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Synthesis and *in vitro* pharmacological evaluation of indolyl carboxylic amide analogues as D₃ dopamine receptor selective ligands[†]

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Abstract

A series of substituted 1*H*-indolyl carboxylic acid amides that contain a *N*-(2-methoxyphenyl)piperazine or *N*-(2-fluoroethoxy)piperazine group were synthesized and their affinities for human dopamine D₂, D₃, and D₄ receptors were determined. Two of these compounds, **14a** and **14b**, displayed high binding affinity at D₃ ($K_i = 0.18$ and 0.4 nM, respectively), and selectivity for D₃ vs. D₂ receptors (87-fold and 60-fold, respectively). These two compounds had low binding affinity at D₄ receptors and receptor sites. The intrinsic activity of these compounds at D₂ and D₃ receptors was determined using a forskolin-dependent adenylyl cyclase inhibition assay; both **14a** and **14b** were found to be partial agonists. Furthermore, for compound **14a**, the log *D* value of 2.85 suggested it has suitable lipophilicity for crossing the blood–brain-barrier.

Introduction

Dopamine receptors are members of the G protein-coupled receptor (GPCR) family of proteins which consist of seven putative transmembrane domains. Dopamine receptors are expressed in both the vertebrate central nervous system (CNS) and in the kidney.^{1,2} They are classified into two major types, the D₁-like and D₂-like receptors based on their sequence and similarity in signal transduction. D₁-like and D₂-like receptors can be further divided into five subtypes. D₁ (D_{1a}) and D₅ (D_{1b}) receptors belong to the D₁-like receptor type and the D₂, D₃ and D₄ receptors belong to the D₂-like receptor type. Agonist activation of D₁-like dopamine receptors results in an increase in adenylyl cyclase activity. Stimulation of the D₂-like receptors results in an inhibition of adenylyl cyclase activity, an increase in the release of arachidonic acid, activation of G protein-coupled inwardly-rectifying potassium channels (GIRKs), activation of phospholipase D (PLD) and an increase in phosphatidylinositol hydrolysis.³ D₂ and D₃ receptors have ~46% overall amino acid sequence homology and 78% sequence homology within the transmembrane spanning segments.⁴

The D₃ receptor subtype was first cloned and characterized in 1990.⁴ Initial neuroanatomical studies indicated that D₃ receptors are expressed primarily in the nucleus accumbens and the

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islands of Calleja. Subsequent studies suggested that D₃dopamine receptors play a role in various neurological and neuropsychiatric disorders,^{5,6} including Parkinson's disease schizophrenia and drug addiction.^{4,7,8} It has been suggested that even moderately selective D₃ versus D₂ dopamine receptor selective compounds might offer a favorable therapeutic profile compared to currently available antipsychotics.^{9,10}

The D₃ receptor has been reported to up-regulate in animal models of L-dopa induced dyskinesia (LID).^{11–13} D₃ receptor selective ligands have also been shown to attenuate L-DOPA-induced abnormal involuntary movements in hemi-parkinsonian rats, which suggest that the D₃ receptor may be an important therapeutic target for the treatment of LID.^{14–18} Finally, the positive reinforcing effects of psychostimulants, such as cocaine and methamphetamine, may be mediated partially by the stimulation of D₃ receptors. Thus, D₃ receptor selective partial agonists and/or antagonists may be useful pharmacotherapeutic agents for the treatment of substance abuse.^{19–24}

Although Positron Emission Tomography (PET) radiotracers have been used to study the expression of dopamine receptors in the brain, the lack of highly selective D₃ PET radioligands has prevented the quantification of D₃ receptor expression in humans.^{3,5,23,25–28} Our group and others have reported a number of conformationally-flexible benzamide analogues with high binding affinity and high selectivity for the D₃ receptor.^{3,27,29} Recently, we reported the radiotracer [¹⁸F]**1** (K_i = 0.17 nM for D₃ receptor, D₂/D₃ ratio = 163) as a promising PET probe for quantifying D₃ density in the nonhuman primate brain; the validation of [¹⁸F]**1** for clinical imaging studies of the D₃ receptor is currently ongoing.^{29–31} Amides **2–8** (Fig. 1) of indole-benzofuran or benzothiofuran carboxylic acids and 4-(4-(2-methoxy)phenyl)piperazin-1-yl)butan-1-amine, or 4-(4-(2-(2-fluoroethoxy))phenyl)piperazin-1-yl)butan-1-amine, were previously reported to have high D₃ affinity and good selectivity for D₃ versus D₂ and D₄.^{27,32,33}

Although indolyl carboxamides containing substituted phenylpiperazinyl groups have been previously reported as D₃ receptor selective ligands,³⁴ exploration of the structure–activity relationships regarding substitution of the indolyl ring is relatively unknown. In this article, we report the synthesis and *in vitro* evaluation of a series of substituted 1*H*-indolyl-carboxylic acid, phenylpiperazine amides in which the structure is altered by (1) introducing a different substitution at the 5 position of the 1*H*indole-carboxylic group of the amides that contain a (2-substituted-phenyl piperazin-1-yl)butan-1-amine or (2-substituted-phenyl piperazin-1-yl)buten-1-amine moiety, (2) using a PEGylation strategy to extend the fluoroethoxy in the 5-position of 1*H*-indolyl-2-carboxylic group to reduce the lipophilicity of the target compounds, (3) replacing the substituted indole-2-carboxylic group with a substituted indole-3-carboxylic group and (4) replacing the saturated four-carbon spacer with a *trans* double bond. The results of this study indicate that substituting the 5-position of the indolyl carboxamide with relatively bulky substituents such as a PEGylated group results in only a minor reduction in affinity to the D₃ receptor.

Results and discussion

Chemistry

The target compounds were synthesized according to Schemes 1 and 2. The substituted 4-(4-phenyl)piperazinyl primary amines (Fig. 2), 4-(4-(2-methoxy)phenyl)piperazin-1-yl)butan-1-amine (**9a**), 4-(4-(2-(2-fluoroethoxy))phenyl)piperazin-1-yl)butan-1-amine (**9b**), 4-(4-(2-methoxy)phenyl)piperazin-1-yl)-*trans*-but-2-en-1-amine (**9c**), and 4-(4-(2-(2-fluoroethoxy))phenyl)piperazin-1-yl)-*trans*-but-2-en-1-amine (**9d**) were synthesized by following literature procedures and the *trans* structure of the double bond in **9c** and **9d** was confirmed by their NMR data.²⁹ In this manuscript, we focused on the synthesis of substituted carboxylic acid

amides obtained by coupling substituted indole carboxylic acids and different substituted *N*-phenylpiperazine amines. The syntheses of the substituted indolyl carboxylic acids (**13a–c**) were accomplished as outlined in Scheme 1. Ethyl 5-hydroxy-1*H*-indole-2-carboxylate (**10**) was reacted with 1-bromo-2-fluoroethane *via* *O*-alkylation in acetone using potassium carbonate as the base to afford intermediate compound **12a**. Hydrolysis of the ethyl ester in a solution of sodium hydroxide in methanol–water afforded the corresponding 5-(2-fluoroethoxy)-1*H*-indole-2-carboxylic acid **13a**. The syntheses of the fluoro-PEGylated 1*H*-indole-2-carboxylic acids (**13b** and **13c**) were accomplished in three steps as shown in Scheme 1. First, *O*-alkylation of the phenol group of 5-hydroxy-1*H*-indole-2-carboxylic acid ethyl ester (**10**) using either 2-(2-chloroethoxy)ethanol or 2-(2-(2-chloroethoxy)-ethoxy)-ethanol in the presence of potassium carbonate in dimethylformamide (DMF) afforded 5-hydroxy-PEGylated-1*H*-indole-2-carboxylic acid ethyl esters (**11b** and **11c**). Second, the conversion of hydroxy compounds **11b** and **11c** to fluoro compounds **12b** and **12c** was accomplished in moderate yields with diethylamino sulphur trioxide (DAST). Third, hydrolysis of **12b** and **c** with sodium hydroxide in aqueous methanol solution afforded PEGylated-1*H*-indole-2-carboxylic acids **13b** and **c**.

The desired substituted indolyl-carboxylic acid amides, **14a–d**, **15a–d**, **16a–c** and **17a–e**, were prepared by coupling substituted 4-phenylpiperazine amines **9a–d** with corresponding substituted indolyl-carboxylic acids, using *N,N*-dicyclohexyl-carbodiimide (DCC) as coupling agent in dichloromethane with 1-hydroxybenzotriazole (HOBt) as a catalyst (Scheme 2). All final compounds were converted into the corresponding oxalic acid salts for *in vitro* binding determination.

In vitro binding studies

Compounds were first evaluated for affinity at human D₂ and D₃ dopamine receptors expressed in stably transfected HEK cells. Analogues which exhibited high binding affinity at D₃ receptor sites and a highly selective ratio for D₃ *versus* D₂ receptor were further evaluated for affinity at (a) D₄ dopamine receptors and (b) α_1 and α_2 receptors. We performed binding assays for the α_1 receptor sites due to the concern that piperazine containing compounds may have high affinity for α_1 receptor site; high α_1 receptor binding affinity could potentially lead to misinterpret the PET imaging measurement of the D₃ expression in the brain.

The binding affinities (K_i values) of the synthesized compounds at D₂ and D₃ receptors were determined using stably transfected cell lines and the high affinity D₂-like dopamine radioligand [¹²⁵I]IABN (Table 1). IC₅₀ values were converted to K_i values according to Cheng and Prusoff equation,³⁵ where the affinity of [¹²⁵I]IABN for the D₂, D₃ and D₄ dopamine receptor subtypes is 0.03 nM, 0.04 nM and 0.60 nM, respectively.³⁶ The ligand binding selectivity index is calculated as K_i (D₂)/ K_i (D₃). For the ensuing discussion, binding affinities are characterized as very high ($K_i < 1.0$ nM), high ($K_i = 1–10$ nM), moderate ($K_i = 11–50$ nM) or low ($K_i > 50$ nM). The log *D* value (pH = 7.4) was calculated by using ACD/log *D* software (Advanced Chemistry Development Inc, Toronto, Canada). Depending on the accuracy of the software used for calculating the log *D* value, there may be differences between the calculated log *D* and experimentally measured log *D* values. However, the ACD/log *D* software is frequently used in the design of D₃ PET tracers.^{23,29,37,38} In this manuscript, the calculated log *D* value serves as the lipophilicity index of a compound. Although other physicochemical properties of a compound impact the blood–brain-barrier (BBB) penetration, those ligands with moderate lipophilicity often exhibit highest brain uptake. For most central nervous system drugs, it was found that the blood–brain-barrier (BBB) penetration is optimal with the log *D* values in the range of 1.5–3.0, with a mean value of 2.5.^{39–41}

The *in vitro* binding data identified a number of potentially useful structure–activity trends. For example, in compounds **14a–d**, the 2-methoxy group was retained in the substituted 4-(4-phenyl)piperazin-1-yl moiety. The single bond that linked the carboxylic acids and the substituted 4-(4-phenyl)piperazin-1-yl amine moiety, and the hydroxyl group in 5-position of the 1*H*-indole-2-carboxy acid was replaced by a 2-fluoroethoxy group or PEGylated with one or two ethoxy units. All four compounds (**14a–d**) displayed high binding affinities for D₃ (K_i values ranges from 0.18 to 1.5 nM) and had a good selectivity for D₃ versus D₂ receptors, with selectivity ratios ranging from 20- to 87-fold. Compound **14a** (K_i values of 15.7 nM for D₂, 0.18 nM for D₃ receptors, 87-fold selectivity) replaces the hydrogen atom in the 5-position of the indolyl-2-carboxylic group of compound **2** with a hydroxyl group. Comparing compound **14a** to compound **2** (K_i values of 37.4 nM for D₂, 0.33 nM for D₃ receptors, 123-fold selectivity), the affinities of compound **14a** binding to D₂ and D₃ receptor sites increased 2.4-fold and 1.7-fold, respectively. However, the ratios for D₃ versus D₂ decreased slightly from 123-fold to 87-fold. Introduction of the hydroxyl group resulted in a calculated log *D* value reduction from 3.6 (**2**) to 2.85 (**14a**). A high log *D* value means the molecule has a high lipophilicity and may bind strongly to plasma proteins, thereby lowering its free fraction and diminishing its ability to cross the BBB. Assuming other properties being equal (*i.e.*, metabolism, substrate for PgP) compound **14a** should have a higher brain uptake than compound **2** because of its lower log *D* value. For compounds **14b–d**, the hydroxyl group in **14a** was replaced with a fluoroethoxy or PEGylated fluoroethoxy group with one or two ethoxy units. The binding affinities for both D₂ and D₃ receptors of compounds **14b–d** are slightly decreased when compared to **14a**; overall selectivity ratios for D₃ versus D₂ were 20-fold. More importantly, the D₃ receptor binding affinity remained high (K_i values of 0.4, 1.1, and 1.5 nM for **14b**, **14c**, and **14d** respectively). Therefore, substitution of the hydroxyl group with a fluoroethoxy or PEGylated fluoroethoxy group does not alter the affinities of compounds to bind to D₃ receptors. However, PEGylation of the 2-fluoroethoxy group decreases the log *D* values of ligands to make the target compound less lipophilic, which should improve the ability of the compound to cross the BBB. Among the four compounds (**14a–d**), compound **14a** not only displays sub-nanomolar affinity for D₃ receptor (K_i value = 0.18 nM) and had 87-fold selectivity for D₃ versus D₂ receptors, the methoxy group in **14a** provides a site for carbon-11 labeling with [¹¹C]methyl iodide.

Compared to compound **2** and **14a**, compound **15a** and **15b** were synthesized by introducing a *trans* double bond in the 4-carbon linker between the carboxyl group and the 4-phenylpiperazine moiety. In addition, the methoxy group in the substituted 4-phenylpiperazine moiety was replaced with a fluoroethoxy group. The *in vitro* binding data indicate that **15a** (2.0 nM) and **15b** (4.0 nM) are able to retain a similar affinity at D₃ receptors with moderate selectivity for D₃ versus D₂ receptors. These changes had little impact on the affinity at D₂ receptor but decreased the D₃ binding potency approximately 6.7-fold (**15a** versus **2**) and 22-fold (**15b** versus **14a**) respectively. Compound **15c** was made *via* a 5-methoxy group replacement of the 5-hydroxy group in the indole-2-carboxylic moiety of **15b**. Compared to compound **15b**, the D₂ binding affinity of **15c** (K_i value of 66.3 nM) decreased from **15b** (K_i value of 33.6 nM). Concurrently, the D₃ binding affinity of **15c** (K_i = 3.1 nM) increased slightly from **15b** (K_i = 4.0 nM). Together, the D₂/D₃ selectivity ratio improved from **15b** (8.4-fold) to **15c** (21.4-fold).

Compounds **15a–c** contain a double bond linker between the indole-2-carboxyl acid and the piperazinyli moiety. The difference in the molecular structures of compounds **15a**, **15b**, and **15c** is the substituted group in 5-position of the indole-2-carboxylic section. As shown in Table 1, the D₃ binding affinities displayed the following trend: OH < OCH₃ < H with K_i values of 4.0, 3.1, and 2 nM for **15b**, **15c** and **15a** respectively. The D₂ binding affinities displayed a different trend: OCH₃ < H < OH with K_i values of 66.3, 44.4, and 33.6 nM, for

15c, **15a** and **15b** respectively. Overall, **15a**, **15b** and **15c** bind with subnanomolar affinity at D₃ receptors (K_i value for D₃ < 5 nM) and their binding selectivity ratios are 20-, 8-, and 21-fold for **15a**, **15b** and **15c** respectively.

Compound **15d** (K_i = 119.1 nM for D₂ and 4.4 nM for D₃ receptors) is structurally similar to **15c**. The difference between these two analogues is that the fluoroethoxy in the substituted 4-phenylpiperazinyl fragment of **15c** was replaced with a methoxy group. This resulted in an almost 2-fold reduction in D₂ binding affinity (K_i of 66.3 nM for **15c** versus 119.1 nM for **15d**) while the K_i value for the D₃ binding was slightly improved (3.1 nM for **15c** and 4.4 nM for **15d**). Thus, the selectivity ratio for D₃ versus D₂ receptor improved only slightly from 21-fold to 27-fold.

Compounds **16a–c** were synthesized to evaluate the effect of introducing a double bond linkage between the carboxylic group and the 4-phenylpiperazinyl moiety with respect to binding to D₂ and D₃ receptors. Compounds **16a**, **16b**, and **16c** are *trans* double bond analogues of compounds **14b**, **14c** and **14d** which have a single bond linkage. The D₂ K_i values were reduced ~2.5-, 5.5-, and 3.6-fold respectively. For the D₃ receptor, the K_i values were also reduced 9.3-, 3.5-, and 5.6-fold respectively. Overall, replacing the single bond with a double bond linkage increased selectivity for compounds binding to D₃ versus D₂ receptors.

A series of compounds were prepared to test if the position of the carboxylic group in the substituted indolyl carboxylic acids impacts the affinity of compounds for binding to D₂ and D₃ receptor sites (Table 1 and Scheme 2). Compounds **17a–e** are composed of a substituted-indole-3-carboxylic fragment and either a (4-(4-(2-substituted)phenyl)piperazin-1-yl)butyl or (*E*)-(4-(4-(2-substituted)-phenyl)piperazin-1-yl)but-2-en-1-amino fragment. Compounds **17a** and **17b** have a single bond 4-carbon spacer while compounds **17c–e** contain a double bond spacer. All of these compounds displayed high binding affinity for both D₂ and D₃ receptors with K_i values ranging from 2.0 to 11.7 nM for D₂ receptors and from 0.4 to 3.7 nM for D₃ receptors.

Comparing the binding data between **17b** and **17c**, it was observed that **17b** with a single bond spacer had higher affinity at both D₂ and D₃ receptors than **17c**, which contains a *trans* double bond spacer. Comparing **17c** and **17d**, compound **17d** has a 2-fold lower selectivity ratio for D₃ versus D₂ than **17c**. The structural difference between **17c** and **17d** is that **17c** contains a methoxy moiety while **17d** has a 2-fluoroethoxy group at the 4-position of the phenylpiperazinyl group. Compounds **17c** and **17d** have similar binding affinity at D₂ and D₃ receptors. The similar binding properties for **17c** and **17d**, as well as **15c** and **15d**, are somewhat surprising because the phenylpiperazine is thought to occupy the orthosteric portion of the binding site of the D₃ receptor.⁴²

The difference in structure between **17d** and **17e** is that the methoxy group in the substituted indole-3-carboxylic group was moved from the 5-position to 6-position. Both compounds were found to have a similar affinity for dopamine D₂ and D₃ receptors.

In vitro binding studies were also conducted to determine the affinity of our most potent D₃ ligands for dopamine D₄ receptors. As with other compounds in this structural class, our compounds had a relatively low affinity for dopamine D₄ receptors (Table 2).

The intrinsic activity of five promising compounds, **14a**, **14b**, **14d**, **15d** and **16b**, was determined using a forskolin-dependent adenylyl cyclase inhibition assay. This evaluation was performed using stably transfected HEK-293 cells expressing human D₂ or D₃ dopamine receptors. For these cell lines we routinely observed approximately 90%

inhibition of adenylyl cyclase activity at D₂ receptors and 35–40% inhibition at D₃ receptors.⁴³ For each compound, the results were normalized to the percent inhibition obtained using the full agonist quinpirole at human D₂ (1 μ M) and D₃ (100 nM) receptors expressed in stably transfected HEK 293 cells. All five compounds were partial agonists at both human D₂ and D₃ dopamine receptors; for D₂ dopamine receptors, the efficacy ranges from approximately 30% to 60% of a full agonist; for D₃ dopamine receptors, the efficacy ranges from ~40% to 70% of a full agonist; the magnitude of the response (% inhibition of forskolin stimulation) is greater for D₂ receptors expressed in HEK cells than for D₃ receptors as shown in Fig. 3.

Previous studies have shown that some *N*-phenylpiperazine analogs have a high affinity for sigma receptors.⁴⁴ In addition, our group has reported a number of conformationally-flexible benzamide derivatives to bind with high affinity to σ_2 receptors.⁴⁵ Therefore, *in vitro* binding studies were conducted to measure the affinity of our most potent compounds for sigma receptors. The *in vitro* data shows that all five partial agonists **14a**, **14b**, **14d**, **15d**, and **16b**, exhibited low binding affinity at σ_1 and σ_2 receptors (Table 2). Partial agonist **14a** has the highest D₃ affinity (0.18 nM), good selectivity for D₃ vs. D₂ receptors (80-fold selective for D₃) and reasonable log *D* value; therefore, radiolabeling **14a** with carbon-11 (*t*_{1/2} = 20.4 min) at the 2-methoxy position could potentially lead to a useful PET radiotracer for imaging D₃ receptors. Similarly, partial agonist **14b** could be radiolabeled with fluorine-18 (*t*_{1/2} = 109.8 min) and evaluated as a potential PET radiotracer although its log *D* value is somewhat higher than that of **14a**, which could lead to a higher level of nonspecific binding in PET imaging studies.

Conclusion

In the present study, we have reported the synthesis and pharmacological evaluation of 16 new indolyl carboxylic acid amides. It was observed that six compounds **14a–d**, **15a** and **16b** are very potent for D₃ receptor (*K*_i < 5 nM) and have at least 20-fold selectivity for D₃ over D₂ receptor site; they displayed very weak binding for D₄ site and σ_1 , σ_2 sites. The intrinsic efficacy assay revealed **14a** and **b**, **14d**, **15d** and **16b** are partial agonists at D₂ and D₃ receptors. The *in vitro* data of this work will provide very useful SAR information to guide further design and synthesis of new analogues that having potency and selectivity for D₃ receptor. In addition, within this series, two partial agonists (**14a** and **14b**) exhibited high D₃ binding affinity (<0.5 nM) and 60-fold selectivity for D₃ vs. D₂ receptors. These two partial agonists differ by a fluoroethoxy group or a methoxy group at the 2-position of the phenyl group of the phenylpiperazine moiety, thus providing candidate ligands for PET imaging studies *via* the corresponding ¹⁸F-labeled or ¹¹C-labeled analogues. Partial agonist **14a** displayed the highest D₃ affinity (*K*_i value of 0.18 nM) and selectivity for D₃ vs. D₂ receptors (87-fold). In addition, it has suitable log *D* value of 2.85, indicating that it has favorable lipophilicity in penetrating the BBB and resulting in potentially high uptake in the brain. Therefore, [¹¹C]**14a** is a potential radiotracer for conducting *in vivo* imaging studies of dopamine D₃ receptors with PET.

Supplementary Material

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Acknowledgments

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Abbreviations

Anal	Analysis
BBB	Blood–brain barrier
CDI	1,1-Carbonyldiimidazole
CIMS	Chemical ionization mass spectrometry
CNS	Central nervous system
DAST	Diethylaminosulfur trifluoride
DCC	<i>N,N</i> -Dicyclohexylcarbodiimide
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DTG	1,3-Ditolyguanidine
GIRKs	G protein-coupled inwardly-rectifying potassium channels
HEK cells	Human Embryonic Kidney 293 cells
[¹²⁵I]IABN	[¹²⁵ I] <i>N</i> -Benzyl-5-iodo-2,3-dimethoxy-[3.3.1]azabicyclononan-3-yl-benzamide
MeOH	Methanol
LID	L-DOPA-induced dyskinesia
PLD	Phospholipase D
NBS	<i>N</i> -Bromosuccinimide
PET	Positron emission tomography
NaOCH₃	Sodium methoxide
SPECT	Single photon emission computed tomography
TBAF	Tetra- <i>n</i> -butylammonium fluoride
THF	Tetrahydrofuran
TEA	Triethylamine

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Fig. 1.
Representative D₃ ligands containing benzo-fused unsaturated five-membered heterocyclic ring carboxylic amides.

Fig. 2.
Substituted piperazine amines.

Fig. 3.

Intrinsic efficacy of selected analogues at dopamine D₂ and D₃ receptors. The intrinsic efficacy of selected test compounds was evaluated by determining the percent inhibition of a forskolin-dependent whole cell adenylyl cyclase assay for both human D₂ (black bars) and D₃ (gray bars) dopamine receptors. The results were normalized to the percentage inhibition obtained using the full agonist quinpirole at human D₂ (1 μ M) and D₃ (100 nM) receptors expressed in stably transfected HEK 293 cells. For D₂ receptors the maximum inhibition was 85% and for D₃ receptors the maximum inhibition was 35%. The test drug was used at a concentration equal to approximately 10 $\times$ the K_i value that was determined from the radioligand binding analysis (Table 1). The mean $\pm$ S.E.M. values are reported for $n = 3$.

Scheme 1.

Syntheses of fluoro-PEGylated indolyl carboxylic acids. *Reagents and conditions:* (a) 1-bromo-2-fluoroethane, K_2CO_3 , acetone, reflux overnight; (b) 2-(2-chloroethoxy)ethanol or 2-(2-(2-chloroethoxy)ethoxy)ethanol, K_2CO_3 , DMF, 100 °C, overnight; (c) DAST, CH_2Cl_2 , 0 °C; (d) NaOH, methanol– H_2O .

9a, R = CH₃, single bond
9b, R = CH₂CH₂F, single bond
9c, R = CH₃, double bond
9d, R = CH₂CH₂F, double bond

14, R = CH₃, single bond
15, R = CH₂CH₂F, single bond
16, R = CH₃, double bond
17, R = CH₂CH₂F, double bond

14a, X = OH
14b, X = OCH₂CH₂F
14c, X = OCH₂CH₂OCH₂CH₂F
14d, X = (OCH₂CH₂)₂OCH₂CH₂F

15a, X = H, R = CH₂CH₂F
15b, X = OH, R = CH₂CH₂F
15c, X = OCH₃, R = CH₂CH₂F
15d, X = OCH₃, R = CH₃

16a, X = OCH₂CH₂F
16b, X = OCH₂CH₂OCH₂CH₂F
16c, X = (OCH₂CH₂)₂OCH₂CH₂F

17a, X = H, Y = H, single bond, R = CH₂CH₂F
17b, X = OCH₃, Y = H, single bond, R = CH₂CH₂F
17c, X = OCH₃, Y = H, double bond, R = CH₃
17d, X = OCH₃, Y = H, double bond, R = CH₂CH₂F
17e, X = H, Y = OCH₃, double bond, R = CH₂CH₂F

Scheme 2.
Syntheses of final compounds.

Table 1D₂ and D₃ affinities ($K_i \pm \text{SD}$, nM) of new analogues

No.	D ₂	D ₃	D ₂ /D ₃ ratio	Log D^a
2b	37.0 $\pm$ 6.2	0.33 $\pm$ 0.03	123	3.6
3b	35.1 $\pm$ 2.1	0.93 $\pm$ 0.03	39	3.5
4b	21.5 $\pm$ 1.6	0.20 $\pm$ 0.04	108	4.8
14a	15.7 $\pm$ 1.1	0.18 $\pm$ 0.02	87	2.9
14b	23.9 $\pm$ 2.2	0.43 $\pm$ 0.10	60	4.2
14c	21.8 $\pm$ 1.3	1.1 $\pm$ 0.2	20	3.8
14d	42.4 $\pm$ 0.5	1.5 $\pm$ 0.1	28	3.4
15a	40.4 $\pm$ 4.6	2.0 $\pm$ 0.2	20	4.4
15b	33.6 $\pm$ 5.9	4.0 $\pm$ 0.5	8	3.2
15c	66.3 $\pm$ 3.0	3.1 $\pm$ 0.2	21	4.3
15d	119 $\pm$ 4.7	4.4 $\pm$ 0.7	27	4.0
16a	60 $\pm$ 7.2	3.7 $\pm$ 0.6	16	4.3
16b	120 $\pm$ 17.5	3.8 $\pm$ 0.8	31	3.9
16c	155 $\pm$ 14	8.4 $\pm$ 1.2	18	3.5
17a	5.1 $\pm$ 1.2	0.90 $\pm$ 0.1	6	3.7
17b	2.0 $\pm$ 0.2	0.39 $\pm$ 0.1	5	3.6
17c	11.7 $\pm$ 0.5	3.0 $\pm$ 0.3	4	3.4
17d	7.5 $\pm$ 0.1	3.2 $\pm$ 0.3	2.3	3.6
17e	5.9 $\pm$ 0.8	3.7 $\pm$ 0.5	1.6	3.6

^aCalculated using ACD log D software, Advanced Chemistry Development, Toronto, Canada.^b*Bioorg. Med. Chem.*, 2005, **13**(1), 77–87.

Table 2

D₄, α_1 and α_2 affinities ($K_i \pm$ SD, nM) of **14a** and **b**, **14d**, **15d** and **16b**

No.	D ₄	α_1	α_2
14a	444 $\pm$ 67	>10 000	11 300 $\pm$ 2000
14b	284 $\pm$ 28	>10 000	2550 $\pm$ 350
14d	565 $\pm$ 91	>10 000	3180 $\pm$ 560
15d	162 $\pm$ 27	>10 000	1884 $\pm$ 21
16b	360 $\pm$ 71	>10 000	1358 $\pm$ 98