



Published in final edited form as:

ChemMedChem. 2014 December ; 9(12): 2653–2660. doi:10.1002/cmdc.201402398.

Pramipexole Derivatives as Potent and Selective Dopamine D₃ Receptor Agonists with Improved Human Microsomal Stability

Dr. Jianyong Chen^[a], Dr. Cheng Jiang^[a], Prof. Beth Levant^[b], Dr. Xiaoqin Li^[c], Dr. Ting Zhao^[c], Dr. Bo Wen^[c], Dr. Ruijuan Luo^[c], Prof. Duxin Sun^[c], and Prof. Shaomeng Wang^[a]

Jianyong Chen: jiachen@umich.edu; Shaomeng Wang: Shaomeng@umich.edu

^[a]Departments of Internal Medicine, Pharmacology, and Medicinal Chemistry, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109

^[b]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, Kansas 66160-7417

^[c]Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109

Abstract

We report herein the synthesis and evaluation of a series of new pramipexole derivatives as highly potent and selective dopamine-3 (D₃) receptor agonists. A number of these new compounds bind to the D₃ receptor with subnanomolar affinities and show excellent selectivity (>10,000) for the D₃ receptor over the D₁ and D₂ receptors. Compound **23** for example, binds to the D₃ receptor with a K_i value of 0.53 nM and shows a selectivity of >20,000 over the D₂ receptor and the D₁ receptor in the binding assays using a rat brain preparation. It has excellent stability in human liver microsomes and *in vitro* functional assays showed it to be a full agonist for the human D₃ receptor.

Keywords

pramipexole derivatives; dopamine 3; receptor; agonists; microsomal stability

Introduction

The dopamine 3 (D₃) receptor subtype has been identified as a major target for several agents currently used clinically for the treatment of schizophrenia, Parkinson's disease, depression, and other neurological diseases.^[1–3] Because all of the clinically approved drugs target not only the D₃ receptor, but also have high affinities for the D₂ receptor and have other off-target effects^[4, 5] there is a need to design potent and selective D₃ ligands. Such new compounds can be used to further investigate the role of the D₃ receptor in different biological and pharmacological processes and to validate unambiguously the D₃ receptor as an important therapeutic target in preclinical and clinical studies.

Due to the high degree of sequence homology between the D₂ and D₃ receptors and nearly identical amino acid sequences that form their binding sites, design of potent and highly selective D₃ ligands has been a challenge for many years.^[4, 5] However, recent studies, including those from our laboratory, have shown that it is possible to design highly selective and potent D₃ ligands.^[6–8] For example, based upon pramipexole (**1**), a potent D₃ agonist with only a modest selectivity over the D₂ receptor, we have developed CJ-1368 (**2**) and CJ-1639 (**3**) as potent and selective D₃ agonists (Figure 1).^[8] More recently, we reported the design of highly selective D₃ antagonists based upon the structure of tranylcypromine.^[9]

Both compounds **2** and **3** display a high affinity for the D₃ receptor and excellent selectivity over the D₂ and D₁ receptors, and thus are promising lead compounds. Toward identification of highly selective D₃ agonists that can be used for investigation *in vivo*, we have performed further evaluations of compounds **2** and **3**. It was found that these compounds have moderate or poor human liver microsomal stability, a shortcoming for their use *in vivo*. In the present study, we have performed modifications of these compounds with the objective of improving their metabolic stability, as well as further enhancing their selectivity for the D₃ over the D₂ receptor, while maintaining high affinity to the D₃ receptor.

Results and Discussion

Microsomal stability testing showed that compound **2** has a $t_{1/2}$ of 26 minutes and compound **3** has a $t_{1/2}$ of 9.5 minutes in human liver microsomes (Table 1). In order to improve the metabolic stability of the compounds, we analyzed the metabolites of compound **2** in human liver microsomes. Our results suggest that hydroxylation of the electron-rich naphthalene group is the major metabolic biotransformation in human liver microsomes (Figure 2 and Supporting Information- SI). Accordingly, in this study we focused our modifications on the naphthalene group.

We hypothesized that replacement of the naphthyl group in compound **2** with less electron-rich groups may improve the metabolic stability and accordingly, a series of new compounds (**11–23**) with groups less electron-rich than the naphthyl group, such as unsubstituted or substituted phenyl rings, were synthesized and evaluated.

These compounds were first evaluated for their binding affinities to the rat D₁-like, D₂-like and D₃ receptors using a rat brain preparation (Table 2). Our binding data showed that all compounds with a phenyl ring, substituted or unsubstituted, have high affinities for the D₃ receptor, with K_i values ranging from 0.43–1.9 nM. Compound **11**, which has a phenyl group in place of the naphthyl group, binds to the D₃ receptor with a K_i value of 0.74 nM and is 301 times more selective for the D₃ receptor over the D₂ receptor. Chlorine substitution at the *o*-, *m*- and *p*-positions on the phenyl group results in compounds **12**, **13** and **14**, respectively. These have similar affinities to the D₃ receptor and also similar selectivities over the D₂ receptor, as compound **11**. Fluorine substitution at the three different positions on the phenyl ring yields compounds **15–17**. While compounds **16** and **17** with a *m*-fluoro or a *p*-fluoro substituent have similar affinities for the D₃ receptor and also similar selectivities over the D₂ receptor, as compound **11**, compound **15** with the *o*-fluoro

substituent has a high affinity for the D₃ receptor and a selectivity of >30,000-fold over the D₂ receptor. The *m*-chloro *p*-fluoro compound (**18**) also has a high affinity for the D₃ receptor ($K_i = 0.43$ nM) and an outstanding selectivity (>15,000-fold) over the D₂ receptor. Methoxyl substitution at three different positions on the phenyl ring generates compounds **19-21** which all have similar binding affinities to the D₃ receptor (0.70–1.0 nM). The *m*-methoxy compound (**21**) has a selectivity of >12,000-fold over the D₂ receptor, highest among these three compounds. Hence, it appears that while substitution on the phenyl ring has modest effect on the affinity for the D₃ receptor, it can have a major effect on the selectivity for the D₃ receptor over the D₂ receptor.

In addition to these simple substituents, we also synthesized compounds **22** and **23** containing a 5-methyl-1,2,4-oxadiazol-3-yl substituent at the *m*- or *p*-position respectively, of the phenyl ring. Compounds **22** and **23** bind to the D₃ receptor with K_i values of 0.96 nM and 0.53 nM, respectively, and both show very high selectivities (>15,000) over the D₂ receptor.

In addition to their high selectivity for the D₃ receptor over the D₂ receptor, compounds **15**, **18**, **21**, **22** and **23** are also highly selective for the D₃ receptor over the D₁ receptor.

We next synthesized a series of compounds (**24-35**) in which the amide group in compounds **11-23** was replaced by a sulfonamide group (Table 3). These compounds retain high affinities to the D₃ receptor, but they are much less selective than the most selective compounds with the amide group. Compound **34** is the most selective compound among compounds **24-35** containing a sulfonamide group but has a selectivity of only 1052-fold for the D₃ receptor over the D₂ receptor and consequently, this series of compounds was not pursued.

We next evaluated the human liver microsomal stability for compounds **20**, **21** and **23**, three potent and highly selective D₃ ligands, in direct comparison with compounds **2** and **3**. The results are provided in Table 1. Our data showed that compounds **20**, **21** and **23** all have $t_{1/2}$ >60 minutes, indicating that the microsomal stability of these compounds is indeed much improved over that of compounds **2** or **3**.

We next evaluated compounds **15**, **18**, **21**, **23**, **33** and **34** for their functional activity at the human D₃ receptor in a quinpirole-induced mitogenesis assay in hD₃-transfected CHOP cells. The results, presented in Table 4 show that all these compounds behave as highly potent full D₃ agonists.

Synthesis of designed compounds

The synthetic route to compounds **11-23** is shown in Scheme 1. The commercially available cyclobutanone (**5**) was reacted with allylmagnesium bromide at -78 °C to give *cis*-allylcyclobutanol (**6**). The stereochemistry of **6** was confirmed by transformation to a known compound **7**, whose stereochemistry was determined by x-ray crystallographic analysis.^[8] The aldehyde (**8**) was obtained by oxidation of **6** with NaIO₄ and a catalytic amount of OsO₄. Pramipexole (**1**) was allowed to react with **8** to give the key intermediate **9** which, treated with trifluoroacetic acid afforded the amine **10**. This amine (**10**) was reacted with

commercially available acid chlorides in the presence of *N,N*-diisopropylethylamine (DIPEA) and the resulting crude amides **11-23** were purified by preparative HPLC.

The synthesis of the sulfonamide-containing compounds **24-35** is shown in Scheme 2. Briefly, these compounds were synthesized by reaction of the intermediate amine **10** at room temperature with the appropriate sulfonyl chlorides in *N,N*-diisopropyl-ethylamine and dichloromethane with 40–56% yield.

Summary

Modifications of the naphthyl group in our previously reported D₃ ligand (**2**) have yielded a series of new compounds with high binding affinities to the D₃ receptor and high selectivity over the D₁ and D₂ receptors and significantly, with improved microsomal stability. Compound **23**, for example, binds to the D₃ receptor with a *K_i* value of 0.53 nM, shows a selectivity of >25,000 over the D₁ and D₂ receptors and superior microsomal stability to that of compound **2** in human liver microsomes. Compound **23** is a full agonist to the human D₃ receptor in the quinpirole-induced mitogenesis assay and represents a potent and highly selective D₃ agonist.

Experimental Section

Biology

***In vitro* dopamine receptor binding assays**—The binding affinities of all the synthetic compounds were determined at the D₁-like, D₂-like and D₃ receptors in membranes prepared from the brains of adult, male Sprague-Dawley rats (Pel-Freez, Rogers, AR). For these assays, all compounds were dissolved in 100% EtOH at a concentration of 5 mM.

[³H]R-(+)-7-OH-DPAT binding assays—[³H]R-(+)-7-OH-DPAT binding assays for the D₃ dopamine receptors were performed as described previously under conditions that yield selective labeling of the D₃ receptor.^[10] Rat ventral striatal (nucleus accumbens and olfactory tubercles) membrane was prepared in an assay buffer (50 mM Tris, 1 mM EDTA; pH 7.4 at 23 °C) to yield a final concentration of 10 mg original wet weight (o.w.w./ml). Membranes were incubated with [³H]R-(+)-7-OH-DPAT (0.15 nM, SA = 163 Ci/mmol, GE Healthcare or SA = 143 Ci/mmol, Perkin-Elmer) and different concentrations of the test compounds (10⁻¹⁰ to 10⁻⁴ M). Nonspecific binding was defined by 1 μM spiperone. Assay tubes were incubated at 23 °C for 90 min. The reaction was terminated by rapid vacuum filtration. Data were analyzed using SigmaPlot 8.0.2. using *K_D* = 0.15 nM for [³H]7-OH-DPAT.^[10] *K_i* values are expressed as the mean ± SEM of 3–6 independent determinations.

[³H]Spiperone binding assays—[³H]Spiperone binding assays for D₂-like receptors were performed as described previously^[11] for [³H] 7-OH-DPAT with the following exception. Assays were performed using membranes prepared from rat caudate-putamen, which expresses D₂ receptors in high density but with very low levels of D₃ receptors, and the final membrane homogenate concentration was 1.5 mg o.w.w./mL. The assay buffer, 50 mM Tris-HCl, 5 mM KCl, 2 mM MgCl₂, and 2 mM CaCl₂, pH 7.4 at 23 °C, was used to

optimize conditions for agonist binding.^[12] The concentration of [³H]spiperone (24 Ci/mmol; SA = 105/mmol, GE Healthcare; SA = 60, American Radiolabeled Chemicals; or SA = 83.4; Perkin-Elmer) was 0.2 nM and the incubation time was 90 min at 23 °C. Nonspecific binding was determined in the presence of 1 μM (+)-butaclamol. K_i values were calculated using the experimentally determined K_D value for [³H]spiperone of 0.4 nM. For those compounds that produced a competition curve consistent with inhibition at 2 sites, which is consistent with the behavior of agonists in this assay, the K_i value for the high affinity component is reported.

[³H]SCH 23390 binding assays—[³H]SCH 23390 (7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol) binding assays for D₁-like dopamine receptors were performed as described previously^[13] for [³H]spiperone binding except the concentration of [³H]SCH 23390 (SA = 73 Ci/mmol, GE Healthcare or SA = 60 Ci/mmol, American Radiolabeled Chemicals) used was 0.3 nM. K_i values were calculated using the K_D value for [³H]SCH 23390 of 0.3 nM.

DA D₃ Mitogenesis Functional Assay—CHOp-D₃ cells were maintained in alpha-MEM with 10% fetal bovine serum (FBS, Atlas Biologicals), 0.05% pen-strep, and 400 μg/ml of G418. To measure D₃ stimulation of mitogenesis (agonist assay) or inhibition of quinpirole stimulation of mitogenesis (antagonist assay), CHOp-D₃ cells were seeded in a 96-well plate at a concentration of 5,000 cells/well. The cells were incubated at 37 °C in alpha-MEM with 10% FBS. After 48–72 h, the cells were rinsed twice with serum-free alpha-MEM then incubated for 24 h at 37 °C. Serial dilutions of test compounds were made by the Biomek robotics system in serum-free alpha-MEM. In the functional assay for agonists, the medium was removed and replaced with 100 μl of a solution of the test compound in serum-free alpha-MEM. In the antagonist assay, the serial dilution of the putative antagonist test compound was added in 90 μl of the solution (1.1X of final concentration) and 300 nM quinpirole (30 nM final) was added in 10 μl of the solution. After a further 16-h incubation at 37 °C, 0.25 μCi of [³H]thymidine in alpha-MEM supplemented with 10% FBS was added to each well and the plates were further incubated for 2 h at 37 °C. The cells were trypsinized by addition of 10X trypsin solution (1% trypsin in calcium-magnesium-free phosphate-buffered saline) and the plates were filtered and counted. Quinpirole is assayed daily as an internal control and dopamine is included for comparative purposes.

Data analysis—For functional assays, GraphPAD Prism was used to calculate either EC₅₀ (agonists) or IC₅₀ (antagonists) values using data expressed as pg cAMP for adenylate cyclase activity and % quinpirole-stimulation for mitogenesis.

Human Liver Microsomal Stability Assay—1 μM of each test compound was metabolized at 37°C by incubating the test compound with 0.5 mg/mL human liver microsomes and 1 mM cofactor NADPH in a total volume of 400 μL of 100 mM potassium phosphate buffer (pH 7.4 containing 3.3 mM MgCl₂). The reactions were stopped at 0, 5, 10, 15, 30, and 60 min, by adding 3 fold the volume of CH₃CN containing 100 nM of internal standard. The collected fractions were centrifuged to collect the supernatant for LC-MS/MS

analyses, from which the amount of parent compound remaining was determined. The natural log of the amount of parent compound remaining was plotted against time to calculate the rate of disappearance of the tested compounds and the half-life of the tested compounds. The intrinsic clearance of each compound was calculated using the equation:

$$CL_{\text{int}} = \frac{\text{volume of incubation}}{\text{protein in the incubation}} \times \frac{0.693}{t_{1/2}}$$

Hydrolysis of **2**, **3**, **20**, **21**, and **23** in human liver microsomes and a PBS-only solution was monitored under similar conditions. 1 μM of each compound was incubated with 0.5 mg/mL human liver microsomes in PBS solution or with PBS only for 60 min. No change in the concentration of three compounds at 60 min compared to that at time zero suggests little hydrolysis had occurred for the three compounds in PBS solution with or without human liver microsomes.

Chemistry

General Methods—Solvents and reagents were purchased and used without further purification. Reactions were monitored by TLC carried out on 250 μm E. Merck silica gel plates (60F-254) using UV light as visualizing agent. E. Merck silica gel (60, particle size 15–40 μm) was used for flash column chromatography. NMR spectra were recorded on a Bruker Avance300 spectrometer (300 MHz). Chemical shifts (δ) are reported as δ values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the standard form. All final compounds have purities > 95%, as determined by HPLC (UV detection at 254 nm).

***t*-Butyl (3-allyl-3-hydroxycyclobutyl)carbamate (6):** 1M Allylmagnesium bromide solution in ether (64.8 mL, 64.8 mmol) was added dropwise to a solution of *t*-butyl (3-oxocyclobutyl)-carbamate **29** (6.0 g, 32.4 mmol) in anhydrous THF at $-78\text{ }^{\circ}\text{C}$ and the reaction mixture was stirred at $-78\text{ }^{\circ}\text{C}$ for 2 h. Then the mixture was allowed to warm up slowly to room temperature. The reaction was quenched by slow addition of aqueous NH_4Cl solution. The mixture was extracted with EtOAc (3 \times 40 mL) and organic layers were combined. The organic solvents were removed under vacuum and the residue was purified by column chromatography (SiO_2 , hexane/EtOAc 2:1) to give **6** (5.0 g, 68%) as a colorless oil. ^1H NMR (CDCl_3 , 300 MHz) δ 5.90-5.70 (m, 1H), 5.25-5.10 (m, 2H), 4.70 (broad, 1H), 3.80-3.70 (m, 1H), 2.35 (d, $J=3.2$ Hz, 2H), 2.20 (s, 1H), 2.00-1.80 (m, 2H), 1.44 (s, 9H).

***N*-(*cis*-3-Allyl-3-hydroxycyclobutyl)-2-naphthamide (7):** TFA (1 mL) was added to a solution of **30** (500 mg, 2.20 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred at room temperature for 12 h. Solvent and TFA were removed under vacuum and the residue was dissolved in DCM (5 mL). Diisopropylethylamine (568 mg, 4.40 mmol) and 2-naphthoyl chloride (502 mg, 2.64 mmol) were added and the mixture was stirred at room temperature for 2 h. The reaction was quenched with H_2O and the pH was adjusted to 9–10 by addition of aqueous Na_2CO_3 . The mixture was extracted with CH_2Cl_2 (30 mL \times 3). The organic layer was separated, combined, dried, and evaporated. The residue was purified by chromatography (SiO_2 , hexane/EtOAc 1:1) to give **7** as a colorless solid (445 mg, 72% over

two steps). $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ 8.27 (s, 1H), 7.95-7.75 (m, 4H), 7.65-7.50 (m, 2H), 6.51 (d, $J = 6.5$ Hz, 1H), 6.00-5.80 (m, 1H), 5.30-5.20 (m, 2H), 4.45-4.27 (m, 1H), 2.80-2.70 (m, 2H), 2.43 (d, $J = 7.1$ Hz, 2H), 2.36 (s, 1H), 2.25-2.15 (m, 2H).

***t*-Butyl (3-hydroxy-3-(2-oxoethyl)cyclobutyl)carbamate (8)**: OsO_4 (223 mg, 0.881 mmol) was added to a solution of **30** (2.0 g, 8.81 mmol) in THF- H_2O (80 mL, 1:1 ratio) at room temperature and the mixture was stirred at room temperature for 30 min. Then, NaIO_4 (4.71 g, 22 mmol) was added and the mixture was stirred for 30 min. The mixture was extracted with EtOAc (40 mLx3) and organic layers were combined. Evaporation of organic solvents under vacuum gave crude product **8** (1.2 g, crude yield 60%) as a slightly yellow oil. This crude product was used directly for the next step without further purification. Purification of this crude product by silica gel column chromatography led to its decomposition.

***t*-Butyl (3-(2-(((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)ethyl)-3-hydroxycyclobutyl)carbamate (9)**: Compound **8** (8.93 g, 39.0 mmol), acetic acid (3.51 g, 58.5 mmol) and sodium triacetoxyborohydride (12.4 g, 58.5 mmol) were added to a solution of pramipexole (8.3 g, 39.0 mmol) in CH_2Cl_2 (60 mL) and the reaction mixture was stirred at room temperature for 6 h. The reaction was quenched with H_2O and the pH was adjusted to 9–10 by addition of aqueous Na_2CO_3 solution. The mixture was then extracted 3 times with CH_2Cl_2 . The organic layers were separated, combined, and evaporated. The residue was chromatographed (SiO_2 , ethyl acetate/methanol 95:5) to give compound **9** (4.5 g, 27% yield). $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ 4.90 (s, 2H), 4.77 (d, $J = 7.8$ Hz, 1H), 3.80-3.60 (m, 1H), 3.25-3.10 (m, 1H), 2.80-2.35 (m, 10H), 2.00-1.45 (m, 8H), 1.43 (s, 9H), 0.89 (t, $J = 7.2$ Hz, 3H).

3-Amino-1-(2-(((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)ethyl)-cyclobutanol (10): TFA (5 mL) was added to a solution of **9** (4.5 g, 10.6 mmol) in CH_2Cl_2 (30 mL) and the mixture was stirred at room temperature for 12 h. Solvent and TFA were removed under vacuum. Water was added to the residue and pH was adjusted to 9–10 by addition of aqueous Na_2CO_3 solution. The mixture was extracted with CH_2Cl_2 (30 mLx3). The organic layers were separated, combined, dried, and evaporated. The residue was chromatographed (SiO_2 , $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 80:20) to give compound **10** as a colorless oil (3.1 g, 90% yield). $^1\text{H NMR}$ (CD_3OD , 300 MHz) δ 4.02-3.83 (m, 1H), 3.60-2.60 (m, 11H), 2.40-1.75 (m, 8H), 1.06 (t, $J = 7.2$ Hz, 3H).

General procedure for the synthesis of compounds 11-23: Diisopropylethylamine (52 mg, 0.4 mmol) and the appropriate acid chloride (0.24 mmol) were added to a suspension of **10** (65 mg, 0.2 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at room temperature for 2 h. The reaction was quenched with H_2O and pH was adjusted to 9–10 by addition of aqueous Na_2CO_3 . The mixture was extracted three times with CH_2Cl_2 (30 mLx3). The organic layer was separated, combined, dried, and evaporated. The residue was purified by preparative HPLC to give the product. This procedure was used to prepare compounds **11-23**, as detailed below.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)benzamide (11):** Colorless solid (47 mg, 55%). ¹H NMR (CD₃OD, 300 MHz) δ 7.83 (d, *J*=7.1 Hz, 2H), 7.60-7.40 (m, 3H), 4.20-3.90 (m, 2H), 3.60-2.60 (m, 10 H), 2.40-1.80 (m, 8H), 1.08 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.76, 170.03, 135.43, 134.63, 132.77, 129.57, 128.35, 112.93, 69.74, 60.02, 54.44, 44.10, 38.12, 34.42, 23.87, 23.39, 22.94, 19.82, 11.17; *m/z* 429 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-2-chlorobenzamide (12):** Colorless solid (42 mg, 45%). ¹H NMR (CD₃OD, 300 MHz) δ 7.50-7.37 (m, 4H), 4.18-3.82 (m, 2H), 3.60-2.60 (m, 10H), 2.40-1.70 (m, 8H), 1.07 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.77, 169.62, 137.46, 134.36, 132.20, 131.94, 130.99, 129.79, 128.15, 112.92, 69.64, 59.98, 54.42, 44.00, 37.88, 34.34, 23.83, 23.34, 22.85, 19.79, 11.16; *m/z* 463 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)-amino)ethyl)-3-hydroxycyclobutyl)-3-chlorobenzamide (13):** Colorless solid (52 mg, 56%). ¹H NMR (CD₃OD, 300 MHz) δ 7.83-7.70 (m, 2H), 7.60-7.40 (m, 2H), 4.18-3.83 (m, 2H), 3.60-2.60 (m, 10H), 2.40-1.72 (m, 8H), 1.08 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.76, 168.32, 137.37, 135.60, 134.46, 132.64, 131.22, 128.50, 126.75, 112.92, 69.71, 59.99, 54.45, 44.01, 38.22, 34.41, 23.85, 23.36, 22.88, 19.81, 11.16; *m/z* 463 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-4-chlorobenzamide (14):** Colorless solid (54 mg, 58%). ¹H NMR (CD₃OD, 300 MHz) δ 7.81 (d, *J*=8.6 Hz, 2H), 7.47 (d, *J*=8.6 Hz, 2H), 4.16-3.83 (m, 2H), 3.60-2.60 (m, 10H), 2.40-1.72 (m, 8H), 1.06 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.77, 168.73, 138.81, 134.41, 134.05, 130.08, 129.72, 112.92, 69.69, 59.99, 54.42, 44.03, 38.17, 34.41, 23.84, 23.35, 22.86, 19.80, 11.16; *m/z* 463 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-2-fluorobenzamide (15):** Colorless solid (40 mg, 45%). ¹H NMR (CD₃OD, 300 MHz) δ 7.81-7.77 (m, 1H), 7.67-7.65(m, 1H), 7.42-7.30 (m, 2H), 4.25-4.08 (m, 2H), 3.55-2.83 (m, 10H), 2.46-1.96 (m, 8H), 1.19 (t, *J*=7.1 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.88, 166.72, 163.01 (d, *J*_{F-C}=247.9 Hz), 134.49, 134.25 (d, *J*_{F-C}=8.6 Hz), 131.37 (d, *J*_{F-C}=2.5 Hz), 125.76 (d, *J*_{F-C}=3.5 Hz), 124.54 (d, *J*_{F-C}=13.8 Hz), 117.47 (d, *J*_{F-C}=22.7 Hz), 113.04, 69.82, 60.10, 54.58, 44.30, 38.09, 34.46, 23.95, 23.45, 22.96, 19.92, 11.24; *m/z* 447 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-fluorobenzamide (16):** Colorless solid (32 mg, 36%). ¹H NMR (CD₃OD, 300 MHz) δ 7.70-7.43 (m, 3H), 7.35-7.24 (m, 1H), 4.16-3.85 (m, 2H), 3.60-2.62 (m, 10H), 2.42-1.74 (m, 8H), 1.08 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.78, 168.37, 164.07 (d, *J*_{F-C}=244.0 Hz), 137.78 (d, *J*_{F-C}=6.8 Hz), 134.35, 131.54 (d, *J*_{F-C}=7.9 Hz), 124.22 (d, *J*_{F-C}=2.7 Hz), 119.48 (d, *J*_{F-C}=21.5 Hz), 115.29 (d, *J*_{F-C}=23.1

Hz), 112.91, 69.71, 59.98, 54.46, 44.01, 38.19, 34.41, 23.83, 23.34, 22.84, 19.78, 11.16; m/z 447 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-4-fluorobenzamide (17):** Colorless solid (59 mg, 66%). ¹H NMR (CD₃OD, 300 MHz) δ 7.90-7.80 (m, 2H), 7.18 (t, *J*=8.8 Hz, 2H), 4.16-3.83 (m, 2H), 3.60-2.62 (m, 10H), 2.42-1.70 (m, 8H), 1.06 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.77, 168.75, 166.21 (d, *J*_{F-C}=248.8 Hz), 134.34, 131.75 (d, *J*_{F-C}=2.9 Hz), 130.97 (d, *J*_{F-C}=9.0 Hz), 116.36 (d, *J*_{F-C}=22.1 Hz), 112.90, 69.69, 59.97, 54.43, 44.05, 38.14, 34.41, 23.83, 23.33, 22.84, 19.79, 11.16; m/z 447 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-chloro-4-fluorobenzamide (18):** Colorless solid (39 mg, 40%). ¹H NMR (CD₃OD, 300 MHz) δ 8.01-7.98 (m, 1H), 7.86-7.81(m, 1H), 7.40-7.34 (m, 1H), 4.30-3.80 (m, 2H), 3.65-2.63 (m, 10H), 2.46-1.76 (m, 8H), 1.08 (t, *J*=7.4 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 170.35, 165.97, 161.60 (d, *J*_{F-C}=251.3 Hz), 133.16, 131.51 (d, *J*_{F-C}=3.7 Hz), 129.83, 127.89 (d, *J*_{F-C}=8.2 Hz), 120.83 (d, *J*_{F-C}=18.2 Hz), 116.56 (d, *J*_{F-C}=21.8 Hz), 111.53, 68.28, 58.62, 53.02, 42.60, 36.87, 33.03, 22.46, 21.99, 21.52, 18.42, 9.76; m/z 481 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-2-methoxybenzamide (19):** Colorless solid (50 mg, 54%). ¹H NMR (CD₃OD, 300 MHz) δ 7.84 (dd, *J*=1.7, 7.7 Hz, 2H), 7.53-7.45 (m, 1H), 7.14 (d, *J*=8.3 Hz, 1H), 7.08-7.00 (m, 1H), 4.18-3.83 (m, 2H), 3.92 (s, 3H), 3.60-2.60 (m, 10H), 2.40-1.70 (m, 8H), 1.06 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.77, 167.81, 159.00, 134.33, 134.18, 131.81, 122.99, 121.92, 112.89, 69.86, 59.97, 56.49, 54.43, 44.54, 37.68, 34.39, 23.82, 23.33, 22.83, 19.79, 11.16; m/z 459 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-4-methoxybenzamide (20):** Colorless solid (41 mg, 45%). ¹H NMR (CD₃OD, 300 MHz) δ 7.80 (d, *J*=8.8 Hz, 2H), 6.97 (d, *J*=8.8 Hz, 2H), 4.18-3.85 (m, 2H), 3.84 (s, 3H), 3.60-2.60 (m, 10H), 2.40-1.70 (m, 8H), 1.05 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.76, 169.49, 163.96, 134.26, 130.23, 127.39, 114.69, 112.87, 69.72, 59.94, 55.92, 54.41, 44.12, 38.01, 34.39, 23.81, 23.30, 22.80, 19.77, 11.16; m/z 459 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-methoxybenzamide (21):** Colorless solid (53 mg, 58%). ¹H NMR (CD₃OD, 300 MHz) δ 7.42-7.30 (m, 3H), 7.13-7.05 (m, 1H), 4.18-3.81 (m, 2H), 3.84 (s, 3H), 3.60-2.60 (m, 10H), 2.40-1.70 (m, 8H), 1.06 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.77, 169.76, 161.22, 136.77, 134.32, 130.66, 120.45, 118.46, 113.70, 112.89, 69.72, 59.96, 55.87, 54.39, 44.05, 38.11, 34.40, 23.83, 23.32, 22.83, 19.78, 11.16; m/z 459 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-4-(5-methyl-1,2,4-oxadiazol-3-yl)benzamide (22):** Colorless

solid (46 mg, 45%). ^1H NMR (CD_3OD , 300 MHz) δ 8.17 (d, $J=8.1$ Hz, 2H), 7.98 (d, $J=8.1$ Hz, 2H), 4.20-3.85 (m, 2H), 3.60-2.70 (m, 10H), 2.69 (s, 3H), 2.45-1.82 (m, 8H), 1.10 (t, $J=7.5$ Hz, 3H); ^{13}C NMR (CD_3OD , 75 MHz) δ 179.21, 171.75, 169.06, 168.87, 137.90, 134.70, 131.09, 129.05, 128.37, 112.97, 69.74, 60.05, 54.46, 44.08, 38.26, 34.43, 23.89, 23.41, 22.98, 19.85, 12.08, 11.17; m/z 511 $[\text{M}+\text{H}]^+$.

***N*-(*cis*-3-(2-(((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-(5-methyl-1,2,4-oxadiazol-3-yl)benzamide (23):** Colorless solid (58 mg, 57%). ^1H NMR (CD_3OD , 300 MHz) δ 8.52 (m, 1H), 8.23-8.21 (m, 1H), 8.02-7.99 (m, 1H), 7.67-7.62 (m, 1H), 4.25-3.85 (m, 2H), 3.60-2.70 (m, 10H), 2.69 (s, 3H), 2.45-1.75 (m, 8H), 1.09 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD , 75 MHz) δ 179.13, 171.77, 169.05, 168.99, 136.43, 134.55, 131.20, 131.00, 130.39, 128.63, 127.28, 112.94, 69.75, 60.03, 54.46, 44.07, 38.25, 34.45, 23.86, 23.40, 22.92, 19.82, 12.09, 11.17; m/z 511 $[\text{M}+\text{H}]^+$.

General procedure for the synthesis of compounds 24-35: Diisopropylethylamine (52 mg, 0.4 mmol) and appropriate sulfonyl chlorides (0.24 mmol) were added to a suspension of **10** (65 mg, 0.2 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at room temperature for 2 h. The reaction was quenched with H_2O and pH was adjusted to 9–10 by addition of aqueous Na_2CO_3 . The mixture was extracted 3 times with CH_2Cl_2 (30 mLx3). The organic layer was separated, combined, dried, and evaporated. The residue was purified by preparative HPLC to give the product. This procedure was used to prepare compounds **24-35** as described below.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)benzenesulfonamide (24):** Colorless solid (37 mg, 40%). ^1H NMR (CD_3OD , 300 MHz) δ 7.86 (d, $J=6.9$ Hz, 2H), 7.80-7.50 (m, 3H), 4.00-3.80 (m, 1H), 3.50-2.65 (m, 9H), 2.50-1.60 (m, 10H), 1.03 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD , 75 MHz) δ 171.70, 142.43, 134.48, 133.74, 130.27, 127.97, 112.87, 69.33, 59.90, 54.31, 44.76, 40.50, 34.15, 23.76, 23.29, 22.85, 19.71, 11.11; m/z 465 $[\text{M}+\text{H}]^+$.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-2-methoxybenzenesulfonamide (25):** Colorless solid (42 mg, 42%). ^1H NMR (CD_3OD , 300 MHz) δ 7.80 (d, $J=8.3$ Hz, 1H), 7.62 (t, $J=7.6$ Hz, 1H), 7.21 (d, $J=8.3$ Hz, 1H), 7.08 (t, $J=7.6$ Hz, 1H), 3.96 (s, 3H), 3.94-3.80 (m, 1H), 3.40-2.60 (m, 9H), 2.35-1.65 (m, 10H), 1.02 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD , 75 MHz) δ 171.71, 158.14, 136.04, 134.27, 131.06, 129.28, 121.40, 113.59, 112.87, 69.28, 59.87, 56.57, 54.26, 44.51, 40.68, 34.07, 23.73, 23.25, 22.78, 19.73, 11.10; m/z 495 $[\text{M}+\text{H}]^+$.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-methoxybenzenesulfonamide (26):** Colorless solid (49 mg, 50%). ^1H NMR (CD_3OD , 300 MHz) δ 7.50-7.13 (m, 4H), 3.95-3.80 (m, 1H), 3.83 (s, 3H), 3.50-2.60 (m, 9H), 2.42-1.62 (m, 10H), 1.03 (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD , 75 MHz) δ 171.70, 161.49, 143.56, 134.39, 131.42, 120.01, 119.36, 113.15, 112.86, 69.33, 59.89, 56.17, 44.72, 40.53, 34.16, 23.74, 23.27, 22.82, 19.73, 11.11; m/z 495 $[\text{M}+\text{H}]^+$.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-4-methoxybenzenesulfonamide (27):** Colorless solid (44 mg, 44%). ¹H NMR (CD₃OD, 300 MHz) δ 7.78 (d, *J*=8.9 Hz, 2H), 7.07 (d, *J*=8.9 Hz, 2H), 3.97-3.83 (m, 1H), 3.85 (s, 3H), 3.42-2.60 (m, 9H), 2.42-1.65 (m, 10H), 1.03 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.71, 164.49, 134.35, 133.76, 130.16, 115.33, 112.87, 69.39, 59.89, 56.20, 44.75, 40.47, 34.19, 23.74, 23.27, 22.82, 19.70, 11.12; *m/z* 495 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-2-chlorobenzenesulfonamide (28):** Colorless solid (56 mg, 56%). ¹H NMR (CD₃OD, 300 MHz) δ 8.04 (d, *J*=7.8 Hz, 1H), 7.62-7.42 (m, 3H), 3.98-3.80 (m, 1H), 3.48-2.63 (m, 9H), 2.40-1.70 (m, 10H), 1.02 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.73, 139.49, 135.13, 134.19, 132.94, 132.83, 132.17, 128.52, 112.85, 69.18, 59.87, 54.32, 44.57, 40.52, 34.06, 23.73, 23.24, 22.76, 19.72, 11.10; *m/z* 499 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-chlorobenzenesulfonamide (29):** Colorless solid (47 mg, 47%). ¹H NMR (CD₃OD, 300 MHz) δ 7.81-7.66 (m, 2H), 7.64-7.43 (m, 2H), 3.95-3.78 (m, 1H), 3.40-2.60 (m, 9H), 2.44-1.65 (m, 10H), 1.01 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.74, 144.51, 136.14, 134.22, 133.69, 131.99, 127.88, 126.38, 112.88, 69.29, 59.89, 54.36, 44.75, 40.51, 34.16, 23.76, 23.26, 22.79, 19.74, 11.12; *m/z* 499 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-4-chlorobenzenesulfonamide (30):** Colorless solid (49 mg, 49%). ¹H NMR (CD₃OD, 300 MHz) δ 7.84 (d, *J*=8.6 Hz, 2H), 7.58 (d, *J*=8.6 Hz, 2H), 3.95-3.80 (m, 1H), 3.50-2.65 (m, 9H), 2.49-1.70 (m, 10H), 1.02 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.73, 141.25, 139.93, 134.19, 130.47, 129.75, 112.87, 69.33, 59.89, 54.32, 44.70, 40.50, 34.14, 23.74, 23.25, 22.77, 19.76, 11.11; *m/z* 499 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-2-fluorobenzenesulfonamide (31):** Colorless solid (39 mg, 40%). ¹H NMR (CD₃OD, 300 MHz) δ 7.87-7.80 (m, 1H), 7.70-7.60 (m, 1H), 7.40-7.25 (m, 2H), 3.95-3.80 (m, 1H), 3.52-2.63 (m, 9H), 2.45-1.70 (m, 10H), 1.01 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.72, 160.25 (d, *J*_{F-C}=252.3 Hz), 136.42 (d, *J*_{F-C}=8.4 Hz), 134.37, 131.21, 130.23 (d, *J*_{F-C}=13.7 Hz), 125.79 (d, *J*_{F-C}=3.8 Hz), 118.12 (d, *J*_{F-C}=24.2 Hz), 112.87, 69.22, 59.90, 54.29, 44.71, 40.52, 34.11, 23.75, 23.27, 22.82, 19.73, 11.11; *m/z* 483 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-fluorobenzenesulfonamide (32):** Colorless solid (42 mg, 43%). ¹H NMR (CD₃OD, 300 MHz) δ 7.70-7.50 (m, 3H), 7.42-7.30 (m, 1H), 3.95-3.82 (m, 1H), 3.50-2.60 (m, 9H), 2.45-1.70 (m, 10H), 1.01 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.74, 163.86 (d, *J*_{F-C}=147.9 Hz), 144.73 (d, *J*_{F-C}=6.5 Hz), 134.21, 132.47 (d, *J*_{F-C}=7.9 Hz), 123.95, 120.67 (d, *J*_{F-C}=21.4 Hz), 115.04 (d, *J*_{F-C}=24.5 Hz), 112.87, 69.29, 59.88, 54.31, 44.72, 40.53, 34.15, 23.74, 23.26, 22.77, 19.73, 11.12; *m/z* 483 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-4-fluorobenzenesulfonamide (33):** Colorless solid (49 mg, 51%). ¹H NMR (CD₃OD, 300 MHz) δ 7.89 (dd, *J*=5.1, 8.9 Hz, 2H), 7.29 (dd, *J*=8.9, 8.9 Hz, 2H), 3.95-3.80 (m, 1H), 3.48-2.65 (m, 9H), 2.49-1.70 (m, 10H), 1.01 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.72, 166.44 (d, *J*_{F-C}=250.8 Hz), 138.73 (d, *J*_{F-C}=3.2 Hz), 134.35, 130.94 (d, *J*_{F-C}=9.3 Hz), 117.26 (d, *J*_{F-C}=22.8 Hz), 112.88, 69.33, 60.03, 59.89, 54.35, 44.73, 40.49, 34.16, 23.75, 23.27, 22.82, 19.73, 11.12; *m/z* 483 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3-chloro-4-fluorobenzenesulfonamide (34):** Colorless solid (50 mg, 48%). ¹H NMR (CD₃OD, 300 MHz) δ 7.98 (dd, *J*=2.2, 6.8 Hz, 1H), 7.83-7.75 (m, 1H), 7.47 (t, *J*=8.8 Hz, 1H), 3.96-3.80 (m, 1H), 3.50-2.63 (m, 9H), 2.45-1.70 (m, 10H), 1.03 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.70, 161.66 (d, *J*_{F-C}=254.7 Hz), 140.06 (d, *J*_{F-C}=3.8 Hz), 134.52, 130.83, 129.09 (d, *J*_{F-C}=8.6 Hz), 122.89 (d, *J*_{F-C}=18.8 Hz), 118.68 (d, *J*_{F-C}=22.4 Hz), 112.88, 69.29, 59.93, 54.32, 44.72, 40.49, 34.15, 23.77, 23.30, 22.88, 19.73, 11.13; *m/z* 517 [M+H]⁺.

***N*-(*cis*-3-(2-(((*S*)-2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)-3-hydroxycyclobutyl)-3,4-difluorobenzenesulfonamide (35):** Colorless solid (40 mg, 40%). ¹H NMR (CD₃OD, 300 MHz) δ 7.80-7.70 (m, 2H), 7.55-7.43 (m, 1H), 3.95-3.80 (m, 1H), 3.50-2.60 (m, 9H), 2.45-1.70 (m, 10H), 1.02 (t, *J*=7.3 Hz, 3H); ¹³C NMR (CD₃OD, 75 MHz) δ 171.73, 154.41 (dd, *J*_{F-C}=12.5, 212.0 Hz), 151.05 (dd, *J*_{F-C}=12.5, 209.8 Hz), 139.75 (t, *J*_{F-C}=4.2 Hz), 134.31, 125.58 (dd, *J*_{F-C}=3.9, 7.6 Hz), 119.45 (d, *J*_{F-C}=18.5 Hz), 117.79 (d, *J*_{F-C}=19.9 Hz), 112.88, 69.29, 60.34, 54.30, 44.69, 40.50, 34.15, 23.75, 23.26, 22.81, 19.72, 11.12; *m/z* 501[M+H]⁺.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by a grant from the National Institute on Drug Abuse, National Institutes of Health (R01DA020669). We are grateful to the Addiction Treatment Discovery Programs at the NIDA and NIH for evaluation of ligands for their functional activity in cells transfected with cloned human dopamine receptors under the contract NIDA Y1-DA-0101-02, performed by Dr. Aaron Janowsky at the Oregon Health & Science University (Portland, Oregon).

References

- Joyce JN. *Pharmacol Ther.* 2001; 90:231–259. [PubMed: 11578658]
- Volkow ND, Fowler JS, Wang GJ. *Behav Pharmacol.* 2002; 13:355–366. [PubMed: 12394411]
- Shimohama S, Sawada H, Kitamura Y, Taniguchi T. *Trends Mol Med.* 2003; 9:360–365. [PubMed: 12928038]
- Newman AH, Grundt P, Nader MA. *J Med Chem.* 2005; 48:3663–3679. [PubMed: 15916415]
- Heidbreder CA, Newman AH. *Ann N Y Acad Sci.* 2010; 1187:4–34. [PubMed: 20201845]
- Newman AH, Beuming T, Banala AK, Donthamsetti P, Pongetti K, LaBounty A, Levy B, Cao J, Michino M, Luedtke RR, Javitch JA, Shi L. *J Med Chem.* 2012; 55:6689–6699. [PubMed: 22632094]

7. Keck TM, Burzynski C, Shi L, Newman AH. *Adv Pharmacol.* 2014; 69:267–300. [PubMed: 24484980]
8. Chen J, Collins GT, Levant B, Woods J, Deschamps JR, Wang S. *ACS Med Chem Lett.* 2011; 2:620–625. [PubMed: 22125662]
9. Chen J, Levant B, Jiang C, Keck TM, Newman AH, Wang S. *J Med Chem.* 2014; 57:4962–4968. [PubMed: 24848155]
10. Bancroft GN, Morgan KA, Flietstra RJ, Levant B. *Neuropsychopharmacology.* 1998; 18:305–316. [PubMed: 9509498]
11. Levant B, Grigoriadis DE, DeSouza EB. *J Pharmacol Exp Ther.* 1992; 262:929–935. [PubMed: 1356154]
12. Grigoriadis D, Seeman P. *J Neurochem.* 1985; 44:1925–1935. [PubMed: 3157782]
13. Levant, B. *Current Protocols in Pharmacology.* John Wiley & Sons, Inc; 2001.

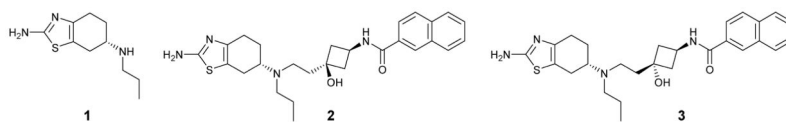


Figure 1.
Chemical structures of pramipexole (1) and two potent and selective D₃ agonists.

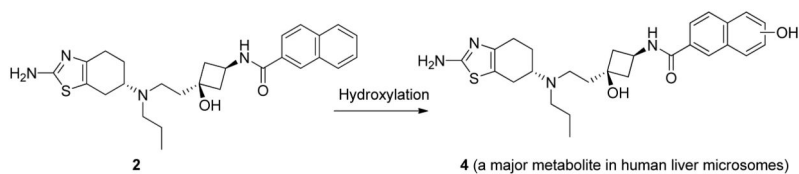
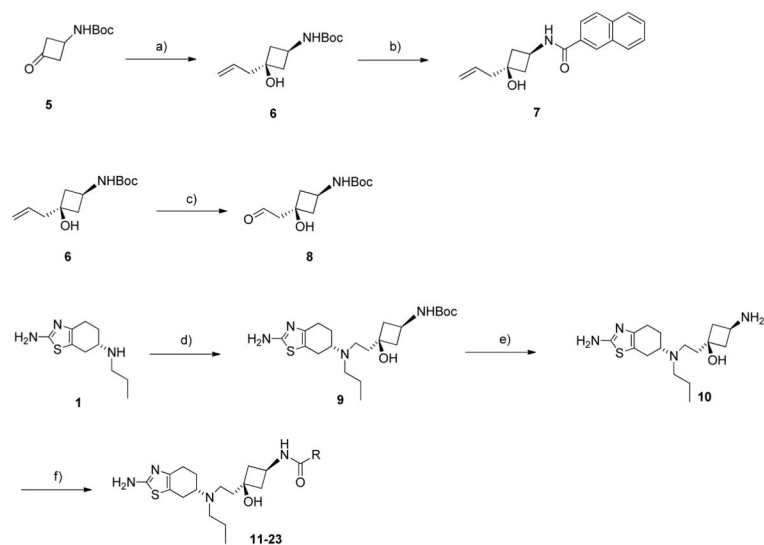
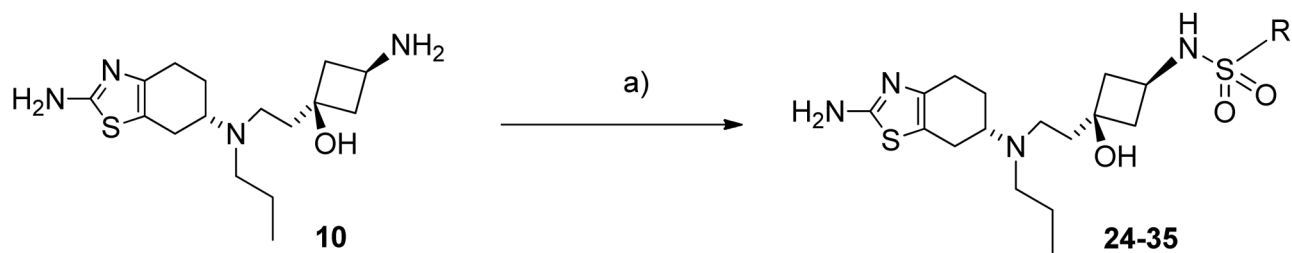


Figure 2.
Major biotransformation of compound **2** in human liver microsomes.

**Scheme 1.**

Synthesis of compounds **11-23**. Conditions and reagents: a) Allylmagnesium bromide, THF, $-78\text{ }^{\circ}\text{C}$, 4 h, 68%; b) 1. TFA, DCM, RT, 12 h, 2. 2-naphthoyl chloride, DIPEA, DCM, RT, 2 h, 72%; c) OsO_4 , NaIO_4 , THF- H_2O , RT, 30 min, 60%; d) **8**, $\text{NaBH}(\text{OAc})_3$, HOAc, DCM, 27%; e) TFA, DCM, RT, 12 h, 90%; f) appropriate acid chlorides, DIPEA, DCM, RT, 2 h, 36–66%.

**Scheme 2.**

Synthesis of compounds **24-35**. Conditions and reagents: a) appropriate sulfonyl chlorides, DIPEA, DCM, RT, 2 h, 40–56%.

Table 1

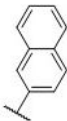
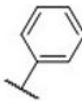
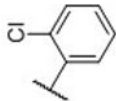
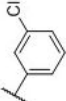
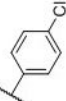
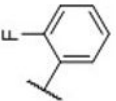
Human liver microsomal stability of representative compounds. Percentage remaining of compounds in human liver microsomes at selected time points.^a








Time (min)	Compounds						
	2	3	20	21	23		
0	100	100	100	100	100		
5	84.6	79.2	97.7	99.7	96.6		
10	72.2	49.9	94.1	98.5	99.2		
15	63.6	34.9	94.0	92.5	99.0		
30	41.1	11.7	85.5	83.0	90.0		
60	20.1	2.1	76.7	74.1	65.5		
t _{1/2} (min)	26.1	9.48	>60	>60	>60		

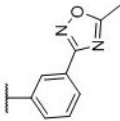
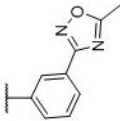
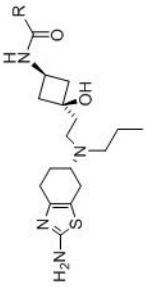
^aData represent a single determination.

Table 2

Binding affinities of original lead compound **2** and new compounds **11-23** at the D₁-like, D₂-like, and D₃ receptors in binding assays using rat brain preparation.

ligand	R	K _i ±SEM (nM)			Selectivity		
		D ₃	D ₂ -like	D ₁ -like	D ₂ -like/D ₃	D ₁ -like/D ₃	D ₁ -like/D ₂
2		0.40±0.087	725±45	1,610±167	1,827	4,025	
11		0.74±0.038	224±9	1,970±179	301	2,662	
12		0.62±0.068	72±11	49,700±2,807	117	80,161	
13		0.58±0.062	101±12	28,700±2,640	175	49,482	
14		1.6±0.19	244±46	15,113±780	157	9,751	
15		0.46±0.06	17,000±4,092	53,600±8,380	36,956	116,521	

ligand	R	$K_i \pm \text{SEM}$ (nM)			Selectivity		
		D ₃	D ₂ -like	D ₁ -like	D ₂ -like/D ₃	D ₁ -like/D ₃	D ₁ -like/D ₂
16		1.9±0.087	264±46	56,600±2,730	138	29,789	
17		0.89±0.17	367±27	50,000±4,890	412	56,180	
18		0.43±0.06	6,950±1,095	28,200±5,308	16,272	65,581	
19		1.0±0.094	158±25	43,500±711	160	44,500	
20		0.70±0.055	2,660±461	42,800±3,279	3,779	61,143	
21		0.76±0.036	9,790±1,500	64,200±1,760	12,836	84,474	
22		0.96±0.10	14,600±1,280	19,200±2,550	15,208	20,000	

ligand	R		$K_i \pm$ SEM (nM)			Selectivity		
			D ₃	D ₂ -like	D ₁ -like	D ₂ -like/D ₃	D ₁ -like/D ₃	
23			0.53±0.07	15,800±5,910	15,000±2,920	29,800	28,300	

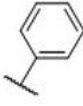
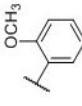
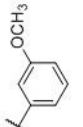
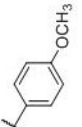
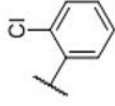
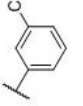
D₃ receptor binding was determined using [³H]7-OH-DPAT and membranes prepared from ventral striatum.

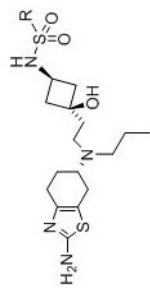
D₂-like receptor binding (D₂, D₃, and D₄) was determined using [³H]spiperone and striatal membranes. D₂-like receptor binding (D₂, D₃, and D₄) was determined using [³H]spiperone and striatal membranes. For those compounds that produced a competition curve consistent with inhibition at 2-sites in this assay, which is consistent with the behavior of agonists in this assay, the K_i value for the high affinity component is reported. D₁-like receptor binding (D₁ and D₅) was determined using [³H]SCH23390 and striatal membranes.

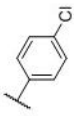
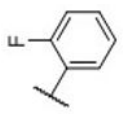
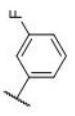
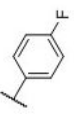
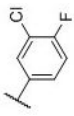
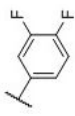
Data are the mean ± SEM of 3–6 independent determinations.

Table 3

Binding affinities of new compounds **24-35** at the D₁-like, D₂-like, and D₃ receptors in binding assays using rat brain preparation.

ligand	R	K _i ±SEM (nM)			Selectivity		
		D ₃	D ₂ -like	D ₁ -like	D ₂ -like/D ₃	D ₁ -like/D ₃	D ₁ -like/D ₂
24		1.9±0.17	81±11	57,000±2,830	42		30,000
25		2.3±0.22	170±18	1,810±93	75		800
26		1.6±0.16	160±8.5	36,500±2,243	98		22,812
27		1.6±0.090	102±15	2,610±255	65		1,631
28		0.59±0.057	286±49	94,900±7,620	485		160,847
29		2.5±0.15	71±4.8	68,000±2,300	28		27,200



ligand	R	$K_i \pm \text{SEM}$ (nM)				Selectivity		
		D ₃	D ₂ -like	D ₁ -like	D ₂ -like/D ₃	D ₁ -like/D ₃	D ₂ -like/D ₁	
30		2.6±0.20	542±79	45,900±2,490	205	17,654		
31		1.0±0.072	619±68	109,000±2,500	614	109,000		
32		1.2±0.091	110±14	117,000±10,170	91	97,500		
33		1.9±0.25	789±36	102,000±5,390	414	53,684		
34		2.3±0.25	2,420±118	41,900±2,764	1,052	18,217		
35		3.1±0.26	57±7.4	76,000±6,810	19	24,516		

D₃ receptor binding was determined using [³H]7-OH-DPAT and membranes prepared from ventral striatum.

D₂-like receptor binding (D₂, D₃, and D₄) was determined using [³H]spiperone and striatal membranes. D₂-like receptor binding (D₂, D₃, and D₄) was determined using [³H]spiperone and striatal membranes. For those compounds that produced a competition curve consistent with inhibition at 2-sites in this assay, which is consistent with the behavior of agonists in this assay, the K_i value for the high affinity component is reported. D₁-like receptor binding (D₁ and D₅) was determined using [³H]SCH23390 and striatal membranes.

Data are the mean \pm SEM of 3–6 independent determinations.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 4

Functional activity of representative D₃ ligands in the quinpirole-induced mitogenesis assay in CHO cells transfected with the human D₃ receptor.^a

Ligands	Agonist activity: IC ₅₀ (nM)	% of Stimulation compared to Quinpirole as the standard agonist
15	2.0±0.49	100
18	2.0±0.34	100
21	18±2.7	100
23	19±1.5	100
33	17±4.4	100
34	26±4.1	100

^aData are the mean ± SEM of 3–6 independent determinations.