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2-(Anilinomethyl)imidazolines as α_1-Adrenoceptor Agonists: The Identification of α_{1A} Subtype Selective 2′-Carboxylic Acid Esters and Amides

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Abstract—2-(Anilinomethyl)imidazolines with 2′-esters or 2′-amides are potent agonists of the cloned human α_1-adrenoceptors in vitro. The size and shape of the *ortho* substituent can have significant effects on the potency, efficacy, and subtype selectivity of these 2-(anilinomethyl)imidazolines. α_{1A}-subtype selective agonists have been identified.

In 1971, a Warner Lambert patent specifically claimed 2′-carboxylic acid and methyl ester 2-(anilinomethyl)-imidazolines (e.g., **1**) as potent pressor agents.[1] The stated mechanism of action, consistent with related known pressor agents, was α-adrenergic activation. Since that time, α-adrenoceptors have been subdivided into two families based on structure and pharmacology (α_1 and α_2). More recently, three subtypes have been isolated, cloned, and expressed for both α_1 and α_2.[2] Among other functions, α_1-adrenoceptors are involved in smooth and cardiac muscle contraction. The three different α_1-subtypes have varying expression levels in different tissues, and therefore subtype selective agents may be useful as pharmacological tools and possibly as improved therapeutic agents.[3] Due to evidence indicating that α_{1A} is the primary α_1-subtype found in the urethra, we are particularly interested in evaluating selective α_{1A}-agonists for the treatment stress urinary incontinence.[4]

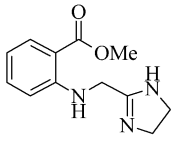

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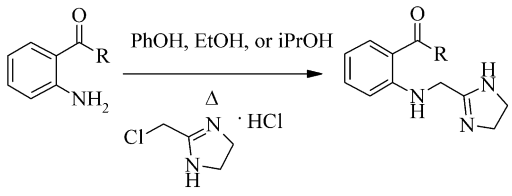
Figure 1.

Our evaluation of methyl ester **1** at the three cloned human α_1-adrenoceptor subtypes in a cell-based functional assay[5] revealed that it is a sub-nanomolar, full agonist at α_{1A} ($pEC_{50} = 9.1$), α_{1B} ($pEC_{50} = 9.4$), and α_{1D} ($pEC_{50} = 9.3$) adrenoceptors (Fig. 1).[6]

This molecule can achieve favorable interactions with all three α_1-subtypes to facilitate binding to and activation of the receptors, and is therefore a useful starting point in the search for novel subtype selective agonists. We have synthesized a series of 2-(anilinomethyl)imidazolines with simple 2′-ester and 2′-amide substituents to evaluate the effect of subtle *ortho* substituent variation on the ability of these ligands to activate the individual α_1-adrenoceptor subtypes.

2-(Anilinomethyl)imidazolines were synthesized from *o*-substituted anilines and 2-(chloromethyl)imidazoline hydrochloride[7] by heating in a protic solvent, such as phenol, isopropanol, or ethanol (Scheme 1).[8]



Scheme 1.

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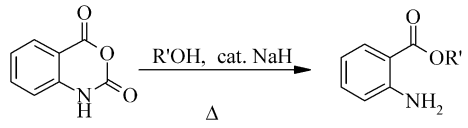
Non-commercial anthranilic acid esters were prepared by heating isatoic anhydride and the appropriate alcohol with catalytic sodium hydride (Scheme 2).[9] Anthranilamides were prepared by opening isatoic anhydride with primary or secondary amines.[10]

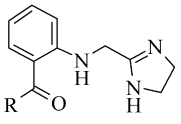


Scheme 2.

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.[5] 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 select 2-(anilinomethyl)imidazolines with 2′-esters and 2′-amides are listed in Tables 1 and 2.

As previously mentioned, the 2′-methyl ester reported by Warner Lambert was a potent full agonist at all three α_1-adrenoceptor subtypes. Simple changes to the ester alkyl group led to subtype selectivity. The ability to activate α_{1D} was significantly reduced (both potency and efficacy) with every other ester, including straight chain, α-branched, and β-branched alkyl groups. Lengthening the ester alkyl chain from methyl to ethyl produced a ligand that was still a full agonist at α_{1A} and α_{1B}, however potency dropped off more than 10-fold at α_{1A} and more than 100-fold at α_{1B}. Further lengthening had an effect on α_{1B} efficacy also, as the butyl ester was only a partial agonist at the α_{1B}-subtype. α-Branched esters resulted in even greater subtype selectivity. The isopropyl, *sec*-butyl, and 3-pentyl esters were potent, full agonists at α_{1A}, but were inactive at α_{1B} and α_{1D} at the highest concentrations tested. Curiously, the *t*-butyl ester was still a reasonably potent agonist ($pEC_{50} = 7.2$) at α_{1B}. Constraining the α-branched ester in the form of a cyclopentyl group resulted in a loss of efficacy at all three α_1-receptor subtypes, and the larger cyclohexyl ester was even inactive at α_{1A}. The smallest β-branched ester, isobutyl, had only partial agonist activity at α_{1A} and α_{1D}, and was more potent and efficacious at α_{1B} than at α_{1A}. This is the only ester (larger than methyl) tested that exhibited subtype selectivity (albeit slight) for α_{1B}.

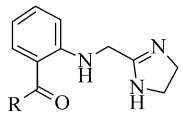
Table 1.[a]



Compd	R	α_{1A}		α_{1B}		α_{1D}	
		pEC_{50}	% Max[b]	pEC_{50}	% Max[b]	pEC_{50}	% Max[b]
Oxymetazoline		7.7	74	< 5.3	—	< 5.3	—
Naphazoline		7.2	72	< 5.3	—	< 5.3	—
Cirazoline		7.9	93	7.2	72	6.9	31
1	OMe	9.1	110	9.4	107	9.3	80
2	OEt	7.9	104	6.8	105	7.2	41
3	OBu	8.0	114	7.6	63	7.8	31
4	O*i*Pr	8.5	100	< 5.3	—	< 5.3	—
5	O*sec*-Bu	8.9	96	< 5.3	—	< 5.3	—
6	O(3-pentyl)	7.7	94	< 5.3	—	< 5.3	—
7	Ocyclopentyl	7.4	58	< 5.3	—	< 5.3	—
8	Ocyclohexyl	< 5.3	—	< 5.3	—	< 5.3	—
9	O*t*-Bu	8.1	96	7.2	81	< 5.3	—
10	O*i*Bu	6.3	68	7.1	90	6.6	59

[a]See ref 5 for a description of the assay. Each entry represents the mean of at least two experiments, with an average SEM of ±0.16.
[b]% of phenylephrine response (40 μM).

Table 2.[a]



Compd	R	α_{1A}		α_{1B}		α_{1D}	
		pEC_{50}	% Max[b]	pEC_{50}	% Max[b]	pEC_{50}	% Max[b]
11	NHMe	8.6	104	8.8	99	8.0	115
12	NHEt	7.9	102	6.7	64	7.1	33
13	NHPr	8.1	104	7.1	77	6.8	69
14	NHButyl	7.7	103	< 5.3	—	< 5.3	—
15	NH*i*Pr	7.3	102	6.9	95	6.5	59
16	NH*sec*-Bu	6.3	82	< 5.3	—	< 5.3	—
17	NHcyclopropyl	7.8	99	6.6	50	< 5.3	—
18	NHcyclobutyl	< 5.3	—	< 5.3	—	< 5.3	—
19	NHcyclopentyl	< 5.3	—	< 5.3	—	< 5.3	—
20	NH*i*Bu	7.1	58	7.9	90	7.8	31
21	$N(Me)_2$	7.7	104	7.1	93	6.7	58
22	N(Me)(Et)	7.3	105	6.6	98	6.2	69
23	$N(Et)_2$	7.5	105	5.7	32	< 5.3	—
24	N(Pr)(Et)	7.0	115	< 5.3	—	< 5.3	—
25	Pyrrolidinyl	6.9	105	6.5	79	< 5.3	—
26	Piperidinyl	6.4	105	7.1	106	6.4	88

[a]See ref 5 for a description of the assay. Each entry represents the mean of at least two experiments, with an average SEM of ±0.17.
[b]% of phenylephrine response (40 μM).

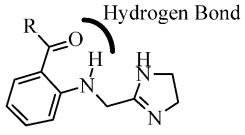


Figure 2.

The structure–activity relationship in the secondary *ortho*-amide series closely resembled the ester pattern. The methylamide was potent and nonselective, and lengthening the alkyl chain on secondary amides had a similar, but somewhat greater, negative effect on the ability of the ligands to activate the α_{1B} and α_{1D} receptors. The ethylamide was only a partial agonist at α_{1B} compared to the full agonism of the ethyl ester, and the *n*-butylamide was inactive at both the α_{1B} and α_{1D} receptors. The ethyl, *n*-propyl and *n*-butyl amides were full agonists at α_{1A} with little difference in potency. Simple α-branching on secondary amides also delivered selectivity, but not as profoundly as in the ester series. The isopropylamide was still a potent full agonist at α_{1B} and a potent partial agonist at α_{1D}, while the *sec*-butylamide was only efficacious at the α_{1A}-receptor. Also, the

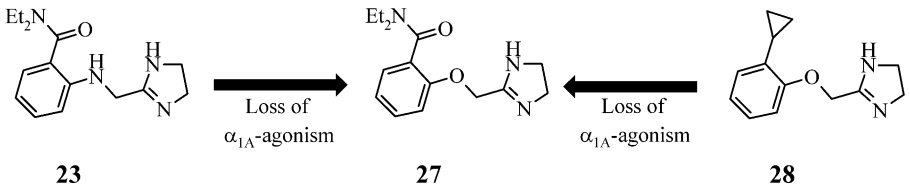


Figure 3.

sec-butylamide was approximately 400 times less potent than the *sec*-butyl ester at α_{1A}. While the cyclopropylamide was a potent full agonist at α_{1A} and a partial agonist at α_{1B}, cycloalkylamides larger than cyclopropyl showed no agonist activity at any of the three α_1-subtypes. The isobutylamide exhibited the same type of selectivity as the isobutyl ester, with greater potency and efficacy at α_{1B} than at α_{1A} or α_{1D}.

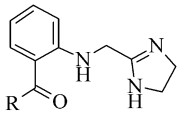
Tertiary amides were also evaluated for their ability to activate α_1-adrenoceptors. Steric bulk proved to be important in accomplishing subtype selectivity in this class as well, as long as the alkyl groups were not constrained. The diethylamide and the *N*-ethyl-*N*-propylamide were quite selective for the α_{1A}-subtype, showing little agonist activity at α_{1B} or α_{1D}. Constraining the alkyl groups reduced selectivity for the α_{1A}-subtype. The pyrrolidino amide regained potency and efficacy at α_{1B} and the larger piperidino amide was a potent full agonist at α_{1B} and a relatively potent partial agonist at α_{1D}. The constraint had little effect on α_{1A} activation.

While the agonist activities of the isobutyl ester, isobutylamide, and the piperidino amide indicate that the α_{1B}-subtype (and, to a lesser extent, α_{1D}) can achieve favorable interactions that lead to receptor activation with certain larger alkyl groups at the 2′-position, steric bulk at the 2′-position of 2-(anilinomethyl)imidazolines generally causes a more significant reduction in the ability of ligands to activate the α_{1B} and α_{1D}-subtypes than the α_{1A}-subtype.[11] The relative ability of the subtypes to tolerate bulky 2′-esters and 2′-amides may be influenced by their ability to tolerate a more rigid ligand in the active ligand–receptor complex. An intramolecular hydrogen bond between the anilino N–H and the carbonyl of the *ortho* substituent is a likely contributor to the actual ligand conformation in the hydrophobic environment of the transmembrane regions of adrenoceptors (Fig. 2).[12] This hydrogen bond would constrain the molecule, reducing the rotational freedom of the ester or amide and the rotational freedom of the anilino bond.

The importance of this intramolecular hydrogen bond to α_{1A}-subtype activation by 2-(anilinomethyl)imidazolines with larger 2′-esters and 2′-amides is supported by the fact that the *o*-diethylamide in a 2-(phenoxymethyl)imidazoline (**27**) lacks agonist activity at all three α_1-subtypes, while the *o*-diethylamide in the 2-(anilinomethyl)imidazoline series is a potent full agonist at the α_{1A}-subtype (cirazoline, **28**, a potent α_{1A} agonist, shows that the 2-(phenoxymethyl)imidazoline is acceptable for α_1-adrenoceptor activation) (Fig. 3).[13,14]

The size and shape of 2′-substituents on 2-(anilinomethyl)imidazolines have significant and variable effects on interactions with α_1-adrenoceptor subtypes. Generally, steric bulk at this position leads to selective agonism at the α_{1A}-subtype. The 2′-*n*-butylamide was greater than 100-fold selective for α_{1A} versus α_{1B} and α_{1D}, and the 2′-isopropyl and 2′-*sec*-butyl esters were greater than 1000-fold selective for the α_{1A}-subtype. Notably, consistent with literature reports, the α_{1A}-subtype selectivity observed in the agonist functional assays did not correlate with affinities in binding assays, as illustrated by select 2-(anilinomethyl)imidazolines in Table 3.[15]

Table 3.[a]



Compd	R	α_{1A}	α_{1B}	α_{1D}
		pIC_{50} (±SEM)[b]	pIC_{50} (±SEM)	pIC_{50} (±SEM)
4	O*i*Pr	6.65 (±0.01)	5.75 (±0.08)	5.70 (±0.07)
11	NHMe	6.67 (±0.04)	6.16 (±0.17)	7.05 (±0.19)
14	NHButyl	6.33 (±0.04)	5.71 (±0.09)	5.83 (±0.06)
17	NHcyclopropyl	5.98 (±0.03)	5.65 (±0.04)	5.77 (±0.07)
22	N(Me)(Et)	5.69 (±0.02)	4.98 (±0.11)	5.17 (±0.03)
23	$N(Et)_2$	6.07 (±0.11)	5.23 (±0.01)	5.18 (±0.10)

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

References and Notes

1. Brown, R. E. US Patent 3,754,002, 1971; *Chem. Abstr.* **1971**, *79*, 92219.
2. (a) The pharmacologically-defined native α_1-adrenoceptors are identified as α_{1A}, α_{1B} and α_{1D}. The corresponding subtypes characterized by molecular cloning techniques are designated as α_{1a}, α_{1b} and α_{1d}. For a brief review of α_1-adrenoceptor molecular pharmacology and a recent discussion of adrenoceptor classification, see: Zhong, H.; Minneman, K. P. *Eur. J. Pharmacol.* **1999**, *375*, 261. (b) Guarino, R. D.; Perez, D. M.; Piascik, M. T. *Cell. Signal* **1996**, *8*, 323. (c) Hieble, J. P. *Pharm. Acta Helv.* **2000**, *74*, 163. (d) Alexander, S.; Peters, J.; Mead, A. *Trends Pharmacol. Sci.* **1998**, 1.
3. (a) Hieble, J. P.; Ruffolo, R. R., Jr. *Drugs Pharm. Sci.* **1998**, *89*, 231. (b) Ruffolo, R. R.; Hieble, J. P., Jr. *Eur. Urol.* **1999**, *36*, 17.
4. (a) Taniguchi, N.; Hamada, K.; Ogasawara, T.; Ukai, Y.; Yoshikuni, Y.; Kimura, K. *Eur. J. Pharmacol.* **1996**, *318*, 117. (b) Taniguchi, N.; Ukai, Y.; Tanaka, T.; Yano, J.; Kimura,

K.; Moriyama, N.; Kawabe, K. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1997**, *355*, 412. (c) Alberts, P.; Bergstrom, P. A. C.; Fredrickson, M. G. *Eur. J. Pharmacol.* **1999**, *371*, 31. (d) There have been several literature reports that a fourth α_1-adrenoceptor subtype, the α_{1L}-subtype, is involved in urethral smooth muscle contraction. The α_{1L}-subtype has not been cloned, but has been pharmacologically defined: Flavahan, N. A.; Vanhouette, P. M. *Trends Pharmacol. Sci.* **1986**, *7*, 347. (e) The α_{1L} receptor may not be a distinct subtype, but a different affinity state of the α_{1A}-subtype. For a discussion, see: Ford, A. P. D. W.; Daniels, D. V.; Chang, D. J.; Gever, J. R.; Jasper, J. R.; Lesnick, J. D.; Clarke, D. E. *Br. J. Pharmacol.* **1997**, *121*, 1127.

5. 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 oxymetazoline, naphazoline and cirazoline are shown for comparative purposes.

6. The potency and maximal response of 1 at the α_{1B} and α_{1D}-adrenoceptors is of interest, as most reported imidazoline-type α_1 agonists show some degree of α_{1A} selectivity. See ref 2a, and see: Minneman, K. P.; Theroux, T. L.; Hollinger, S.; Han, C.; Esbenshade, T. A. *Mol. Pharmacol.* **1994**, *46*, 929.

7. Prepared from chloroacetonitrile via ester imidate formation and treatment with ethylenediamine, see: Klarer, W.; Urech, E. *Helv. Chim. Acta* **1944**, *27*, 1762.

8. These compounds can be synthesized in parallel using a Stemblock (available from STEM Corporation, Essex, UK) for heating with stirring under nitrogen, followed by purification on a MultiElute chromatography system (available from Biotage Corporation, Charlottesville, VA, USA). However, aqueous work up and extraction from basic solution, followed by hydrochloride or fumarate salt formation often gave material of high purity without chromatography.

9. The *t*-butyl ester was prepared slightly differently, see: DiBiase, S. A.; Gokel, G. W. *J. Org. Chem.* **1978**, *43*, 447.

10. For example: Jacobs, R. *J. Heterocycl. Chem.* **1970**, *7*, 1337.

11. Via elegant site-directed mutagenesis studies using hamster α_1-adrenoceptors, Perez and co-workers have shown that the differences in agonist binding affinities between the α_{1A} and α_{1B} is almost entirely due to two non-conserved residues in the transmembrane domains of the two receptor subtypes (Ala^{204} and Leu^{314} in α_{1b} versus Val^{185} and Met^{293} in α_{1A}) Hwa, J.; Graham, R. M.; Perez, D. M. *J. Biol. Chem.* **1995**, *270*, 23189.

12. (a) IR and Raman spectroscopic analyses were performed to corroborate literature evidence: Dandarova, M.; Kovac, S. *Zb. Pr. Chemickotechnol. Fak. SVST* **1978**, 61. (b) Denisov, G. S.; Kuzina, L. A.; Shchepkin, D. N. *Croat. Chem. Acta* **1992**, *65*, 89. Alternative hydrogen bonds involving the other ester oxygen or the amide nitrogen with the aniline are also possible.

13. Compound **27** did not activate ($pEC_{50} < 5.3$) any of the three α_1-receptors. It was prepared from *N*,*N*-diethylsalicylamide and 2-chloromethylimidazoline hydrochloride.

14. Najer, H; Giudicelli, J. F. Ger. Offen. DE 2234714, 1973; *Chem. Abstr.* **1973**, *78*, 111312. Cirazoline was a full agonist at the cloned human α_{1A}-receptor in our cell-based functional assay with a $pEC_{50} = 7.9$.

15. Other researchers have noted this phenomenon, and usually attribute it to the compounds having different intrinsic activities at the different subtypes. For a discussion, see p 264 of ref 2a.

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.