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Communications to the Editor

4-Amino-2-[4-[1-(benzyloxycarbonyl)-2(*S*)-[[(1,1-dimethylethyl)amino]carbonyl]-piperazinyl]-6,7-dimethoxyquinazoline (L-765,314): A Potent and Selective α_{1b} Adrenergic Receptor Antagonist

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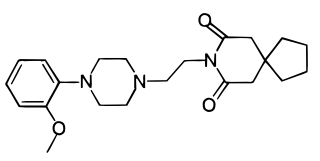
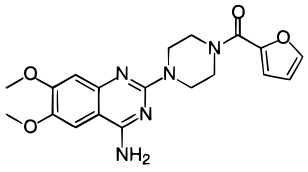
Research efforts in the α_1 adrenergic receptor antagonist area have led to the discovery of some marketed antihypertensive drugs.[1] These agents apparently function by relaxing vascular smooth muscle which contains high concentrations of α_1 receptors. Researchers also found that α_1 receptors are abundant in the human prostate, bladder neck, and urethra,[2] and demonstrated that the use of nonselective α_1 receptor antagonists could provide symptomatic relief from the dynamic component of urinary flow problems resulting from increased adrenergic tone in males with hyperplastic prostates.[3]

Subsequent to these findings, pharmacological and binding studies indicated there are three subclasses of α_1 receptors. The existence of these receptor subtypes, the α_{1a}, α_{1b}, and α_{1d}, has been confirmed through the use of molecular biological cloning techniques.[4] The study of a variety of tissue preparations led to the discovery of a heterogeneous distribution of the three α_1 receptors within animal and human tissues. Later, it was discovered that the α_{1a} receptor was the putative target for the treatment of benign prostatic hyperplasia (BPH).[5]

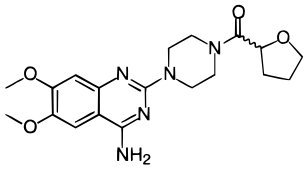
The effort to synthesize agents selective for each of the three α_1 receptor subtypes has been an active area of research. As a result, the structures of α_{1a} selective antagonists,[6] α_{1a} selective agonists,[7] the α_{1b} selective antagonist, (+)-cyclazosin,[8] and an α_{1d} selective antagonist **1**, BMY-7378,[9] have been disclosed. Despite these advances, the physiological roles of the α_{1b} and α_{1d} receptors in blood pressure or other physiological functions remain undefined.



1, BMY-7378



2, Prazosin



3, Terazosin

Our goal at the outset of this research was to synthesize novel, potent, and subtype selective α_1 antagonists. The strategy was to convert the subtype nonselective α_1 receptor antagonist **2**, prazosin, into a subtype selective antagonist. Our approach was to incorporate new structural elements into the prazosin piperazine subunit. Herein, we describe our preliminary results focusing on the synthesis and pharmacological evaluation of some novel, potent, and selective α_{1b} receptor antagonists.

Synthesis. The synthesis of the targeted substituted piperazine derivatives was accomplished as outlined in Scheme 1. The starting material, 2(*S*)-(*tert*-butylcarboxamido)piperazine,[10] was regioselectively N-4 BOC-

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Scheme 1

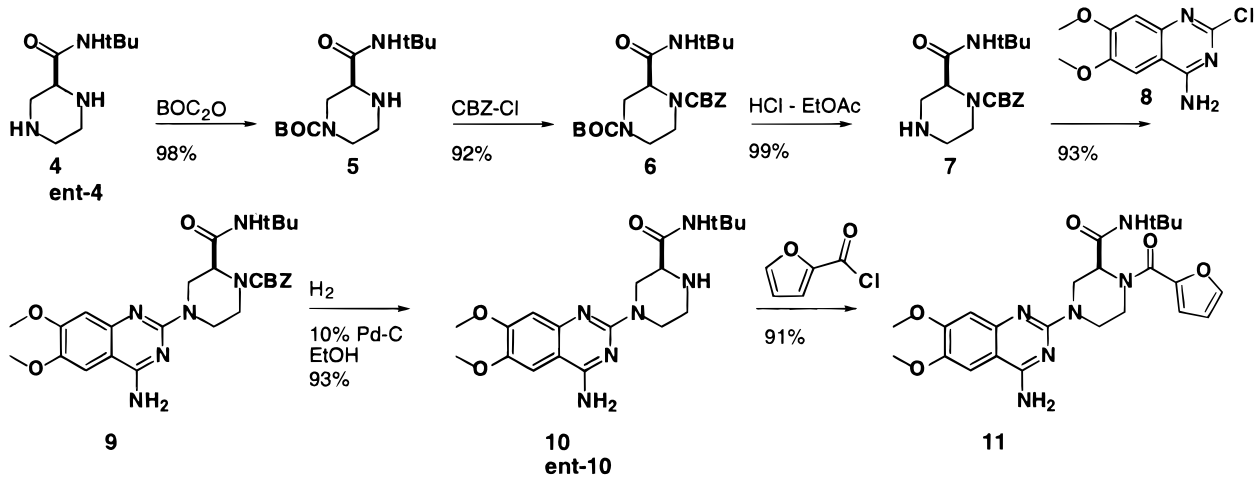

Table 1. Receptor Binding Affinity

		K_i (nM)[a] ($n \geq 2$)		
no.	species	α_{1a}	α_{1b}	α_{1d}
1	rat	620 ± 190	328 ± 78	2.4 ± 0.7
	human	294 ± 61	191 ± 52	1.6 ± 0.4
2	rat	0.30 ± 0.06	0.20 ± 0.02	0.30 ± 0.02
	human	0.39 ± 0.21	0.21 ± 0.045	0.20 ± 0.059
3	rat	3.9 ± 0.05	1.9 ± 0.06	3.4 ± 0.07
	human	4.2 ± 0.46	1.2 ± 0.15	2.0 ± 0.63
9	rat	500 ± 20	5.4 ± 0.6	50 ± 8
	human	420 ± 62	2.0 ± 0.66	34 ± 6.0
10	rat	510 ± 43	1500 ± 690	630 ± 69
	human	830 ± 170	25 ± 1.6	250 ± 6.8
ent-**10**	human	2900 ± 300	1050 ± 50	2250 ± 450
11	rat	580 ± 150	7.0 ± 1.9	78 ± 7.8
	human	1700 ± 590	3.5 ± 0.29	73 ± 28

[a] [^{125}I]HEAT used as radioligand. Only small shifts (<1×) in receptor binding affinity were observed in the presence of 5% dog plasma.

protected, N-1 CBZ-protected and N-4 BOC-deprotected, which provided the appropriate monoprotected piperazine **7**. Nucleophilic addition of piperazine **7** to the 4-amino-2-chloroquinazoline derivative **8** produced **9**, which after hydrogenation yielded **10**. Acylation of **10** with freshly distilled 2-furoyl chloride completed the synthesis of 3(*S*)-(*tert*-butylcarboxamido)prazosin, **11**. The synthesis of the enantiomer of **10** (ent-**10**) possessing the 3(*R*) configuration was accomplished directly by heating ent-**4** and **8**. These refined reaction conditions were also applied to the preparation of **10**.

Receptor Binding Experiments. The binding affinity to the rat and human α_1 receptors for the synthetic compounds was measured utilizing cloned receptor binding assays (Table 1).[5b] The more potent compounds, **9** (L-765,314) and **11**, were ≥20-fold selective when compared to the other G-protein-coupled receptors (human α_2, histamine 1 and 2, and 5HT receptors) tested.

The results of a series of tissue binding experiments are summarized in Table 2. The antagonists, **9**, **10**, and **11**, exhibited tissue binding values which were in good agreement with their corresponding cloned receptor binding affinities.

Binding to Rat Thoracic Spinal Cord Membrane. The rat thoracic spinal cord membrane has been shown to be comprised primarily of α_{1a} and α_{1b} receptors.[11] Therefore, as an assay for α_1 receptor binding affinity and selectivity, the binding to rat

Table 2. Tissue Binding Affinity

	IC_{50} (nM) ($n \geq 2$)				
no.	rat prostate[a] (α_{1a})	rat liver[a] (α_{1b})	rat spleen[a,c] (α_{1b})	human prostate[a] (α_{1a})	human aorta[b] (α_{1b})
9	340 ± 40	9.3 ± 1.2	9.3	590 ± 100	8.8 ± 1.2 (87−100%) 5800 (0−13%)
10	ND[d]	2700 ± 190	1500	1700 ± 1100	31[c]
11	2000 ± 550	15 ± 2.6	14	1900 ± 310	3.2[c] (62%) 2600 (38%)

[a] [^{3}H]Prazosin used as radioligand. [b] [^{125}I]HEAT used as radioligand. [c] $n = 1$. [d] ND = not determined.

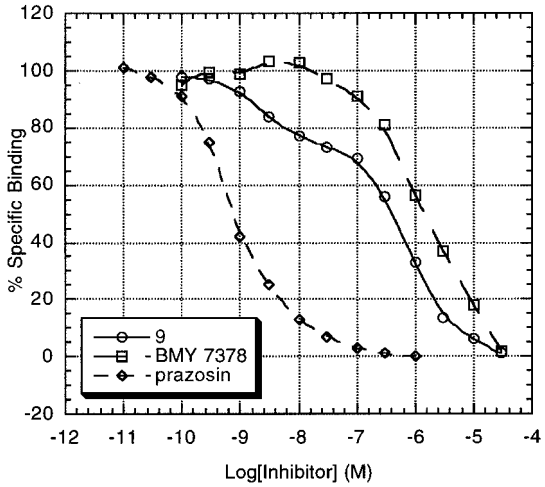

Figure 1. Inhibition of [^{3}H]prazosin binding to rat thoracic spinal cord membrane.

thoracic spinal cord membrane of three compounds was measured, and the inhibition curves were graphed in Figure 1. The three compounds chosen were as follows: (1) the subtype nonselective α_1 antagonist, prazosin, (2) the α_{1d} selective antagonist, **1**, and (3) the α_{1b} selective antagonist, **9**. The data for prazosin exemplifies monophasic, potent inhibition (IC_{50} = 0.64 nM) consistent with a subtype nonselective antagonist. The highly selective α_{1d} antagonist, **1**, was a weak inhibitor due to its low affinity for predominant receptor subtypes (α_{1a} and α_{1b}), and therefore inhibition is only observed at higher concentrations (IC_{50} = 1550 nM). The α_{1b} selective antagonist, **9**, exhibits two displacement sites. The high-affinity site accounted for approximately 25%

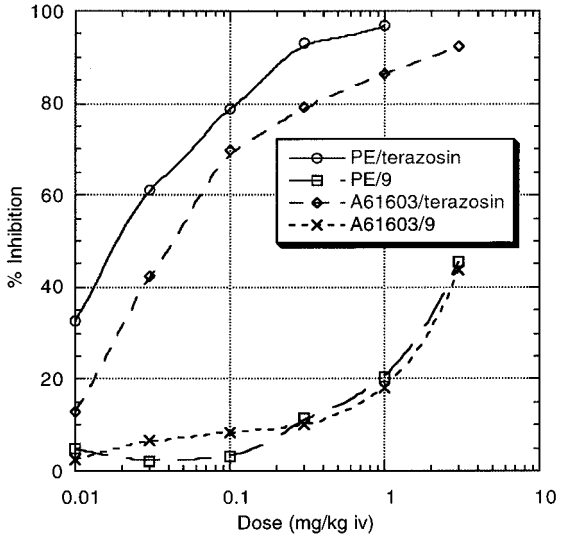

Figure 2. Effect of antagonists on pressor responses of phenylephrine (PE, 10 μg/kg iv) or A-61603 (0.05 mg/kg iv).

of binding (IC_{50} = 1.90 nM) and represented binding to the α_{1b} sites. The low-affinity site accounted for the residual 75% of binding (IC_{50} = 790 nM) and represented binding to the α_{1a} sites. Similar biphasic inhibition curves were observed for other α_{1a} selective antagonists tested in this model (data not shown here). Here, presumably the high-affinity site corresponded to the α_{1a} component and the low-affinity site represented the α_{1b} component. The high-affinity component accounted for approximately 75% of the total displaceable [^{3}H]prazosin binding.

In Vivo Pharmacology. Inhibition of Pressor Responses to Exogenous α_1 Adrenergic Agonists. Mean arterial pressure is increased in response to administration of either the α_1 subtype nonselective agonist phenylephrine or the α_{1a} subtype selective agonist A-61603 (Figure 2). The potency of terazosin and antagonist **9** for inhibiting the pressor responses to phenylephrine and A-61603 was evaluated in anesthetized male Sprague−Dawley rats (n = 4). Using an ascending antagonist dose protocol, the inhibition of phenylephrine or A-61603 pressor responses was measured and the dose of antagonist eliciting a 50% inhibition of the pressor response (AD_{50}) was determined. Terazosin inhibited in a dose-dependent manner the increase in mean arterial pressure elicited by the subtype nonselective agonist, phenylephrine, and the α_{1a} selective agonist A-61603. Terazosin was more potent inhibiting phenylephrine (AD_{50} = 0.019 mg/kg) as compared to A-61603 (AD_{50} = 0.042 mg/kg). Antagonist **9**[12] showed weak potency for inhibiting the pressor response to either phenylephrine or A-61603 (AD_{50} > 3 mg/kg for each). On the basis of the inhibition of pressor responses to the α_{1a} subtype selective agonist A-61603, antagonist **9** appeared to be selective versus the α_{1a} receptor up to a dose of 0.3 mg/kg. However, at 1 and 3 mg/kg, inhibition of the A-61603 pressor response was observed, suggesting that at these doses **9** did not discriminate between α_{1a} and α_{1b} receptor subtypes.

Hypotensive Potency. Subtype nonselective α_1 receptor antagonists, which were originally developed as antihypertensive agents, decrease blood pressure in hypertensive animals. The hypotensive potency of **9** and terazosin was compared in anesthetized male spontaneously hypertensive rats (SHR, n = 4). The rats were dosed iv with either vehicle or ascending doses of test compounds, and the peak changes in mean arterial pressure were measured. The dose of antagonist eliciting a 25 mmHg decrease in mean arterial pressure (AD_{25}) was calculated as an index of hypotensive potency. Terazosin elicited a dose-dependent decrease in mean arterial pressure (AD_{25} = 0.011 mg/kg) (Figure 3). Antagonist **9** also decreased mean arterial pressure in a dose-related manner but was 80-fold less potent than terazosin (AD_{50} = 0.89 mg/kg) (Figure 3). Both terazosin and **9** tended to decrease heart rate (about 25 bpm at 1 mg/kg iv).

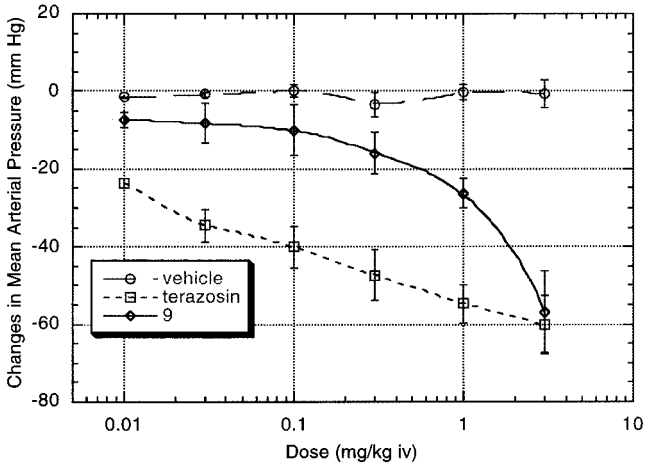

Figure 3. Changes in mean arterial pressure.

Discussion. Moderate α_{1b} selectivity was induced by incorporating an (S)-*tert*-butylcarboxamido group at the piperazine 3-position, relative to the biogenic amine, in prazosin. This result suggests that a key secondary interaction unique to the α_{1b} receptor has been realized. One plausible explanation is that the 3-carboxamido group has formed a hydrogen bond with a binding site within the α_{1b} receptor. Another possibility is that a steric discrimination has been established. Such a discrimination could indicate that access may have been gained to a binding domain somewhat unique to the α_{1b} receptor. This explanation is consistent with the α_{1b} selectivity observed for the (+)-enantiomer of cyclazosin, which like **9** bears a bulky substituent on the piperazine core ring. Whatever the cause of the preferential interaction, it is clearly stereochemically biased (**10** vs ent-**10**). Antagonist **10** is somewhat more potent at the cloned human α_{1b} receptor than for that of the rat clone. This binding data suggests that some difference may exist between the binding domains of the human α_{1b} receptor and the rat α_{1b} receptor.[13]

Compound **9** behaved predictably in the rat thoracic spinal cord membrane binding experiment. The data confirmed the subtype selectivity, $\alpha_{1b} > \alpha_{1d} \gg \alpha_{1a}$, observed in the cloned receptor and tissue binding studies, and illustrated its potential utility as a pharmacological tool.

Antagonist **9** appears to be a selective α_{1b} antagonist based on the lack of inhibition of A-61603 induced increases in mean arterial pressure. On the basis of the lack of effect of antagonist **9** on phenylephrine-induced pressor responses at doses which do not affect

A-61603 pressor responses, it appears that α_{1b} receptors do not contribute to the phenylephrine pressor response. By similar reasoning, α_{1b} receptors do not appear to play a significant role in the maintenance of basal mean arterial pressure in SHR. However, at present, to clearly demonstrate in vivo selectivity versus pure α_{1b}- or α_{1d}-mediated physiological responses is problematic due to the lack of selective agonists for these subtypes. Therefore, the lack of in vivo potency versus phenylephrine- or A-61603-mediated increases in mean arterial pressure, or lack of hypotensive potency in SHR should be interpreted cautiously. The lack of in vivo potency may be due to factors other than subtype selectivity such as plasma protein binding or rapid metabolism. Nevertheless, if the in vivo potency and selectivity parallels the in vitro potency and selectivity, then these data suggest that the α_{1b} receptor subtype may not be important in mediating phenylephrine-induced increases in mean arterial pressure in rats, nor may it be important for the maintenance of basal blood pressure in anesthetized SHR.

Conclusion. We have described the synthesis of some novel, potent, and selective α_{1b} receptor antagonists related to prazosin. The effects induced by the modest structural changes to the 2-*N*-piperazinyl-4-aminoquinazoline containing α_1 antagonists described herein suggest a viable strategy for introducing subtype selectivity in other series of nonselective or less-selective α_1 receptor antagonists.

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(12) Three rats were dosed iv with **9** at 3 mg/kg, and the plasma was assayed by LCMS for parent compound. The mean C_{max} was 1.05 μM and the $t_{1/2}$ was 0.5 h.

(13) Although we have no explanation for this difference, a similar species effect was observed for rauwolscine between the α_{2a} adrenergic receptors from human and rodent sources. For a discussion of this example, please see: Heible, J. P.; Bondinell, W. E.; Ruffolo, R. R. *J. Med. Chem.* **1995**, *38*, 3415−3444.

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