McCalet, G. S.; Clayton, S. D.; Frye, J. L.; Krauth, C. A.; Montgomery, J. A. Synthesis of analogues of *N*-(2chloroethyl)-*N*¹-(*trans*-4-methylcyclohexyl)-*N*-nitrosourea for evaluation as anticancer agents. *J. Med. Chem.* **1977**, *20*, 279–290.
- (22) Routledge, C.; Thorn, L.; Ashmeade, T.; Taylor, S. Elucidation of D₃ receptor function in vivo: do D₃ receptors mediate inhibition of dopamine neuronal activity? *Biochem. Soc. Trans.* **1996**, *24*, 199–201.
- (23) Bouthenet, M. L.; Souil, E.; Martres, M. P.; Sokoloff, P.; Giros, B.; Schwartz, J.-C. Localization of dopamine D_3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D_2 receptor mRNA. *Brain Res.* **1991**, *564*, 203–19.

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