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Pramipexole Derivatives as Potent and Selective Dopamine D_3 Receptor Agonists with Improved Human Microsomal Stability

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Herein we report the synthesis and evaluation of a series of new pramipexole derivatives as highly potent and selective agonists of the dopamine-3 (D_3) receptor. A number of these new compounds bind to the D_3 receptor with sub-nanomolar affinity and show excellent selectivity (>10000) for the D_3 receptor over the D_1 and D_2 receptors. For example, compound **23** (*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) binds to the D_3 receptor with a K_i value of 0.53 nM and shows a selectivity of >20000 over the D_2 and D_1 receptors in the binding assays using a rat brain preparation. It has excellent stability in human liver microsomes. Moreover, in vitro functional assays showed it to be a full agonist for the human D_3 receptor.

Introduction

The dopamine-3 (D_3) receptor subtype has been identified as a major target for several agents currently in clinical use 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 D_3 receptor, but also have high affinities for the D_2 receptor and have other off-target effects,[4,5] there is a need to design potent and selective D_3 ligands. Such new compounds can be used to further investigate the role of the D_3 receptor in different biological and pharmacological processes and to validate unambiguously the D_3 receptor as an important therapeutic target in preclinical and clinical studies.

The design of potent and highly selective D_3 ligands has been a challenge for many years given the high degree of sequence homology between the D_2 and D_3 receptors and nearly identical primary sequences that form their binding sites.[4,5] However, recent studies, including those from our research group, have shown that it is possible to design highly selective and potent D_3 ligands.[6–8] For example, based on pramipexole (**1**), a potent D_3 agonist with only a modest selectivity over the D_2 receptor, we have developed CJ-1368 (**2**) and CJ-1639 (**3**) as potent and selective D_3 agonists (Figure 1).[8] More recently, we reported the design of highly selective D_3 antagonists based on the structure of tranylcypromine.[9]

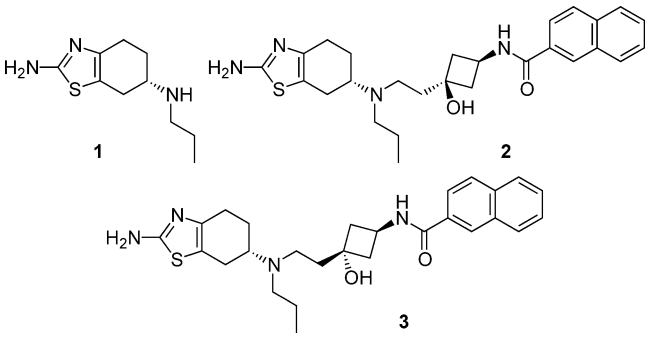

Figure 1. Structures of pramipexole (**1**) and two potent and selective D_3 agonists **2** and **3**.

Both compounds **2** and **3** display a high affinity for the D_3 receptor and excellent selectivity over the D_2 and D_1 receptors, and are thus promising lead compounds. Toward the identification of highly selective D_3 agonists that can be used for investigations in vivo, we 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 modified these compounds with the objective of improving their metabolic stability, as well as further enhancing their selectivity for the D_3 over the D_2 receptor, while maintaining high affinity for the D_3 receptor.

Results and Discussion

Microsomal stability testing showed that compound **2** has a $t_{1/2}$ of 26 min and compound **3** has a $t_{1/2}$ of 9.5 min in human

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Table 1. Human liver microsomal stability of representative compounds.[a]

Compd	Remaining in HLM [%]						$t_{1/2}$ [min]
	0 min	5 min	10 min	15 min	30 min	60 min	
2	100	84.6	72.2	63.6	41.1	20.1	26.1
3	100	79.2	49.9	34.9	11.7	2.1	9.48
20	100	97.7	94.1	94	85.5	76.7	>60
21	100	99.7	98.5	92.5	83	74.1	>60
23	100	96.6	99.2	99	90	65.5	>60

[a] Percentage compound remaining in human liver microsomes at indicated time points; data represent single determinations.

liver microsomes (Table 1). 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). 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.

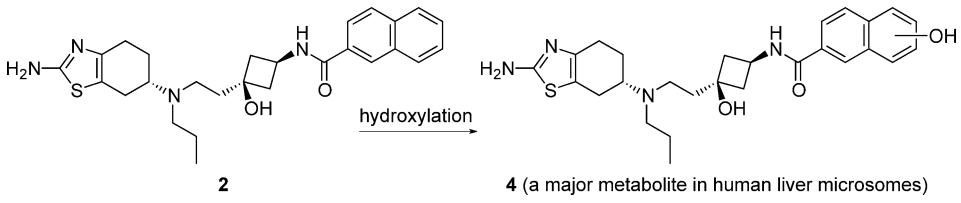

Figure 2. Major biotransformation of compound **2** in human liver microsomes.

These compounds were first evaluated for their binding affinities to the rat D_1-like, D_2-like, and D_3 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 D_3 receptor, with K_i values in the range of 0.43–1.9 nM. Compound **11**, which has a phenyl group in place of the naphthyl group, binds to the D_3 receptor with a K_i value of 0.74 nM and is 301-fold more selective for the D_3 receptor over the D_2 receptor. Chlorine substitution at the *ortho*, *meta*, and *para* positions on the phenyl group resulted in compounds **12**, **13**, and **14**, respectively. These have similar affinities for the D_3 receptor and also similar selectivities over the D_2 receptor, as compound **11**. Fluorine substitution at the three different positions on the phenyl ring yielded compounds **15**–**17**. Whereas compounds **16** and **17**, with a *meta*-fluoro or a *para*-fluoro substituent, have similar affinities for the D_3 receptor and also similar selectivities over the D_2 receptor, as compound **11**, compound **15** with the *ortho*-fluoro substituent, has high affinity for the D_3 receptor and a selectivity of >30000-fold over the D_2 receptor. The *m*-chloro-*p*-fluoro compound **18** also has high affinity for the D_3 receptor (K_i = 0.43 nM) and outstanding selectivity (>15000-fold) over the D_2 receptor. Methoxy group substitution at three different positions on the phenyl ring generated compounds **19**–**21**, all of which have similar binding affinities for the D_3 receptor (0.70–1.0 nM). The *meta*-methoxy compound **21** is >12000-fold selective for D_3 over the D_2 receptor, the highest selectivity among these three compounds. Hence, it appears that while substitution on the phenyl ring has a modest effect on the affinity for the D_3 receptor, it can have a major effect on the selectivity for the D_3 receptor over the D_2 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 *meta* or *para* positions, respectively. Compounds **22** and **23** bind to the D_3 receptor with respective K_i values of 0.96 and 0.53 nM, and both show very high selectivities (>15000) over the D_2 receptor. In addition to their high selectivity for the D_3 receptor over the D_2 receptor, compounds **15**, **18**, **21**, **22**, and **23** are also highly selective for the D_3 receptor over the D_1 receptor.

We next synthesized a series of compounds (**24**–**35**) in which the amide group in compounds **11**–**23** is replaced by a sulfonamide group (Table 3). These compounds retain high affinities for the D_3 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 D_3 receptor over the D_2 receptor; 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 D_3 ligands, in direct comparison with compounds **2** and **3**. The results are listed in Table 1. Our data show that compounds **20**, **21**, and **23** all have $t_{1/2}$ > 60 min, 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 D_3 receptor in a quinpirole-induced mitogenesis assay in hD_3-transfected CHOp cells. The results, presented in Table 4, show that all these compounds behave as highly potent full D_3 agonists.

Synthesis of designed compounds

The synthetic route to compounds **11**–**23** is shown in Scheme 1. The commercially available cyclobutanone **5** was treated with allylmagnesium bromide at −78 °C to give *cis*-allylcyclobutanol **6**. The stereochemistry of **6** was confirmed by transformation into known compound **7**, the stereochemistry of which was determined by X-ray crystallographic analysis.[8]

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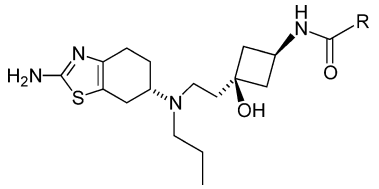
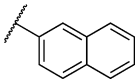
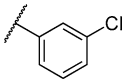
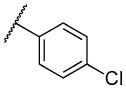
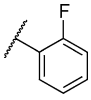
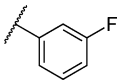
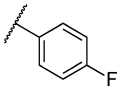
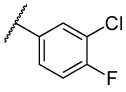
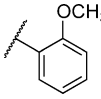
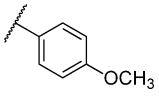
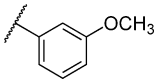
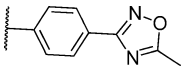
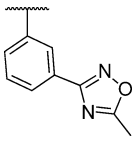
Table 2. Binding affinities of original lead compound **2** and new compounds **11**–**23** at the D_1-like, D_2-like, and D_3 receptors in binding assays using rat brain preparations.



Ligand	R	K_i [nM][a–c] D_3[a]	D_2-like[b]	D_1-like[c]	Selectivity D_2-like/D_3	D_1-like/D_3
2		0.40 ± 0.087	725 ± 45	1610 ± 167	1827	4025
11		0.74 ± 0.038	224 ± 9	1970 ± 179	301	2662
12		0.62 ± 0.068	72 ± 11	49700 ± 2807	117	80161
13		0.58 ± 0.062	101 ± 12	28700 ± 2640	175	49482
14		1.6 ± 0.19	244 ± 46	15113 ± 780	157	9751
15		0.46 ± 0.06	17000 ± 4092	53600 ± 8380	36956	116521
16		1.9 ± 0.087	264 ± 46	56600 ± 2730	138	29789
17		0.89 ± 0.17	367 ± 27	50000 ± 4890	412	56180
18		0.43 ± 0.06	6950 ± 1095	28200 ± 5308	16272	65581
19		1.0 ± 0.094	158 ± 25	43500 ± 711	160	44500
20		0.70 ± 0.055	2660 ± 461	42800 ± 3279	3779	61143
21		0.76 ± 0.036	9790 ± 1500	64200 ± 1760	12836	84474
22		0.96 ± 0.10	14600 ± 1280	19200 ± 2550	15208	20000
23		0.53 ± 0.07	15800 ± 5910	15000 ± 2920	29800	28300

[a] D_3 receptor binding was determined using [^{3}H]7-OH-DPAT and membranes prepared from ventral striatum. [b] D_2-like receptor binding (D_2, D_3, and D_4) was determined using [^{3}H]spiperone and striatal membranes. For those compounds that produced a competition curve consistent with two-site inhibition, which is typical of the behavior of agonists in this assay, the K_i value for the high-affinity component is reported. [c] D_1-like receptor binding (D_1 and D_5) was determined using [^{3}H]SCH23390 and striatal membranes. Data are the mean ± SEM of 3–6 independent determinations.

Aldehyde **8** was obtained by oxidation of **6** with $NaIO_4$ and a catalytic amount of OsO_4. Pramipexole (**1**) was allowed to react with **8** to give the key intermediate **9**, which, upon treatment with trifluoroacetic acid, afforded amine **10**. This amine was treated 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 DIPEA and dichloromethane with 40–56% yield.

Conclusions

In summary, modifications of the naphthyl group in our previously reported D_3 ligand **2** yielded a series of new compounds with high binding affinities for the D_3 receptor and high selectivity over the D_1 and D_2 receptors, and significantly with improved microsomal stability. Compound **23**, for example, binds to the D_3 receptor with a K_i value of 0.53 nM, shows a selectivity of $>25\,000$ over the D_1 and D_2 receptors, and superior microsomal stability to that of compound **2** in human liver microsomes. Compound **23** is a full agonist to the human D_3 receptor in the quinpirole-induced mitogenesis assay and represents a potent and highly selective D_3 agonist.

Experimental Section

Chemistry

General: Solvents and reagents were purchased and used without further purification. Reactions were

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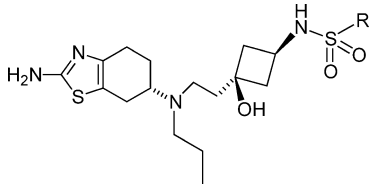
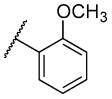
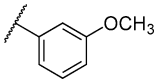
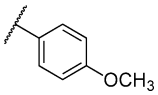
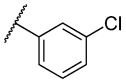
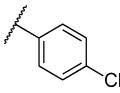
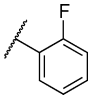
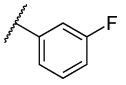
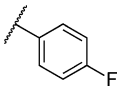
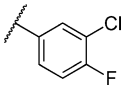
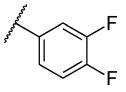
Table 3. Binding affinities of new compounds **24–35** at the D_1-like, D_2-like, and D_3 receptors in binding assays using rat brain preparation.[a]



Ligand	R	K_i [nM][a–c]			Selectivity	
		D_3[a]	D_2-like[b]	D_1-like[c]	D_2-like/D_3	D_1-like/D_3
24		1.9 ± 0.17	81 ± 11	57 000 ± 2830	42	30 000
25		2.3 ± 0.22	170 ± 18	1810 ± 93	75	800
26		1.6 ± 0.16	160 ± 8.5	36 500 ± 2243	98	22 812
27		1.6 ± 0.090	102 ± 15	2610 ± 255	65	1631
28		0.59 ± 0.057	286 ± 49	94 900 ± 7620	485	160 847
29		2.5 ± 0.15	71 ± 4.8	68 000 ± 2300	28	27 200
30		2.6 ± 0.20	542 ± 79	45 900 ± 2490	205	17 654
31		1.0 ± 0.072	619 ± 68	109 000 ± 2500	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 ± 5390	414	53 684
34		2.3 ± 0.25	2420 ± 118	41 900 ± 2764	1052	18 217
35		3.1 ± 0.26	57 ± 7.4	76 000 ± 6810	19	24 516

[a] D_3 receptor binding was determined using [^{3}H]7-OH-DPAT and membranes prepared from ventral striatum. [b] D_2-like receptor binding (D_2, D_3, and D_4) was determined using [^{3}H]spiperone and striatal membranes. For those compounds that produced a competition curve consistent with two-site inhibition, which is typical of the behavior of agonists in this assay, the K_i value for the high-affinity component is reported. [c] D_1-like receptor binding (D_1 and D_5) was determined using [^{3}H]SCH23390 and striatal membranes. Data are the mean ± SEM of 3–6 independent determinations.

monitored by thin-layer chromatography (TLC) carried out on 250 μm silica gel plates (E. Merck, 60 F_{254}) using UV light as visualizing agent. Silica gel 60 (E. Merck, particle size: 15–40 μm) was used for flash column chromatography. NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz). Chemical shifts (δ) are reported in ppm downfield relative to tetramethylsilane (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 λ 254 nm).

***tert*-Butyl (3-allyl-3-hydroxycyclobutyl)carbamate (6)**. Allylmagnesium bromide solution (1 M) in ether (64.8 mL, 64.8 mmol) was added dropwise to a solution of *tert*-butyl (3-oxocyclobutyl)carbamate **29** (6.0 g, 32.4 mmol) in anhydrous THF at −78 °C, and the reaction mixture was stirred at −78 °C for 2 h. Then the mixture was allowed to warm slowly to room temperature. The reaction was quenched by slow addition of aqueous saturated NH_4Cl. The mixture was extracted with EtOAc (3×40 mL) and organic layers were combined. The organic solvents were removed under vacuum, and the residue was purified by column chromatography (SiO_2, hexanes/EtOAc 2:1) to give **6** (5.0 g, 68%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 5.90–5.70 (m, 1H), 5.25–5.10 (m, 2H), 4.70 (br, 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 ppm (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 CH_2Cl_2 (5 mL). DIPEA (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 (3×30 mL). The organic layer was separated, combined, dried, and evaporated. The residue was purified by chromatography (SiO_2, hexanes/EtOAc 1:1) to give **7** as a colorless solid (445 mg, 72% over two steps). ^{1}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 ppm (m, 2H).

***tert*-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,

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Table 4. Functional agonist activity of representative D_3 ligands in the quinpirole-induced mitogenesis assay in hD_3-transfected CHOp cells.

Ligand	IC_{50} [nM][a]	Stimulation [%][b]
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

[a] Data are the mean ± SEM of 3–6 independent determinations. [b] Relative to quinpirole as the standard agonist.

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 (3×40 mL) and organic layers were combined. Evaporation of organic solvents under vacuum gave crude product **8** (1.2 g, crude yield 60%) as a pale-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.

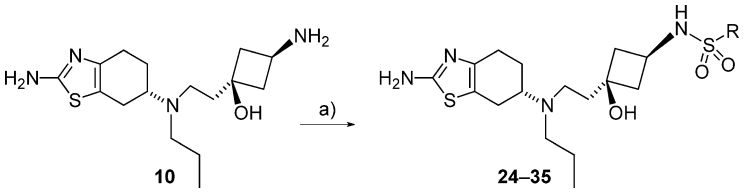
***tert*-Butyl (3-(2-(((*S*)-2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)ethyl)-3-hydroxycyclobutyl)carbamate (9)**. Compound **8** (8.93 g, 39.0 mmol), acetic acid (3.51 g, 58.5 mmol) and sodium triacetoxyborohydride (12.4 g, 58.5 mmol) were added to a solution of pramipexole (8.3 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 with CH_2Cl_2 (3×30 mL). The organic layers were separated, combined, and evaporated. The residue was subjected to chromatography (SiO_2, EtOAc/MeOH 95:5) to give compound **9** (4.5 g, 27% yield). ^{1}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 ppm (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. H_2O 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 (3×30 mL). The organic layers were separated, combined, dried, and evaporated. The residue was subjected to chromatography (SiO_2, CH_2Cl_2/MeOH 80:20) to give compound **10** as a colorless oil (3.1 g, 90% yield). ^{1}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 ppm (t, J = 7.2 Hz, 3H).

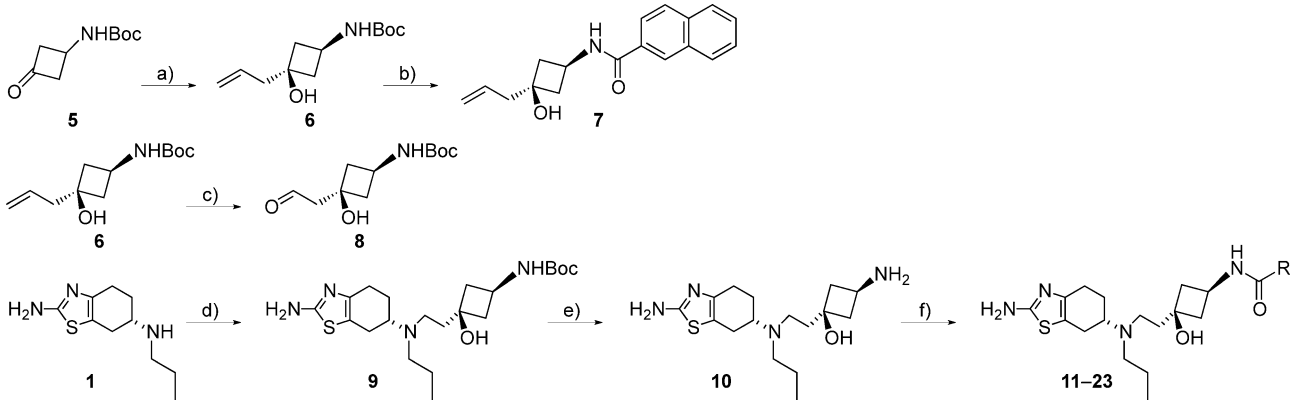
General procedure for the synthesis of compounds 11–23. DIPEA (52 mg, 0.4 mmol) and the appropriate acid chloride (0.24 mmol) were added to a suspension of **10** (65 mg, 0.2 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred at room temperature for 2 h. The reaction was quenched with H_2O and pH was adjusted to 9–10 by addition of aqueous Na_2CO_3. The mixture was extracted with CH_2Cl_2 (3×30 mL). 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%). ^{1}H NMR (CD_3OD, 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, 10H), 2.40–1.80 (m, 8H), 1.08 ppm (t, J = 7.3 Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): δ = 171.8, 170.0, 135.4, 134.6, 132.8, 129.6, 128.4, 112.9, 69.7, 60.0, 54.4, 44.1, 38.1, 34.4, 23.9, 23.4, 22.9, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 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



Scheme 2. Synthesis of compounds **24–35**. *Reagents and conditions*: a) appropriate sulfonyl chlorides, DIPEA, CH_2Cl_2, RT, 2 h, 40–56%.



Scheme 1. Synthesis of compounds **11–23**. *Reagents and conditions*: a) allylmagnesium bromide, THF, −78 °C, 4 h, 68%; b) 1. TFA, CH_2Cl_2, RT, 12 h, 2. 2-naphthoyl chloride, DIPEA, CH_2Cl_2, RT, 2 h, 72%; c) OsO_4, $NaIO_4$, THF/H_2O, RT, 30 min, 60%; d) **8**, $NaBH(OAc)_3$, HOAc, CH_2Cl_2, 27%; e) TFA, CH_2Cl_2, RT, 12 h, 90%; f) appropriate acid chlorides, DIPEA, CH_2Cl_2, RT, 2 h, 36–66%.

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(m, 8H), 1.07 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$ 171.8, 169.6, 137.5, 134.4, 132.2, 131.9, 131.0, 129.8, 128.2, 112.9, 69.6, 60.0, 54.4, 44.0, 37.9, 34.3, 23.8, 23.3, 22.9, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.8, 168.3, 137.4, 135.6, 134.5, 132.6, 131.2, 128.5, 126.8, 112.9, 69.7, 60.0, 54.5, 44.0, 38.2, 34.4, 23.9, 23.4, 22.9, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.8, 168.7, 138.8, 134.4, 134.1, 130.1, 129.7, 112.9, 69.7, 60.0, 54.4, 44.0, 38.2, 34.4, 23.9, 23.4, 22.9, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=$ 7.1 Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.9, 166.7, 163.0 (d, $J_{F-C}=247.9$ Hz), 134.5, 134.3 (d, $J_{F-C}=8.6$ Hz), 131.4 (d, $J_{F-C}=$ 2.5 Hz), 125.8 (d, $J_{F-C}=3.5$ Hz), 124.5 (d, $J_{F-C}=13.8$ Hz), 117.5 (d, $J_{F-C}=22.7$ Hz), 113.0, 69.8, 60.1, 54.58, 44.3, 38.1, 34.5, 24.0, 23.5, 23.0, 19.9, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.8, 168.4, 164.1 (d, $J_{F-C}=244.0$ Hz), 137.8 (d, $J_{F-C}=6.8$ Hz), 134.4, 131.5 (d, $J_{F-C}=7.9$ Hz), 124.2 (d, $J_{F-C}=$ 2.7 Hz), 119.5 (d, $J_{F-C}=21.5$ Hz), 115.3 (d, $J_{F-C}=23.1$ Hz), 112.9, 69.7, 60.0, 54.5, 44.0, 38.2, 34.4, 23.8, 23.3, 22.8, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.8, 168.8, 166.2 (d, $J_{F-C}=$ 248.8 Hz), 134.3, 131.8 (d, $J_{F-C}=2.9$ Hz), 131.0 (d, $J_{F-C}=9.0$ Hz), 116.4 (d, $J_{F-C}=22.1$ Hz), 112.9, 69.7, 60.0, 54.4, 44.1, 38.1, 34.4, 23.8, 23.3, 22.8, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$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 ppm (t, $J=7.4$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$170.4, 166.0, 161.6 (d, $J_{F-C}=251.3$ Hz), 133.2, 131.5 (d, $J_{F-C}=3.7$ Hz), 129.8, 127.9 (d, $J_{F-C}=8.2$ Hz), 120.8 (d, $J_{F-C}=18.2$ Hz), 116.6 (d, $J_{F-C}=$ 21.8 Hz), 111.5, 68.3, 58.6, 53.0, 42.6, 36.9, 33.0, 22.5, 22.0, 21.5, 18.4, 9.8 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.8, 167.8, 159.0, 134.3, 134.2, 131.8, 123.0, 121.9, 112.9, 69.9, 60.0, 56.5, 54.4, 44.5, 37.7, 34.4, 23.8, 23.3, 22.8, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=$ 7.3 Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.8, 169.5, 164.0, 134.3, 130.2, 127.4, 114.7, 112.9, 69.7, 59.9, 55.9, 54.4, 44.1, 38.0, 34.4, 23.8, 23.3, 22.8, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.8, 169.8, 161.2, 136.8, 134.3, 130.7, 120.5, 118.5, 113.7, 112.9, 69.7, 60.0, 55.9, 54.4, 44.05, 38.1, 34.4, 23.8, 23.3, 22.8, 19.8, 11.2 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$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 ppm (t, $J=7.5$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$ 179.2, 171.8, 169.1, 168.9, 137.9, 134.7, 131.1, 129.1, 128.4, 113.0, 69.7, 60.1, 54.5, 44.1, 38.3, 34.4, 23.9, 23.4, 23.0, 19.9, 12.1, 11.2 ppm; MS *m/z* 511 $[M+H]^+$.

***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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$179.1, 171.8, 169.1, 169.0, 136.4, 134.6, 131.2, 131.0, 130.4, 128.6, 127.3, 112.9, 69.8, 60.0, 54.5, 44.1, 38.3, 34.5, 23.9, 23.4, 22.9, 19.8, 12.1, 11.2 ppm; MS *m/z* 511 $[M+H]^+$.

General procedure for the synthesis of compounds 24–35. DIPEA (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 with CH_2Cl_2 (3×30 mL). The organic lawyer 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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=$ 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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=$171.7, 142.4, 134.5, 133.7, 130.3,

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128.0, 112.9, 69.3, 59.9, 54.3, 44.8, 40.5, 34.2, 23.8, 23.3, 22.9, 19.7, 11.1 ppm; MS *m/z* 465 $[M+H]^+$.

***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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.80$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 158.1, 136.0, 134.3, 131.1, 129.3, 121.4, 113.6, 112.9, 69.3, 59.9, 56.6, 54.3, 44.5, 40.7, 34.1, 23.7, 23.3, 22.8, 19.7, 11.1 ppm; MS *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)-3-methoxybenzenesulfonamide (26)**. Colorless solid (49 mg, 50%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.50$–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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 161.5, 143.6, 134.4, 131.4, 120.0, 119.4, 113.2, 112.9, 69.3, 59.9, 56.2, 44.7, 40.5, 34.2, 23.7, 23.3, 22.8, 19.7, 11.1 ppm; MS *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)-4-methoxybenzenesulfonamide (27)**. Colorless solid (44 mg, 44%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.78$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 164.5, 134.4, 133.8, 130.2, 115.3, 112.9, 69.4, 59.9, 56.2, 44.8, 40.5, 34.2, 23.7, 23.3, 22.8, 19.7, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=8.04$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 139.5, 135.1, 134.2, 132.9, 132.8, 132.2, 128.5, 112.9, 69.2, 59.9, 54.3, 44.6, 40.5, 34.1, 23.7, 23.2, 22.8, 19.7, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.81$–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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 144.5, 136.1, 134.2, 133.7, 132.0, 127.9, 126.4, 112.9, 69.3, 59.9, 54.4, 44.8, 40.5, 34.2, 23.8, 23.3, 22.8, 19.7, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.84$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 141.3, 139.9, 134.2, 130.5, 129.8, 112.9, 69.3, 59.9, 54.3, 44.7, 40.5, 34.1, 23.7, 23.3, 22.8, 19.8, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.87$–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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 160.3 (d, $J_{F-C}=252.3$ Hz), 136.4 (d, $J_{F-C}=8.4$ Hz), 134.4, 131.2, 130.2 (d, $J_{F-C}=13.7$ Hz), 125.8 (d, $J_{F-C}=3.8$ Hz), 118.1 (d, $J_{F-C}=24.2$ Hz), 112.9, 69.2, 59.9, 54.3, 44.7, 40.5, 34.1, 23.8, 23.3, 22.8, 19.7, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.70$–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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 163.9 (d, $J_{F-C}=147.9$ Hz), 144.7 (d, $J_{F-C}=6.5$ Hz), 134.2, 132.5 (d, $J_{F-C}=7.9$ Hz), 124.0, 120.7 (d, $J_{F-C}=21.4$ Hz), 115.0 (d, $J_{F-C}=24.5$ Hz), 112.9, 69.3, 59.9, 54.3, 44.7, 40.5, 34.2, 23.7, 23.3, 22.8, 19.7, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.89$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 166.4 (d, $J_{F-C}=250.8$ Hz), 138.7 (d, $J_{F-C}=3.2$ Hz), 134.4, 130.9 (d, $J_{F-C}=9.3$ Hz), 117.3 (d, $J_{F-C}=22.8$ Hz), 112.9, 69.3, 60.0, 59.9, 54.4, 44.7, 40.5, 34.2, 23.8, 23.3, 22.8, 19.7, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.98$ (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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 161.7 (d, $J_{F-C}=254.7$ Hz), 140.1 (d, $J_{F-C}=3.8$ Hz), 134.5, 130.8, 129.1 (d, $J_{F-C}=8.6$ Hz), 122.9 (d, $J_{F-C}=18.8$ Hz), 118.7 (d, $J_{F-C}=22.4$ Hz), 112.9, 69.3, 59.9, 54.32, 44.7, 40.5, 34.2, 23.8, 23.3, 22.9, 19.7, 11.1 ppm; MS *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%). ^{1}H NMR (CD_3OD, 300 MHz): $\delta=7.80$–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 ppm (t, $J=7.3$ Hz, 3H); ^{13}C NMR (CD_3OD, 75 MHz): $\delta=171.7$, 154.4 (dd, $J_{F-C}=12.5$, 212.0 Hz), 151.1 (dd, $J_{F-C}=12.5$, 209.8 Hz), 139.8 (t, $J_{F-C}=4.2$ Hz), 134.3, 125.6 (dd, $J_{F-C}=3.9$, 7.6 Hz), 119.5 (d, $J_{F-C}=18.5$ Hz), 117.8 (d, $J_{F-C}=19.9$ Hz), 112.9, 69.3, 60.3, 54.3, 44.7, 40.5, 34.2, 23.8, 23.3, 22.8, 19.7, 11.1 ppm; MS *m/z* 501 $[M+H]^+$.

Biology

In vitro dopamine receptor binding assays. The binding affinities of all synthetic compounds were determined at the D_1-like, D_2-like, and D_3 receptors in membranes prepared from the brains of adult male Sprague–Dawley rats (Pel-Freez Biologicals, Rogers, AR, USA). For these assays, all compounds were dissolved in 100% EtOH at a concentration of 5 mM.

[^{3}H]R-(+)-7-OH-DPAT binding assays. [^{3}H]*R*-(+)-7-OH-DPAT binding assays for the D_3 dopamine receptors were performed as described previously under conditions that yield selective labeling of the D_3 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.) per mL. Membranes were incubated with [^{3}H]*R*-(+)-7-OH-DPAT (0.15 nM, SA = 163 Ci mmol^{-1}, GE Healthcare, or SA = 143 Ci mmol^{-1}, PerkinElmer) and various concentrations of the test compounds (10^{-10} to 10^{-4} M). Nonspecific binding

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was defined by 1 μM spiperone. Assay tubes were incubated at 23 °C for 90 min. Reactions were terminated by rapid vacuum filtration. Data were analyzed using SigmaPlot 8.0.2, with $K_D = 0.15$ nM for [^{3}H]7-OH-DPAT.[10] K_i values are expressed as the mean ± SEM of 3–6 independent determinations.

[^{3}H]Spiperone binding assays. [^{3}H]Spiperone binding assays for D_2-like receptors were performed as described previously[11] for [^{3}H]7-OH-DPAT with the following exception. Assays were performed with membranes prepared from rat caudate-putamen, which expresses D_2 receptors at high density, but with very low levels of D_3 receptors, and the final membrane homogenate concentration was 1.5 mg o.w.w. per mL. The assay buffer, 50 mM Tris·HCl, 5 mM KCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$, pH 7.4 at 23 °C, was used to optimize conditions for agonist binding.[12] The concentration of [^{3}H]spiperone (24 Ci mmol^{-1}, SA = 105 mmol, GE Healthcare; SA = 60, American Radiolabeled Chemicals; or SA = 83.4, PerkinElmer) 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 from the experimentally determined K_D value for [^{3}H]spiperone of 0.4 nM. For those compounds that produced a competition curve consistent with two-site inhibition, which is consistent with the behavior of agonists in this assay, the K_i value for the high-affinity component is reported.

[^{3}H]SCH23390 binding assays. [^{3}H]SCH23390 (7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benxazepin-8-ol) binding assays for D_1-like dopamine receptors were performed as described previously[13] for [^{3}H]spiperone binding, except the concentration of [^{3}H]SCH23390 (SA = 73 Ci mmol^{-1}, GE Healthcare or SA = 60 Ci mmol^{-1}, Americal Radiolabeled Chemicals) used was 0.3 nM. K_i values were calculated from the K_D value for [^{3}H]SCH23390 of 0.3 nM.

DA D_3 mitogenesis functional assays. CHOp-D_3 cells were maintained in alpha-MEM with 10% fetal bovine serum (FBS, Atlas Biologicals), 0.05% penicillin/streptomycin, and 400 μg mL^{-1} of G418. To measure D_3 stimulation of mitogenesis (agonist assay) or inhibition of quinpirole stimulation of mitogenesis (antagonist assay), CHOp-D_3 cells were seeded in a 96-well plate at a concentration of 5000 cells per 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 solution (1.1× 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 [^{3}H]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 10× trypsin solution (1% trypsin in calcium-/magnesium-free phosphate-buffered saline [PBS]) and the plates were filtered and counted. Quinpirole was assayed daily as an internal control, and dopamine was included for comparative purposes.

Data analysis: For functional assays, GraphPad Prism 6 was used to calculate either EC_{50} (agonists) or IC_{50} (antagonists) values using data expressed as pg cAMP for adenylate cyclase activity and percent quinpirole stimulation for mitogenesis.

Human liver microsomal stability assays. Each test compound (1 μM) was metabolized at 37 °C by incubating the test compound with 0.5 mg mL^{-1} human liver microsomes and 1 mM NADPH cofactor in a total volume of 400 μL of 100 mM potassium phosphate buffer (pH 7.4 containing 3.3 mM $MgCl_2$). The reactions were stopped at 0, 5, 10, 15, 30, and 60 min, by adding threefold the volume of CH_3CN containing 100 nM of internal standard. The collected fractions were centrifuged for 10 min at 21130 *g* to collect the supernatant for LC–MS/MS analyses, from which the amount of parent compound remaining was determined. The natural logarithm of the amount of parent compound remaining was plotted against time to calculate the rate of disappearance and half-life of the tested compounds. Hydrolysis of **2**, **3**, **20**, **21**, and **23** in human liver microsomes and a PBS-only solution was monitored under similar conditions. Each compound (1 μM) was incubated with 0.5 mg mL^{-1} human liver microsomes in PBS solution or with PBS only for 60 min. No change in the concentration of three compounds at 60 min relative to that at time zero suggests little hydrolysis had occurred for the three compounds in PBS solution with or without human liver microsomes.

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