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Design, synthesis and structure–activity relationship studies of hexahydropyrazinoquinolines as a novel class of potent and selective dopamine receptor 3 (D_3) ligands

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Abstract—A hexahydropyrazinoquinoline (compound **5c**) was previously discovered as a novel D_3 ligand with a moderate binding affinity to the D_3 receptor (K_i = 304 nM) but no selectivity over the D_1-like and D_2-like receptors. In this study, we wish to report the design, synthesis and structure–activity relationship studies of a series of novel hexahydropyrazinoquinolines. Our efforts resulted in new compounds with improved binding affinity and selectivity. Among them, compound **12d** has a K_i value of 2.6 nM for its binding affinity to the D_3 receptor and has >2000- and 99-fold selectivity over the D_1-like and D_2-like receptors, respectively, representing a potent and selective D_3 ligand.

The dopamine 3 (D_3) subtype receptor has been implicated in several neurological conditions and potent and selective D_3 ligands may have the therapeutic potential for the treatment of drug addiction, Parkinson's disease and schizophrenia.[1–5] Design and development of highly potent and selective D_3 ligands is currently a very active research area.[6–12]

Although several classes of D_3 ligands have been designed and synthesized in the last decade, many of the previously reported D_3 ligands were based upon a very limited number of basic core structures.[7] Indeed, the majority of those recently reported potent and selective D_3 ligands[8–13] were based upon the core structure of BP 897.[6] Hence, we believe that D_3 ligands with novel chemical core structures or scaffolds would have considerable value to increase the chemical diversity in D_3 ligand design and may lead to the development of potent and highly selective D_3 ligands with unique in vitro and in vivo pharmacological properties. To this end, we have employed a novel computational three-dimensional (3D) database screening strategy to discover novel D_3 ligands.[14] Our efforts led to the identification of several classes of new D_3 ligands.[14]

Among those new D_3 ligands we have discovered,[14] compound **5c** contains hexahydropyrazinoquinoline as the basic core structure, which was not found in previously reported D_3 ligands. Hence, compound **5c** represents a promising initial lead compound with a novel core structure for further design and optimization.[7–13] Structurally, compound **5c** may be viewed as a conformationally constrained analogue of BP 897.

The initial lead compound **5c** has a K_i value of 304 nM to the D_3 receptor and no selectivity over the D_1-like and D_2-like receptors. Hence, its potency and selectivity need to be significantly improved. In this paper, we wish to report our design, synthesis and preliminary structure–activity relationship studies for this class of D_3 ligands.

Based upon its chemical structure, compound **5c** may be divided into three regions, the tricyclic hexahydropyrazinoquinoline core structure as the 'head', the phenyl ring

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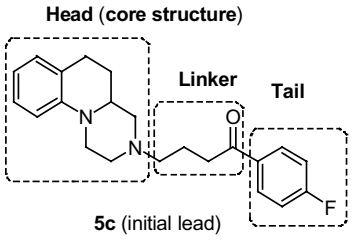
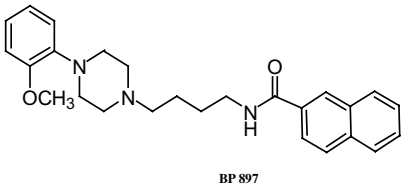




as the 'tail' and the linker between the 'head' and the 'tail' groups. Our current study provides initial structure–activity relationship with modifications made on each of the three regions for this class of compounds aiming at improving both its binding affinity to the D_3 receptor and its selectivity over other closely related dopamine receptor subtypes.

In view of the numerous dopamine receptors at which binding affinity can be determined for these compounds, we have designed our screening protocol to make the most important comparison (i.e., D_2-like/D_3) as well as comparison between the major subclasses of dopamine receptors (i.e., D_1-like/D_2-like and D_1-like/D_3) in our initial investigation. Because of the relatively low sequence homology between the D_1 and D_3 receptors, many of the previously reported D_3 ligands were shown to have an excellent selectivity over the D_1 receptor.[7] In contrast, the D_2 and D_3 receptors have close sequence homology. Until very recently, most of the previously reported D_3 ligands also displayed good affinities to the D_2 receptor.[7] Hence, a high-affinity D_3 ligand displaying an excellent selectivity between the D_2 and D_3 receptor subtypes is likely to be highly selective over other dopamine receptors. Hence, in this study, all the new compounds were evaluated for their binding affinities at the D_1-like, D_2-like and D_3 receptors using previously established methods.[15–17] Because the affinities of compounds at the dopamine receptor subtypes have been shown to vary depending on the in vitro assay conditions used and the source of receptors (i.e., human or rat and the expression system used),[18] the assay conditions were designed to favour agonist binding and used receptors expressed in their native tissue, brain.

First, compounds **5a**–**d** were designed and synthesized to determine the optimal length of the linker. As can be seen from Table 1, compounds **5a** and **5b** with either a 1- or 2-carbon linker are 26- and 9-times less potent than compound **5c** with a 3-carbon linker. In comparison, compound **5d** with a 4-carbon linker has a similar binding affinity to the D_3 receptor as compound **5c**, indicating either a 3- or a 4-carbon linker is optimal for binding to the D_3 receptor.

Table 1. Binding affinities at the D_1-like, D_2-like and D_3 receptors in binding assays using rat brain

Compounds	$K_i \pm$ SEM (nM)			Selectivity	
	D_1-like [^{3}H]SCH 23390	D_2-like [^{3}H]spiperone	D_3 [^{3}H]PD 128907	D_1-like/D_3	D_2-like/D_3
5a	7947 ± 597	3887 ± 664	7487 ± 591	1.1	0.5
5b	8893 ± 568	3643 ± 459	2755 ± 475	3.2	1.3
5c	904 ± 100	243 ± 30	304 ± 53	3.0	0.8
5d	2467 ± 303	852 ± 49	381 ± 59	6.5	2.2
9a	>100,000	>100,000	22,967 ± 6846	>4	>4
9b	356 ± 47	906 ± 190	2523 ± 692	0.1	0.34
9c	258 ± 52	220 ± 21	22 ± 6	12	10
10a	1218 ± 145	1389 ± 111	1650 ± 424	0.7	0.8
10b	152,567 ± 17,284	2443 ± 403	1535 ± 81	10	1
10c	791 ± 187	1568 ± 338	18 ± 2.4	44	87
12a	4602 ± 287	762 ± 51	5.8 ± 1.3	793	131
12b	>250,000	>250,000	244 ± 59	>1000	>1000
12c	5802 ± 422	1125 ± 207	45 ± 7	130	25
12d	6051 ± 570	258 ± 41	2.6 ± 0.4	>2000	99
BP 897	636 ± 103	162 ± 48	1.1 ± 0.2	578	147

Data represent the mean ± SEM of three to five independent determinations. [^{3}H]SCH 23390 binding assays for D_1-like dopamine receptors were performed as previously described in detail[17] using membranes prepared from the caudate-putamen of adult male Sprague–Dawley rats (Harlan, Indianapolis, IN). All compounds were dissolved in 100% EtOH at a concentration up to 5 mM. The assay buffer was 50 mM Tris–HCl, 5 mM KCl, 2 mM $MgCl_2$ and 2 mM $CaCl_2$, pH 7.4 at 23 °C; the concentration of [^{3}H]SCH 23390 (73 Ci/mmol; Amersham) was 0.3 nM; and nonspecific binding was determined in the presence of 1 μM (+)-butaclamol. SigmaPlot was used to determine K_i values using the K_D value for [^{3}H]SCH 23390 of 0.3 nM.[17] [^{3}H]spiperone binding assays for D_2-like dopamine receptors were performed as previously described in detail and as described for [^{3}H]SCH 23390 except the concentration of [^{3}H]spiperone (24 Ci/mmol; Amersham) was 0.2 nM.[15,17] K_i values were determined using the K_D value for [^{3}H]spiperone of 0.1 M.[15] [^{3}H]PD 128907 binding assays D_3-like dopamine receptors were performed as previously described in detail[16,17] using ventral striatal (nucleus accumbens and olfactory tubercles) membranes prepared in assay buffer (50 mM Tris, 1 mM EDTA; pH 7.4 at 23 °C). The concentration of [^{3}H]PD 128907 was 0.3 nM (116 Ci/mmol; Amersham, Arlington Heights, IL) and nonspecific binding was defined by 1 μM spiperone. K_i values were determined using the K_D value for [^{3}H]PD 128907 of 0.3 nM.[16]

Compounds **9a–c** were designed to investigate if a much longer biphenyl can be used to replace the 4-flurophenyl ring in compound **5c** as the tail. Consistent with the data obtained for compound **5b**, compound **9a** with a 2-carbon linker only has a weak affinity (K_i = 23 μM) to the D_3 receptor. In contrast to **5c** and **5d**, compounds **9b** and **9c** with a 3-carbon and 4-carbon linker, have very different affinities to the D_3 receptor. While compound **9b** has a relatively weak binding affinity (K_i = 2.5 μM) to the D_3 receptor, compound **9c** is a potent D_3 ligand (K_i = 22 nM). Interestingly, compound **9b** has higher affinities at the D_1-like and D_2-like receptors than at the D_3 receptor. In contrast, compound **9c** has a higher affinity at the D_3 receptor than at the D_1-like and D_2-like receptors, thus displaying a selectivity of 12- and 10-fold.

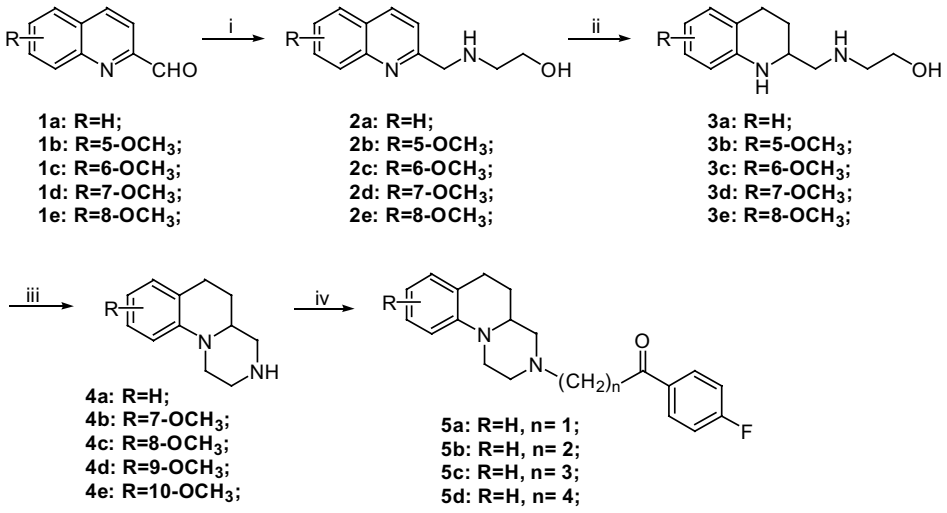
Previously, it was shown that a naphthyl ring can be used as the tail in D_3 ligands to improve binding affinity and/or selectivity. Accordingly, compounds **10a–c** were designed and synthesized. Consistent with the data obtained for compounds **9a** and **9b**, compounds **10a** and **10b** with either a 2- or 3-carbon linker only has a weak affinity to the D_3 receptor. Similar to **9c**, compound **10c** with a 4-carbon linker is a potent D_3 ligand (K_i = 18 nM). But **10c** is more selective than **9c** and has a selectivity of 44- and 87-fold over the D_1-like and D_2-like receptors. These data indicate that a bulky naphthyl tail and a 4-carbon linker afford compound (**10c**) with a potent binding affinity to the D_3 receptor (K_i = 18 nM) and a good selectivity over both the D_1-like and the D_2-like receptors.

Finally, based upon the potent binding affinity activity of BP 897 to the D_3 receptor and its good selectivity over the D_1 and D_2 receptors, it appears that a methoxyl substituent on the phenyl ring in the hexahydropyrazinoquinoline core structure may further improve the binding and/or selectivity of compound **10c**. This idea is supported by our modeling studies on **10c** (data not shown), which suggests that a methoxyl substituent on the phenyl ring may form hydrogen bonding interaction with one of the three serine residues, namely Ser192, Ser193 and Ser196 in the D_3 receptor. To explore the influence of a methoxyl substituent on binding affinity and selectivity, compounds **12a–d** were synthesized and tested.

Compound **12a** with the 7-methoxyl substituent on the phenyl ring has a K_i value of 5.8 nM at the D_3 receptor, 793-fold selectivity over the D_1-like receptors and 131-fold selectivity over the D_2-like receptors. The 7-methoxyl substituent on the phenyl ring improves the binding affinity by three-fold at the D_3 receptor as compared to **10c**. Furthermore, the 7-methoxyl substituent improves the selectivity by 18 times between the D_3 receptor and the D_1-like receptors as compared to **10c**. This improved selectivity over the D_1-like receptors is the combination of an increased binding affinity at the D_3 receptor and a decreased binding affinity at the D_1-like receptors. The selectivity of **12a** between the D_3 receptor and the D_2-like receptors is only improved marginally by 1.5-fold as compared to that of **10c** (131-fold vs 87-fold). Compound **12b** with the 8-methoxyl substituent on the phenyl ring has a K_i value of 244 nM at the D_3 receptor, 13-time less potent than **10c**. However, compound **12b** was found to be completely inactive at the D_1-like and D_2-like receptors at the highest concentration tested (250 μM). Hence, **12b** is a moderately potent D_3 ligand but has an excellent selectivity over the D_1-like and D_2-like receptors. Compound **12c** with the 9-methoxyl substituent on the phenyl ring has a K_i value of 45 nM at the D_3 receptor, 130-fold selectivity over the D_1-like receptors and 25-fold selectivity over the D_2-like receptors. Thus, 9-methoxyl substituent on the phenyl ring has a marginal influence on both the binding affinity and selectivity. Compound **12d** with the 10-methoxyl substituent on the phenyl ring has a K_i value of 2.6 nM at the D_3 receptor, >2000-fold selectivity over the D_1-like receptors and 99-fold selectivity over the D_2-like receptors. Hence, 10-methoxyl substituent on the phenyl ring of **10c** improves the binding affinity by seven times at the D_3 receptor and selectivity more than 45-fold between the D_3-like receptor and the D_2-like receptors. Compound **12d** is a potent D_3 ligand with an excellent selectivity over the D_1-like receptors and a good selectivity over the D_2-like receptors.

To directly compare **12a** and **12d** with other known D_3 ligands, we have evaluated BP 897, a known selective D_3 ligand,[6] in our assay conditions and the results are provided in Table 1. As can be seen, BP 897 has K_i values of 1.1 nM at the D_3 receptor, 162 nM at the D_2-like receptors, and 636 nM at the D_1-like receptors, respectively. These values are in good agreement with the reported K_i values of 0.92 nM at the D_3 receptor, 61 nM at the D_2 receptor and 3 μM at the D_1 receptor, respectively, using the CHO cells expressing recombinant human D_1, D_2 and D_3 receptors.[6] Of note, although it is known that assay conditions can have a significant influence on the binding affinity of a ligand at the D_3 receptor and the selectivity over other dopamine subtype receptors,[18] our results on BP 897 indicate that our assays using membranes prepared from rat brains and assays using CHO cells expressing recombinant human D_1, D_2 and D_3 appear to produce quite consistent results in both binding affinity and selectivity. Based upon our data, compound **12d** and BP 897 have similar binding affinities at the D_3 receptor (2.6 vs 1.1 nM for their K_i values). Compound **12d** has a better selectivity than BP 897 over the D_1-like receptors (>2000-fold vs 578-fold) and has a slightly worse selectivity than BP 897 (99- vs 147-fold). Taken together, our data indicate that compound **12d** represents a promising new lead compound for further optimization towards our goal of obtaining highly potent D_3 ligands with outstanding selectivity over the D_1-like and D_2-like receptors.

The synthetic route for compounds **5a–d** is outlined in Scheme 1. The key intermediate **4** was synthesized using known methods with some modifications.[19–21] Briefly, condensation of 2-quinoline-carboxaldehyde **1** with ethanolamine generated the Schiff's base, which was reduced to 2-substituted aminomethylquinoline **2** with $NaBH_4$.[19,20] Reduction of **2** with nickel–aluminium alloy in aqueous KOH readily produced 2-substituted



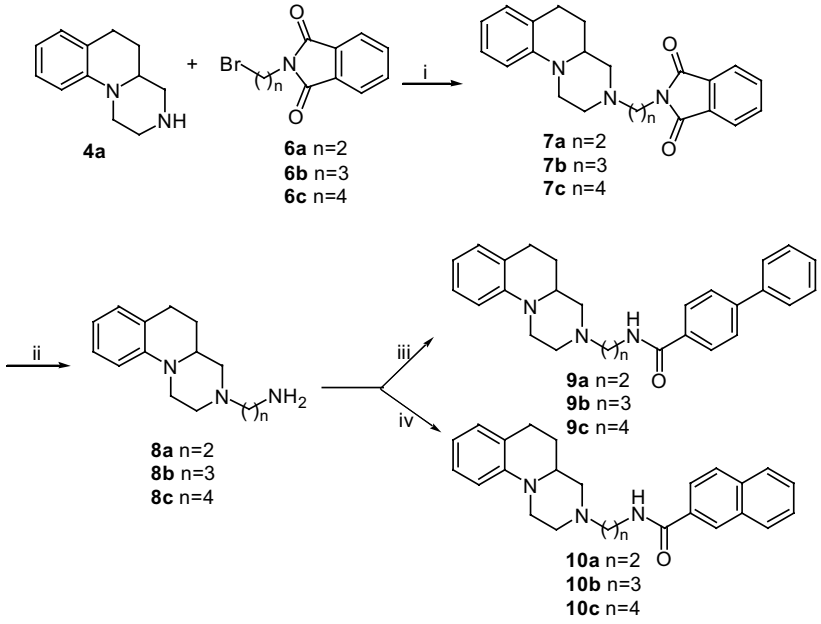
Scheme 1. Synthesis of important intermediates **4a**–**e** and compounds **5a**–**d**. Reagents and conditions: (i) a. 1.1 equiv ethanolamine, benzene, under N_2, reflux, overnight; b. 3.5 equiv $NaBH_4$, absolute ethanol, under N_2, reflux, overnight; (ii) ca. 5 g nickel–aluminium alloy/1 g substrate, 1 M KOH, methanol, rt, overnight; (iii) 3 equiv P_2O_5, xylenes, under N_2, reflux, overnight; (iv) 1.2 equiv p-$FC_6H_4CO(CH_2)_{n(n=1,2,3,4)}$–I, 2 equiv Cs_2CO_3, CH_3CN, under N_2, reflux, overnight.

aminomethyl-1,2,3,4-tetrahydro-quinoline **3** in good yield.[19] The key intermediate **4** was prepared by reflux of **3** with P_2O_5 in xylenes.[19,20] Alkylation of **4** with different alkyl iodides in the presence of Cs_2CO_3 in acetonitrile afforded compounds **5a**–**d**.[20]

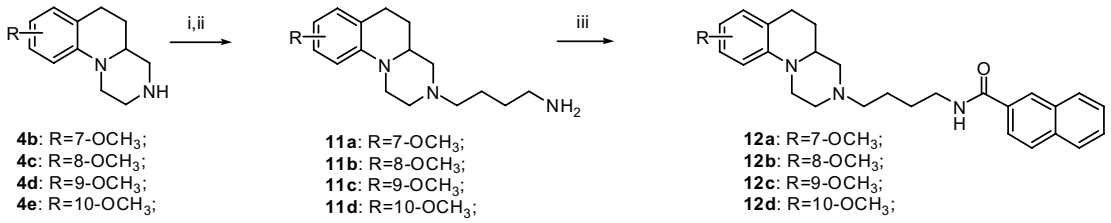
The synthetic route for compounds **9a**–**c** and **10a**–**c** is provided in Scheme 2. Briefly, phthalimido-protected 1-bromoalkylamines **6a**–**c** were reacted with **4** to produce **7a**–**c**. Deprotection of **7a**–**c** with hydrazine generated amines **8a**–**c**. Amidation of **8a**–**c** using 4-biphenylcarbonyl chloride or 2-naphthoyl chloride afforded compounds **9a**–**c** and **10a**–**c**, respectively.[21]

The synthetic route for compounds **12a**–**d** is shown in Scheme 3. Briefly, compounds **11a**–**d** were obtained by treatment of the important intermediates **4b**–**e** obtained in Scheme 1 with *N*-(4-bromobutyl)-phthalimide in CH_3CN followed by hydrazine. Acylation with 2-naphthoyl chloride in the presence of *N*,*N*-diisopropylethylamine afforded the target compounds **12a**–**d** in good yield (>90%).

In summary, compound **5c** was previously identified as a novel D_3 ligand with a moderate binding affinity to the D_3 receptor (K_i value = 304 nM) but no selectivity over the D_1-like and D_2-like receptors. Our present study



Scheme 2. Synthesis of compounds **9a**–**c** and **10a**–**c**. Reagents and conditions: (i) 1.2 equiv phthalimido-1-bromoalkylamines (**6a**–**c**), 2 equiv Cs_2CO_3, CH_3CN, under N_2, reflux, overnight, yield 75–88%; (ii) 2 equiv hydrazine, EtOH, reflux, 2 h, yield 82–87%; (iii) 1.2 equiv 4-biphenylcarbonyl chloride, 3 equiv triethylamine, 0 °C, 4 h, yield 31–48%; (iv) 1.2 equiv 2-naphthoyl chloride, 3 equiv triethylamine, 0 °C, 4 h, yield 83–87%.



Scheme 3. Synthesis of compounds **12a–d**. Reagents and conditions: (i) *N*-(4-bromobutyl)-phthalimide, Cs_2CO_3, CH_3CN, reflux, 3 h; (ii) NH_2NH_2, EtOH, reflux, 2 h; (iii) 2-naphthoyl chloride, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 2 h.

shows that the 4-flurophenylkentone group can be successfully replaced by other hydrophobic groups and the optimal length of the 'linker' between the 'head' and the 'tail' is 4-carbon. Furthermore, introduction of a methoxyl group to the phenyl ring in the hexahydropyrazinoquinoline core structure can significantly improve the binding affinity and/or selectivity. Our preliminary SAR study has thus led to potent and selective D_3 ligands. Of which, compound **12d** is a potent D_3 ligand (K_i = 2.6 nM) and displays an excellent selectivity of >2000-fold between the D_3 receptor and the D_1-like receptors and a good selectivity of 99-fold between the D_3 receptor and the D_2-like receptors. Based upon compound **12d**, extensive modifications are being performed towards achieving novel D_3 ligands with high binding affinity and outstanding binding selectivity and the results will be reported in due course.

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