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1-Arylpiperazinyl-4-cyclohexylamine derived isoindole-1,3-diones as potent and selective α-1a/1d adrenergic receptor ligands

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Abstract—Subtype-selective α-1a and/or α-1d adrenergic receptor antagonists may be useful for the treatment of benign prostatic hyperplasia (BPH) and lower urinary tract symptoms (LUTS) with fewer adverse effects than non-selective drugs. A series of 1-arylpiperazinyl-4-cyclohexylamine derived isoindole-1,3-diones has been synthesized, displaying in vitro α_{1a} and α_{1d} binding affinity K_i values in the range of 0.09–38 nM with $K_i(\alpha_{1b})/K_i(\alpha_{1a})$ and $K_i(\alpha_{1b})/K_i(\alpha_{1d})$ selectivity ratios up to 3607-fold.

Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of the prostate and is the cause of lower urinary tract symptoms (LUTS) in a large segment of the elderly male population. There are two major types of symptoms: the voiding (obstructive) symptoms and the irritative (storage) symptoms. Symptoms such as straining, hesitancy, dribbling, weak stream, and incomplete emptying are classified as voiding or obstructive symptoms. These are primarily due to pressure upon the urethra from the physical mass of the enlarged prostate gland (the static component) and the increased tone of the smooth muscle of the prostate stroma and bladder neck (the dynamic component).[1] Symptoms such as increased frequency, urgency, nocturia, dysuria, and burning sensation belong to irritative or storage symptoms, and patients feel that these symptoms are more disturbing than the obstructive symptoms. LUTS also develop in women of a certain age. As in men, LUTS in women include both filling symptoms such as urgency, incontinence, and nocturia, and voiding symptoms such as weak stream, hesitancy, incomplete bladder emptying, and abdominal straining.

The adrenergic receptors (ARs), through which norepinephrine and epinephrine exert their biological activities, are targets for many therapeutically important drugs. The α_1-ARs are members of the G-protein coupled receptor superfamily, and in most cells the primary functional response to activation of all α_1-AR subtypes is an increase in intracellular Ca^{2+}. They play a dominant role in control of smooth muscle contraction and are important in control of blood pressure, nasal congestion, prostate function, and other processes.[2] To date, α_1-ARs have been characterized as three subtypes (α_{1A}, α_{1B}, and α_{1D}) that represent the receptors from animal or human tissues.[3–5] Three genes encoding different α_1-AR subtypes (α_{1a}, α_{1b}, and α_{1d}) have been cloned.[6–8]

Functional studies have established that prostate smooth muscle tone is maintained through α_1-ARs and that these receptors mediate the dynamic component of obstruction. α_1-AR antagonists have successfully been used to treat the obstructive symptoms associated with BPH.[9] Furthermore, the α_{1a}-AR subtype comprises the majority of α_1-ARs in human prostatic smooth muscle and has been shown to mediate contraction in this tissue. α_1-AR antagonists reduce smooth muscle tone

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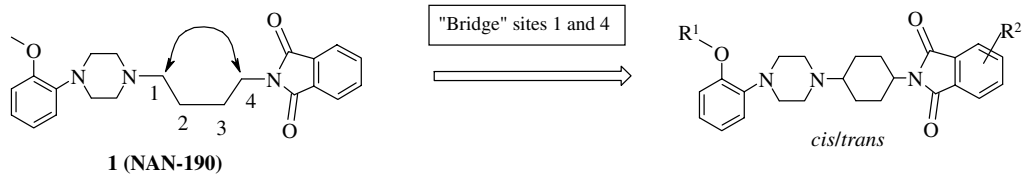
in the prostate and lower urinary tract, thereby relaxing the bladder outlet and increasing urinary flow. The major disadvantage of non-selective α_1-blockers is their adverse effect profile, particularly vasodilatation leading to dizziness, postural hypotension, asthenia, and occasionally syncope.

It remains unclear which α_1-AR is dominant in the human bladder. One study reported a predominance of the α_{1a} subtype mRNA in the bladder dome, base, and trigone.[10] Another report found that the α_{1d} subtype is present as 66% of the α_1-ARs at both the mRNA and protein levels, while the α_{1a} subtype is present as 34% of the total, with no evidence of the α_{1b} subtype.[11] Drugs that selectively antagonize only the α_{1a}-AR subtype appear to have little effect upon the irritative symptoms of BPH. Ro-70004, an α_{1a} subtype-selective compound, was reported to be discontinued in clinical studies when it was found to have poor efficacy in treating these symptoms.[12] α_{1d}-ARs may be involved in mediating the irritative symptoms; however, the location of these α_{1d}-ARs is unknown.[13]

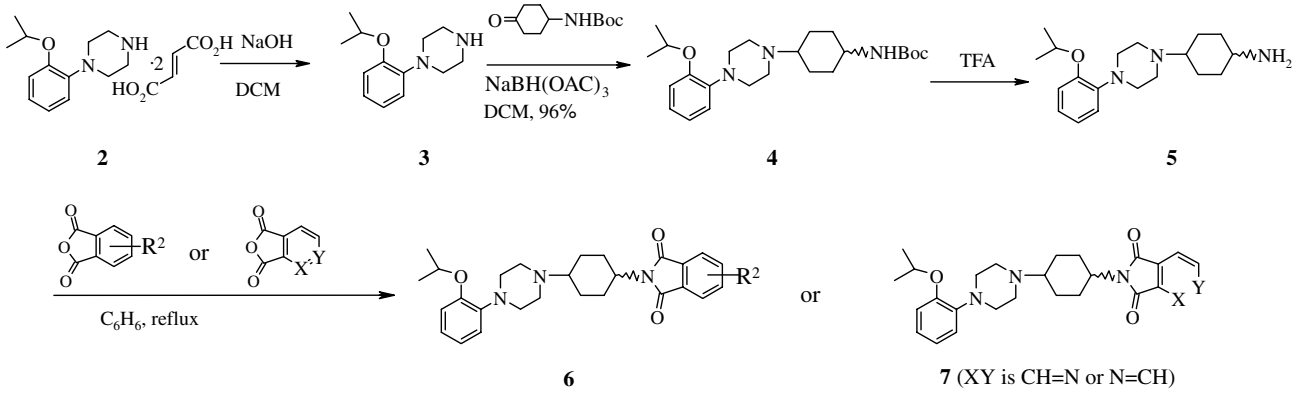
There may be clinical advantages to the pharmacological blockade of the α_{1d}-ARs in the CNS in reducing BPH symptoms. Antagonism of α_{1d}-ARs in the CNS and bladder may be an important activity in reducing the irritative or filling symptoms of BPH and improving patient symptom scores.[14] Tamsulosin (Flomax®, Yamanuchi and Boehringer Ingelheim) is an α_1-AR antagonist that is about 15-fold selective for the α_{1a} and α_{1d} subtypes over the α_{1b} subtype. Large clinical trials of BPH patients with tamsulosin showed improvement in both obstructive and irritative symptoms; however, cardiovascular and erectile dysfunction side effects were seen.[15–17] Ejaculatory dysfunction, or retrograde ejaculation, is a side effect seen in 10–35% of patients using tamsulosin.[18,19] This activity has been attributed to tamsulosin antagonism at the 5-HT_{1a} receptor. Patients treated with non-selective α_1 antagonists also have shown improvement in both obstructive and irritative symptoms, although the risk of vascular side effects is greater. The non-selective α_1-AR antagonists and tamsulosin are contraindicated for use in conjunction with PDE inhibitors. There is likely to be high co-morbidity between LUTS and erectile dysfunction patients. Patients being treated for LUTS with the current α_1-AR blockers will find that they are excluded from using PDE inhibitors.

Generally, the α_{1a} subtype predominates in arteries at the mRNA and protein levels, while all three subtypes are found in veins. The particular vessel bed is important in that α_{1a} is the subtype found primarily in the splanchnic and coronary arteries, while the α_{1d} subtype is the predominant subtype found in the aorta. The α_1-AR subtypes in the vasculature have been found to change with age. Contraction of the mammary artery is mediated by both α_{1a} and α_{1b} subtypes. The number of α_1 receptors in the mammary artery doubles with age; however, the α_{1b} subtype increases to a greater extent than the α_{1a} subtype.[20] The α_{1b} subtype may play a greater role in vascular tone in elderly patients. This suggests that an α_{1a}- and α_{1d}-selective antagonist may have less effects upon the vasculature in elderly BPH patients, resulting in fewer cardiovascular side effects than are seen with non-selective α_1 antagonists and allowing for use in conjunction with PDE inhibitors, while providing relief from both obstructive and irritative symptoms. Such compounds are predicted to be more efficacious treatments for BPH/LUTS patients and may have fewer side effects than existing pharmaceuticals.



1 (NAN-190)

Scheme 1.

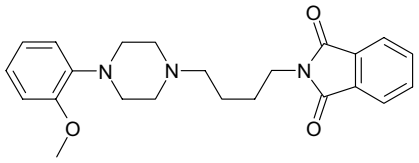
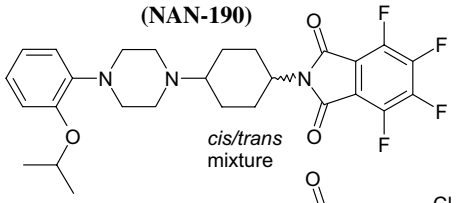
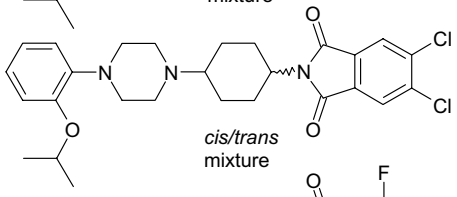
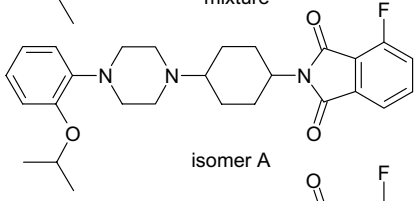
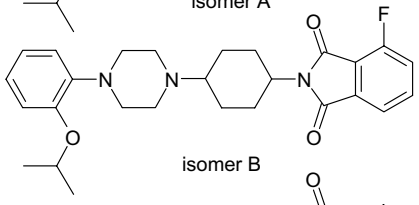
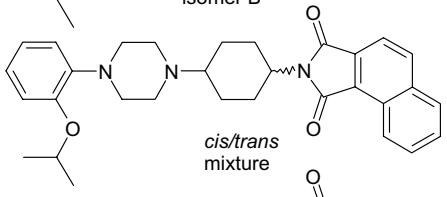
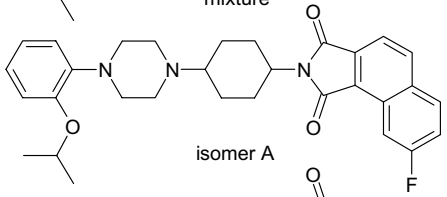
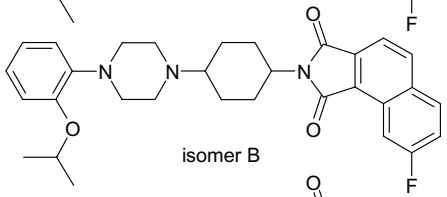
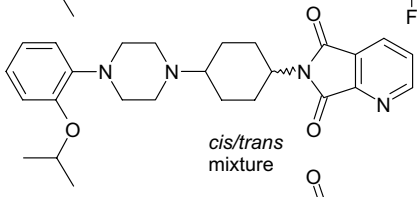
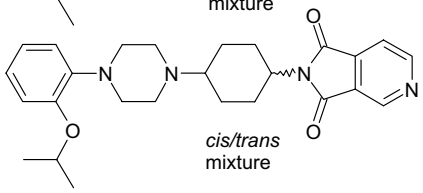


2 **3** **4** **5**

6 **7** (XY is CH=N or N=CH)

Scheme 2. Synthesis of 1-arylpiperazinyl-4-cyclohexylamine derived isoindole-1,3-diones.

Table 1. Binding affinities (K_i, nM) to human α_1-ARs

Compound	Structure[a]	$K_i(\alpha_{1a})$	$K_i(\alpha_{1b})$	$K_i(\alpha_{1d})$	$K_i(\alpha_{1b})/K_i(\alpha_{1a})$	$K_i(\alpha_{1b})/K_i(\alpha_{1d})$
1	 **(NAN-190)**	0.26	0.96	0.3	3.7	3.2
6a	 *cis/trans* mixture	37.8	4108	30.2	109	136
6b	 *cis/trans* mixture	2.7	1082	0.3	401	3607
6c	 isomer A	0.2	18	0.09	90	200
6d	 isomer B	1	127.1	14.9	127	9
6e	 *cis/trans* mixture	1.8	15	0.2	8	75
6f	 isomer A	15	269	16.4	18	16
6g	 isomer B	3.9	46.5	0.5	12	93
7a	 *cis/trans* mixture	0.8	44.6	1.5	56	30
7b	 *cis/trans* mixture	0.4	22.4	0.8	56	28

[a] In some cases, *cis* and *trans* stereoisomers were not separable by silica gel chromatography. **6a**, **6b**, **6e**, **7a**, and **7b** are approximate 1:1 *cis*/*trans* mixtures. For separable isomeric pairs **6c**/**6d** and **6f**/**6g**, NMR spectroscopy failed to allow unambiguous assignment of stereochemistry. In this event, the less polar constituent (**6c** or **6f**) was termed 'isomer A' and the more polar constituent (**6d** or **6g**) was termed 'isomer B'.

A number of studies[21–25] including those from our organization[26,27] have demonstrated that compounds with an open-chain linker between arylpiperazinyl and isoindole-1,3-dione-2-yl groups (e.g., NAN-190, **1**) bind to α_1 adrenergic receptors with high affinity but have very limited subtype selectivity. We hypothesized that bridging sites 1 and 4 of the *n*-butyl linker in compounds like **1**, that is, the incorporation of a cyclohexyl ring into such compounds, will retain binding affinity and improve selectivity among the three α_1-AR subtypes (α_{1a}, α_{1b}, and α_{1d}). Herein, we report a series of 1-arylpiperazinyl-4-cyclohexylamine derived isoindole-1,3-diones as α_{1a}- and α_{1d}-selective ligands (Scheme 1).

The synthesis of 1-arylpiperazinyl-4-cyclohexylamine derived isoindole-1,3-diones **6** or the corresponding heterocyclic diones **7** starting from 1-(2-isopropoxyphenyl)piperazine difumarate **2** is outlined in Scheme 2.[28] The free base **3**, which was generated from **2**, underwent reductive alkylation uneventfully at room temperature to produce the *t*-Boc-protected key intermediate **4** in high yield. Deprotection with trifluoroacetic acid generated the free amine **5** that was subsequently condensed with either the appropriately substituted phthalic anhydride or the anhydride of a pyridine-dicarboxylic acid under reflux in benzene, forming the 1-arylpiperazinyl-4-cyclohexylamine derived isoindole-1,3-diones **6** or aza derivatives **7**.

Binding data (K_i) were determined utilizing a ^{125}I HEAT [(±)-(β-(([^{125}I] 3-iodo-4-hydroxyphenyl)-ethyl)-aminomethyl)-tetralone] radioligand binding assay. In this assay the binding affinities of the synthesized compounds to COS cell membranes expressing the human adrenergic receptor subtypes (α_{1a}-AR, α_{1b}-AR, and α_{1d}-AR) were evaluated. Binding K_i(nM) values as well as ratios of $K_i(\alpha_{1b})$ to $K_i(\alpha_{1a})$ and $K_i(\alpha_{1d})$ are summarized in Table 1.[29]

The open-chain amine derived arylpiperazinyl isoindole-1,3-dione **1** displayed strong affinity toward α_{1a}-AR, α_{1b}-AR, and α_{1d}-AR with sub-nanomolar binding K_i values. The selectivity ratios $K_i(\alpha_{1b})/K_i(\alpha_{1a})$ and $K_i(\alpha_{1b})/K_i(\alpha_{1d})$ were only about 3. By contrast, the cyclohexylamine derived tetrafluoro-isoindole-1,3-dione **6a** showed an improvement in selectivity; ratios $K_i(\alpha_{1b})/K_i(\alpha_{1a})$ and $K_i(\alpha_{1b})/K_i(\alpha_{1d})$ were 109- and 136-fold, respectively, although the binding affinities $K_i(\alpha_{1a})$ and $K_i(\alpha_{1d})$ were 30 and 38 nM, respectively. By changing substituents on the aromatic ring of the isoindole-1,3-dione, $K_i(\alpha_{1b})/K_i(\alpha_{1a})$ and $K_i(\alpha_{1b})/K_i(\alpha_{1d})$ selectivity ratios could be further enhanced. Compound **6b** exemplified such an improvement and retained α_{1a}-AR and α_{1d}-AR binding K_i values that were 2.7 and 0.3 nM, respectively.

The removal of some fluorine atoms on the aromatic ring of the isoindole-1,3-dione had a mixed effect on both the binding affinity and selectivity (**6a** vs **6c**/**6d**). Interestingly, the less polar isomer **6c** showed a better $K_i(\alpha_{1b})/K_i(\alpha_{1d})$ selectivity (200-fold) than $K_i(\alpha_{1b})/K_i(\alpha_{1a})$ selectivity (90-fold). In contrast, the more polar isomer **6d** showed a reverse preference of selectivity ($K_i(\alpha_{1b})/K_i(\alpha_{1a})$ was 127-fold, $K_i(\alpha_{1b})/K_i(\alpha_{1d})$ was only 9-fold). Lastly, the replacement of the phenyl ring of the isoindole-1,3-dione with either fused aromatic rings (e.g., naphthalenyl **6e** or substituted naphthalenyl **6f** and **6g**) or pyridine (**7a** and **7b**) led to compounds that had higher selectivity than NAN-190 (**1**) but were somewhat less selective than **6a**, **6b**, and **6c**. With the exception of isomer **6f**, the naphthalenyl derivatives **6e** and **6g**, and the pyridine analogues **7a** and **7b** maintained high binding affinities toward both α_{1a}-AR and α_{1d}-AR.

In summary, a series of 1-arylpiperazinyl-4-cyclohexylamine derived isoindole-1,3-diones was synthesized. The study of their binding affinities for α_{1a}-AR, α_{1b}-AR, and α_{1d}-AR has led to the discovery of novel family of potent and selective α_{1a}-AR and α_{1d}-AR ligands.

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28. Example. Synthesis of 4,5,6,7-tetrafluoro-2-[4-[4-(2-isopropoxyphenyl)piperazin-1-yl]cyclohexyl]-1*H*-isoindole-1,3(2*H*)-dione (**6a**).
The difumarate of 1-(2-isopropoxyphenyl)piperazine **2** (10 g, 29.7 mmol) was mixed with dichloromethane (DCM, 100 mL) and treated with 1 N NaOH (80 mL). The two layers were separated, the aqueous layer was extracted with DCM (20 mL × 3), and the combined organic extracts were dried over K_2CO_3. The free base **3** (6.5 g) was obtained by evaporating solvent from the filtered dry solution on a rotary evaporator.
At room temperature, 1-(2-isopropoxyphenyl)piperazine **3** (3.00 g, 13.6 mmol), *N*-Boc-4-amino-cyclohexanone (2.90 g, 13.6 mmol), $NaBH(OAc)_3$ (8.6 g, 40.8 mmol), HOAc (1 mL), and anhydrous DCM (80 mL) were mixed together and stirred under nitrogen atmosphere (white slurry became yellowish solution) until no ketone was detected by TLC (100% AcOEt, 18 h). The reaction mixture was diluted with DCM (80 mL), washed with H_2O and NH_4Cl (satd), and dried over Na_2SO_4. Crude product was obtained by removing solvent from the filtered dry solution on a rotary evaporator. Pure product **4** (5.43 g, 13.02 mmol, yield 96%) was obtained by flash chromatography (100% AcOEt, silica gel) as white sticky oil. LC–MS at 2.85 min, *m*/*z* 418.2 (M^++1). ^1H NMR ($CDCl_3$, TMS) δ 1.38 (d, J = 6.0 Hz, 6H), 1.46 (s, 9H), 1.50–2.40 (m, 8H), 2.74 (br s, 4H), 3.13 (br s, 4H), 3.20–4.40 (m, 2H), 4.20–4.90 (m, 2H), 6.80–7.05 (m, 4H).
At room temperature, compound **4** (5.43 g, 13.0 mmol) was dissolved into DCM (25 mL). The resulting yellowish clear solution was stirred with trifluoroacetic acid (TFA, 10 mL) for 0.5 h. The volatiles were removed on a rotary evaporator; the yellow residue was mixed with DCM (80 mL) and was treated with 1 N KOH to pH 10. After separation, the aqueous layer was extracted with DCM (20 mL × 3). The combined organic extracts were dried over K_2CO_3/Na_2SO_4. Crude product **5** (3.08 g, yield 74.6%) was obtained as white sticky oil that was used directly for derivative formation without purification. LC-MS at 2.258 min, *m*/*z* 318.2 (M^++1). ^1H NMR ($CDCl_3$, TMS) δ 1.05–1.20 (m, 1H), 1.20–1.45 (m, 3H), 1.30 (d, J = 6.0 Hz, 6H), 1.48–1.76 (m, 4H), 1.83–2.02 (m, 2H), 2.20–2.50 (m, 1H), 2.55–2.85 (m, 4H), 2.95–3.25 (m, 5H), 4.54–4.60 (m, 1H), 6.80–6.92 (m, 4H).
Compound **5** (0.10 g, 0.32 mmol) and tetrafluorophthalic anhydride (0.070 g, 0.32 mmol) were dissolved in dry benzene (10 mL). The resulting yellowish clear solution was refluxed (80 °C) until no compound **5** was detected by LC–MS (ca. 18 h). Then, the solvent was removed on a rotary evaporator; the residue was dissolved into minimum MeOH and DCM, and loaded on preparative TLC plate (silica gel). The plate was developed in mixed solvents (5% MeOH/DCM). Pure compound **6a** (a *cis* and *trans* mixture) was obtained as yellowish oil. MS, *m*/*z* 520.0 (M^++1). ^1H NMR ($CDCl_3$, TMS) δ 1.36 (d, J = 6 Hz, 6H), 1.42–1.60 (m, 2H), 1.60–1.93 (m, 5H), 2.00–2.58 (m, 4H), 2.65–2.88 (m, 3H), 2.95–3.28 (m, 3H), 4.00–4.20 (m, 1H), 4.50–4.70 (m, 1H), 6.8–7.05 (m, 4H).
29. Although compounds **6** and **7** were not tested in a functional assay, they share key structural features such as the 1-(2-isopropoxyphenylpiperazine) moiety with literature α_1-AR antagonists (Ref. 26) and hence are also expected to have antagonist activity.