N-[3-(1*H*-Imidazol-4-ylmethyl)phenyl]-ethanesulfonamide (ABT-866, 1),[1] a Novel α_1-Adrenoceptor Ligand with an Enhanced in Vitro and in Vivo Profile Relative to Phenylpropanolamine and Midodrine

Robert J. Altenbach,* Albert Khilevich,[†] Michael D. Meyer, Steven A. Buckner, Ivan Milicic, Anthony V. Daza, Michael E. Brune, Alyssa B. O'Neill, Donna M. Gauvin, John C. Cain, Masaki Nakane, Mark W. Holladay,[‡] Michael Williams,[§] Jorge D. Brioni, and James P. Sullivan

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064

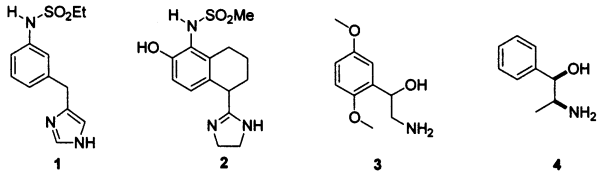
Received June 5, 2002

Abstract: *N*-[3-(1*H*-Imidazol-4-ylmethyl)phenyl]ethanesulfonamide (ABT-866, **1**) is a novel α_1 agent having the unique profile of α_{1A} (rabbit urethra, $EC_{50} = 0.60\ \mu M$) agonism with α_{1B} (rat spleen, $pA_2 = 5.4$) and α_{1D} (rat aorta, $pA_2 = 6.2$) antagonism. An in vivo dog model showed **1** to be more selective for the urethra over the vasculature than A-61603 (**2**), ST-1059 (**3**, the active metabolite of midodrine), and phenylpropanolamine (**4**).

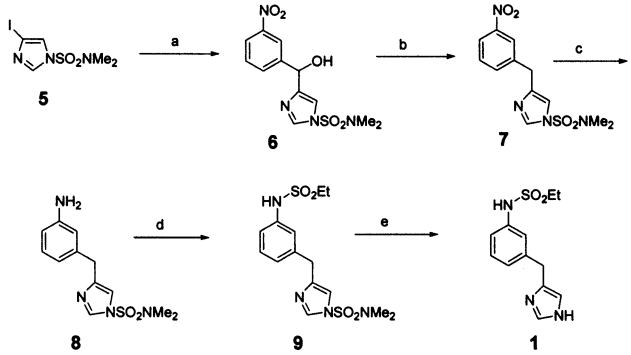
Introduction. Stress urinary incontinence (SUI) is the involuntary leakage of urine due to a stress on the abdomen such as coughing or sneezing. In the human, urethral tone is largely maintained by activation of postsynaptic α-adrenoceptors.[2] Nonselective α_1-adrenoceptor agonists such as midodrine[3] and **4**[4] (see Chart 1) have been assessed clinically for the pharmaceutical treatment of SUI. These agents increase intraurethral pressure and reduce urine leakage but suffer from side effects that can include blood pressure elevation.[2−5]

The discovery of subtypes of the α_1-adrenoceptor (α_{1A}, α_{1B}, and α_{1D})[6] has allowed for the identification of the α_{1A}-adrenoceptor as the primary subtype in the human urethra and the receptor most likely to be responsible for the contraction of the urethra.[7] There is conflicting information on the role of the α_{1A} subtype in blood pressure regulation. α_{1A}-Adrenoceptors have been reported to be present in the cardiovasculature in humans and other species.[8] However, in vitro radioligand binding selectivity of antagonists for the α_{1A} over the α_{1B} subtype has been shown to correspond with selectivity in vivo for blockade of agonist-induced increases in intraurethral versus arterial pressure.[9,10] Evidence in support of a prominent role for the α_{1B} receptor in the regulation of blood pressure is derived from a recent study where α_{1B} knockout mice displayed a substantially reduced responsiveness to phenylephrine-induced increases in blood pressure.[11] Although the physiological role of the α_{1D} subtype remains uncertain, the α_{1D} subtype has recently been demonstrated to play a part in the pressor responses to sympathetic stimulation.[12] Given the evidence that the α_{1A}- and α_{1B}-adrenoceptors participate in the control of urethral and vascular tone, respectively, we hypothesized that an agent that selectively activated the α_{1A}-adrenoceptor, especially versus the α_{1B} subtype, would constrict the urethra with reduced cardiovascular side effects.

Chart 1. Selective and Nonselective α_1 Agonists



Scheme 1. Synthesis of Compound **1**[a]



[a] Reagents and conditions: (a) EtMgBr, CH_2Cl_2, 3-nitrobenzaldehyde; (b) TFA, triethylsilane, reflux (33% yield, two steps); (c) H_2, Pd/C, EtOAc (quantitative); (d) ethanesulfonyl chloride, pyridine, CH_2Cl_2; (e) 2 M HCl, reflux (75% yield, two steps).

In the course of our studies, we discovered **1**,[1,13] an α_{1A} agonist that possesses antagonistic activity for the α_{1B}- and α_{1D}-adrenoceptor subtypes. The in vitro and in vivo profile of **1** will be discussed and compared to the highly selective α_{1A} agonist **2** as well as the nonselective α_1 agonists **3** and **4**.

Chemistry. Scheme 1 depicts the synthesis of **1**. Treatment of the Grignard reagent,[14] generated in situ from compound **5**, with 3-nitrobenzaldehyde provided benzyl alcohol **6**, which was reduced to 3-nitrobenzylimidazole **7** using excess triethylsilane in refluxing trifluoroacetic acid. Catalytic reduction of the nitro group provided aniline **8**, which was treated with ethanesulfonyl chloride in the presence of pyridine to yield the ethanesulfonamide **9**. Deprotection of the imidazole of compound **9** in 2 M HCl provided **1**, which was converted to the maleic acid salt.

Results and Discussion. Radioligand binding assays were performed on compounds **1**−**4** essentially as described by Knepper et al.[15] Results are summarized in Table 1. The order of potencies of these agents for the α_{1A}-adrenoceptor is **2** > **1** > **3** > **4**. The order of selectivities of these agents for the α_{1A} vs α_{1B} and α_{1D} subtypes is **2** ≫ **1** > **3** > **4**.

The functional agonism of **1**−**4** for the pharmacologically defined α_1-adrenoceptor subtypes[16] was evaluated and is reported in Table 2. All were agonists at rabbit

* To whom correspondence should be addressed. Phone: 847-935-4194. Fax: 847-937-9195. E-mail: Robert.j.altenbach@abbott.com.
[†] Present address: Pharmacia, Skokie, IL 60077.
[‡] Present address: Siddco Inc., Tucson, AZ 85747.
[§] Present address: Molecumetics, Ltd., Bellevue, WA 98005.

10.1021/jm025550h CCC: $22.00
Published on Web 08/22/2002

Table 1. Radioligand Binding Profile

compd	binding[a] (K_i, μM) α_{1A}	α_{1B}	α_{1D}	selectivity α_{1B}/α_{1A}	α_{1D}/α_{1A}
1	0.14 (0.12, 0.15)	0.88 (0.76, 1.0)	0.28 (0.24, 0.32)	6	2
2	0.012 (0.009, 0.015)	2.9 (2.0, 4.4)	1.5 (1.3, 1.6)	240	120
3	2.0 (1.6, 2.5)	6.9 (6.2, 7.7)	1.7 (1.5, 1.9)	3.5	1
4	9.8 (6.2, 15)	9.6 (6.4, 14)	8.4 (5.5, 13)	1	1

[a] α_{1A}, rat submaxillary gland; α_{1B}, hamster clone; α_{1D}, rat clone. Number of determinations is ≥4. Values in parentheses are the upper and lower limits derived as a result of the standard error of the mean.

Table 2. In Vitro Functional Profile

compd	potency and efficacy for tissues[a] (EC_{50}, μM) rabbit urethra α_{1A}	rat spleen α_{1B}	rat aorta α_{1D}	functional selectivity α_{1B}/α_{1A}	α_{1D}/α_{1A}
1	0.60 ± 0.07 (80%)	inactive[b]	inactive[b]		
2	0.0093 ± 0.003 (88%)	0.32 ± 0.07 (91%)	2.6 ± 0.4 (100%)	34	280
3	7.1 ± 1.2 (133%)	85 ± 14 (68%)	1.9 ± 0.6 (106%)	12	0.3
4	230 ± 60 68%	280 ± 90 34%	58 ± 15 91%	1	0.3

[a] Agonist dose−response curves were determined against rabbit urethra (α_{1A}), rat spleen (α_{1B}), and rat aorta (α_{1D}). The dose ± SEM that contracted the tissue 50% (EC_{50}) and the percent efficacy (in parentheses) relative to phenylephrine are reported. Number of determinations is ≥4. [b] EC_{50} < 15% at 10 μM.

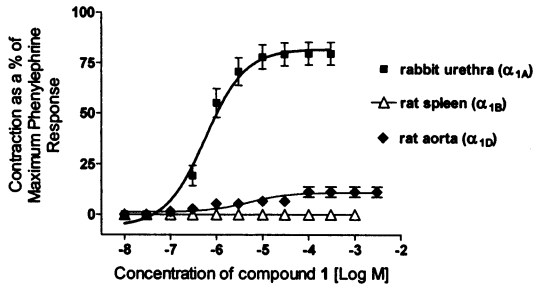


Figure 1. Compound **1** in vitro functional profile.

urethra (α_{1A}), and the relative potencies of these agents for the rabbit urethra correlate with the relative α_{1A} binding affinities.

Compound **2**, a full agonist at all of the α_1 tissue subtypes, was highly selective for the rabbit urethra (α_{1A}). Compound **3** demonstrated moderate selectivity for rabbit urethra (α_{1A}) over rat spleen (α_{1B}), but **4** was nonselective in this regard. Both **3** and **4** displayed some preference for the rat aorta (α_{1D}). Compound **1** was found to be inactive at both rat spleen (α_{1B}) and rat aorta (α_{1D}) and therefore was highly selective for the rabbit urethra (α_{1A}) (see Figure 1). The lack of efficacy of **1** for the rat spleen (α_{1B}) and rat aorta (α_{1D}) was surprising in light of the radioligand binding results where it displayed greater binding affinity for the α_{1B} and α_{1D} subtypes than **2**. This discrepancy between the relatively high binding affinity and low functional efficacy led us to examine **1** for antagonism of the α_{1B} and α_{1D} subtypes. Compound **1** was indeed found to be an antagonist of the α_{1B}- and α_{1D}-adrenoceptors with pA_2 values of 5.4 and 6.2, respectively (see Table 3).

Table 3. In Vitro Antagonism Profile (pA_2)[a]

compd	rat spleen α_{1B}	rat aorta α_{1D}
1	5.4 ± 0.2 (1.2) [18]	6.2 ± 0.4 (0.96) [16]

[a] Antagonistic activity was determined for each compound using phenylephrine challenge in rat spleen (α_{1B}) and rat aorta (α_{1D}) isolated tissues. Data are expressed as a pA_2 ± SEM. The Schild slope is shown in parentheses. The number of determinations is shown in brackets.

Table 4. In Vivo Assessment of Agonist Uroselectivity

compd	IUP ED_5 [a]	MAP ED_{20} [a]	MAP/IUP ratio[b]
1	12 ± 1	80 ± 10	6.5 ± 0.5
2	0.16 ± 0.02	0.27 ± 0.05	1.7 ± 0.4
3	205 ± 32	250 ± 20	1.3 ± 0.2
4	1100 ± 400	330 ± 80	0.40 ± 0.10

[a] Data expressed as nmol/kg ± SEM. Number of determinations is ≥4. [b] Data expressed as the mean ± SEM of the calculated MAP/IUP ratios for each determination.

In vivo assessments of agonist uroselectivity were performed in a dog model similar to one used to evaluate α_1 antagonists.[9] Briefly, mean arterial pressure (MAP) and intraurethral pressure (IUP)[17] were measured simultaneously in isoflurane-anesthetized female beagles using a chronically implanted telemetry transducer/transmitter and a urethral catheter, respectively. Increasing doses of the target agents were administered via iv injection, and the maximal effect[18] of each dose was determined. The doses corresponding to a 5 mmHg increase in IUP (IUP ED_5)[19] and a 20 mmHg increase in MAP (MAP ED_{20})[20] were calculated. For the purposes of comparing compounds, the ratios of MAP ED_{20} to IUP ED_5 (MAP/IUP ratio) for **1**−**4** are shown in Table 4.

The rank order of in vivo potency for constriction of the urethra is **2** ≫ **1** > **3** > **4**, which, as expected, parallels the relative potencies seen in α_{1A} binding and functional studies. Somewhat surprisingly, the rank order of potencies for increases in MAP also parallels the relative potencies seen in α_{1A} binding and functional studies rather than α_{1B} activity. The MAP/IUP ratio indicates that the order of uroselectivity for these agents is **1** > **2** > **3** > **4**.

Our original hypothesis was that an α_1 agonist selective for α_{1A} over the α_{1B} subtype would have increased uroselectivity over a nonselective α_1 agonist. This was observed with **1**, a full agonist for rabbit urethra (α_{1A}) and inactive at both rat spleen (α_{1B}) and rat aorta (α_{1D}). Compound **1** displayed greater uroselectivity than the α_{1A} selective agonist **2** as well as the nonselective agents **3** and **4**. The fact that **1** and **2** still had significant effects on mean arterial pressure lends support for a prominent role of the α_{1A}-adrenoceptor in the control of vascular pressure.[21] In view of the intrinsic pressor effects mediated by the α_{1A} receptor, absolute urethral selectivity may not be achievable with agents that act via the α_{1A} mechanism.[22] The antagonism of the α_{1B} and α_{1D} adrenoceptors by **1** may be indirectly blunting the apparent α_{1A}-mediated increases in MAP, thus providing the observed enhancement in the in vivo selectivity.

Compound **1** is a novel α_1 agent possessing a unique pharmacological profile of α_{1A} agonism with α_{1B} and α_{1D} antagonism. Compound **1** demonstrates greater selectivity for constricting the urethra over increasing MAP

than the highly α_{1A} selective agonist **2**, as well as midodrine and **4**, two agents that have been clinically tested for the treatment of stress incontinence.

Acknowledgment. We thank William Carroll for his critical assessment of this manuscript and for his scientific advice.

Supporting Information Available: Figure showing pressor effect of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Altenbach, R. J.; Khilevich, A.; Meyer, M. D.; Buckner, S. A.; Milicic, I. V.; Daza, A. V.; Brune, M.; O'Neill, A.; Cain, J. C.; Nakane, M.; Brioni, J.; Holladay, M. W.; Sullivan, J. P. ABT-866, a Novel α_1-Adrenoceptor Ligand with an Enhanced in Vitro and in Vivo Profile Relative to Phenylpropanolamine (PPA) and Midodrine. Presented at the 220th ACS National Meeting, Washington, DC, August 20−24, 2000; Paper Medi 294. (b) Buckner, S. A.; Milicic, I.; Daza, A. V.; Meyer, M. D.; Altenbach, R. J.; Williams, M.; Sullivan, J. P.; Brioni, J. D. ABT-866, a novel α_{1A}-adrenoceptor agonist with antagonist properties at the α_{1B}- and α_{1D}-adrenoceptor subtypes. *Eur. J. Pharmacol.*, in press.

(2) Anderson, K. E. Pharmacology of Lower Urinary Tract Smooth Muscles and Penile Erectile Tissues. *Pharmacol. Rev.* **1993**, *45*, 253−308.

(3) (a) Jonas, D. Treatment of Female Stress Incontinence with Midodrine: Preliminary Report. *J. Urol.* **1977**, *118*, 980−982. (b) Garofalo, F.; Lalanne, G. M.; Nanni, G. Midodrine for Female Incontinence: A Preliminary Report. *Clin. Ther.* **1986**, *9*, 44−46. (c) Nito, H. Clinical effect of midodrine hydrochloride on the patients with urinary incontinence. *Hinyokika Kiyo−Acta Urol. Jpn.* **1994**, *40*, 91−94. (d) Weil, E. H.; Eerdmans, P. H.; Dijkman, G. A.; Tamussino, K.; Feyereisl, J.; Vierhout, M. E.; Schmidbauer, C.; Egarter, C.; Kolle, D.; Plasman, J. E.; Heidler, H.; Abbuhl, B. E.; Wein, W. Randomized Double-Blind Placebo-Controlled Multicenter Evaluation of Efficacy and Dose Finding of Midodrine Hydrochloride in Women with Mild to Moderate Stress Urinary Incontinence: A Phase II Study. *Int. Urogyn. J.* **1998**, *9*, 145−50. (e) Andersson, K. E.; Ekman, G.; Henriksson, L.; Ulmsten, U. The effect of midodrine and its active metabolite ST 1059 on the human urethra in vitro and in vivo. *Scand. J. Urol. Nephrol.* **1983**, *17*, 261−265.

(4) Collste, L.; Lindskog, M. Phenylpropanolamine in treatment of female stress urinary incontinence. Double-blind placebo controlled study in 24 patients. *Urology* **1987**, *30*, 398−403.

(5) McClellan, K. J.; Wiseman, L. R.; Wilde, M. I. Midodrine. A Review of its Therapeutic Use in the Management of Orthostatic Hypotension. *Drugs Aging* **1998**, *12*, 76−86.

(6) (a) Hieble, J. P.; Bylund, D. B.; Clarke, D. E.; Eikenburg, D. C.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Ruffolo, R. R. International Union of Pharmacology. X. Recommendation for Nomenclature of Alpha 1-Adrenoceptors: Consensus Update. *Pharmacol. Rev.* **1995**, *47*, 267−270. (b) Bylund, D. B.; Eikenberg, D. C.; Hieble, J. P.; Langer, S. Z.; Lefkowitz, R. J.; Minneman, K. P.; Molinoff, P. B.; Ruffolo, R. R.; Trendelenburg, U. International Union of Pharmacology Nomenclature of Adrenoceptors. *Pharmacol. Rev.* **1994**, *46*, 121−136.

(7) Nasu, K.; Moriyama, N.; Fukasawa, R.; Tsujimoto, G.; Tanaka, T.; Yano, J.; Kawabe, K. Quantification and Distribution of Alpha1-Adrenoceptor Subtype mRNAs in human proximal urethra. *Br. J. Pharmacol.* **1998**, *123*, 1289−1293.

(8) (a) Rudner, X. L.; Berkowitz, D. E.; Booth, J. V.; Funk, B. L.; Cozart, K. L.; D'Amico, E. B.; El-Moalem, H.; Page, S. O.; Richardson, C. D.; Winters, B.; Marucci, L.; Schwinn, D. A. Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. *Circulation* **1999**, *100*, 2336−2343. (b) Guimaraes, S.; Moura, D. Vascular adrenoceptors: An update. *Pharmacol. Rev.* **2001**, *53*, 319−356. (c) Schwinn, D. A. Novel role for alpha(1)-adrenergic receptor subtypes in lower urinary tract symptoms. *BJU Int.* **2000**, *86* (Suppl. 2), 11−22.

(9) Brune, M. E.; Katwala, S. P.; Milicic, I.; Buckner, S. A.; Ireland, L. M.; Kerwin, J. F.; Hancock, A. A. Effects of Selective and Nonselective Alpha-1-Adrenoceptor Antagonists on Intraurethral and Arterial Pressures in Intact Conscious Dogs. *Pharmacology* **1996**, *53*, 356−368.

(10) Hancock, A. A.; Brune, M. E.; Witte, D. G.; Marsh, K. C.; Katwala, S.; Milicic, I.; Ireland, L. M.; Crowell, D.; Meyer, M. D.; Kerwin, J. F. Actions of A-131701, a Novel, Selective Antagonist for Alpha-1A Compared with Alpha-1B Adrenoceptors on Intraurethral and Blood Pressure Responses in Conscious Dogs and a Pharmacodynamic Assessment of in Vivo Prostatic Selectivity. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 628−642.

(11) Cavalli, A.; Lattion, A. L.; Hummler, E.; Nenniger, M.; Pedrazzini, T.; Aubert, J. F.; Michel, M. C.; Yang, M.; Lembo, G.; Vecchione, C.; Mostardini, M.; Schmidt, A.; Beermann, F.; Cotecchia, S. Decreased Blood Pressure Response in Mice Deficient of the Alpha1b-Adrenergic Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11589−11594.

(12) (a) Castillo, E. F.; Lopez, R. M.; Rodriguez-Silverio, J.; Bobadilla, R. A.; Castillo, C. Alpha 1D-Adrenoceptors Contribute to the Neurogenic Vasopressor Response in Pithed Rats. *Fundam. Clin. Pharmacol.* **1998**, *12*, 584−589. (b) Villalobos-Molina, R.; Ibarra, M. Vascular Alpha(1D)-Adrenoceptors: Are They Related to Hypertension? *Arch. Med. Res.* **1999**, *30*, 347−352.

(13) Brioni, J. D.; Brune, M. E.; Buckner, S. A.; Milicic, I.; Daza, A. V.; Meyer, M. D.; O'Neill, A.; Reinhart, G.; Cox, B.; Altenbach, R. J.; Holladay, M. W.; Williams, M.; Sullivan, J. P. ABT-866: A novel α_{1A}-adrenoceptor agonist with $\alpha_{1B/D}$ antagonist properties. *J. Urol.* **2001**, *165* (Suppl. 5), 34.

(14) Turner, R. M.; Lindell, S. D.; Ley, S. V. A facile route to imidazol-4-yl anions and their reaction with carbonyl compounds. *J. Org. Chem.* **1991**, *56*, 5739−5740.

(15) Knepper, S. M.; Buckner, S. A.; Brune, M. E.; DeBernardis, J. F.; Meyer, M. D.; Hancock, A. A. A-61603, A Potent Alpha 1-Adrenergic Receptor Agonist, Selective for the Alpha 1A Receptor Subtype. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 97−103.

(16) (a) Aboud, R.; Shafii, M.; Docherty, J. R. Investigation of the subtypes of alpha 1-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. *Br. J. Pharmacol.* **1993**, *109*, 80−87. (b) Buckner, S. A.; Oheim, K. W.; Morse, P. A.; Knepper, S. M.; Hancock, A. A. Alpha 1-adrenoceptor-induced contractility in rat aorta is mediated by the alpha 1D subtype. *Eur. J. Pharmacol.* **1996**, *297*, 241−248. (c) Auguet, M.; Delaflotte, S.; Chabrier, P. E. Different alpha1-adrenoceptor subtypes mediate contraction in rabbit aorta and urethra. *Eur. J. Pharmacol.* **1995**, *287*, 153−161.

(17) Brune, M. E.; O'Neill, A. B.; Gauvin, D. M.; Katwala, S. P.; Altenbach, R. J.; Brioni, J. D.; Hancock, A. A.; Sullivan, J. P. Comparison of alpha1-adrenoceptor agonists in canine urethral pressure profilometry and abdominal leak point pressure models. *J. Urol.* **2001**, *166*, 1555−1559.

(18) Measurements were made within 1 min of dosing to alleviate any PK effects.

(19) See ref 4. The 5 mmHg increase in IUP was chosen as a minimally therapeutically relevant effect. The mean urethral closure pressure in a phenylpropanolamine-treated group of 24 women with slight or moderate stress incontinence increased 7 cmH_2O (or 5 mmHg) from 48 to 55 cmH_2O and resulted in a significant decrease in leakage episodes from 5 per 24 h to 2 per 24 h.

(20) The 20 mmHg increase in MAP was chosen as a consistently measurable response above the noise of the assay.

(21) Since compound **1** has affinity for both α_1- and α_2-adrenoceptors, the possibility that the MAP effects of compound **1** may in part be due to activation of α_2-adrenoceptors was evaluated. In binding studies, the affinity of compound **1** for the α_{2a} (human clone), α_{2B} (neonatal rat lung), and α_{2c} (human clone) adrenoceptor subtypes was 171, 973, and 213 nM, respectively. α_2-Adrenoceptors are known to exist postsynaptically in the periphery, and the α_{2B}-adrenoceptor subtype has been shown by transgenic models to be responsible for the immediate hypertensive response to intraveneously administered α_2 agonists. See the following references. (a) Lahdesmaki, J.; Sallinen, J.; MacDonald, E.; Kobilka, B. K.; Fagerholm, V.; Scheinin, M. Behavioral and neurochemical characterization of alpha(2A)-adrenergic receptor knockout mice. *Neuroscience* **2002**, *113*, 289−299. (b) Hein, L. Transgenic models of alpha 2-adrenergic receptor subtype function. *Rev. Physiol. Biochem. Pharmacol.* **2001**, *142*, 161−185. An in vivo blockade experiment supported the role of α_1- over α_2-adrenoceptors in the control of MAP by **1**. The pressor effects in conscious dogs of an iv administered 100 nM/kg dose of **1** were significantly attenuated by the α_1 antagonist prazosin (0.3 mg/kg) but not by the α_2 antagonist idazoxan (1 mg/kg).

(22) The following reference describes a study in which the α_{1A} vs α_{1B} selective agonist methoxamine increased systolic blood pressure in the absence of a significant rise in maximal urethral pressure. The authors suggest a limited potential for subtype selective α_1-adrenoceptor agonists in the treatment of stress incontinence. Radley, S. C.; Chapple, C. R.; Bryan, N. P.; Clarke, D. E.; Craig, D. A. Effect of methoxamine on maximum urethral pressure in women with genuine stress incontinence: A placebo-controlled, double-blind crossover study. *Neurourol. Urodyn.* **2001**, *20*, 43−52.

JM025550H