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Design of novel hexahydropyrazinoquinolines as potent and selective dopamine D_3 receptor ligands with improved solubility

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Abstract—We have recently reported hexahydropyrazinoquinolines as a new class of dopamine 3 (D_3) receptor ligands with high-affinity to the D_3 receptor and excellent selectivity over the closely related D_1-like and D_2-like receptors. However, our previously reported most potent and selective D_3 ligands have poor aqueous solubility, which greatly hinders our in vivo studies aimed at evaluation of their therapeutic potential in animal models. In this study, we wish to report the design, synthesis, and evaluation of a series of new hexahydropyrazinoquinolines as D_3 ligands with improved solubility. Among them, compound **4g** has a K_i value of 9.7 nM for the D_3 receptor and displays a selectivity of >5000 and 466 times over the D_1-like and D_2-like receptors, respectively. Importantly, the hydrochloride salt form of compound **4g** has a good aqueous solubility (>50 mg/mL) and represents a promising D_3 ligand for further in vivo evaluations of its therapeutic potential for the treatment of drug abuse, restless legs syndrome, schizophrenia, Parkinson's disease, and depression.

Recent studies[1–5] have suggested that the dopamine 3 subtype receptor (D_3) is a promising therapeutic target for a variety of conditions including drug abuse, restless legs syndrome, schizophrenia, Parkinson's disease, and depression. Potent and selective D_3 ligands may have therapeutic potential for the treatment of these conditions. Accordingly, there is a strong research interest in the design of potent and selective D_3 ligands.[6]

Our laboratory has recently reported the design, synthesis, and evaluation of hexahydropyrazinoquinolines as a new class of potent and selective D_3 ligands.[7,8] Based upon its chemical structure, the initial lead compound **1** was divided into three structural regions, the tricyclic hexahydropyrazinoquinoline core structure as the 'head', the naphthyl ring as the 'tail', and the linker between the 'head' and the 'tail' groups. In our previous studies,[7,8] we have designed and synthesized analogues to explore the structure–activity relationships for the 'head' and 'linker' regions. Those modifications have yielded compounds **2** and **3** with high affinity for the D_3 receptor and excellent selectivity over the D_1-like and D_2-like receptors. However, compounds **2** and **3** have a limited aqueous solubility, which greatly hinders our in vivo studies for evaluation of their therapeutic potential in animal models. Of note, many of those potent and selective D_3 ligands recently reported by other laboratories are also highly hydrophobic in nature and will likely encounter a similar solubility problem.[5,9–11] In a recent study, efforts have been made toward improvement of the aqueous solubility in the design of potent and selective D_3 ligands.[12] In this paper, we report the design, synthesis, and evaluation of new analogues of compounds **2** and **3** with modifications at the 'tail' region with a goal to derive potent and selective D_3 ligands with improved aqueous solubility.

Our previous molecular modeling analysis showed that the hydrophobic naphthyl 'tail' occupies a large binding pocket primarily formed by hydrophobic residues in the D_3 receptor.[8] For this reason, a hydrophobic tail may be required for achieving high binding affinities to the D_3 receptor. Hence, in our design, we have chosen to

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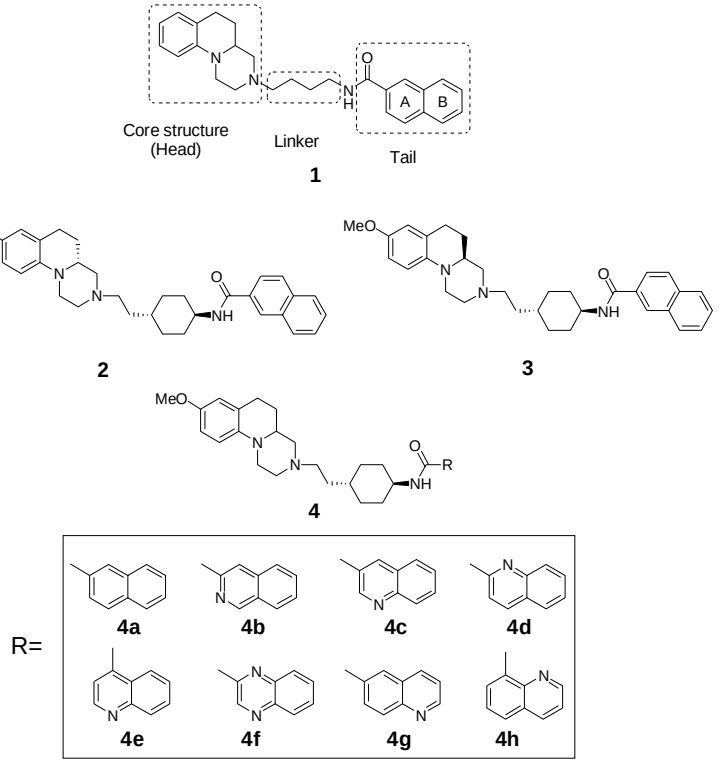
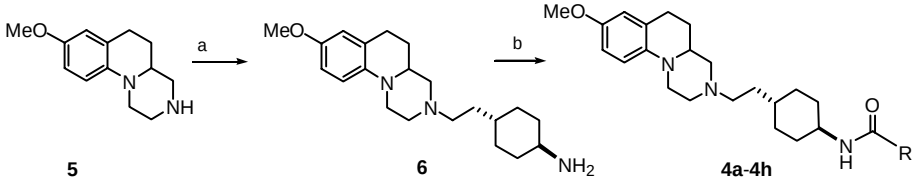


replace the naphthyl ring in our lead compounds with either a quinolinyl ring, or an isoquinolinyl ring, or quinoxalinyl ring. We reasoned that these ring systems are still primarily hydrophobic in nature but have improved aqueous solubility over the naphthyl ring. Thus, the resulting compounds (**4a**–**h**) will have better aqueous solubility than compounds **2** and **3**. Since enantiomers **2** and **3** have very similar binding affinities to the D_3 receptor and also very similar selectivities over the D_1-like and D_2-like receptors,[8] we have decided to employ the racemic template for rapid exploration of the structure–activity relationship in the tail region. A series of new analogues **4a**–**h** were synthesized and evaluated at the dopamine receptors.

The synthesis for compounds **4a**–**h** is straightforward and outlined in Scheme 1. Briefly, compound **5** was synthesized using our previously published method.[7] Compound **6** was obtained by condensation of compound **5** with *trans*-(4-*tert*-butoxycarbonylamino-cyclohexyl)-acetic acid, followed by removal of the Boc-protecting group and reduction with lithium alumina hydride. Compound **6** was then coupled with the requisite acids using EDCI as coupling reagent in dichloromethane to produce the target compounds **4a**–**4h**.[17]

In view of the numerous dopamine receptors at which binding affinity might 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 compare between the major subclasses of dopamine receptors (i.e., D_1-like/D_2-like, and D_1-like/D_3). Accordingly, 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.[13–15] 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),[5] the assay conditions used in these assays were designed to favor agonist binding and used receptors expressed in their native tissue, brain. Our receptor binding assay enable selective assessment of each dopamine receptor subtype of interest by using brain regions that predominantly express the receptor of interest (e.g., the caudate puta-



Scheme 1. Synthesis of hexahydropyrazinoquinolines. Reagents and conditions: (a) i. *trans*-(4-*tert*-butoxycarbonylamino-cyclohexyl)-acetic acid, EDCI, HOBT, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 12 h; ii. 4 M HCl in dioxane, rt, 4 h; iii. $LiAlH_4$, THF, reflux, 2 h; (b) RCO_2H, EDCI, HOBT, *N*,*N*-diisopropylethylamine, CH_2Cl_2, rt, 2 h.

men, which expresses very low levels of D_3 sites, for the D_1-like and D_2-like receptor assays) and/or in vitro conditions that affect the binding of the radioligand to a particular binding site (e.g., [^{3}H]PD 128907 binding to D_2 sites is disfavored in the absence of Mg^{2+}, thus enabling selective labeling of D_3 sites in ventral striatal membranes).[14] These assay conditions have been extensively validated and compared with those of other in vitro assay systems.[13] Of note, the non-selective antagonist haloperidol, and the D_3-selective antagonists (+)-S14297 and U99194A exhibited binding affinities and selectivities in our assays and are highly similar to those previously reported using cloned human receptors expressed in transfected cells.[5,16] The binding affinities for compounds **4a**–**h**, together with reference compounds haloperidol, (+)-S14297, and U99194A at the D_3, D_1-like, and D_2-like receptors determined in our assay conditions are summarized in Table 1.

Replacement of the naphthyl ring by a 3-isoquinolinyl, 3-quinolinyl, or 2-quinolinyl ring results in compounds **4b**, **4c**, and **4d** in which a carbon atom in ring A of the naphthyl ring is replaced by a nitrogen atom at three different positions. These replacements reduce the binding affinity to the D_3 receptor by 10, 10, and 36 times, respectively, as compared to the initial lead compound **4a**. These replacements also significantly reduce the selectivity of these compounds for the D_3 receptor versus the D_2-like receptors. To investigate the effect of the substitution position for the aromatic ring, we changed the substitution position from β- to α-position for the quinolinyl in compound **4c**, which resulted in compound **4e**. This change results in an improvement of three times in both binding affinity to the D_3 receptor and selectivity over the D_2-like receptors as compared to compound **4c**. But compound **4e** is still three times less potent than **4a** and also much less selective over the D_2-like receptors than **4a**. Replacement of the naphthyl ring by a 2-quinoxalinyl ring resulted in compound **4f**, which is significantly less potent and selective than compound **4a**. The significant reduction in binding affinity and selectivity for these new analogues as compared to compound **4a** further confirms the importance of the hydrophobic interaction between the naphthyl ring in compound **4a** and the hydrophobic residues in the D_3 receptor, as suggested in our molecular modeling studies.[8]

Based upon our previous modeling results,[8] although the naphthyl ring in compound **4a** interacts with several hydrophobic residues, part of the ring B in the naphthyl ring is exposed to solvent. It suggests that a nitrogen atom may be inserted into the ring B in the naphthyl ring without causing significant reduction in binding affinity to the D_3 receptor. To test this idea, we have synthesized compounds **4g** and **4h**, in which the 2-naphthyl ring in **4a** is replaced by either 6-quinolinyl or 8-quinolinyl ring. As can be seen from Table 1, although compound **4h** is >10 times less potent than **4a** in its binding affinity to D_3 and has no selectivity between the D_3 receptor and D_2-like receptors, compound **4g** is a potent D_3 ligand with a K_i value of 9.7 nM. Furthermore, compound **4g** has an excellent selectivity over the D_1-like and D_2-like receptors, being >5000 times between the D_3 and D_1-like receptors, and 466 times between the D_3 and D_2-like receptors. Importantly, compound **4g** was found to have a much better aqueous solubility than compound **4a** in our solubility testing. For example, the hydrochloride salt form of **4g** has an aqueous solubility greater than 50 mg/mL, whereas the hydrochloride salt form of **4a** has an aqueous solubility less than 1 mg/mL. Therefore, compound **4g** represents a potent, selective, and soluble D_3 ligand for our further in vivo studies.

In summary, a series of new hexahydropyrazinoquinolines were designed, synthesized, and evaluated as D_3 ligands. Among them, compound **4g** has a K_i value of 9.7 nM for the D_3 receptor and displays a selectivity of >5000 and 466 times over the D_1-like and D_2-like receptors, respectively. Importantly, compound **4g** has a good aqueous solubility (>50 mg/mL) and represents a promising D_3 ligand for further in vivo evaluations of its therapeutic potential for the treatment of drug abuse and several other conditions.

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

Compound	K_i ± 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
1	>250,000	>250,000	244 ± 59	>1000	>1000
4a	>50,000	3660 ± 594	5.1 ± 0.67	>9804	717
4b[19]	>50,000	188 ± 39	48 ± 1.8	>1041	3.9
4c	>50,000	1360 ± 345	51 ± 6.5	>980	27
4d	55,000 ± 4640	4760 ± 797	183 ± 17	301	26
4e	19,700 ± 2130	1470 ± 132	16 ± 2.7	1259	94
4f	24,800 ± 2800	2830 ± 171	152 ± 24	163	19
4g	>50,000	4500 ± 841	9.7 ± 1.8	>5154	466
4h[19]	>50,000	162 ± 48	87 ± 12	>575	1.9
Haloperidol	42 ± 3.4	7.2 ± 1.7	24 ± 3.1[14]	1.75	0.30
S12947	>100,000	256 ± 52	9.8 ± 1.6	>10,000	26
U99194A	>100,000	11,200 ± 513	498 ± 72	>200	22

Validated in vitro assay conditions were optimized to enable selective assessment of each receptor of interest and to favor agonist binding.[18] Data represent means ± SEM of 3–5 independent determinations.

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17. The NMR data for compound **4c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10–1.40 (m, 5H), 1.45–1.57 (m, 2H), 1.72–2.00 (m, 5H), 2.16–2.30 (m, 3H), 2.41–2.47 (m, 2H), 2.72–3.05 (m, 6H), 3.67–3.74 (m, 1H), 3.76 (s, 3H), 4.00–4.10 (m, 1H), 6.07 (d, J = 8.0 Hz, 1H), 6.61 (d, J = 3.0 Hz, 1H), 6.68 (dd, J = 9.0, 3.0 Hz, 1H), 6.77 (d, J = 9.0 Hz, 1H), 7.60–7.67 (m, 1H), 7.80–7.85 (m, 1H), 7.93 (d, J = 8.5 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.58 (d, J = 2.2 Hz, 1H), 9.25 (d, J = 2.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 27.6, 27.8, 32.3, 33.5, 34.1, 35.9, 47.7, 49.8, 53.9, 55.8, 56.0, 56.9, 60.2, 112.6, 114.0, 115.3, 126.4, 127.3, 127.8, 127.9, 129.1, 129.8, 131.6, 135.8, 140.9, 148.5, 149.6, 152.6, 165.3.
18. [^{3}H]SCH 23390 binding assays for D_1-like dopamine receptors were performed as previously described in detail[15] 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 non-specific 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.[15] [^{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 that the concentration of [^{3}H]spiperone (24 Ci/mmol; Amersham) was 0.2 nM.[13,15] K_i values were determined using the K_D value for [^{3}H]spiperone of 0.1 nM.[15] [^{3}H]PD 128907 binding assays D_3-like dopamine receptors were performed as previously described in detail[14,15] 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 non-specific 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.[15] Haloperidol and U99194A were purchased from Sigma (St. Louis, MO). (+)-S12497 was the generous gift of Dr. Mark Millan of Institut de Recherches Servier.
19. Competition data for these compounds in the [^{3}H]spiperone binding assay was consistent with interactions at two binding sites as analyzed by SigmaPlot, suggesting potential agonist activity at D_2-like receptors. K_i values presented here are for the high-affinity component.