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Communications to the Editor

Synthesis and Pharmacologic Evaluation of 2-*endo*-Amino-3-*exo*-isopropylbicyclo[2.2.1]heptane: A Potent Imidazoline$_1$ Receptor Specific Agent

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The imidazoline (I receptors) receptor family has received extensive attention since its identification by Bousquet *et al.*, Coupry *et al.*, and Ernsberger *et al.* in the 1980s.[1] Recently, agmatine (**1**, Figure 1) has been proposed to be the endogenous ligand for the I receptors.[2] There is some question whether agmatine is the sole endogenous agent.[3] These receptors do not interact with catecholamines including norepinephrine (**2**), the natural ligand for adrenoceptors. The imidazoline family of receptors does, however, recognize many imidazoline-containing α_2 adrenergic agents including clonidine (**4**) and moxonidine (**5**). The I_1 receptor subtype in particular has been suggested to mediate a variety of physiologic functions including the reduction of blood pressure.[4] To date, the evidence for the imidazoline binding site functioning as a receptor has been indirect as all of the ligands described interact with other receptors including the α_2 receptors. Thus, agents including clonidine and moxonidine interact with both receptor families precluding confirmation of the specific receptor responsible for the observed physiologic response. Our interest in this area arose from our search for selective α_2 agonists possessing no imidazoline receptor activity. Our hypothesis was that enhanced receptor selectivity could be achieved through preparation of a conformationally restrained framework. An α_2 agonist without imidazoline receptor activity should reduce elevated intraocular pressure, a condition often associated with glaucoma, without untoward side effects including reduction of blood pressure.

During the course of our studies, we discovered AGN 192403 (**3**), the first agent that is equipotent to moxonidine in imidazoline receptor binding assays and nonpotent in adrenergic binding assays. To our surprise, this potent, I_1 specific agent was devoid of all physiologic response. Compound **3** did not affect blood pressure, intraocular pressure, or induce sedation in our animal models. Further, **3** did not antagonize the effects of agonists thought to mediate their physiologic effects through imidazoline receptors nor did **3** potentiate the effects of putative imidazoline receptor agonists. This may suggest that we have identified a new binding site. *Alternatively, this may imply that the imidazoline site defined by this binding assay is not a functional receptor but rather simply a nonfunctional binding site.*

Compound **3** was prepared as shown in Scheme 1. 2-Methylpropionaldehyde was condensed with nitromethane in the presence of potassium hydroxide[5] and then treated with methanesulfonyl chloride in the presence of triethylamine[6] to afford (*E*)-1-nitro-3-methyl-1-butene as the sole isomer in 50% yield. Diels–Alder reaction of the nitro olefin and freshly cracked cyclopentadiene in dichloroethane in a sealed tube at 80 °C afforded the [2.2.1] system bearing an *endo* nitro group and an *exo* isopropyl group in 60% yield as the major product.[7] This was confirmed by a single-crystal X-ray analysis of the subsequent reduction product, **3**. The reduction was conducted in ethanol and an atmosphere of hydrogen using catalytic Pd–C to afford a quantitative yield of the amine. Those conditions reduced both the nitro group and the double bond in a single step.

Compound **3** was evaluated in a variety of binding assays. Binding studies were conducted using adrenergic membrane preparations to determine the subtype selectivity. The α_1 preparation was from human brain and has a mixed population of subtypes. The α_2 membranes were all from CHO cells transfected with appropriate constructs: the α_{2A} was the human C-10 receptor; the α_{2B} was the rat RNG receptor; the α_{2C} was the human C-2 receptor. The imidazoline I_1 preparation was from the bovine ventrolateral medulla.[8] Binding affinity for the I_1 binding site was determined by the displacement of [^{3}H]clonidine binding with concurrent

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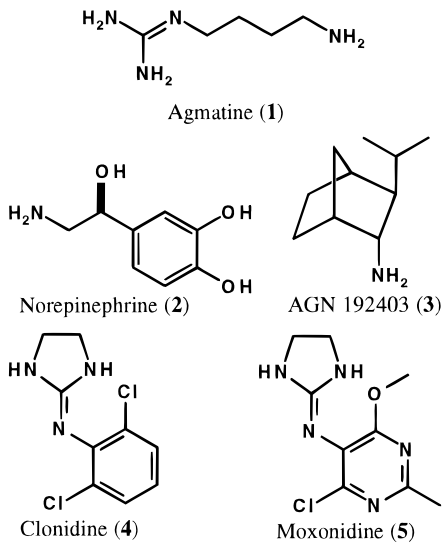
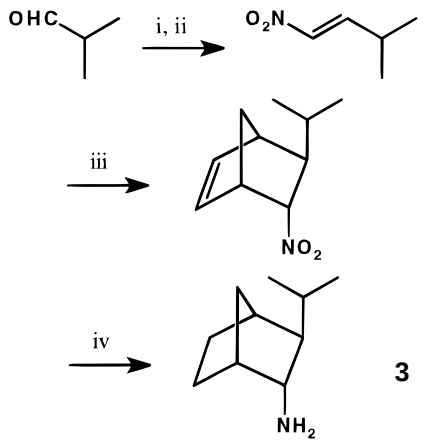


Figure 1.

Scheme 1[a]



[a] Reagents: (i) CH_3NO_2, 3 N methanolic KOH; (ii) MsCl, Et_3N; (iii) cyclopentadiene, $Cl(CH_2)_2Cl$; (iv) Pd−C, H_2, EtOH.

Table 1. Binding Affinities

	K_i (nM, ± SEM)[a]				
agent	α_1	α_{2A}	α_{2B}	α_{2C}	I_1
2	2600 ± 290	66 ± 4.2	78 ± 6.5	63 ± 7.2	>100000
3	>100000	>20000	>20000	>20000	42 ± 17
4	510 ± 110	3.8 ± 0.4	8.3 ± 0.2	30 ± 2	8.9 ± 2.2
5	>30000	150 ± 25	1000 ± 34	2000 ± 200	56 ± 8.7

[a] In all cases where the value is listed as "greater than", the SEM is not reported as all measurements were greater than the number reported.

masking of the α_2 receptors with norepinephrine. The results of this evaluation are presented in Table 1.

Compound **3** had an affinity of 42 nM for the I_1 preparation. This proved comparable to that of moxonidine, an agent thought to mediate its effect on blood pressure through the I_1 site.[8] Compound **3** was only 5-fold less potent than clonidine at the I_1 binding site. In sharp contrast, the agent was devoid of binding activity at any α adrenoceptor evaluated. **3** is the first ligand reported to bind selectively at the I_1 binding site. While the data are not presented, the agent is devoid of affinity for the I_2 binding sites as well. Clonidine was potent in all α_2 and in I_1 binding assays while moxonidine displayed moderate affinity for the α_{2A} receptor and had a high affinity for the I_1 binding site.

Upon intravenous administration to cynomologus monkeys, **3** proved to have no affect on blood pressure at a dose as high as 5000 μg/kg while clonidine reduced blood pressure at a dose of 17 μg/kg and moxonidine reduced blood pressure at a dose of 167 μg/kg. When the animals were pretreated with **3** and then treated with clonidine, there was no affect on clonidine's ability to reduce blood pressure. The agent also had no affect on blood pressure upon direct administration of the agent to the central nervous system of rabbit.[9] We demonstrated that the agent penetrated the cornea.[10] We therefore attempted to reduce elevated intraocular pressure with topical administration of this agent to the rabbit.[11] Again, there was no observed physiologic response to the agent. The agent also proved to be nonsedating in both monkey and rat.

We have discovered an agent that is specific for binding to the I_1 binding site. On the basis of the potency and selectivity of this agent in binding assays, we evaluated it using *in vivo* models for reduction of blood pressure, reduction of intraocular pressure, and sedation. Compound **3** was devoid of both agonist and antagonist activity in all functional assays used. Our data suggest that the I_1 site is a unique binding site. It may not, however, be a functional receptor. These conclusions may be supported by recent pharmacologic studies suggesting that the effects of rilmenidine, another putative I_1 agent, may also be mediated by α_2 adrenoceptors.[12]

Acknowledgment. We thank Professor Paul Ernsberger (Division of Hypertension, Case Western Reserve School of Medicine) for confirming our binding selectivity and Dr. Marilyn Olmstead (Department of Chemistry, U. C. Davis) for the X-ray analysis.

Supporting Information Available: Details of the agent synthesis, X-ray crystallographic analysis, and binding assays (12 pages) are available. Ordering information is given on any current masthead page.

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