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α_1-Adrenoceptor Activation: A Comparison of 4-(Anilinomethyl)imidazoles and 4-(Phenoxymethyl)imidazoles to Related 2-Imidazolines

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Abstract—Literature reports suggest that disruption of an interhelical salt bridge is critical for α_1-adrenoceptor activation, and the basic amine found in adrenergic receptor ligands is responsible for the disruption. Novel 4-(anilinomethyl)imidazoles and 4-(phenoxymethyl)imidazoles are agonists of the cloned human α_1-adrenoceptors in vitro, and potent, selective α_{1A}-adrenoceptor agonists have been identified in this series. These imidazoles demonstrate similar potencies and α_1-subtype selectivities as the corresponding 2-substituted imidazolines. The extremely close SAR *suggests* that, in spite of the large difference in basicity, these imidazoles and imidazolines may establish the same interactions to activate α_1-adrenoceptors.

The three known α_1 adrenoceptor subtypes (α_{1A}, α_{1B}, and α_{1D}) are 7TM G-protein coupled receptors (GPCRs) that are activated by the neurotransmitter norepinephrine and the neurohormone epinephrine. The mechanism of receptor activation of 7TM GPCRs is not well-established, and is an area of much investigation. Many GPCRs have an acidic residue on the third transmembrane helix (an aspartic or glutamic acid) and binding of the basic amine of an agonist to this acid appears to be a key step in receptor activation. Studies on the mechanism of α_1-adrenoceptor activation by Perez and co-workers using site-directed mutagenesis indicate that an interhelical salt bridge exists in the basal state between an aspartic acid (D^{125}) on the third transmembrane helix and a lysine (K^{331}) on the seventh transmembrane helix of the α_{1B}-adrenoceptor.[1] Their elegant studies suggest that α_1-adrenoceptor agonists such as epinephrine (a phenethylamine) disrupt this salt bridge via competition of the protonated amine for the aspartate, leading to receptor activation. The lysine on helix seven is conserved in the other two α_1-adrenoceptor subtypes, and recent modeling studies on the α_{1D}-adrenoceptor by Carotti and co-workers support the Perez hypothesis.[2] Based on a model where competition with a lysine for an aspartate is a key step in receptor activation, the basicity of a ligand's amine could be expected to effect its ability to activate the receptor.

Subtype-selective ligands may separate the cardiovascular and urogenital effects of α_1 agonists. Literature evidence suggesting that α_{1A} may be the predominant subtype in urethral tissue led us to seek α_{1A}-selective agonists (vs α_{1B} and α_{1D}) as a possible improved treatment for stress urinary incontinence.[3] We have recently reported on a diverse set of 2-(anilinomethyl)imidazolines and 2-(phenoxymethyl)imidazolines that are novel, potent and selective α_{1A} agonists (e.g., **1** and **2**, respectively).[4] We wanted to identify other novel structural types that would provide selective α_{1A} agonists to improve our chances of clinical success, especially in light of literature data that indicates that some imidazolines have solution stability issues (the imidazoline can be hydrolyzed to the *N*-(aminoethyl)amide, generally at neutral to high pH).[5] Accordingly, we were interested in exploring related series that would not possess this potential development liability. One simple change was to replace the 2-imidazoline with a 4-imidazole, as

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imidazoles are known adrenergic receptor agonists.[6] While 4-(anilinomethyl)imidazoles and 4-(phenoxymethyl)imidazoles have been reported as adrenergic receptor ligands, they are typically described as α_2 agonists.[7] One report does indicate that some of these compounds also have affinity for α_1 adrenoceptors, such as compounds **3** and **4**, which demonstrated IC_{50}s at α_1 of 102 and 263 nM, respectively[8] (Fig. 1).

While both imidazoles and imidazolines are known to be α_1 agonists, imidazoles are significantly less basic than imidazolines (for instance, the calculated pK_a of imidazoline compound **5** is 10.3 while the calculated pK_a for the corresponding 4-imidazole, compound **6**, is 6.5).[9] Given the differences in pK_a, aromaticity, and planarity (the aromatic imidazole ring is planar, whereas low-energy conformations of imidazolines are not), it is interesting to consider whether α_1 agonists containing imidazoles and imidazolines bind and activate receptors in a similar manner.[10]

To quickly assess whether 4-(anilinomethyl)imidazoles and 4-(phenoxymethyl)imidazoles would provide potent, α_{1A}-selective α_1 agonists, we utilized the structure–activity relationships developed in our imidazoline series. Several representative 4-(anilinomethyl)imidazoles and 4-(phenoxymethyl)imidazoles were synthesized and tested in a cellular assay for functional agonism at the α_{1A}, α_{1B} and α_{1D} adrenoceptor subtypes for comparison to the corresponding imidazolines (Fig. 2).

Chemistry

4-(Anilinomethyl)imidazoles were prepared by alkylating the appropriate anilines with 4-(chloromethyl)imidazole hydrochloride, typically by heating in a protic solvent (Scheme 1).[11]

4-(Phenoxymethyl)imidazoles were synthesized from the appropriate phenols using a protected 4-(hydroxymethyl)imidazole under Mitsunobu conditions (Scheme 2).[12]

The anilines required for the preparation of compounds **15**–**18** were synthesized from isatoic anhydride as previously described.[13] The aniline needed for compounds **13** and **14** was obtained via reduction of the commercially available 5-(2-nitrophenyl)oxazole using hydrazine and catalytic palladium on carbon. The phenols and anilines required for the remainder of the target compounds were available from commercial sources.[14]

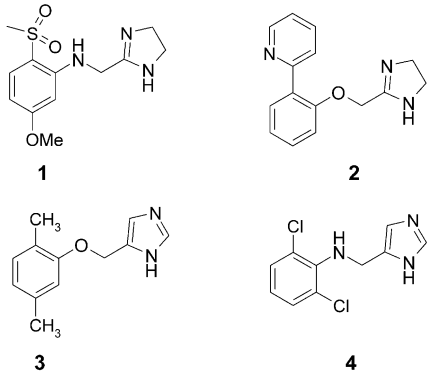
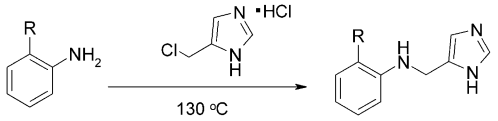
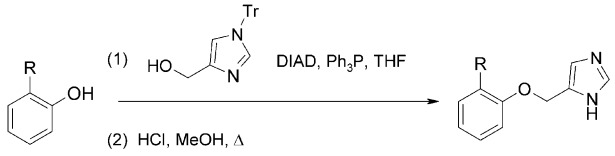


Figure 1.



Scheme 1.



Scheme 2.

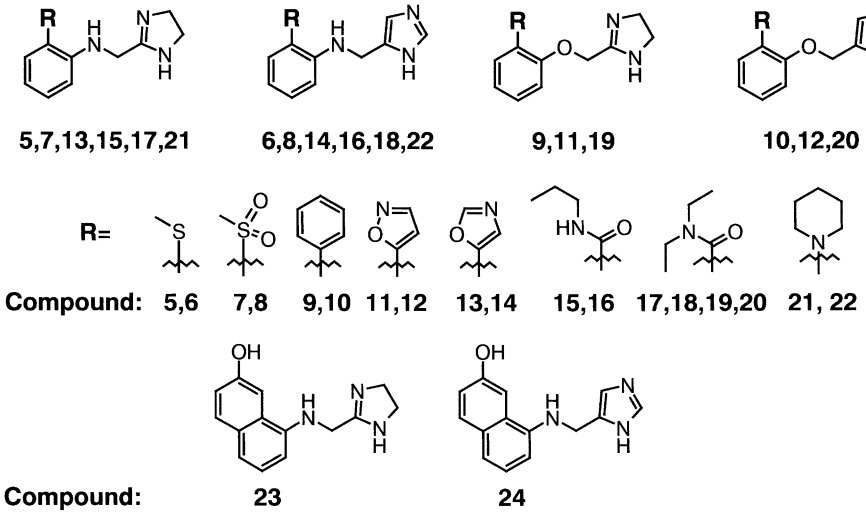


Figure 2.

Results

To model the potential ability of ligands to activate the individual α_1-subtypes in humans, all compounds were evaluated in a cell-based functional assay using the cloned human receptors expressed in rat-1 fibroblasts.[15] The agonist potency (expressed as the pEC_{50}) and efficacy (expressed as a percent of the maximal effect of the α_1-adrenoceptor agonist standard, phenylephrine) of both the imidazolines and the novel imidazole analogues are reported in Table 1. The affinity data for select imidazolines and imidazoles is shown in Table 2.[16]

2-(Anilinomethyl)imidazolines with 2′-sulfides and 2′-sulfones are potent α_{1A} agonists.[14] Small sulfides such as compound **5** lacked subtype selectivity while sulfones such as compound **7** possessed reduced potency but much better functional agonism selectivity for the α_{1A} receptor. The corresponding imidazoles, compounds **6** and **8**, had slightly reduced α_{1A} potencies compared to the imidazolines, with similar selectivity profiles.

2′-Aryl and 2′-heteroaryl substituents were also found to provide potent α_{1A} agonists in both the 2-(anilinomethyl)imidazoline and 2-(phenoxymethyl)imidazoline series.[17] To rapidly assess the viability of these substituents in the corresponding imidazole series, 2′-phenyl (**10**) and 2′-isoxazole (**12**) analogues were made in the 4-(phenoxymethyl)imidazole series and a 2′-oxazole analogue (**14**) was synthesized in the 4-(anilinomethyl)imidazole series. While the 2′-phenyl (**10**) was slightly less potent but had similar selectivity as the corresponding imidazoline (**9**), the 2′-isoxazole (**12**) showed improved selectivity versus **11**, as it had no functional agonist activity at the highest concentrations tested at the α_{1B}- and α_{1D}-subtypes. The 2′-oxazole (**14**) had slightly less α_{1A} activity than **13**, and gained α_{1D} partial agonist activity.

Small 2′-amines, 2′-amides and fused bicyclic aromatic compounds (such as naphthalenes) have also been identified as potent α_{1A} agonists in the 2-(anilinomethyl)imidazoline series, however the corresponding 2′-amides in the 2-(phenoxymethyl)imidazoline series were devoid of α_1 agonist activity.[18] The functional agonism data on representative analogues in the 4-imidazole series (**16**, **18**, **20**, **22**, **24**) demonstrated that the 4-imidazoles and 2-imidazolines possess similar activity profiles across all three α_1-adrenoceptors for these sub-series.

Table 1. In vitro functional agonism activity[a]

	α_{1A}		α_{1B}		α_{1D}	
	pEC_{50}	%Max[b]	pEC_{50}	%Max[b]	pEC_{50}	%Max[b]
NS-49	6.5	86	<4.0	—	<4.0	—
Cirazoline	7.9	93	7.2	72	6.9	31
1	7.9	102	<4.0	—	<4.0	—
2	7.9	104	<4.0	—	<4.0	—
5	8.3	105	8.7	105	8.1	55
6	8.0	94	7.6	78	7.7	36
7	7.3	105	<4.0	—	<4.0	—
8	6.9	97	<4.0	—	<4.0	—
9	8.5	96	<4.0	—	<4.0	—
10	7.8	102	<4.0	—	<4.0	—
11	8.2	91	7.0	81	7.0	34
12	6.8	91	<4.0	—	<4.0	—
13	7.7	107	<5.3	—	<5.3	—
14	7.3	120	<5.3	—	6.6	51
15	8.1	104	7.1	77	6.8	69
16	8.4	100	6.5	89	7.1	77
17	7.5	105	5.7	32	<5.3	—
18	7.2	114	<5.3	—	<5.3	—
19	<4.0	—	<4.0	—	<4.0	—
20	<4.0	—	<4.0	—	<4.0	—
21	7.9	114	<5.3	—	<5.3	—
22	7.3	95	<5.3	—	<5.3	—
23	8.2	110	<5.3	—	7.5	53
24	7.2	100	<5.3	—	<5.3	—

[a]See ref 15 for a description of the assay. Each entry represents the mean of at least two experiments, with pEC_{50}s having an average SEM of ±0.12.
[b]% of phenylephrine response (40 μM).

Table 2. Binding affinities for select imidazolines and imidazoles[a]

	α_{1A}	α_{1B}	α_{1D}
	pIC_{50} (±SEM)[b]	pIC_{50} (±SEM)	pIC_{50} (±SEM)
5	6.97 (±0.07)	6.66 (±0.01)	7.71 (±0.04)
6	6.77 (±0.02)	6.05 (±0.08)	7.24 (±0.06)
9	7.18 (±0.03)	5.62 (±0.03)	6.26 (±0.02)
10	6.52 (±0.07)	5.23 (±0.09)	6.03 (±0.17)
15	6.55 (±0.08)	5.77 (±0.16)	6.29 (±0.29)
16	7.11 (±0.15)	5.52 (±0.02)	6.06 (±0.08)
19	<4.52	<4.52	<4.52
20	<4.52	<4.52	<4.52

[a]See ref 16 for a description of the assay.
[b]Each entry is the mean of at least two experiments.

Conclusions

Novel 4-(anilinomethyl)imidazoles and 4-(phenoxymethyl)imidazoles are agonists of the cloned human α_1-adrenoceptors in vitro, and potent, selective α_{1A}-adrenoceptor agonists have been identified. The molecules examined in this series generally retain the potency and selectivity of the analogous 2-imidazolines. Considering this in light of the Perez hypothesis of the mechanism of agonist-induced α_1-adrenoceptor activation, it is possible that in the microenvironment of the receptor a relatively non-basic imidazole may be as effective as a basic imidazoline in disrupting the α_1-adrenoceptor interhelical salt bridge, leading to receptor activation. There is considerable data indicating that imidazoles on histidines are frequent and effective salt bridge partners for aspartic acids in proteins.[19] Once the imidazole of a 4-(anilinomethyl)imidazole or a 4-(phenoxymethyl)imidazole comes in contact with the aspartic acid on the third transmembrane helix, it should be able to establish favorable interactions. While further studies probing the ligand–receptor interactions are needed to support the hypothesis, the extremely close SAR exhibited by these imidazolines and imidazoles across all three subtypes may indicate that they bind and activate adrenoceptors via similar interactions, forming similar ligand–receptor complexes.

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15. Human α_{1A} (clone #137-12), α_{1B} (clone #37-11) and α_{1D} (clone #16–7)-adrenoceptors were expressed in Rat-1 fibroblast cells. Receptor activation was determined via calcium mobilization through the Gq coupled PLC pathway using calcium-sensitive fluorescent dyes (Calcium Green, Molecular Probes C 3011), measured by a Fluorescent Light Imaging Plate Reader (FLIPR). Eleven point concentration–response curves were calculated as percent of the 40 μM phenylephrine response, with the highest sample concentration typically 5 μM. The assay results for NS-49 and cirazoline are shown for comparative purposes.

16. Affinity of compounds at α_1-adrenoceptor subtypes was determined by radioligand binding techniques using membranes prepared from Rat-1 fibroblasts expressing human α_{1A}, α_{1B}, and α_{1D}-adrenoceptors as previously described. See: Gobel, J.; Saussy, D. L.; Goetz, A. S. *J. Pharmacol. Toxicol.* **1999**, *42*, 237 Consistent with literature reports and receptor theory, the α_{1A}-subtype selectivity observed in the cell-based agonist functional assays did not correlate directly with subtype selectivity in receptor binding assays.

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18. For amides in the 2-(anilinomethyl)imidazoline series, a hydrogen bond between the amide carbonyl and the N-H of the aniline appears to be important for achieving the active conformation with the receptor, and 2-(phenoxymethyl)imidazoline amide compounds such as **19** cannot make this type of hydrogen bond. The same phenomena was observed for amides in the 4-imidazole series, indicated that the potential for hydrogen bonding between the amide carbonyl and the linker N-H may be important in the 4-(anilinomethyl)imidazole series also. See: Bishop, M. J.; Berman, J.; Bigham, E. C.; Garrison, D. T.; Gobel, M. J.; Hodson, S. J.; Irving, P. E.; Liacos, J. A.; Minick, D. J.; Navas, F.; Saussy, D. L., Jr.; Speake, J. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2871.

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