Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1375–1377

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Potent and Selective Dopamine D_4 Antagonists as Candidate Radioligands

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Received 30 January 2001; accepted 23 March 2001

Abstract—A series of dopamine D_4 antagonists was synthesized and evaluated as potential candidates for development as positron emission tomography (PET) radioligands. All new compounds display high affinity and selectivity for the D_4 receptors and compounds **5b**, **5d**, and **5e** were identified as candidates for radioligand development.

The dopamine D_4 receptor is a member of the dopamine D_2-like family of receptors, which include the D_2, D_3, and D_4 receptor subtypes.[1,2] The D_4 receptor differs from the D_2 and D_3 receptors in that it is mostly localized in the extrastriatal areas of the brain such as cortex, hippocampus and thalamus and thus does not follow the dopamine concentration gradient in the CNS, which has the highest DA concentrations in the striatum.[1,3] The initial interest in the D_4 receptor stemmed from the findings that the atypical antipsychotic clozapine had a higher affinity for the dopamine D_4 receptor compared to the D_2 and that postmortem studies reported results consistent with an up-regulation of D_4 receptors in the striatum of patients with schizophrenia.[4,5] Although this initial enthusiasm was somewhat dampened due to the failure to replicate the postmortem findings, more recently published results have in general supported the importance of the D_4 receptor in brain functions and its involvement in neuropsychiatric disorders.[6,7] For example, a recent autoradiographic study reported a selective increase of D_4 receptors in the entorhinal cortex of patients with schizophrenia.[8] Jentch et al.[9] reported the reversal of PCP-induced cognitive deficits in monkeys by the selective D_4 antagonist NGD 94-1 (K_i 3.0 nM at hD_4), suggesting a possible role for the D_4 receptors in the cognitive and memory impairments seen in schizophrenia and Alzheimer's disease. Preclinically, several D_4-selective antagonists, notably NRA 0160 (K_i 0.5 nM at hD_4) and CI 1030 (K_i 4.3 at hD_4), have been shown to be active in animal models predictive of antipsychotic efficacy and thus, may be useful atypical antipsychotics.[10,11] In addition, the investigation of the D_4 receptor's involvement in attention deficit hyperactivity disorder (ADHD), drug and alcohol abuse, Parkinsonism, and depression remain active areas of research.[12]

Despite the unique localization of the D_4 receptor and its possible involvement in a number of neuropsychiatric disorders, the study of this receptor subtype in vivo has so far been hindered by the lack of selective radioligands. To date only two selective D_4 radioligands, [^{3}H]NGD 94-1 and [^{3}H]PNU-101958, have been reported and utilized in binding and autoradiography studies in vitro.[12,13] However, there are no positron emission tomography (PET) radioligands available for in vivo study of the D_4 receptor, despite some recent development efforts in this direction.[14,15] The successful development of a selective D_4 PET radioligand will allow for the study of this receptor in vivo to probe its functions and possible involvement in psychiatric diseases. Herein we report our effort to synthesize selective D_4 receptor antagonists as candidate compounds for possible development as PET radioligands.

The synthetic program was initiated based on the lead compound L745,870 (Fig. 1), the first potent and selective D_4 receptor antagonist reported in the literature.[16]

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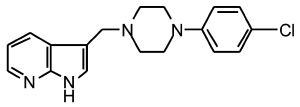
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00241-4

In binding studies L745,870 displays a high affinity (K_i 0.43 nM at hD_4, 1.53 nM at rD_4) and excellent selectivity for D_4 over other receptors.[17,18] Based on this lead compound, a number of derivatives bearing functional groups amenable to labeling with positron-emitting isotopes were prepared and screened in a comprehensive in vitro binding study. Other molecules with alkyl substituent of various length and type were also synthesized and screened to probe the structure–activity relation of this class of compounds.



L-745, 870

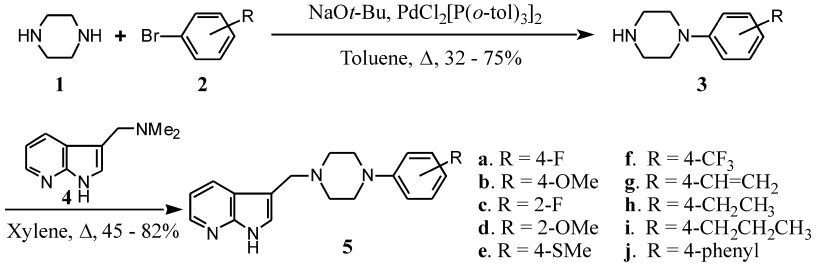
Figure 1. Structural representation of L-745,870.

Preparation of these compounds is described in Scheme 1 and was achieved by the condensation of substituted phenylpiperazines **3** and azagramine **4**.[14] Synthesis of azagramine followed the literature procedures,[19] while the preparation of substituted phenylpiperazine, if not commercially available, was accomplished by the palladium catalyzed aromatic amination of substituted phenylbromides **2** with piperazine **1**.[20] Monosubstituted phenylpiperazines **3e–j** were obtained in 32–75% yields. Desired compounds **5a–j** were prepared in 45–82% yields following purification by recrystallization.[21] All new compounds were characterized by ^1H NMR, MS, HRMS and/or elemental analysis.

All compounds were tested in receptor binding studies. Binding to dopamine D_2 and D_4 receptors was performed by displacement of the radioligand [^{3}H]spiperone at the cloned rat D_2 and D_4 receptors with the test compounds. For binding to cloned rat D_3 receptor the radioligand [^{3}H]sulpiride was used and for D_1 and D_5 [^{3}H]SCH23982 was used. In addition to dopamine receptors, binding affinities of these compounds at various cloned human serotonin (5-HT) receptors, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{5A}, 5-HT_6, and 5-HT_7, as well as a large number of other biogenic amine receptors were also determined as previously described.[22] Selected biological data are presented in Table 1.

As is evident from Table 1 the test compounds demonstrated high affinity for the dopamine D_4 receptors and excellent selectivity over other DA and 5-HT receptors. All compounds display negligible affinity ($K_i > 10\,\mu M$) for the following cloned receptors: D_1, D_5, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2C}, 5-HT_{5A}, κ, δ, μ H_1, NMDA, M_1–M_5, nicotinic acetylcholine receptors and the NMDA PCP site.

From the biological data it is clear that substituents as diverse as fluorine, methoxy, thiomethyl, vinyl and trifluoromethyl, placed at either the *ortho* or *para* position of the phenylpiperazine moiety in L745,870, are well tolerated by the D_4 binding site and the resulting compounds all retain their high binding affinity and selectivity for the D_4 receptor. Replacement of the chlorine in L745,870 with an alkyl group such as ethyl, propyl, or vinyl group also resulted in compounds with high affinity and selectivity for the D_4 receptor (**5h–i**). However, with a bulky group such as the phenyl in the *para* position (**5j**) the binding affinity at D_4 is diminished, although the selectivity remains.



Scheme 1. Synthesis of compounds **5a–j**.

Table 1. In vitro binding affinities (K_i, nM) of compounds **5a–j** at selected DA and 5-HT receptors[a]

Compound	rD4	rD2	rD3	5-HT_{1A}	5-HT_{2A}	5-HT_6	5-HT_7
L745,870	3.7±0.6	>10,000	>10,000	6620.9	453.0	1656.2	226.3
5a	2.3±0.7	>10,000	>10,000	2759.7	232.2	>10,000	412.3
5b	3.6±1.1	>10,000	>10,000	>10,000	>10,000	>10,000	>10,000
5c	3.3±0.5	>10,000	>10,000	990.7	242.0	>10,000	212.9
5d	1.4±0.2	438.0	668.0	405.6	2311.6	7385.0	102.8
5e	4.7±0.5	>10,000	4507.0	>10,000	>10,000	>10,000	335.9
5f	34.1±6.8	>10,000	>10,000	>10,000	2791.0	7109.5	318.2
5g	2.8±0.5	>10,000	>10,000	3967.7	509.0	>10,000	>10,000
5h	5.0±1.0	>10,000	1692.0	>10,000	627.0	4460.7	411.0
5i	6.7±1.1	>10,000	921.0	>10,000	>10,000	6486.5	713.0
5j	148.0±22.2	>10,000	>10,000	>10,000	1932.0	>10,000	>10,000

[a]All assays were conducted in duplicate. For each K_i value at rD_4 the data are the mean±SD of computer-derived estimates for $n=4$ separate determinations. K_i's for other receptors are the means of $n=4$ separate determinations.

Among the analogues of L745,870, the 4-methoxyphenyl derivative (**5b**) has an affinity for D_4 receptor that is comparable to the lead (K_i 3.6 at rD_4 vs 3.7 nM for L745,870), yet with a slightly better selectivity, while the 2-methoxyphenyl analogue (**5d**) has a slightly higher affinity (K_i 1.4 nM) for the D_4 receptor than the lead and somewhat diminished selectivity. Another compound, **5e**, shows affinity and selectivity very similar to the lead (K_i 4.7 nM). These affinities are determined using the cloned rat dopamine receptors. It has been demonstrated that L745,870 displays lower affinity at the rat D_4 receptor than at the human D_4 receptor.[18] Therefore, the affinity of **5b**, **5d**, and **5e** at the human D_4 receptor might also be in the subnanomolar range, similar to that of L745,870 (K_i 0.43 at hD_4). Compounds with nanomolar or subnanomolar affinity at the target receptor are in general good candidates for development as in vivo radioligands for PET imaging. As all these three compounds have a group (methoxy or methylthio) that is amenable to labeling with a positron-emitting [^{11}C]methyl group, they are suitable candidates for radiolabeling and development as potential PET radioligands.

In summary, compounds with high affinity and selectivity for the dopamine D_4 receptors are synthesized and assayed in receptor binding studies. Three compounds (**5b**, **5d**, and **5e**) were identified as potential candidates for development as PET radioligands to investigate the dopamine D_4 receptors in vivo.

Acknowledgements

This study was supported by the National Alliance for Research in Schizophrenia and Depression (NARSAD), the Lieber Center for Schizophrenia Research, and the Public Health Service (NIMH MH59342-01 and K02-MH01603-01). In vitro binding studies were supported by NO1-MH80005 and KO2-MH01366.

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21. The preparation of compound **5b** is typical: Azagramine **4** (175 mg, 1.0 mmol) and 4-(4-methoxyphenyl)piperazine **3b** (1.2 mmol) were dissolved in xylene, stirred at 150 °C for 36 h, and cooled to room temperature. The solid was collected, rinsed twice with ether, and dried to give the product **5b** (245 mg, 75%). Recrystallization from acetone afforded an analytical sample as a colorless solid, mp 209–212 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 8.34 (dd, 1H, J = 1.38, 4.75 Hz), 8.14 (dd, 1H, J = 1.38, 7.86 Hz), 7.32 (s, 1H), 7.12 (dd, 1H, J = 4.75, 7.86 Hz), 6.92 (d, 2H, J = 9.2 Hz), 6.84 (d, 2H, J = 9.2 Hz), 3.83 (s, 5H), 3.13 (m, 4H), 2.70 (m, 4H). Anal. calcd: C, 70.78; H, 6.88; N, 17.38; found: C, 70.91; H, 6.87; N, 17.35.
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