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Highly Selective Dopamine D₃ Receptor Antagonists with Arylated Diazaspiro Alkane Cores

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Abstract

A series of potent and selective D₃ receptor (D₃R) analogues with diazaspiro alkane cores were synthesized. Radioligand binding of compounds **11**, **14**, **15a**, and **15c** revealed favorable D₃R affinity ($K_i = 122\text{--}25.6$ nM) and were highly selective for D₃R vs D₂R (ranging from 264–905-fold). Variation of these novel ligand architectures can be achieved using our previously reported 10–20 minute benchtop C–N cross-coupling methodology, affording a broad range of arylated diazaspiro pre-cursors.

Graphical abstract

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications. Molecular formula strings (CSV), along with ¹H and ¹³C NMR spectra, and mass spectral data of isolated compounds **6**, and **8–16** (PDF).

Author Contributions

The manuscript was written through contributions of all authors./All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Keywords

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INTRODUCTION

Dopamine is a crucial neurotransmitter that acts by stimulating G-protein coupled receptors responsible for many neurological processes such as emotion, reward, and motivation.¹ These receptors are classified into two subtypes, D₁-like and D₂-like, based on sequence identity and similarity in signal transduction. The D₂-like dopamine D₃ receptor (D₃R) is a protein of interest for various neurological and neuropsychiatric disorders including schizophrenia, Parkinson's disease, dementia, anxiety and depression.^{2–5} The high appeal of the D₃R stems from the high density of its expression within the mesolimbic pathway of the CNS.^{6–9} This region of the brain is responsible for the reward and motivational mechanisms associated with drug addiction, thus, making the D₃R an attractive target for pharmacological therapy in substance abuse disorders (SUD).^{10–13} However, the similarities shared between the D₃R and D₂R, such as an overall ~46% amino acid sequence homology, a 78% sequence identity within the transmembrane-spanning segments,¹⁴ and the near-identical binding site residues of these receptors,¹⁵ have made the development of a FDA approved D₃R selective therapeutic quite challenging.

Many previously reported ligand architectures designed to selectively target the D₃R employ the classic arylpiperazine amino template to enhance ligand affinity to the receptor (**1–3**, Figure 1).^{2, 16} In 2010, Reichert and co-workers used computational modeling to illustrate the extensive interactions between the amino component of the ligand and the D₃R orthosteric binding site (OBS).¹⁷ Their modeling experiments indicated ligand binding efficacy is due to a salt bridge formation between the Asp^{3.32} of the receptor and the protonated nitrogen on the amino moiety. This binding interaction was later confirmed in the D₃R crystal structure, with later reports correlating ligand affinity to OBS interactions, and D₃R selectivity to secondary binding pocket interactions.¹⁸ As a result, these molecular determinants provide a challenging path for medicinal chemists to develop potent and

selective D₃R ligand scaffolds with amino moiety alternatives to the arylpiperazine pharmacophore.¹⁹

Over the past several years, reports have emerged with 1,2,4-triazole-based scaffolds with notable D₃R vs. D₂R efficacy and selectivity.^{1, 11, 20} GlaxoSmithKline (GSK) disclosed several 1,2,4-triazole-based compounds with excellent D₃R affinity and selectivity profiles,^{21–23} most notably, **GSK598,809 (4)**, the first D₃R selective antagonist to exhibit clinical evidence as a potential therapeutic for substance abuse disorders.²⁴ More recently, Micheli and co-workers reported a D₃R antagonist with an azaspiro alkane moiety (**5**), which displays sub-nanomolar affinity and excellent D₃R selectivity over the D₂R.²⁵ Encouraged by these reports, we designed a new class of thiotriazole D₃R selective scaffolds using commercially available diazaspiro alkanes **A–H** (Figure 2) as modified amino cores. Spiro synthons provide spatial modifications that are otherwise non-accessible to saturated six-membered rings such as piperazine, thus, affording unique interactions in the D₃R binding pocket. Moreover, spirocyclic compounds exhibit lower lipophilicity compared to their monocyclic counterparts, resulting in increased bioavailability.^{26, 27} Herein we report the synthesis and *in vitro* studies of diazaspiro-containing 1,2,4-triazole-3-thiol ligand systems.

RESULTS AND DISCUSSION

Initial synthesis of the target compounds began with arylation of amino cores **A–H** outlined in Scheme 1. Arylated diazaspiro synthons (**I**) and **6** were afforded in high yields in just 10–20 min following our previously reported one-pot Pd C–N cross-coupling methodology.^{28, 29} This catalytic protocol can be conducted under aerobic conditions, thus eliminating the need for an inert atmosphere or anhydrous solvents.

S-Alkylation of **K** with 1-bromo-3-chloropropane afforded **7** in high yield (92%) using a modified synthetic route from a previous report (Scheme 2).³⁰ Compound **7** was then reacted with the appropriate arylated diazaspiro free amine intermediate (**J**) to form derivatives **8–16** illustrated in Figure 3. Target compounds can also be developed using the alternative synthetic route depicted in Scheme 2, by reacting the appropriate alkylated intermediate **L** with **K** in the presence of trimethylamine in ethanol.

Our initial radioligand binding studies revealed diazaspirocycles **A–B** as poor amino core candidates in the 1,2,4-triazole scaffold (Table 1, compounds **8–9**). Attempts to improve D₃R efficacy with **B** were also unsuccessful with ortho-substituted fluorobenzene, resulting in diminished D₃R affinity of **9b**. Binding activity and selectivity improved with **10**, containing slightly bulkier core **C**, when compared to **A** and **B**. When investigating **D** in compound **11**, we observed moderate affinity ($K_i = 24.2$ nM) and good selectivity (D₃R/D₃R = 264) at the D₃R. Replacing **D** with amino cores **E** and **F** resulted in lower D₃R potency and selectivity compared to **11**. Compounds **13** and **14**, however, displayed more promising binding profiles than **8–9**, scaffolds also with azetidine containing diazaspiro cores (**A–C**).

When investigating bulkier diazaspiro cores **G–H** as potential amino surrogates with the selected 1,2,4-triazole system, a noticeable increase in D₃R potency and selectivity was

observed. For example, compounds **14** and **15a** exhibited good binding D₃R affinity (K_i = 19.6 nM and 12.0 nM, respectively), with **15a** demonstrating over 900-fold selectivity. Introduction of the –OCH₃ functional group in *para* position (**15b**) led to a reduction in receptor affinity (K_i = 97.7 nM), and a slight increase in D₃R selectivity. Replacing the aryl group with a para-substituted fluorobenzene on the **H** amino moiety (compound **15c**) resulted in moderate D₃R binding affinity (K_i = 25.6 nM) and selectivity (383-fold selectivity) in contrast to **15a**. The insertion of a benzonitrile and pyridine moiety (**15d** and **15e**, respectively) led to significant regression in D₃R affinity and selectivity. Diazaspiro synthon **6** was evaluated as a potential bioisostere for the classical dichlorophenylpiperazine displayed in many antipsychotics. However, the binding profile for **15f** revealed decreased D₃R affinity (K_i = 82.4 nM) and selectivity (91.0-fold) in comparison to lead compound **15a**.

We also examined the D₃R binding profile of compound **16**, a piperazine congener of lead compound **15a**. Although compound **16** demonstrated a slightly higher affinity at the D₃R (K_i = 6.5 nM), a significant decrease in D₃R/D₂R selectivity was observed (~40-fold) in contrast to **15a**. This direct comparison illustrates the potential of spiro system **H** to act as a viable alternative to the classical arylpiperazine pharmacophore in D₃R ligand frameworks.

Compounds **14**, **15a**, **15c**, and **16** were then evaluated for human serotonin 5-HT_{1A} receptor (5-HT_{1A}R) binding affinity (Figure 4), a common off-target binding receptor with piperazine containing D₃R scaffolds.^{31–35} As suspected, we observed low binding profiles at the 5-HT_{1A} receptor with selected spiro compounds **14** (K_i = 724 nM), **15a** (K_i = 931 nM), and **15c** (K_i = 587 nM). It should also be noted, we briefly furthered the profiles for compounds **14**, **15a**, and **15c** at the 5-HT_{2A} and 5-HT_{2C} receptors as well, and observed low binding affinities comparable to those obtained at the 5-HT_{1A}R (data not shown). Piperazine analogue **16**, however, expressed a 0.22 nM binding affinity to the 5-HT_{1A}R, in contrast to the receptor affinity observed with lead compound **15a**. In regards to our current research focus in PET probes development, eliminating off-target affinities of D₂-like radioligands, such as for serotonin receptors, is pivotal in developing a radiotracer that can image the D₃R in clinical settings.

Finally, we evaluated compounds **11**, **14**, **15a**, and **15c** for functional activity using adenylyl cyclase and β -arrestin recruitment assays (Figure 5). With the exception of **15a** acting as a partial agonist for adenylyl cyclase, compounds **11**, **14**, and **15c** had low activity in these assays, thus, consistent with their being antagonists at D₃R.

CONCLUSION

We have identified several ligand architectures containing modified amino cores that exhibit excellent selectivity and good affinity at the D₃R. In addition, diazaspiro cores **D**, **G**, and **H** alleviates serotonin binding, a common off-target effect of piperazine containing D₃R ligands, illustrated by **16**. Access to arylated diazaspiro synthons can be readily achieved in just 10–20 minutes using our previously reported C–N cross-coupling conditions, affording a convenient synthetic route to these novel D₃R scaffolds. Investigation is currently ongoing

to further improve D₃R binding profiles of these compounds and will be reported in due course.

EXPERIMENTAL SECTION

General

Chemical compounds **A–H** and **K** were purchased and used without further purification. NMR spectra were taken on a Bruker DMX 500 MHz. Compound structures and identity were confirmed by ¹H and ¹³C NMR, and mass spectroscopy. Compound purity greater than 95% was determined by LCMS analysis using a 2695 Alliance LCMS. All other commercial reagents were purchased and used without further purification. Purification of organic compounds were carried out on a Biotage Isolera One with a dual-wavelength UV-VIS detector. Chemical shifts (δ) in the NMR spectra (¹H and ¹³C) were referenced by assigning the residual solvent peaks.

Synthesis for Compound 7

3-((3-chloropropyl)thio)-4-methyl-5-phenyl-4H-1,2,4-triazole (7). A mixture of **K** (2.00 g, 10.46 mmol), 1-bromo-3-chloropropane (10.34 mL, 104.60 mmol), and K₂CO₃ (2.17 g, 15.00 mmol) were stirred in acetone (30.00 mL) at room temperature for 20 h. The crude reaction mixture was then filtered and solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel eluting with hexane/EtOAc (5:2) affording 2.59 g, 92% yield (white solid). ¹H NMR (500 MHz, CDCl₃) δ 7.55–7.53 (m, 2H), 7.41–7.39 (m, 3H), 3.63 (t, *J* = 6.1 Hz, 2H), 3.52 (s, 3H), 3.32 (t, *J* = 6.8 Hz, 2H), (quint, *J* = 6.4, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 155.9, 151.2, 130.0, 128.8, 128.5, 127.0, 53.4, 43.1, 31.9, 31.5, 29.9; LC-MS (ESI) *m/z*: 268.05 [M+H].

General Method for Preparing Diazaspiro Analogues 8–16

The appropriate arylated diazaspiro and piperazine compounds (**I**), obtained following our previous report,^{28, 29} were dissolved in CH₂Cl₂ (2 mL), followed by dropwise addition of CF₃COOH (2 mL), and stirred at room temperature for 3 h. Volatiles were then removed under reduced pressure and the crude product was neutralized with a saturated NaHCO₃ (aq) solution (10 mL). The reaction mixture was extracted with CH₂Cl₂ (3 × 20 mL), and the organic layers were combined, dried, and concentrated to afford the free-amine intermediates (**J**) that were used as such in the following steps.

An equimolar mixture of the appropriate intermediate **J** (0.50 mmol), **7** (0.50 mmol), and Cs₂CO₃ (1.0 mmol) was stirred in acetonitrile (5 mL) at 70 °C for 12 h. The crude reaction mixture was then filtered and solvent was removed under reduced pressure. The residue was loaded onto a Biotage SNAP flash purification cartridge and eluted with 10% 7N NH₃ in MeOH solution/CH₂Cl₂ to give the target compounds **8–16**.

Alternative Method for Preparing Diazaspiro Analogues 8–16

A mixture of **J** (1 mmol), 1-bromo-3-chloropropane (2 mmol), and K₂CO₃ (1.5 mmol) was stirred in acetone (5 mL) at room temperature for 20 h. The crude reaction mixture was then filtered and solvent was removed under reduced pressure. The residue was loaded onto a

Biotage SNAP flash purification cartridge and eluted with 5% MeOH in CH₂Cl₂ affording intermediates **L**.

A mixture of **K** (1 mmol), TEA (1.5 mmol), and ethanol (10 mL) was stirred at 75 °C for 15 min. The appropriate intermediate **L** (1 mmol) was then added, and the solution was stirred at 75 °C for 12 h. Solvent from the crude reaction mixture was then removed under reduced pressure. The residue was loaded onto a Biotage SNAP flash purification cartridge and eluted with 10% 7N NH₃ in MeOH solution/CH₂Cl₂ to give the target compounds **8–16**.

Compounds **8–16** were taken up with CH₂Cl₂ followed by dropwise addition of a 2.0M HCl solution in diethyl ether. After stirring at rt for 1 h, the solvent was removed under reduced pressure to afford the desired compound as a hydrochloride salt for *in vitro* studies.

Receptor Binding Assays

The binding properties of membrane-associated receptors were characterized by a filtration binding assay.³⁶ For human D₂R (long isoform) and D₃R expressed in HEK 293 cells, membrane homogenates were suspended in 50 mM Tris-HCl/150 mM NaCl/10 mM EDTA buffer, pH 7.5 and incubated with [¹²⁵I]IABN³⁶ at 37 °C for 60 min, using 20 μM (+)-butaclamol to define the nonspecific binding. Human 5-HT_{1A}R binding was assessed using membranes from heterologously expressing CHO-K1 cells (PerkinElmer, Waltham, MA), suspended in buffer containing 50 mM Tris-HCl, 10 mM MgSO₄, 0.5 mM EDTA, and 0.1% (w/v) ascorbic acid, pH 7.4 and incubated with [³H]-8-OH-DPAT (PerkinElmer) at 27 °C for 60 min. Nonspecific binding was determined in the presence of 10 μM metergoline (Tocris Bioscience, Bristol, UK).

The radioligand concentration was equal to approximately 0.5 (D_{3/2}R) or 1.5–2 (5-HT_{1A}R) times the K_d value, and the concentration of the competitive inhibitor ranged over 5 orders of magnitude for competition experiments. For each competition curve, two concentrations of inhibitor per decade were used, and triplicates were performed. Binding was terminated by the addition of ice cold wash buffer (D_{2/3}R; 10 mM Tris-HCl, 150 mM NaCl, pH 7.5, 5-HT_{1A}R; 10 mM TrisHCl, pH 7.4) and filtration over a glass-fiber filter (D_{3/2}R; Schleicher and Schuell No. 32, 5-HT_{1A}R; Whatman grade 934-AH, GE Healthcare Bio-Sciences, Pittsburgh, PA). Packard Cobra (D_{3/2}R) or PerkinElmer MicroBeta2 (5-HT_{1A}R) scintillation counters were used to measure the radioactivity. The equilibrium dissociation constant and maximum number of binding sites were generated using unweighted nonlinear regression analysis of data modeled according to the equation describing mass R-binding. The concentration of inhibitor that inhibits 50% of the specific binding of the radioligand (IC₅₀ value) was determined by using nonlinear regression analysis to analyze the data of competitive inhibition experiments. Competition curves were modeled for a single site, and the IC₅₀ values were converted to equilibrium dissociation constants (K_i values) using the Cheng and Prusoff³⁷ correction. Mean K_i values ± SEM are reported for at least three independent experiments.

β-Arrestin Assay

The PathHunter eXpress human D₃ dopamine receptor-expressing human bone osteosarcoma epithelial cell line-based (U2OS cell line) β-Arrestin GPCR Assay kit (DiscoverX) was used to determine the efficacy of test compounds for β-arrestin-2 binding. The PathHunter® β-Arrestin D₃ receptor cell line was genetically engineered to co-express a ProLink™ (PK) tagged receptor and the Enzyme Acceptor (EA) tagged β-Arrestin. Activation of the Dopamine D₃ receptor-PK chimeric protein induces β-Arrestin-EA binding, leading to complementation of two β-galactosidase enzyme fragments (EA and PK), resulting in a functional enzyme that is capable of hydrolyzing substrate and generating a chemiluminescent signal. Following the manufacturer's protocol, the D₃ dopamine receptor expressing U2OS cells are seeded at a concentration of 10,000 cells per well, in white, 96-well, clear-bottomed plates that are provided with the kit. After a 48 hour incubation (37°C, 5% CO₂), test compound or control compounds (quinpirole included as a prototypical full agonist and haloperidol as a prototypical antagonist) are added (at a dose of 10x the K_i value) to the appropriate wells and incubated for 90 minutes (37°C, 5% CO₂). Kit substrate buffer is added (room temperature, 60 min in the dark) to each well and the luminescence is determined using an EnSpire Alpha 2390 multilabel plate reader (Perkin Elmer).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

ACN	acetonitrile
AMP	adenosine monophosphate
CNS	central nervous system
DCM	dichloromethane
D₂R	dopamine D ₂ receptor
D₃R	dopamine D ₃ receptor
HEK cells	human embryonic kidney 293 cells
[¹²⁵I]IABN	[¹²⁵ I]-N-benzyl-5-iodo-2,3-dimethoxy[3.3.1]azabicyclononan-3-β-ylbenzamide
Pd	palladium
PET	positron emission tomography

TFA

trifluoroacetic acid

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Figure 1.
Chemical structure and binding data of D₃R selective antagonists as reported in the literature.

Figure 2.
Diazaspiro compounds evaluated as amino cores.

Figure 3.
Chemical structures of synthesized target compounds.

Figure 4. Competition curve of **14**, **15a**, **15c**, and **16** at the 5-HT_{1A}R. [³H]8-OH-DPAT used as competitive substrate in binding assay. Data shown are determined by at least three experiments.

Figure 5.

The efficacy of compounds to inhibit forskolin-dependent stimulation of adenylyl cyclase and β-arrestin binding is shown. Each test compound was used at a concentration equal to 10x the K_i value. The bar graph represents the mean percent efficacy $\pm$ SEM relative to the full agonist Quinpirole. Mean $\pm$ SEM values were determined by at least three experiments. Haloperidol was included as a prototypical antagonist.

Scheme 1.

^aReagents and conditions: (a) $\text{Pd}_2(\text{dba})_3$, RuPhos, aryl halide, **A-H**, $\text{NaO}t\text{-Bu}$, dioxane, 100 °C, 20 min; (b) TFA, DCM, RT, 3h.

Scheme 2.

^aReagents and conditions: (a) 1-bromo-3-chloropropane, K₂CO₃, acetone, RT, 20 h; (b) Cs₂CO₃, ACN, 70 °C, 12 h; (c) 1-bromo-3-chloropropane, K₂CO₃, acetone, RT, 20 h; (d) TEA, EtOH, 75 °C, 12 h.

Table 1

D₃ and D₂ Receptor Binding Affinities of Diazaspiro Analogues^a

Compound	Ar	$K_i^b \pm SEM$ (nM)		D ₃ R/D ₂ R Ratio ^e	cLog P ^f
		D ₃ R ^c	D ₂ R ^d		
8	2-OCH ₃	833 ± 154	21,216 ± 4,636	25.5	3.95
9^a	2-OCH ₃	4,790 ± 280	114,945 ± 31,960	24.0	3.85
9^b	4-F	18,188 ± 2,284	>109,574 ± NA	>6.0	4.23
10	2-OCH ₃	192 ± 2.3	60,152 ± 13,327	313	3.90
11(rac)^g	2-OCH ₃	24.2 ± 5.6	6,370 ± 1,145	264	4.16
12	2-OCH ₃	235 ± 41.1	37,579 ± 1,806	160	3.76
13	2-OCH ₃	169 ± 20.2	29,300 ± 3,139	173	3.76
14	2-OCH ₃	19.6 ± 4.7	6,168 ± 939	315	4.32
15^a	2-OCH ₃	12.0 ± 2.8	10,895 ± 2,069	905	4.88
15^b	4-OCH ₃	97.7 ± 17.4	104,847 ± 29,076	1,073	4.88
15^c	4-F	25.6 ± 5.6	9,792 ± 1,790	383	5.32
15^d	pyridine-2-yl	871 ± 66.9	83,671 ± 62,789	96.1	4.05
15^e	3-CN	438 ± 33.6	57,101 ± 29,507	130	4.83
15^f	2,3-Cl	82.4 ± 1,053	7,501 ± 12.9	91.0	6.54
16	2-OCH ₃	6.5 ± 0.88	260 ± 44.2	40.2	4.09

^aSpiperone assayed under the same conditions as a reference blocker D₂R 0.06 (nM) ± 0.001; D₃R 0.33 (nM) ± 0.02; D₄R 0.45 (nM) ± 0.01.

^bMean ± SEM, K_i values were determined by at least three experiments.

^cK_i values for D₃ receptors were measured using human D₃ expressed in HEK cells with [¹²⁵I]ABN as the radioligand.

^dK_i values for D₂ receptors were measured using human D₂ expressed in HEK cells with [¹²⁵I]ABN as the radioligand.

^eK_i for D₃ receptors/K_i for D₂ receptors.

^fCalculated using ChemDraw Professional 15.1.

$\vec{g}_{rac} = \text{racemate.}$

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