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Bioisosteric phentolamine analogs as potent α-adrenergic antagonists

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Abstract—The synthesis and biological evaluation of a new series of bioisosteric phentolamine analogs are described. Replacement of the carbon next to the imidazoline ring of phentolamine with a nitrogen atom provides compounds (**2**, **3**) that are about 1.6 times and 4.1 times more potent functionally than phentolamine on rat α_1-adrenergic receptors, respectively. In receptor binding assays, the affinities of phentolamine and its bioisosteric analogs were determined on the human embryonic kidney (HEK) and Chinese Hamster ovary (CHO) cell lines expressing the human α_1- and α_2-AR subtypes, respectively. Analogs **2** and **3**, both, displayed higher binding affinities at the α_2- versus the α_1-ARs, affinities being the least at the α_{1B}-AR. Binding affinities of the methoxy ether analog **2** were greater than those of the phenolic analog **3** at all six α-AR subtypes. One of the nitrogen atoms in the imidazoline ring of phentolamine was replaced with an oxygen atom to give compounds **4** and **5**, resulting in a 2-substituted oxazoline ring. The low functional antagonist activity on rat aorta, and binding potencies of these two compounds on human α_{1A}- and α_{2A}-AR subtypes indicate that a basic functional group is important for optimum binding to the α_1- and α_{2A}-adrenergic receptors.

The initial classification of adrenoceptors (AR) into alpha (α-AR) and beta (β-AR) was described by Ahlquist in 1948 on the basis of their pharmacological characteristics.[1] Cloning and expression of α-AR have confirmed the presence of multiple subtypes of both α_1-(α_{1a}, α_{1b}, α_{1d}) and α_2-(α_{2a}, α_{2b}, α_{2c})AR.[2–5] The membrane-spanning protein subunits among the α-AR family contain seven putative transmembrane helices, and α-ARs are members of the G-protein-coupled superfamily of receptors.[6]

Since phentolamine (**1**), a non-selective α-AR antagonist, was discovered by Urech et al.,[7] this drug has been used in a number of clinical situations: (a) for the treatment of pheochromocytoma-related hypertension, (b) for the diagnosis of pheochromocytoma, and (c) for norepinephrine-related dermal necrosis.[8,9] Phentolamine is not useful in the treatment of systemic hypertension or in patients with myocardial infarctions since it induces cardiac stimulation. This side-effect is thought to be caused by the presynaptic α_2-AR blockade, which results in an enhanced neuronal release of norepinephrine. The released norepinephrine activates adrenergic receptors in the heart, producing cardiac stimulation.[8] Recently, phentolamine mesylate (Vasomax®) has been marketed in Mexico for use in the treatment of male erectile dysfunction (ED).[6,10] The drug induces relaxation of corpus cavernosum erectile tissue by direct antagonism of α_1- and α_2-adrenergic receptors and by indirect functional antagonism via a non-adrenergic, endothelium-mediated mechanism, suggesting nitric oxide synthase activation.[11,12] A better understanding of the structural requirements for the subtypes of α-adrenergic receptors could result in the design and

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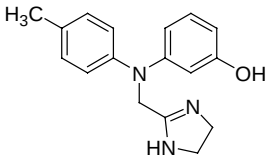
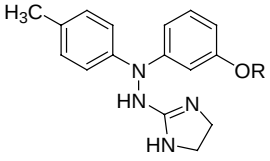
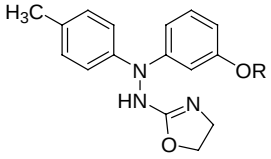


Phentolamine (1)



R = CH_3 (2), H (3)

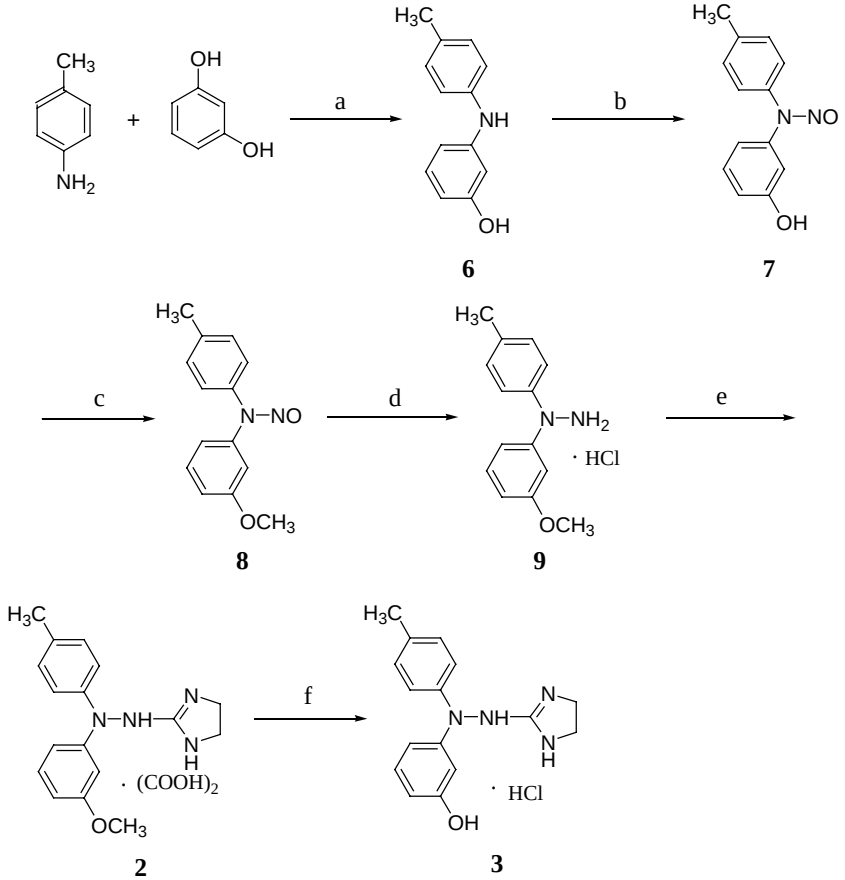


R = CH_3 (4), H (5)

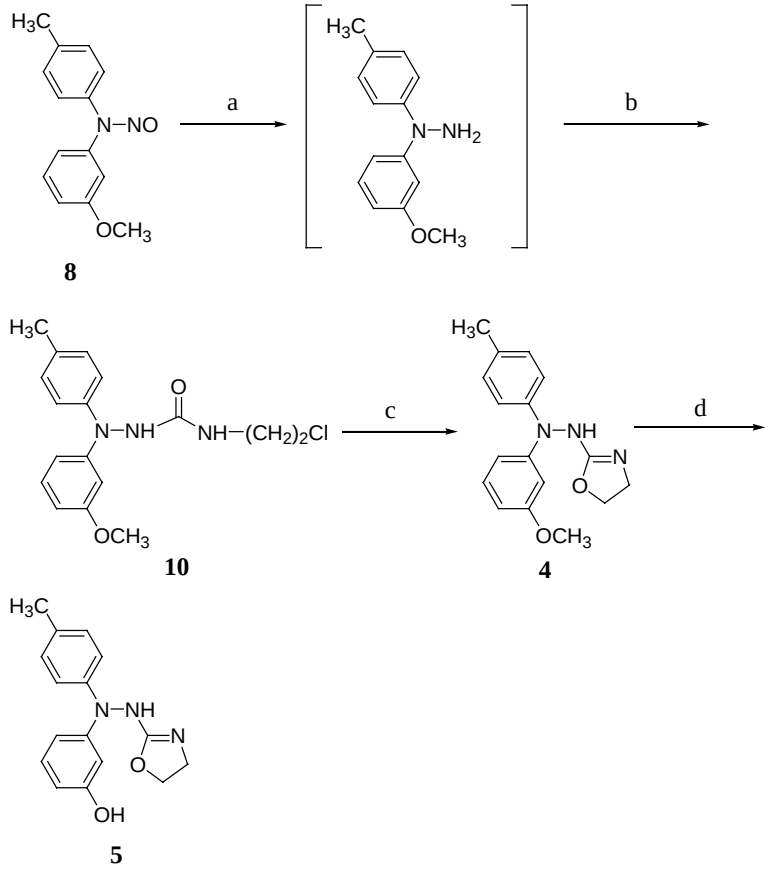
synthesis of more selective drugs for treating erectile dysfunction and a number of other diseases, including nasal decongestion, hypertension, hypotension, hyperglycemia, depression, liver cell proliferation, hyperaggregability of platelets, and benign prostatic hyperplasia (BPH).

In a previous paper,[13] we reported the preparation of geometrical isomers by replacing the nitrogen atom with a carbon at the benzylic position of phentolamine; the ethylene analog having the *Z* configuration was less potent than phentolamine, but the α_1-AR selectivity was considerably increased. Our next goal was to see if bioisosteric analogs (**2**, **3**) of phentolamine, in which the carbon atom bridging the 2-position of the imidazoline ring with the diphenylamine nitrogen is replaced by nitrogen, might bind to α-ARs with an enhanced affinity. As an additional test modification, intended to partially evaluate the importance of basicity, one of the nitrogen atoms in the imidazoline ring of phentolamine was replaced with an oxygen atom to give the corresponding oxazoline analogs (**4, 5**). The pharmacological properties of phentolamine (**1**) and newly synthesized bioisosteric analogs (**2**–**5**) were compared for their functional activities on rat thoracic aorta and for binding potencies on human α_1- and α_2-AR subtypes expressed in HEK and CHO cells, respectively.

The synthesis of imidazoline derivatives **2** and **3** is shown in Scheme 1. The hydrazine **9** was the key intermediate in the synthesis of all of the target compounds (Schemes 1 and 2). This hydrazine was synthesized with the initial condensation of *p*-toluidine and resorcinol in the presence of a catalytic amount of *p*-toluidine HCl to give diphenylamine **6** in 74% yield.[14] N-Amination of **6** was not successful with hydroxylamine-*O*-sulfonic acid. Diphenylamine **6** in ethanol was treated with concentrated HCl, followed by the



Scheme 1. Reagents and conditions: (a) heat at 160 °C; (b) $NaNO_2$, concd HCl; (c) dimethyl sulfate, K_2CO_3; (d) 1—$LiAlH_4/Et_2O$, 2—HCl; (e) 1—*N*-acetyl imidazolinone, $POCl_3$, 2—oxalic acid; (f) 1—satd $NaHCO_3$, 2—BBr_3, 3—satd $NaHCO_3$, 4—HCl.



Scheme 2. Reagents and conditions: (a) $LiAlH_4/Et_2O$; (b) $Cl(CH_2)_2NCO$; (c) CH_3OH/H_2O, reflux; (d) BBr_3.

addition of a cold solution of sodium nitrite in water, to give nitrosamine **7** in 73% yield,[15] and this intermediate was then treated with dimethyl sulfate in acetone in the presence of K_2CO_3 to provide the desired methoxy compound **8** in 91% yield. In earlier attempts, treatment of *N*-nitrosamine **8** with Zn in the presence of acetic acid gave extensive decomposition (N–N bond cleavage) and the yield was very poor. The *N*-nitroso group was more successfully reduced by $LiAlH_4$ in diethyl ether; the optimum reaction time was 5 h because slow N–N bond cleavage became significant thereafter. The hydrazine free base readily decomposed, which could not be purified by flash chromatography, so it was directly converted to the HCl salt (70% yield from nitrosamine **8**) using excess HCl gas. The hydrazine **9** was treated with *N*-acetyl-2-imidazolinone[16] in the presence of $POCl_3$, followed by base workup, to give imidazoline **2** in 69% yield, obtained as the oxalic acid salt. The methoxy-protecting group of **2** in anhydrous methylene chloride was deprotected by BBr_3, and base workup, followed by conversion to the hydrochloride salt, gave **3** in 69% yield.

Scheme 2 outlines the synthesis of oxazoline derivatives **4** and **5**. *N*-Nitrosamine **8** in diethyl ether was treated with $LiAlH_4$ as described above. The resulting mixture of the hydrazine and *N*-nitrosamine was directly treated with 2-chloroethylisocyanate in tetrahydrofuran to provide chloroethylurea **10** in 67% yield. The urea **10** was heated in aqueous MeOH solution to give a cyclized compound, oxazoline **4**, in 40% yield. This methyl ether group was also cleaved with BBr_3, and subsequent base workup gave phenol **5** in 47% yield.

Radioligand binding analyses of phentolamine and its bioisosteric analogs were performed in CHO and HEK293 cells stably expressing homogeneous populations of human α_{2A}-, α_{2B}-, and α_{2C}-ARs, and human α_{1A}-, α_{1B}-, and α_{1D}-ARs, respectively. Initial studies were conducted on human α_{1A}- and α_{2A}-AR subtypes, and the rank order of affinities of these compounds (K_i values) was phentolamine, **1** (4.1 nM) > **2** (20 nM) > **3** (27 nM) >> **5** (5620 nM) = **4** (5740 nM), and **2** (6.3 nM) > **3** (24 nM) > phentolamine, **1** (56 nM) >> **5** (18,600 nM) > **4** (24,600 nM), respectively (Table 1). These data revealed that the parent compound, phentolamine, had a 14-fold higher binding affinity at the α_{1A}- versus the α_{2A}-AR. Bioisosteric analogs of phentolamine in which the original imidazoline ring was retained (**2** and **3**), however, exhibited a different binding pattern. When compared to phentolamine (**1**), both the analogs (**2**, **3**) showed decreased binding potencies at the α_{1A}-AR, whereas the binding potencies at the α_{2A}-AR improved slightly. The α_{1A}- affinity decreases were 5- and 7-fold, while the α_{2A}- affinity increases were 9- and 2-fold for analogs **2** and **3**, respectively. Analog **2** showed a 3-fold greater binding affinity at the α_{2A}- versus the

Table 1. Binding affinities of phentolamine (1), bioisosteric analogs (2, 3) and 2-substituted oxazoline ring analogs (4, 5) on human α_1- and α_2-AR subtypes stably expressed in human embryonic kidney (HEK293) and Chinese Hamster ovary (CHO) cells, respectively[a,b,c]

Compound	α_{1A}	α_{1B}	α_{1D}
	$K_i \pm$ SEM[b,c] (nM)	$K_i \pm$ SEM[b] (nM)	$K_i \pm$ SEM[b] (nM)
1	4.12 ± 0.49	47.4 ± 4.4	19.4 ± 4.2
2	19.9 ± 1.6	102.8 ± 27.3	17.5 ± 1.5
3	26.8 ± 1.7	843 ± 220.1	49.7 ± 6.8
	α_{2A}	α_{2B}	α_{2C}
1	55.9 ± 3.6	48.0 ± 5.8	80.3 ± 19.0
2	6.3 ± 0.2	7.3 ± 0.6	3.8 ± 0.4
3	23.8 ± 3.7	18.0 ± 2.1	12.8 ± 1.0

[a] [^{3}H]Prazosin and [^{3}H]rauwolscine were used as the radioligands in the equilibrium competition radioligand binding assays for the α_1- and α_2-ARs, respectively, and the nonspecific binding was measured in the presence of 10 μM of phentolamine or yohimbine.

[b] K_i was calculated according to the Cheng–Prusoff equation[17] and the data for all analogs are means ± SEM of $N = 4$–6.

[c] The oxazoline ring analogs were evaluated for binding only in human α_{1A}- and α_{2A}-AR subtypes and the mean pK_i ± SEM (K_i, nM) values for analogs **4** and **5** on human α_{1A}- and α_{2A}-ARs are 5.24 ± 0.05 (5740 nM) and 4.61 ± 0.17 (24,600 nM), and 5.25 ± 0.04 (5620 nM) and 4.73 ± 0.04 (18,600 nM), respectively.

Table 2. Functional activities of phentolamine (**1**) and bioisosteric analogs (**2**–**5**) as assessed by the antagonism of phenylephrine-induced contractions of rat aorta

Compound	Rat aorta (α_{1D}-AR[18–20])	
	K_B (nM) ± 95% CL[a]	Potency ratio[b]
1	11.8 ± 0.8	1.0
2	7.4 ± 0.6	1.6
3	2.9 ± 0.7	4.1
4	>30,000	—
5	13,000 ± 0.8	—

[a] The K_B value for each compound, as determined by rightward shifts in the agonist (phenylephrine) concentration–response curves, was calculated according to the Schild equation:[21] K_B = antagonist concentration/dose ratio-1 where dose ratio is the ratio of the EC_{50} for phenylephrine in the presence and absence of the antagonist. Data were analyzed using GraphPad Prism software [GraphPad Prism, San Diego, CA] to obtain EC_{50} values and are expressed as means ± 95% confidence limits (CL) of $N = 4$–6 experiments.

[b] Potency ratio = K_B(phentolamine, **1**)/K_B(bioisosteric analog).

α_{1A}-AR, whereas analog **3** was equipotent in binding to the α_{1A}- and α_{2A}-AR. Bioisosteric analogs of phentolamine containing an oxazoline ring, analogs **4** and **5**, showed much lower binding affinities at both the α_{1A}- as well as the α_{2A}-AR, indicating that replacement of one of the nitrogen atoms of the imidazoline ring by oxygen is detrimental for α-AR binding. This likely suggests that the basicity of this ring system is important for binding to the α-ARs.

With these results, we extended the evaluation of phentolamine **1** and bioisosteric analogs **2**, **3** to all six α-AR subtypes (Table 1). The rank order of binding affinities exhibited by phentolamine at human α-ARs was $\alpha_{1A} > \alpha_{1D} > \alpha_{1B} = \alpha_{2A} = \alpha_{2B} \geqslant \alpha_{2C}$. Analogs **2** and **3**, both, displayed higher binding affinities at the α_2- versus the α_1-ARs, affinities being the least at the α_{1B}-AR. Binding affinities of the methoxy ether analog **2** were greater than those of the phenolic analog **3** at all six α-AR subtypes.

The parent compound, phentolamine (**1**), and its bioisosteric analogs (**2**–**5**) were also tested for functional activities on rat aorta. Responses in the rat aorta have been known to be mediated predominantly by the α_{1D}-AR.[20] All test compounds blocked the phenylephrine-induced contractions of rat aorta and produced parallel shifts in the concentration-response curve of the α-AR agonist, phenylephrine. Table 2 shows the potency of the test compounds expressed as their K_B values. As compared to phentolamine (K_B, 11.8 nM), analog **3** was 4.1 times more potent blocker of the vascular response (K_B, 2.9 nM). Methoxy analog **2** (K_B, 7.4 nM) was also more potent than phentolamine. As observed in our binding studies, oxazoline analogs **4** (K_B, >30,000 nM) and **5** (K_B, 13,000 nM) were much less potent than phentolamine or analogs **2** and **3**; and these findings suggest that the basicity of this ring system is important for binding to α_1-adrenoceptors. However, the binding and functional data for analogs **2** and **3** at cloned human α_{1D} and rat aorta α_{1D} are not in complete agreement. Our binding studies suggest that analog **2** binds with a 2.8-fold higher affinity than analog **3** at cloned human α_{1D}-AR (Table 1), whereas the functional potency of analog **3** is 2.6-fold higher than that of analog **2** (Table 2). A possible explanation for this discrepancy is that the organization of cloned receptors in host cell lines such as the one used for determining binding affinities in the present case may be different from those of native receptors in integrated tissue systems, such as the rat aorta, used for our functional assays. However, there may exist other possibilities leading to this disparity (see Table 3).

Table 3. Elemental analyses

Compound	Calculated (%)			Formula	Found (%)		
	C	H	N		C	H	N
7	68.41	5.30	12.27	$C_{13}H_{12}N_2O_2$	68.32	5.30	12.24
9	63.51	6.47	10.58	$C_{14}H_{16}N_2O{\cdot}HCl$	63.31	6.45	10.51
2	57.71	5.86	14.17	$C_{17}H_{20}N_4O{\cdot}(COOH)_2{\cdot}0.5H_2O$	58.03	5.70	14.01
3	60.28	6.01	17.57	$C_{16}H_{18}N_4O{\cdot}HCl$	60.17	6.04	17.31
10	61.17	6.04	12.59	$C_{17}H_{20}N_3O_2Cl$	61.15	6.04	12.51
4	64.74	6.71	13.32	$C_{17}H_{19}N_3\ O_2{\cdot}1.0H_2O$	64.35	6.71	13.25
5	62.84	6.43	13.73	$C_{16}H_{17}N_3\ O_2{\cdot}1.25H_2O$	62.85	6.38	13.68

Overall, these results indicate that replacement of the carbon bridging the 2-position of the imidazoline ring with the diphenylamine nitrogen by a nitrogen atom as in compounds (**2** and **3**) maintains or modestly increases the activity on selected α-AR subtypes. Replacing one of the nitrogen atoms of the imidazoline ring of phentolamine with an oxygen atom drastically reduces activity. Compounds **2** and **3** serve as promising lead compounds for further investigations.

Acknowledgments

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