

## PHARMACOLOGICAL EVALUATION OF PYRROLIDINES AS POTENT $\alpha_1$ -ADRENERGIC RECEPTOR ANTAGONIST WITH URO-SELECTIVE PROFILE

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### Abstract

Continuing our efforts in developing potent  $\alpha_1$ -adrenoceptor antagonists with uroselective profile, a series of derivatives of pyrrolidines was biologically evaluated *in vitro* for their affinity for  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors. Result from binding assays allowed the identification of compounds with the highest affinity and selectivity for  $\alpha_1$ -adrenoceptors behaving as potent antagonists at those sites in cellular functional assays. Among tested derivatives, compound V [1-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)pyrrolidin-2-one], displayed a 152-fold functional preference to  $\alpha_{1A}$ -adrenoceptor versus  $\alpha_{1B}$  subtype. Finally, the most promising compound V at the doses of 2, 5 and 10 mg/kg after *i.v.* administered, in contrast to tamsulosin (at a dose of 2 mg/kg, *i.v.*) did not significantly decrease systolic and diastolic blood pressure in normotensive anesthetized rats. This selected  $\alpha_{1A}$ -adrenoceptor antagonist with stronger uroselective profile, requires further research.

**Keywords:** derivatives of pyrrolidines,  $\alpha_1$ -adrenoceptors antagonists,  $\alpha_{1A/B}$  receptor selectivity, uroselective activity

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## Introduction

$\alpha_1$ -Adrenergic receptors ( $\alpha_1$ -ARs) are members of the G-protein-coupled receptor superfamily activated by adrenaline and noradrenaline.  $\alpha_1$ -Adrenoceptors generally mediate their actions through  $G_{q/11}$  proteins that stimulate the activation of phospholipase C, generation of the inositol triphosphate and diacylglycerol, liberation of calcium from the endoplasmic reticulum, and activation of genes [1, 2]. Three highly homologous subtypes of  $\alpha_1$ -adrenoceptor have been identified in human tissues:  $\alpha_{1A}$ ,  $\alpha_{1B}$  and  $\alpha_{1D}$  which differ and differing in biological structure, tissue distributions and pharmacological properties [1, 2]. Distribution studies have shown that  $\alpha_{1A}$  - and  $\alpha_{1D}$  - adrenoceptor subtypes are three to 9- fold greater expressed in the prostatic gland in benign prostatic hyperplasia (BPH), comparing to healthy tissue [1, 3].

$\alpha_1$ -ARs are found to play a prominent role in the control of blood pressure, prostatic function and other processes in the organism [1, 2, 4, 5].  $\alpha_1$ -ARs may therefore be involved in the pathogenesis of hypertension or BPH [4, 5, 6]. BPH is a common disease that affects men as they age [7, 8]. Aim of pharmacological therapy of BPH is to relieve lower urinary tract symptoms (LUTS), [1, 7, 8, 9, 10, 11, 12]. In particular  $\alpha_1$ -adrenoceptor blockers are considered the first-line drug treatment [2, 5, 6, 8, 13, 14]. Population-based cohort study using an administrative prescription database and hospital discharge codes for 1.5 million men aged  $\geq 40$  year treated with  $\alpha_1$ -blockers and 5- $\alpha$  reductase inhibitors alone or in combination [14, 15, 16].

Various  $\alpha_1$ -adrenoceptor blockers have significantly expanded over the years, giving rise to the receptor-specific  $\alpha_{1A}$ - and  $\alpha_{1D}$ -antagonists with high efficacy and low risk of

side-effect [17]. Consequently  $\alpha_{1A}$ - and  $\alpha_{1D}$ -adrenoceptor blockade relieve obstructive and voiding symptoms by the smooth muscle relaxation of prostate and bladder detrusor, respectively [1, 18]. In contrary  $\alpha_{1B}$ -adrenoceptors are predominantly expressed in vascular smooth muscle [1, 19].  $\alpha_{1B}$ -ARs blockade mediates vasodilation in blood vessels and thus is related with cardiovascular side-effects, especially orthostatic hypotension [2].

$\alpha_1$ -adrenoceptor antagonists belong to different chemical classes such as, e.g. quinazolines, phenethylamines, piperidines, dihydropyridines, arylpiperazines and related compounds [1, 5, 7, 14, 18, 20, 21]. The old  $\alpha_1$ -adrenolitics, derived from quinazoline moiety, i.e. doxazosin or terazosin, nonspecifically interact with all  $\alpha_1$ -adrenoceptor subtypes [1, 5, 13, 14, 22].

Contrasting, tamsulosin and silodosin, are the subtype-selective  $\alpha_{1A}$ - and  $\alpha_{1D}$ -adrenoceptor antagonists with minimal undesirable effects on blood pressure regulation, due to the lower interaction with  $\alpha_{1B}$ -adrenoceptor subtype [1, 2, 14, 23, 24, 25]. These new selective  $\alpha_1$ -adrenoceptor blockers decrease risk for cardiovascular side effects comparing to the non-selective representatives [23, 26]. Several studies have reported progress in the development of  $\alpha_1$ -adrenoreceptor ligands [1, 5].

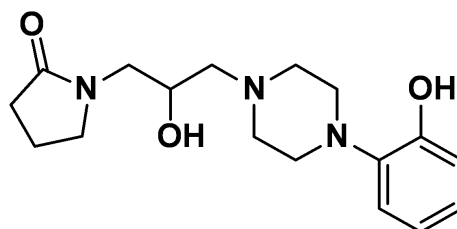
Accordingly  $\alpha_1$ -adrenoreceptor antagonists with stronger uroselective profile (higher selectivity for  $\alpha_{1A}$ - and  $\alpha_{1D}$ - versus  $\alpha_{1B}$ -adrenoceptors) are still intensively sought for the improvement of BPH therapy [1]. There are several classes of medications available for the treatment of BPH, but despite the different mechanisms of action, their beneficial effects are not observed in all patients [13, 15, 16, 27]

Continuing our efforts in development of

potent  $\alpha_{1A}$ -/ $\alpha_{1D}$ -receptor antagonists, we designed a limited series of derivatives of pyrrolidines (Figure 1), [1].

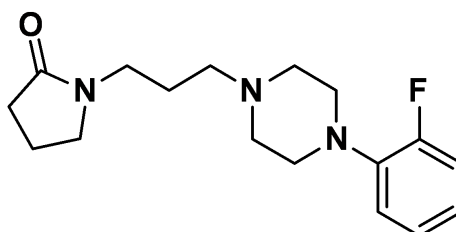
In the present study several pharmacological tests were carried out to determine  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors affinity, as well as  $\alpha_{1A}$  and  $\alpha_{1B}$ -adrenoceptor intrinsic activity in *in vitro* functional assay. Finally an influence on blood pressure and influence on blood vaso-pressor response elicited by methoxamine of most potent compound was evaluated.

I



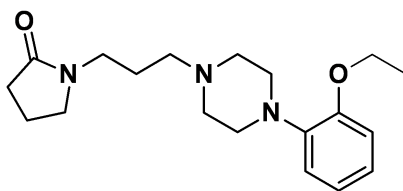
1-(2-hydroxy-3-(4-(2-hydroxyphenyl)piperazin-1-yl)propyl)pyrrolidin-2-one

II



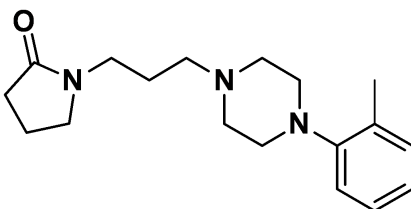
1-(3-(4-(2-fluorophenyl)piperazin-1-yl)propyl)pyrrolidin-2-one

### III



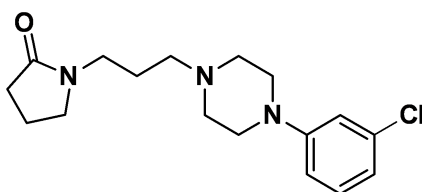
1-(3-(4-(2-ethoxyphenyl)piperazin-1-yl)propyl)pyrrolidin-2-one

### IV



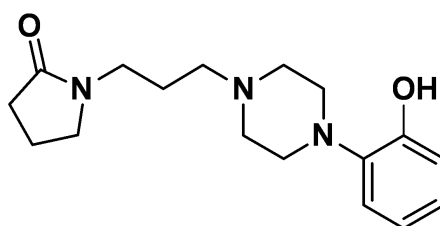
1-(3-(4-(o-tolyl)piperazin-1-yl)propyl)pyrrolidin-2-one

### V



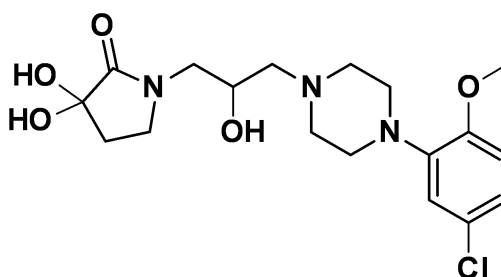
1-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)pyrrolidin-2-one

## VI



1-(3-(4-(2-hydroxyphenyl)piperazin-1-yl)propyl)pyrrolidin-2-one

## VII



1-(3-(4-(5-chloro-2-methoxyphenyl)piperazin-1-yl)-2-hydroxypropyl)-3,3-dihydroxypyrrolidin-2-one

Figure 1. Chemical structures of tested compounds I-VII

## Materials and method

### Determination of the affinity of the tested compounds at the $\alpha_1$ -adrenoreceptors and $\alpha_2$ -adrenoreceptors

The affinity of the obtained compounds was evaluated by radioligand binding assays (the ability to displace [ $^3$ H]-Prazosin and [ $^3$ H]-Clonidine from  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor, respectively) on rat cerebral cortex [28]. The brains were homogenized in 20 volumes of an icecold 50 mM Tris-HCl buffer (pH 7.6) and were centrifuged at 20,000 g for 20 min (0–4 °C). The cell pellet was resuspended in the Tris-HCl buffer and centrifuged again. Radioligand binding assays were performed

in plates (MultiScreen/Millipore). The final incubation mixture (final volume 300  $\mu$ L) consisted of 240  $\mu$ L of the membrane suspension, 30  $\mu$ L of [ $^3$ H]-Prazosin (0.2 nM) or [ $^3$ H]-Clonidine (2 nM) solution and 30  $\mu$ L of the buffer containing seven to eight concentrations ( $10^{-11}$  to  $10^{-4}$  M) of the tested compounds. For measuring the unspecific binding, phentolamine, 10  $\mu$ M (in the case of [ $^3$ H]-Prazosin) and clonidine, 10  $\mu$ M (in the case of [ $^3$ H]-Clonidine) were applied. The incubation was terminated by rapid filtration over glass fiber filters (Whatman GF/C), using a vacuum manifold (Millipore). The filters were then washed twice with the assay buffer and placed in scintillation vials with

a liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter. All the assays were made in duplicate. The inhibition constants ( $K_i$ ) were calculated from the Cheng-Prusoff equation [29, 30].

#### **Determination of the intrinsic activity of the $\alpha_{1A}$ -adrenoreceptors**

Intrinsic activity assay was performed according to the manufacturer of the assay kit (Invitrogen, Life Technologies). The cells were harvested and suspended in Assay Medium to a density of 312,500 cells/mL. 32  $\mu$ L per well of the cell suspension was added to the Test Compound wells, the Unstimulated Control wells, and Stimulated Control wells and incubated per 16–24 h. To perform an agonist assay 8 concentrations of 8  $\mu$ L of the tested compound ( $10^{-4}$ – $10^{-11}$  M), e.g. in 5 fold higher concentration in comparison to the final tested concentration in the well, was added to the cells. To perform an antagonist assay 8 concentrations of 4  $\mu$ L of the tested compound ( $10^{-4}$ – $10^{-11}$  M), e.g. in 10 fold higher concentration in comparison to the final tested concentration in the well, was added to the cells. Then, after 30 min 4  $\mu$ L of standard agonist in  $EC_{80}$  (10 fold higher concentration in comparison to the  $EC_{80}$  in the well), in Assay Medium, was added to the cells. Then both the agonist and the antagonist plate were incubated in a humidified 37 °C/5%  $CO_2$  incubator for 5 h. After the incubation 8  $\mu$ L of LiveBLAzer™-FRET B/G Substrate Mixture (CCF4-AM) was loaded cells in the absence of direct strong lighting, covered and incubated at room temperature for 2h [30].

#### **Determination of the intrinsic activity of the $\alpha_{1B}$ -adrenoreceptors**

Intrinsic activity assay was performed

according to the manufacturer of the ready to use cells with stable expression of the  $\alpha_{1B}$ -adrenoreceptors (Perkin Elmer). For measurement cells (frozen, ready to use) were thawed and re-suspended in 10-ml of assay buffer containing 5  $\mu$ M coelenterazine. This cells suspension was put in a 10-ml Falcon tube, fixed onto a rotating heel and incubated for overnight in the dark (8 rpm; 45° angle). Cells were diluted with Assay Buffer to 5000 cells/20  $\mu$ L. Agonistic ligands 2  $\times$  (50  $\mu$ L/well), diluted in Assay Buffer, were prepared in 1/2 white polystyrene area plates, and the cell suspension was dispensed in 50  $\mu$ L volume on the ligands using the injector. The light emitted was record for 20 s. Cells with antagonist were incubate for 15 min at room temperature. Therefore 50  $\mu$ L of agonist (3  $\times$   $EC_{80}$  final concentration) was injected onto the mix of cells and antagonist and record the light emitted for 20s.

#### ***In vivo* pharmacology**

##### **Animals**

The experiments were carried out on male Wistar rats (body weight 200–250 g). The animals were housed in pairs in plastic cages in constant temperature facilities exposed to 12:12 h light/dark cycle, water and food were available *ad libitum*. Experimental groups consisted of six animals each. All experiments were conducted according to the guidelines of the Animal Use and Care Committee of the Jagiellonian University (2012, Poland).

#### **Determination of the effect of the tested compounds on blood pressure after a single administration in rats**

The normotensive rats were anesthetized with thiopental (70 mg/kg) by *i.p.* injection. The left carotid artery was cannulated

with polyethylene tubing filled with heparin solution in saline to facilitate pressure measurements using PowerLab Apparatus (ADInstruments). Blood pressure was measured: before administration of the compound time 0 min (control pressure) and 60 min thereafter. For compound V, studies were performed in three doses: 2 mg/kg, 5 mg/kg and 10 mg/kg b.w. Compound was dissolved in water and administered intravenously.

### Influence on blood vasopressor response in rats

The influence of studied compound V, given intravenously at the dose of 2 mg/kg on the increase in blood pressure elicited by methoxamine (150 µg/kg), was examined according to the previously described method [31, 32]. Methoxamine were injected in to caudal vein before administration of tested compound (control group) and again 5 min after the studied compound were given.

### Statistical analysis

Statistical calculations were carried out with the GraphPadPrism 6 program. Results are given as the arithmetic means with standard error of the mean (SEM). The statistical significance was calculated using a one-way ANOVA post-hoc Dunnett's Multiple Comparison Test. Differences were considered statistically significant at: \*  $p \leq 0.05$ , \*\*  $p \leq 0.01$ , \*\*\*  $p \leq 0.001$ .

## Results

### *In vitro* radioligand binding

All synthesized compounds were evaluated in *in vitro* binding assays for their affinity toward  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors. Generally, it was found that, tested compounds showed high affinity for  $\alpha_1$ -adrenoceptors ( $K_i = 47.5$ – $195$  nM), and low for  $\alpha_2$ -adrenoceptor subtype ( $K_i = 511.6$ – $2000$  nM), (Table 1). Compounds III, V showed the highest affinity for  $\alpha_1$ -adrenoceptors and selectivity over  $\alpha_2$ -adrenoceptor subtype (Table 1).

Table 1. The binding data of the compounds I–VII for  $\alpha_1$  and  $\alpha_2$ -adrenoceptors

Compound	$K_i$ [nM] <sup>a</sup> ± SEM		
	$\alpha_1$	$\alpha_2$	$\frac{bS}{\alpha_2/\alpha_1}$
I	126.5	1380	11
II	186.3	728	3.9
III	47.5	892.5	18.7
IV	95.5	511.6	5.3
V	118.3	2000	16.9
VI	195.0	1400	7.2
VII	171	1188	7

<sup>a</sup> $K_i$  values based on two independent binding experiments; <sup>b</sup> Ratio of affinity for  $\alpha_2$  and  $\alpha_1$  adrenoceptors

The intrinsic activity of tested compounds for  $\alpha_{1A}$  and  $\alpha_{1B}$  -adrenoceptors

In the next step, all compounds were further *in vitro* tested for intrinsic activity behaving as potent antagonists at  $\alpha_{1A}$  and  $\alpha_{1B}$ -adrenoceptors (Table 2).

Table 2. The intrinsic activity of tested compounds and reference compound for  $\alpha_{1A}$ - and  $\alpha_{1B}$ -adrenoceptors

Compd	$\alpha_{1A}$		$\alpha_{1B}$		<sup>b</sup> S <sub>1B/1A</sub>	<sup>c</sup> S <sub>1A/1B</sub>
	EC <sub>50</sub> [nM] <sup>a</sup>	Profile	EC <sub>50</sub> [nM]	Profile		
<b>I</b>	105.0	ANT	27.8	ANT	0.26	3.7
<b>II</b>	135.2	ANT	207.6	ANT	1.53	0.65
<b>III</b>	178.7	ANT	30.5	ANT	0.17	5.8
<b>IV</b>	42510	ANT	32.6	ANT	0.001	1305
<b>V</b>	54.3	ANT	8254.0	ANT	152	0.007
<b>VI</b>	66090.0	ANT	294.5	ANT	0.004	224
<b>VII</b>	235.9	ANT	287.7	ANT	1.2	0.81
<b>tamsulosin</b>	0.07	ANT	1.3	ANT	19.3	19.3

ANT- antagonist; <sup>a</sup>The means EC<sub>50</sub> values were obtained from three experiments; <sup>b</sup> Ratio of EC<sub>50</sub> for  $\alpha_{1B}$  and  $\alpha_{1A}$  adrenoceptors; <sup>c</sup> Ratio of EC<sub>50</sub> for  $\alpha_{1A}$  and  $\alpha_{1B}$  adrenoceptors

The intrinsic activity of tested compounds and reference compound for  $\alpha_{1A}$ - and  $\alpha_{1B}$ -adrenoceptors showed that, compound **V** displayed the highest selectivity ratio behaving as potent  $\alpha_{1A}$  receptor antagonist (EC<sub>50</sub> = 54.3 nM), (152-fold functional preference to  $\alpha_{1A}$ -adrenoceptor over  $\alpha_{1B}$  subtype) (Table 2). While **tamsulosin** showed 19.3-fold functional preference to  $\alpha_{1A}$ -adrenoceptor. Ratio of EC<sub>50</sub> for  $\alpha_{1B}$  and  $\alpha_{1A}$  adrenoceptors for compounds **II** and **VII** amounted 1.53 and 1.2, respectively. Other compounds i.e. **IV**, **VI**, **III** and **I** displayed 1305-fold, 224-fold, 5.8-fold and 3.7-fold functional preference to  $\alpha_{1B}$ -adrenoceptor over  $\alpha_{1A}$  subtype, respectively (Table 2). In our previous studies, preference to  $\alpha_{1B}$ -adrenoceptor has been confirmed by the hypotensive effect of these compounds after *i.v.*

administration in normotensive rats [5, 32, 33, 34, 35].

#### Influence of the test compounds on blood pressure after a single administration in rats

It is well known that blockade of  $\alpha_{1A}$ -AR relaxes the enhanced prostate and bladder detrusor smooth muscle tone, whereas  $\alpha_{1B}$ -AR antagonism is involved in blood pressure regulation. This latter activity in the context of the BPH treatment leads to undesirable side effects in cardiovascular system. Therefore, with these findings in mind, compound **V** with the highest  $\alpha_{1A}/\alpha_{1B}$  selectivity ratio was chosen for further *in vivo* tests to evaluate their potential influence on blood pressure. The hypotensive activity was determined after one time *i.v.* administration to normotensive anaesthetized rats at single



doses 2.0, 5.0 and 10.0 mg/kg. It was found that tested compound did not significantly decrease systolic (SBP) and diastolic blood pressure (DBP) at mentioned doses in the whole period of observation (60 min).

Compound V at dose of 10 mg/kg reduced SBP from 148 to 133,2 mmHg (10%) and DBP from 105 to 89,5 mmHg (5%). At lower dose (5 mg/kg), the compound reduced SBP from 140 to 134.4 mmHg (4%) and DBP from 100 to 90,5 mmHg (4.5%). At

lower dose (2 mg/kg), the compound reduced SBP from 142 to 137.7 mmHg (3%) and DBP from 109 to 104.6 mmHg (4%).

In comparison, the highly  $\alpha_{1A}$  - selective compound **tamsulosin** administered intravenously at dose of 2 mg/kg decreased significantly SBP from 124 to 103 mmHg (17%) in the first 20 minutes of observation (Figure 2). In contrast, **tamsulosin** slightly decrease the DBP from 94 to 77 mmHg (18%).

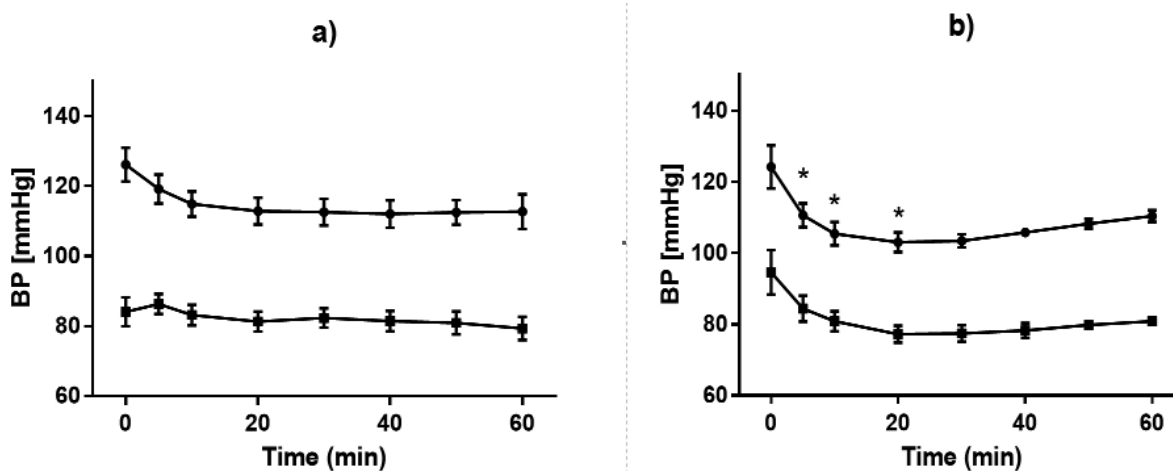


Figure 2. The hypotensive activity of tamsulosin in anaesthetised rats after i.v. administration. (A) tamsulosin at the dose 1 mg/kg b.w., i.v.; (B) tamsulosin at the dose 2 mg/kg b.w., i.v

### Influence on blood vasopressor response in rats

Methoxamine (150  $\mu$ g/kg) were given i.v. to rats to induce vasopressor response. In the control group the increases in systolic blood pressure elicited by methoxamine was from 137+9.81 to 179.25+15.70 ( $p < 0.02$ ). Compound V, given i.v. at the dose 2 mg/kg, statistically significant ( $p < 0.001$ ) at 93% reduced the increase in blood pressure elicited by methoxamine. This study confirmed  $\alpha_1$ -adrenoceptor antagonism of the compound V.

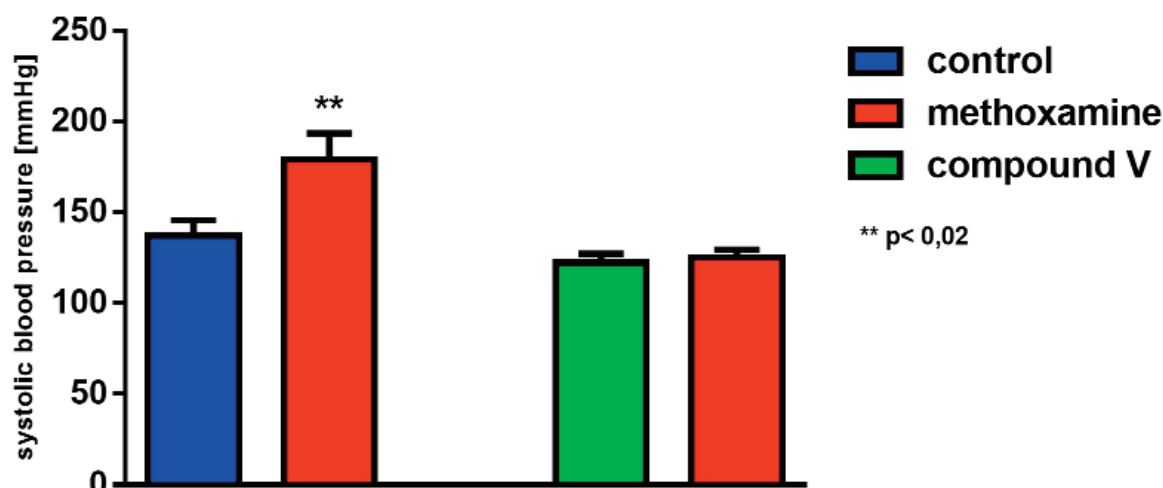


Figure 3. Influence of compound V on blood vasopressor response elicited by methoxamine

## Conclusion

To conclude, all derivatives of pyrrolidines were evaluated *in vitro* binding assays for their affinity toward  $\alpha_1$ -adrenoceptor and selectivity over  $\alpha_2$  subtype. Generally, it was found that, tested compounds showed high-to-moderate affinity for  $\alpha_1$ -adrenoceptors ( $K_i = 47.5\text{--}195$  nM), and low-to-moderate selectivity over  $\alpha_2$ -adrenoceptor subtype. The intrinsic activity of tested compounds and reference compound for  $\alpha_{1A}$ - and  $\alpha_{1B}$ -adrenoceptors showed  $\alpha_{1A}$ - and  $\alpha_{1B}$ -adrenoceptor antagonists. Derivatives of pyrrolidines: I, III, IV and VI displayed the selectivity ratio behaving as potent  $\alpha_{1B}$  receptor antagonist ( $EC_{50} = 27.8\text{--}294.5$  nM). Preference to  $\alpha_{1B}$ -adrenoceptor for these compounds has been confirmed by the hypotensive effect of these compounds in our previous studies. Compound V [1-(3-(4-(3-chlorophenyl) piperazin-1-yl) propyl) pyrrolidin-2-one] has shown higher selectivity toward  $\alpha_{1A}$ - over  $\alpha_{1B}$ - adrenoceptor, comparing it to reference drug tamsulosin. This compound administered in a dose of 2 mg/kg b.w. *i.v.* has shown none significant influence on blood pressure, while tamsulosin in the same dose caused a significant decrease of systolic blood pressure in normotensive rat. The study allowed the

identification of compound V as potent  $\alpha_{1A}$ -adrenoceptor antagonist with uroselective profile, without influence on blood pressure. Additional studies are warrant further confirm their potential application in the treatment of lower urinary tract symptoms associated with BPH.

## Resumo

*Daŭrigante niajn fortostreĉojn evoluigi potencajn antagonistojn de  $\alpha_1$ -adrenoceptoroj kun uroselectiva profilo, serio de derivitaj de pirrolidinoj estis biologie taksita *in vitro* por ilia afineco por  $\alpha_1$ - kaj  $\alpha_2$ -adrenoceptoroj. La rezulto de ligitaj ekzamenoj permesis identigi kemiajn komponaĵojn kun la plej alta afineco kaj specifeco por  $\alpha_1$ -adrenoceptoroj efikantaj kiel potencaj kontraŭiloj ĉe tiuj lokoj en ĉelaj funkciaj ekzamenoj. Inter testitaj derivaĵoj, kemia komponaĵo V [1-(3-(4-(3-clorofenil) piperazin-1-il) propilo) pirrolidin-2-one], montris 152-flankan funkcion prefere al  $\alpha_{1A}$ -adrenoceptoro kontraŭ  $\alpha_{1B}$ -subtipo. Fine, la plej efika kemia komponaĵo V administritaj en la dozo de 2,5 kaj 10 mg/kg post *i.v.*, kontraŭe al tamsulosino (je dozo de 2 mg/kg, *i.v.*) ne signife malpliigis sistolikan kaj diastolikan sangopremon ĉe normale anestezitaj ratoj. Ĉi tiu elektita antagonisto de  $\alpha_{1A}$ -adrenoceptoro kun pli forta uroselectiva profilo postulas pliajn esplorojn.*

## Acknowledgement

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## References

- Rak, Żmudzki, P.; Kotańska, M.; Knutelska, J. Siwek, A. Stachowicz, G.; Bednarski, M.; Nowiński, L.; Zygmunt, M.; Zajdel, P.; Sapa, J.; Bioorg. Med. Chem. 2016, 24(21), 5582-5591.
- Schwinn, D. A.; Roehrborn, C. G.; Int. J. Urol. 2008, 15(3), 193-199.
- Nasu, K.; Moriyama, N.; Kawabe, K.; Tsujimoto, G.; Murai, M.; Tanaka, T.; Yano, J.; Br. J. Pharmacol. 1996, 119(5), 797-803.
- Tatt, F.K.; Singapore Med. 2017, 58(8), 473-480.
- Malawska, B.; Kulig, K.; Gippert, A.; Filipek, B.; Sapa, J.; Maciag, D.; Farmaco. 2005, 60(10), 793-803.
- Gratzke, C.; Bachmann, A.; Descazeaud, A.; Drake, M. J.; Madersbacher, S.; Mamoulakis, C.; Oelke, M.; Tikkinen, K. A. O.; Gravas, S.; Eur. Urol. 2015, 67(6), 1099-1109.
- Priest, R.; Garzotto, M.; Kaufman, J.; Tech. Vasc. Interv. Radiol. 2012, 15(4), 261-264.
- Parsons, J. K.; Bergstrom, J.; Silberstein, J.; Barrett-Connor, E.; Urology 2008, 7(2), 318-321.
- Juliao, A.; Plata, M.; Kazzazi, A.; Bostanci, Y.; Djavaan, B.; Curr. Opin. Urol. 2012, 22(1), 34-39.
- Madersbacher, S.; Alivizatos, G.; Nordling, J.; Sanz, C. R.; Emberton, M.; De La Rosette, J. J.; Eur. Urol. 2004, 46(5), 547-554.
- Ficarra, V.; BJU Int. 2013, 112(4), 421-422.
- Biester, K.; Skipka, G.; Jahn, R.; Buchberger, B.; Rohde, V.; Lange, S.; BJU Int. 2012, 109(5), 722-730.
- McConnell, J.D.; Roehrborn, C.G.; Bautista, O.M.; Andriole, G.L.; McConnell, C. M.; Kusek, J. W.; Ph, D.; Lepor, H.; Mcvary, K. T.; Nyberg, L. M.; Clarke, H. S.; Crawford, E. D.; Diokno, A.; Foley, J. P.; Foster, H. E.; Jacobs, S. C.; Kaplan, S. A.; Kreder, K. J.; Lieber, M. M.; Lucia, M. S.; Miller, G. J.; Menon, M.; Milam, D. F.; Ramsdell, J. W.; Schenkman, N. S.; Slawin, K. M.; Smith, J.A.; N. Engl. J. Med. 2003, 349(25), 2387-2398.
- Yuan, J.; Liu, Y.; Yang, Z.; Qin, X.; Yang, K. M.; Mao, C.; Curr. Med. Res. Opin. 2013, 29(3), 279-287.
- Cindolo, L.; Pirozzi, L.; Fanizza, C.; Romero, M.; Tubaro, A.; Autorino, R.; De Nunzio, C.; Schips, L.; Eur. Urol. 2015, 68(3), 418-425.
- Roehrborn, C. G.; Siami, P.; Barkin, J.; Damião, R.; Major-Walker, K.; Nandy, I.; Morrill, B. B.; Gagnier, R. P.; Montorsi, F.; Eur. Urol. 2010, 57(1), 123-131.
- Li, M. Y.; Tsai, K. C.; Xia, L.; Bioorg. Med. Chem. Lett. 2005, 15(3), 657-664.
- Romeo, G.; Materia, L.; Marucci, G.; Modica, M.; Pittalà, V.; Salerno, L.; Siracusa, M. A.; Bucci, M.; Angeli, P.; Minneman, K. P.; Bioorg. Med. Chem. Lett. 2006, 16(24), 6200-6203.
- 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.; Proc. Natl. Acad. Sci. U. S. A. 1997, 94(21), 11589-11594.
- Betti, L.; Zanelli, M.; Giannaccini, G.; Manetti, F.; Schenone, S.; Strappaghetti, G.; Bioorg. Med. Chem. 2006, 14(8), 2828-2836.
- Manetti, F.; Corelli, F.; Strappaghetti, G.; Botta, M.; Curr Med Chem 2002, 9(13), 1303-1321.
- Chiu, G.; Li, S.; Connolly, P. J.; Pulito, V.; Liu, J.; Middleton, S.; Bioorg. Med. Chem. Lett. 2007, 17(14), 3930-3934.
- Castiglione, F.; Benigni, F.; Briganti, A.; Salonia, A.; Villa, L.; Nini, A.; Di Trapani, E.; Capitanio, U.; Hedlund, P. Montorisi, F.; Curr. Med. Res. Opin. 2014, 30(4), 719-732.
- Lepor, H.; Urology 1998, 51(6), 892-900.
- Kawabe, K.; Yoshida, M.; Homma, Y.; BJU Int. 2006, 98(5), 1019-1024.
- Nickel, J. C.; Sander, S.; Moon, T. D.; Int. J. Clin. Pract. 2008, 62(10), 1547-1559.
- Füllhase, C.; Chapple, C.; Cornu, J.-N.; De Nunzio, C.; Gratzke, C.; Kaplan, S. A.; Marberger, M.; Montorsi, F.; Novara, G.; Oelke, M.; Porst, H.; Roehrborn, C.; Stief, C.; Mcvary, K. T.; Eur. Urol. 2013, 64(2), 228-243.
- Maj, J.; Klimek, V.; Nowak, G.; J. Pharmacol. 1985, 119(1-2), 113-116.
- Cheng, Y.; Prusoff, W. H. Biochemistry 1973, 22(23), 3099-3108.
- Zareba, P.; Dudek, M.; Lustyk, K.; Siwek, A.; Starowicz, G.; Bednarski, M.; Nowiński, L.; Zygmunt, M.; Sapa, J.; Malawska, B.; Kulig, K.; Arch. Pharm.(Weinheim) 2015, 348(12), 861-867.
- Kubacka, M.; Mogilski, S.; Filipek, B., Marona, H.; 2013. Eur. J. Pharmacol. 698(1-3), 335-344.

32. Kulig, K.; Sapa, J.; Nowaczyk, A.; Filipek, B.; Malawska, B.; *Acta Pol. Pharm.* 2009, 66(6), 649-62 (II)
33. Kulig, K.; Sapa, J.; Maciag, D.; Filipek, B.; Malawska, B.; *Arch. Pharm. (Weinheim)* 2007, 340(9), 466-75.
34. Kulig, K.; Sapa, J.; Nowaczyk, A.; Filipek, B.; Malawska, B.; *Eur. J. Med. Chem.* 2009, 44(10), 3994-4003.
35. Kulig, K.; Spieces, C.; Sapa, J.; Caspers, C.; Filipek, B.; Malawska, B.; *Pharmacol. Rep.* 2010, 62(1), 68-85.