Synthesis and Structure–Activity Relationship of Fluoro Analogues of 8-{[2-{4-(4-Methoxyphenyl)piperazin-1-yl}ethyl]-8-azaspiro[4.5]decane-7,9-dione as Selective α₁d-Adrenergic Receptor Antagonists

Michael J. Konkel,* John M. Wetzel, Marie Cahir,† Douglas A. Craig, Stewart A. Noble,‡ and Charles Gluchowski§
Lundbeck Research USA, Inc., 215 College Road, Paramus, New Jersey 07652

Received October 26, 2004

We have discovered high-affinity antagonists (exemplified by 11 and 12) that are the most selective for α₁d-adrenergic receptors (α₁d-AR) reported to date. In cloned receptor assay systems, 12 displays at least 95-fold selectivity for the α₁d-AR over all other G-protein-coupled receptors tested, and the subtype selectivity of 11 was confirmed in pharmacologically defined isolated tissue preparations.

α-Adrenergic receptors (α-ARs) modulate intercellular biochemical processes in response to changes in extracellular concentrations of the neurotransmitter norepinephrine and the circulating hormone epinephrine, leading to widespread physiological actions that make them attractive targets for drug discovery.1 Antagonists that are highly selective for the α₁a-AR are well-known,1c and recent reports have described compounds with modest selectivity for the α₁b-AR.2 Saussy et al. have reported that 5 (Chart 1)3 is selective for the α₁d-AR,4 and this has been confirmed in our assay systems (Table 1). However, 5 (pKᵢ = 8.8 at α₁d) also has comparable or higher affinity for several other G-protein-coupled receptors (GPCRs),1 including the serotonin 5-HT₁A receptor and the dopamine D₂ and D₃ receptors (Table 1). Cystazosin5 has also been reported to be selective for the α₁D -AR and devoid of cross-reactivity to 5-HT₁A and dopamine receptors. Its subtype selectivity, however, is only about 10-fold. Recently, A-315456 has been reported6 to be selective for the α₁β₁-AR with low cross reactivity to 5-HT₁A and D₂. Also, recently a report by a group from Recordati on analogues of 5 with low cross-reactivity has appeared.7 The report did not mention whether the Recordati compounds had significant cross-reactivity to dopamine receptors, a known cross-reactivity of 5.6

We describe herein the synthesis and SAR of fluoro analogues of 5 and novel trifluoro analogues that show decreased affinity for 5-HT₁A, D₂, and D₃ receptors while maintaining high affinity and subtype selectivity for the α₁d-AR.8,9

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Chart 1

Scheme 1. Synthesis of Compounds 6–13

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* Reagents and conditions: (a) 2-ethanolamine or 2-aminopropanol; (b) SOCl₂, 45% from 1; (c) substituted N-phenylpiperazine, 22–50%.

Synthesis

Compounds 6–13 were synthesized as outlined in Scheme 1. 3,3-Tetramethyleneglutaric anhydride (1) was allowed to react with ethanolamine, giving a mixture of imide 2 and amide 3a. The crude mixture of imide 2 and amide 3a was treated with thionyl chloride,
resulting in condensation of the amide to the imide and substitution of the hydroxy group with chloride. The resulting intermediates 4 were treated with the appropriately substituted N-arylpyperazines, giving the desired compounds 6–13. The N-arylpyperazines were commercially available or synthesized by a previously described procedure. For (12) and its enantiomer (13), tetramethylethenyluracil anhydride (1) was allowed to react with optically pure (R)-2-aminopropanol (or (S)-2-aminopropanol), giving imide 3b that was converted to 4b by thiouyl chloride treatment.

**Pharmacology**

Radioligand binding experiments were performed on membranes prepared from cells transiently transfected with DNA for the cloned human α-AR (α1a, α1b, and α1d) and 5-HT1A, as described previously. Membranes for dopamine human D2 and rat D3 receptors were purchased from New England Nuclear Corporation. The binding affinities (Ki) were determined by displacement of the following radioligands: [3H]prazosin (0.3 nM, α1-ARs), [3H]-[3H]-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 0.3 nM, 5-HT1A), or [3H]spiperone (1 nM, D2, ARs), [3H]-8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT, 0.3 nM, 5-HT1A), or [3H]spiperone (1 nM, D2, ARs), [3H]-8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT, 0.3 nM, 5-HT1A), or [3H]spiperone (1 nM, D2, ARs), [3H]-8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT, 0.3 nM, 5-HT1A), or [3H]spiperone (1 nM, D2, ARs), [3H]-8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT, 0.3 nM, 5-HT1A), or [3H]spiperone (1 nM, D2, ARs). The results are presented in Table 1.

The Ki of 11 at native α1-ARs was determined by measuring antagonism of phenylephrine-evoked contractions in three pharmacologically defined isolated rat tissue preparations: vas deferens (α1A), spleen (α1B), and thoracic aorta (α1D). In each of the preparations 11 behaved as a competitive antagonist. The Ki determined for each tissue (α1A, 5.4; α1B, 6.7; α1D, 8.8) correlates well with the corresponding Ki derived from binding experiments.

**Structure–Activity Relationships**

We hypothesized that the selectivity of 5 for the α1d-AR over the α1a- and α1b-ARs could be largely attributed to the imide moiety because many examples were known of 1-substituted 4-(methoxyphenyl)piperazines that were not α1d-selective. Therefore, we focused our initial studies on modification of the piperazine moiety.

It was found that the methoxyl group of 5 could be replaced with fluorine (6), resulting in decreased affinity for the 5-HT1A and D2 receptors (16- and 13-fold, respectively) but essentially unchanged affinity for the α1a and D3 receptors (Table 1). The 4-fluoro-substituted 7 displays a 18-fold decrease in α1d-AR affinity and a decrease in the selectivity for α1d versus 5-HT1A compared to the 2-fluoro-substituted 6. For difluoro substitution, the substitution pattern of the fluorine atoms on the phenyl ring is critical for maximizing the affinity and selectivity for the α1d-AR. For instance, it was found that the affinity for α1d for 2,5-difluoro-substituted 10 is equivalent to the 2-fluoro-substituted 6 while affinity for the 5-HT1A and α1a receptors is decreased. Compared to 2,5-difluoro substitution (10), the binding affinity and selectivity for the α1d-AR decreased with 2,4-difluoro substitution (8) or 3,4-difluoro substitution (9). The trifluorophenyl-substituted 11 exhibits α1a-AR affinity approximately equal to that of 5 while displaying significantly decreased affinity for α1a, 5-HT1A, and D2 receptors (Table 1). The affinity of 11 for the D2_3 receptor was reduced relative to 5 (6-fold) but was still significantly high (Ki = 23 nM).

Placement of a methyl group on the linker (12) resulted in decreased binding affinity at the D3 receptor (Ki = 123 nM) while maintaining high affinity for the α1d-AR (Ki = 1.3 nM) and greater than 100-fold selectivity over other GPCRs including α1a, α1b, α2a, α2b, α3, D3, and 5-HT1A. The (R)-configuration of the methyl group is important because the (S)-isomer was found to have significantly lower binding affinity for the α1d-AR (Ki ≥ 124 nM).

In conclusion, we have discovered high-affinity antagonists (exemplified by 11 and 12) that are the most selective for the α1d-AR reported to date. In cloned receptor assay systems, 12 displays at least 95-fold selectivity for the α1d-AR over all other GPCRs tested, and the subtype selectivity of 11 was confirmed in pharmacologically defined isolated tissue preparations. These compounds display the highest selectivity for the α1d-AR thus far reported and should prove useful for further functional characterization of α1-ARs in vivo models. The information gained through such studies will aid in the understanding of the physiological importance of the individual α1-AR subtypes and potentially lead to the discovery of therapies that benefit from selective modulation of α1-ARs.

**Experimental Section**

**General Methods.** Substituted N-phenylpiperazines were synthesized according to the procedure described by Martin. The syntheses of 4 were carried out as described previously by Y. H. Wu. [3H]-(R)-2-Aminopropanol and (S)-2-aminopropanol were purchased from Aldrich and both are listed as 97% ee. The actual ee purity for the batch of (R)-2-aminopropanol used in the syntheses described herein was 99.9% (GLC) as communicated to us by Aldrich. Enantiomeric purities of 12 and 13 were determined by chiral HPLC, using a Chiralcel OD, 0.46 cm × 25 cm column (Daicel Chemical Industries, LTD), 1 mL/min (5% EtOH/95% hexane with 0.1% TEA). 1H and 13C NMR spectra were obtained at 300 and 75 MHz, respectively, with CDC13 as solvent and referenced to TMS as an internal standard. Coupling constants (J) are reported in Hz.

**Table 1. Binding Affinities at Cloned α-Adrenoceptors, 5-HT1A, and Dopamine Receptors**

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<th>R3</th>
<th>R4</th>
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<th>α1b</th>
<th>5-HT1A</th>
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<td>F</td>
<td>&gt;1.24</td>
<td>NT</td>
<td>NT^b</td>
<td>NT^b</td>
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^a α1a, α1b, α1a, 5-HT1A, and D3 are cloned human receptors. D3 is a cloned rat receptor. Ki determinations are an average of two or more (four to six for key compounds) independent determinations. The margin of error is within 5% of the mean for all data shown. ^b NT: not tested.

8-[1(R)-2-Chloro-1-methylethyl]-8-azaspiro[4.5]decane-7,9-dione (4b). A mixture of 3,3-tetramethyleneglutaric anhydride (7,9-dione) was allowed to react with (R)-2-Chloro-1-methylethyl-8-azaspiro[4.5]decane-7,9-dione (4b) was found to have significantly lower binding affinity for the α1d-AR (Ki ≥ 124 nM).
methanol, transferred to a preparative thin layer chromatographic plate (silica gel), and eluted with ethyl acetate/hexane (1:1). A band at Rf = 0.3 was removed and rinsed with chloroform/methanol (4:1). The solvent was removed, giving the title compound as a pale-yellow oil (69.8 mg, 0.18 mmol, 41%). 1H NMR δ 7.17 (q, 1H, J = 9.3), 6.65 (dd, 1H, J = 13.5, 6.9, 3.0), 6.59–6.54 (m, 1H), 3.97 (t, 2H, J = 6.5), 3.07 (t, 4H, J = 4.8), 2.68 (t, 4H, J = 5.0), 2.60 (s, 4H), 2.58 (t, 2H, J = 6.3), 1.72–1.68 (m, 4H), 1.54–1.50 (m, 4H); ESI-MS m/z 392 (MH+). The title compound was dissolved in ether and precipitated by addition of 1 N HCl in ether, giving white flakes (mp 227–228 °C).

8-(2-[4-(3,4-Difluorophenyl)piperazin-1-yl]ethyl)-8-azaspiro[4.5]decane-7,9-dione (10). A mixture of 1-(2,4,5-trifluorophenyl)piperazine (100 mg, 0.51 mmol) and 8-(2-chloroethyl)-8-azaspiro[4.5]decane-7,9-dione (100 mg, 0.44 mmol) was heated with stirring at 160 °C for 5 h. The residue was dissolved in methanol, transferred to a preparative thin layer chromatographic plate (silica gel), and eluted with ethyl acetate/hexane (1:1). A band at Rf = 0.3 was removed and rinsed with chloroform/methanol (4:1). The solvent was removed, giving the title compound as a pale-yellow oil (64.9 mg, 0.15 mmol, 35%), (mp 237–238 °C).
solvent was removed, giving the title compound as a pale-
yellow oil (86.2 mg, 0.20 mmol, 47%, 100% ee). 1H NMR δ 6.89
(ddd, 1H, J = 11.7, 10.2, 7.5), 6.71 (dt, 1H, J = 12.0, 8.1), 5.08–
4.96 (m, 1H), 3.14 (dd, 1H, J = 12.6, 10.5), 2.92 (t, 4H, J =
4.7), 2.73–2.66 (m, 2H), 2.58 (s, 4H), 2.51–2.44 (m, 2H), 2.36
(dd, 1H, J = 12.6, 5.4), 1.75–1.68 (m, 4H), 1.57–1.50 (m, 4H),
1.34 (d, 3H, J = 6.9); ESI-MS m/z 424 (MH+). The title compound
was dissolved in ether and precipitated by addition of 1 N HCl in ether,
giving a white solid (mp 231–235 °C).

Acknowledgment. We are indebted to the following people
for their support of this research: Y. Z. Zheng for cell
culture and membrane preparation; Thelma Thompson, Dipa Deshpande, and Michelle Iacolina for
radioligand displacement assays; Faye Hsieh and Usha
Yeramilli for mass spectroscopic analysis; Qingping Han
for enantiomeric purity assessments. We are also in-
debted to the National Institutes of Health for financial
support of this project (SBIR Grant 1 R44 NS33418-
02).

Supporting Information Available: Results from el-
mental analysis. This material is available free of charge via
the Internet at http://pubs.acs.org.

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(12) Since the ee of 13 was 98% and its Kᵢ is 100-fold higher than
that of 12, the observed binding for 13 (Kᵢ = 124 nM)
could be accounted for by the 1% of 12 in the sample.

J0491391