# Design and Synthesis of *trans*-*N*-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (SB-277011): A Potent and Selective Dopamine $D_3$ Receptor Antagonist with High Oral Bioavailability and CNS Penetration in the Rat

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*Received February 29, 2000*

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

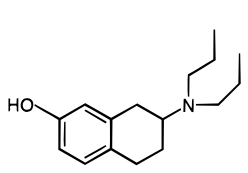
Dopaminergic neurotransmission is mediated by five receptor subtypes ($D_1$−$D_5$), which can be grouped into two receptor families. $D_1$-like receptors include the $D_1$ and $D_5$ subtypes, whereas $D_2$-like receptors include the $D_2$, $D_3$, and $D_4$ subtypes.

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 $D_2$ receptors, and their antipyschotic effects could therefore be mediated via $D_2$ and/or $D_3$ receptors. Blockade of dopamine $D_2$ 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 $D_3$ 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]

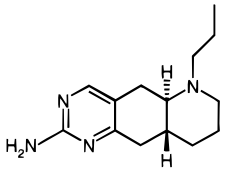
Pharmacological approaches to understanding the role of the dopamine $D_3$ receptor have been hindered by a lack of compounds (either agonists or antagonists) with the required selectivity and pharmacokinetic profiles. The aim of our research was therefore to identify orally bioavailable, CNS penetrant antagonists with high affinity ($pK_i \geq 8$) for the dopamine $D_3$ receptor and high selectivity (≥100-fold) over other receptors and ion channels in order to test the above hypothesis and to further characterize the role of the dopamine $D_3$ 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

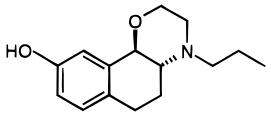
**Chart 1.** Dopamine $D_3$ Receptor Agonists




7-OH-DPAT **1**




quinelorane **2**

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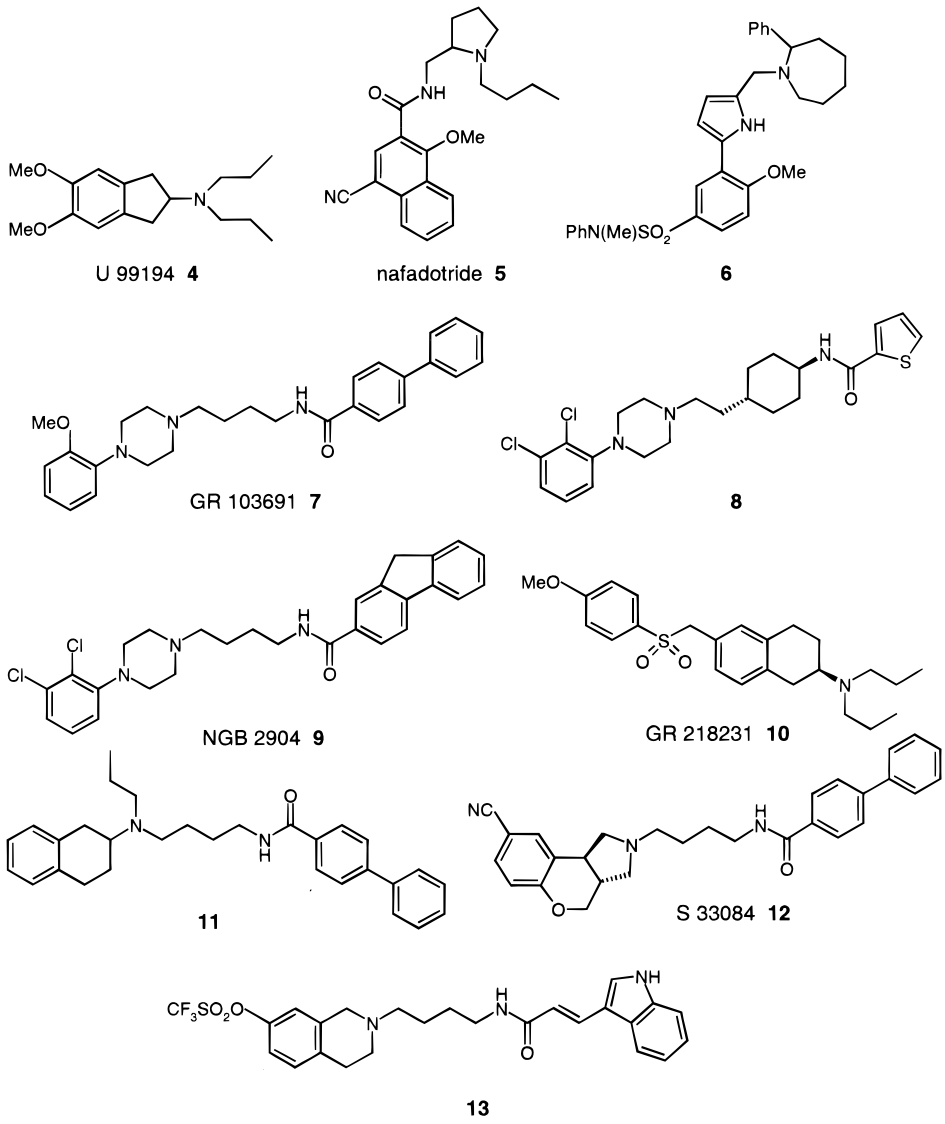
10.1021/jm000090i CCC: $19.00 
Published on Web 04/18/2000

**Chart 2.** Dopamine $D_3$ Receptor Antagonists



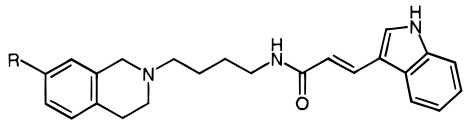

appropriate functional selectivity[7] to allow clear discrimination between $D_3$ and $D_2$ receptor function in vivo.

Antagonists with varying degrees of selectivity for the $D_3$ receptor compared to the $D_2$ 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 $D_3$ 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 $D_3$ receptor[19] 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, p$K_i$ 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-$HT_{1B}$ (p$K_i$ 7.0) and 5-$HT_{1D}$ (p$K_i$ 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 tetrahydroisoquinoline 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 $D_3$ affinity was reduced compared to **13** and selectivity over 5-$HT_{1B}$ and 5-$HT_{1D}$ receptors remained modest.

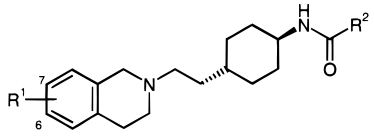
**Table 1.** Receptor Binding Profiles of 7-Substituted 1,2,3,4-Tetrahydroisoquinolines

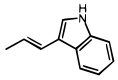
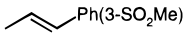
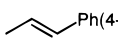


| compd | R | mp °C (HCl salt) | formula | anal.[a] | $D_3$[b] | $D_2$[b] | 5-$HT_{1B}$[b] | 5-$HT_{1D}$[b] |
|---|---|---|---|---|---|---|---|---|
| **13**[17] | $CF_3SO_2O$ | 177-180 | $C_{25}H_{26}F_3N_3O_4S.HCl$ | | 8.4 | 6.2 | 7.0 | 7.2 |
| **14** | NC | 243-245 | $C_{25}H_{26}N_4O.HCl.H_2O$ | C,H,N | 7.9 | 6.1 | 6.8 | 6.6 |

[a] Analyses for the elements indicated were within ±0.4% of the theoretical values. [b] p$K_i$ 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.[a] | $D_3$[b] | $D_2$[b] | 5-$HT_{1B}$[b] | 5-$HT_{1D}$[b] |
|---|---|---|---|---|---|---|---|---|---|
| **15** | 7-CN |  | 288-291 | $C_{27}H_{31}N_3O.HCl$ | $M^+$ | 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 | NT[c] | NT[c] |
| **17** | 7-CN |  | 278-280 | $C_{27}H_{30}N_4O.HCl$ | C,H,N | 8.7 | 7.0 | 6.2 | 7.0 |
| **18** | 6-CN |  | 253-255 | $C_{27}H_{30}N_4O.HCl$ | C,H,N | 7.8 | 6.1 | NT[c] | NT[c] |
| **19** | 6-CN |  | 282-285 | $C_{27}H_{31}N_3O.HCl$ | C,H,N | 8.5 | 6.7 | 6.0 | 6.5 |
| **20** | 6-CN |  | 268-269 | $C_{28}H_{33}N_3O_3S.HCl$ | C,H,N | 8.3 | 6.5 | 5.2 | 5.4 |
| **21** | 6-CN |  | 263-264 | $C_{27}H_{30}FN_3O.HCl$ | H,N;C[d] | 8.5 | 6.7 | <5 | 6.2 |
| **22** | 6-CN | 2-naphthyl | 255-258 | $C_{29}H_{31}N_3O.HCl$ | C,H,N | 8.0 | 5.8 | <5 | 7.0 |
| **23** | 6-CN | 1-naphthyl | 191-194 | $C_{29}H_{31}N_3O.HCl$ | 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 |

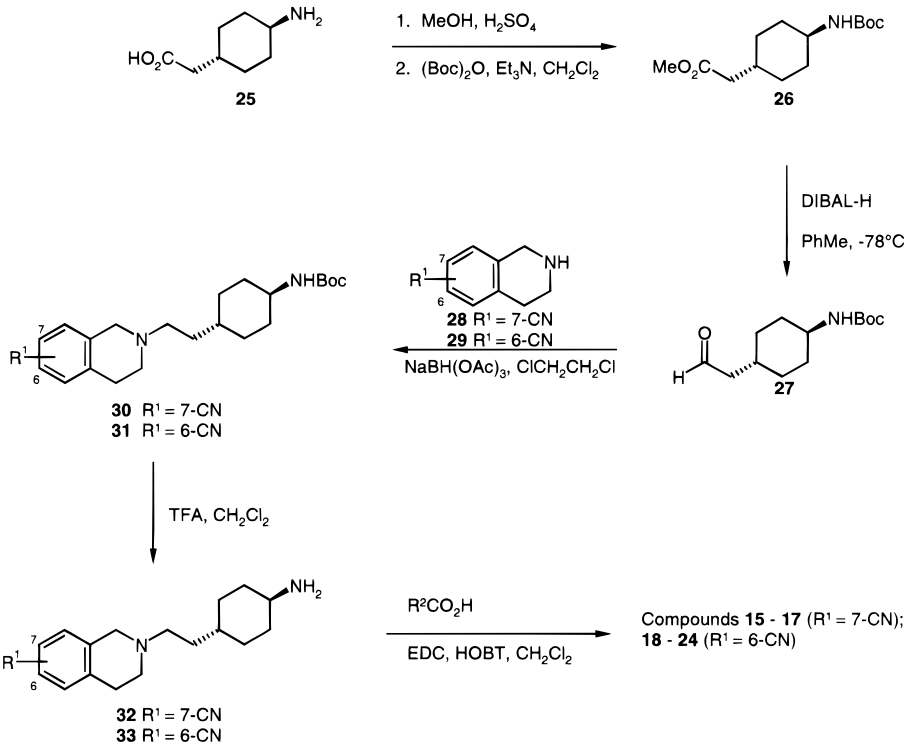
[a,b] See footnotes *a*, *b* in Table 1. [c] NT = not tested. [d] Calcd: C, 69.23; found: C, 68.81. [e] p$K_i$ values represent the mean of 15 determinations.

Inspection of molecular models of compounds **13** and **14** and comparison of these with known selective 5-$HT_{1B}$ and 5-$HT_{1D}$ ligands[20] 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 $D_3$ receptor affinity (p$K_i$ 8.9) and 110-fold selectivity over the $D_2$ 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 $D_3$ 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 1,2,3,4-tetrahydroisoquinolines **28** ($R^1$ = 7-CN) and **29** ($R^1$ = 6-CN) to give the protected amines **30** ($R^1$ = 7-CN) and **31** ($R^1$ = 6-CN), respectively. Deprotection with TFA to the corresponding amines **32** ($R^1$ = 7-CN) and **33** ($R^1$ = 6-CN) followed by coupling to the appropriate acids gave **15**−**17** ($R^1$ = 7-CN) and **18**−**24** ($R^1$ = 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 $D_3$ receptor affinity and selectivity over the $D_2$ 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 $D_3$ receptor. Interestingly, **17** had improved selectivity over the 5-$HT_{1B}$ receptor compared to **15**, but selectivity over the $D_2$ 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 $IC_{50}$ of 0.8 $\mu$M. However, the corresponding 6-cyanotetrahydroisoquinoline derivative **18** had greatly reduced liability to inhibit 2D6 (17% inhibition at 10 $\mu$M), but $D_3$ 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 ≥100-fold selectivity against the $D_2$, 5-$HT_{1B}$, and 5-$HT_{1D}$ receptors. Subsequent cross-screening showed **24** to be ≥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 $D_2$ receptors[7] demonstrated that **24** was devoid of agonist-like activity and was a potent and selective antagonist ($D_3$, p$K_b$ 8.4; $D_2$, p$K_b$ 6.5).

Compound **24** (SB-277011) was shown to have an excellent pharmacokinetic profile in the rat (oral bioavailability 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 $D_3$ 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 $D_3$ 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 $D_3$ 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 $D_3$ 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 $D_3$ receptor, will be reported.

## Experimental Section

**Chemistry.** Melting points were determined on a Buchi melting point apparatus and are uncorrected. $^1$H 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 $\mu$m (30 × 2.1 mm) column was used with elution using the following conditions: eluant A, 0.1% TFA/$H_2O$ v/v, eluant B, 0.1% TFA/$CH_3CN$ 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 sulfate[21] (27.0 g, 106 mmol), concentrated $H_2SO_4$ (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 $NaHCO_3$ (500 mL) and dichloromethane (3 × 200 mL), and the combined extracts were dried ($Na_2SO_4$) and evaporated in vacuo to give **26** (24.6 g, 86%) as a colorless solid. $^1$H NMR ($CDCl_3$) $\delta$: 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 × 1 L). The combined organic extracts were dried ($Na_2SO_4$) 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** (21.77 g, 53%) as a colorless solid. $^1$H NMR ($CDCl_3$) $\delta$: 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-1,2,3,4-tetrahydroisoquinoline (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 °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 ($Na_2SO_4$), 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. $^1$H NMR ($CDCl_3$) $\delta$: 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**. $^1$H NMR ($CDCl_3$) $\delta$: 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-1,2,3,4-tetrahydroisoquinoline (33).** A mixture of *trans*-2-(2-(1-(4-(*N*-*tert*-butyloxycarbonyl)amino)cyclohexyl)ethyl)-6-cyano-1,2,3,4-tetrahydroisoquinoline (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 $K_2CO_3$ (200 mL) and dichloromethane (2 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried ($Na_2SO_4$), and evaporated in vacuo to give **33** (4.99 g, 81%) as a brown oil. $^1$H NMR ($CDCl_3$) $\delta$: 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 ($MH^+$). $C_{18}H_{25}N_3$ requires 283.

***trans*-2-(2-(1-(4-Amino)cyclohexyl)ethyl)-7-cyano-1,2,3,4-tetrahydroisoquinoline (32)** was prepared in a manner similar to compound **33**. $^1$H NMR ($CDCl_3$) $\delta$: 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 ($MH^+$). $C_{18}H_{25}N_3$ requires 283.

***trans*-*N*-[4-[2-(7-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-1*H*-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-2-carboxylic 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%). $^1$H NMR ($CDCl_3$) $\delta$: 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 ($MH^+$). $C_{27}H_{30}N_4O$ 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).** $^1$H NMR (DMSO-$d_6$) $\delta$: 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 ($MH^+$). $C_{27}H_{31}N_3O$ 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).** $^1$H NMR (DMSO-$d_6$) $\delta$: 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 ($MH^+$). $C_{29}H_{32}N_4O$ 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. $^1$H NMR (DMSO-$d_6$) $\delta$: 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 ($MH^+$). $C_{28}H_{33}N_3O_3S$ 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).** $^1$H NMR (DMSO-$d_6$) $\delta$: 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, $J$ = 8 Hz), 7.23 (1H, d, $J$ = 8 Hz), 7.38 (3H, m), 8.01 (1H, d, $J$ = 8 Hz), 11.34 (1H, s). Mass spectrum (API$^+$): Found 427 ($MH^+$). $C_{27}H_{30}N_4O$ requires 426.

***trans*-(*E*)-*N*-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-3-phenyl-2-propenamide (19).** $^1$H NMR (DMSO-$d_6$) $\delta$: 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).** $^1$H NMR ($CDCl_3$ + $CD_3OD$) $\delta$: 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 ($MH^+$). $C_{27}H_{30}FN_3O$ 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).** $^1$H NMR (DMSO-$d_6$) $\delta$: 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 ($MH^+$). $C_{29}H_{31}N_3O$ requires 437.

***trans*-*N*-[4-[2-(6-Cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-1-naphthalenecarboxamide (23).** $^1$H NMR (DMSO-$d_6$) $\delta$: 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, $J$ = 8 Hz), 7.54 (6H, m), 7.99 (2H, m), 8.17 (1H, m), 8.42 (1H, d, $J$ = 8 Hz). Mass spectrum (API$^+$): Found 438 ($MH^+$). $C_{29}H_{31}N_3O$ 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-4-carboxylic 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 × 250 mL). Combined organic extracts were dried ($Na_2SO_4$) 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. $^1$H NMR ($CDCl_3$) $\delta$: 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 ($MH^+$). $C_{28}H_{30}N_4O$ 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. $^1$H NMR (DMSO-$d_6$) $\delta$: 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 $D_2$ and $D_3$ Receptors.** Cloned human $D_2$ (long) receptors expressed in CHO cells were obtained from the Garvan Institute of Medical Research, Sydney, Australia. Human $D_3$ 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 $hD_2$ and $hD_3$ receptors were carried out using membranes from CHO cells. Membranes (5−15 $\mu$g of protein) were incubated with [$^{125}$I]iodosulpride (0.1 nM) in buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (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 $hD_2$ 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 $hD_3$ 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 cm$^2$. 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%$O_2$/5%$CO_2$, 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 $\mu$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 $hD_2$, 7.5 min for $hD_3$) 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 p$K_i$ 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 p$K_b$ (−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; $MgCl_2$, 1.18 mM; $CaCl_2$, 1.26 mM; pH 7.4) at a flow rate of 1 $\mu$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 $\mu$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 $\mu$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 $KH_2PO_4$, 1 mM octane sulfonic acid, 0.1 mM EDTA, 10% methanol, pH 2.5) through a Symmetry C18 analytical column (3.5 $\mu$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.

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JM000090I