Arch. Pharm. Chem. Life Sci. 2017, *350*, e1600394

ARCH PHARM
Archiv der Pharmazie

Full Paper

Synthesis and Biological Evaluation of New Combined α/β-Adrenergic Blockers

Andrej Némethy[1], *Peter Vavrinec*[2], *Diana Vavrincová-Yaghi*[2], *Diana Čepcová*[2], *Svetozár Mišúth*[2], *Eva Král'ová*[2], *Ružena Čižmáriková*[1], *and Eva Račanská*[2]

[1] Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia
[2] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia

The synthesis, characterization, and pharmacological evaluation of new aryloxyaminopropanol compounds based on substituted (4-hydroxyphenyl)ethanone with alterations in the alkoxymethyl side chain in position 2 and with 2-methoxyphenylpiperazine in the basic part of the molecule are reported. For the *in vitro* pharmacological evaluation, isolated aorta and atria from normotensive Wistar rats were used. Compared to naftopidil, compounds with ethoxymethyl, propoxymethyl, butoxymethyl, and methoxyethoxymethyl substituent displayed similar α_1-adrenolytic potency. Compounds with methoxymethyl, ethoxymethyl, and propoxymethyl substituent caused a significant decrease in both spontaneous and isoproterenol-induced beating of isolated rat atria. Naftopidil and the tested substances containing a butoxymethyl and methoxyethoxymethyl substituent had no effect on the spontaneous or isoproterenol-induced beating. The tested substance that had the most pronounced effect was the compound with a propoxymethyl substituent. Its antihypertensive efficacy was investigated *in vivo* on spontaneously hypertensive rats (SHRs). The systolic blood pressure was found to be significantly lower in SHRs subjected to the treatment for 2 weeks than in untreated SHRs. Naftopidil had no significant effect.

Keywords: 4-Hydroxyphenylethanone / α/β-Blockers / Isolated aorta / Isolated atria / Spontaneously hypertensive rats

Received: December 26, 2016; Revised: March 29, 2017; Accepted: April 6, 2017

DOI 10.1002/ardp.201600394

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Introduction

Hypertension is a major human health problem of our times. Several mechanisms can be used to reduce high blood pressure. One of the main approaches is decreasing sympathetic neurotransmission via antagonization of adrenoceptors [1].

Correspondence: Andrej Némethy, Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University in Bratislava, Kalinčiakova 8, 83232 Bratislava, Slovakia.
E-mail: nemethy@fpharm.uniba.sk
Fax: +421250117357

Adrenergic receptors belong to the family of G-protein coupled receptors. They are divided into three subclasses: α_1, α_2, and β, and further into several subtypes (i.e., α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3). The classification is based on structural similarity, localization, and pharmacology as well as on cloning techniques [2, 3]. The α-adrenoceptors participate in the functioning of the central and peripheral nervous system [4], while β-adrenoceptors are mainly involved in the physiological processes in the cardiovascular system, respiratory tract, and in fat tissue [5]. In particular, α_1-receptors play a role in vascular smooth muscle contraction, increasing of blood pressure, dilation of the pupil, the human prostate smooth muscle contraction [6], as well as in regulation of cerebral microcirculation [7].

ARCH PHARM
Archiv der Pharmazie

α_1-Antagonists have been successfully applied in the therapy of hypertension over the past two decades [3]. However, both β- and α-adrenergic mechanisms are involved in the control of blood pressure; α-mediated vasoconstriction regulates the vascular tone, and β-mediated responses stimulate the heart directly or indirectly by renin release, thus affecting vascular smooth muscle tone [8].

The combination of α_1- and β-adrenoceptor antagonists in the therapy of hypertension also makes sense in terms of hemodynamics. α_1-Adrenoceptor blockade leads to vasodilatation and in this way counteracts elevated peripheral vascular resistance which is the most common hemodynamic derangement found in established essential hypertension. One drawback of this approach is the possibility of induction of reflex tachycardia [9]. The reflex cardiostimulation can be conveniently suppressed by simultaneous antagonization of β-adrenoceptors [10]. The blockade of β-adrenoceptors does not implicate a decrease in vascular resistance; the observed hypotensive response seems to be based on a reduction in cardiac output [9]. Adverse consequences of β-blockade, on the other hand, predominantly affect the α-antagonization [8].

Combined α/β-adrenoceptor blockade can be achieved either by simultaneous administration of both types of adrenoceptor antagonists or by employing drugs that possess α- and β-adrenoceptor antagonistic activity in the same molecule [9]. Hybrid drugs consist of different pharmacophoric groups which are linked together in one molecule. The advantage of hybrid drugs is stable pharmacokinetics, ensuring that the concentration of pharmacophores during the entire treatment remains in balance. While concomitantly applied drugs compete with each other for plasma-protein binding sites and in this way influence each other's bioavailability, the total plasma-protein bound fraction and the free drug concentration for hybrid drugs remain constant [11].

In this work, we decided to combine the aryloxyaminopropanol structure of β-blockers with a 2-methoxyphenylpiperazine moiety that provides the α_1-antagonization.

The structural fragment of piperazine occurs in molecules of several potent α-receptor antagonists; 2-methoxyphenylpiperazine is part of the molecules of urapidil and naftopidil [3]. It is worth mentioning that naftopidil contains an aryloxyaminopropanol structure (Fig. 1), but like its metabolites *O*-desmethyl-naftopidil, (phenyl)hydroxy-naftopidil and (naphthyl)hydroxy-naftopidil, it possesses no affinity to α_2- or β-adrenoceptors [12, 13]. Several authors have already tried, with varying degrees of success, to connect the aryloxyaminopropanol structure with a 2-methoxyphenylpiperazine moiety in order to enhance the hypotensive effect. In the aromatic part of aryloxyaminopropanols, fragments of β_1-selective betaxolol, the oxypropanol derivative of labetalol, and benzothiazine derivatives were investigated [14–17]. Likewise, the combination of vasodilating and β-antagonistic effects in the treatment of hypertension was studied in hybrid structures that connect the dihydropyridine pharmacophore of calcium channel blockers and the aryloxyaminopropanol structure [18, 19].

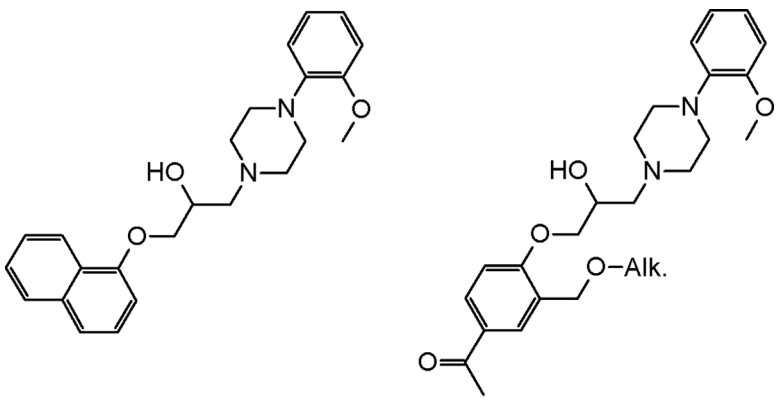
This work investigated the biological activity of newly synthesized aryloxyaminopropanols based on substituted (4-hydroxyphenyl)ethanone with modified alkoxymethyl side chain in position 2 and with 2-methoxyphenylpiperazine in the basic part of the molecule (Fig. 1).

The synthesis and characterization of the compounds are described. For the biological evaluation, isolated aorta and atria from normotensive Wistar rats were used. The antihypertensive efficacy of selected compound was tested on spontaneously hypertensive Wistar rats (SHR).

Results and discussion

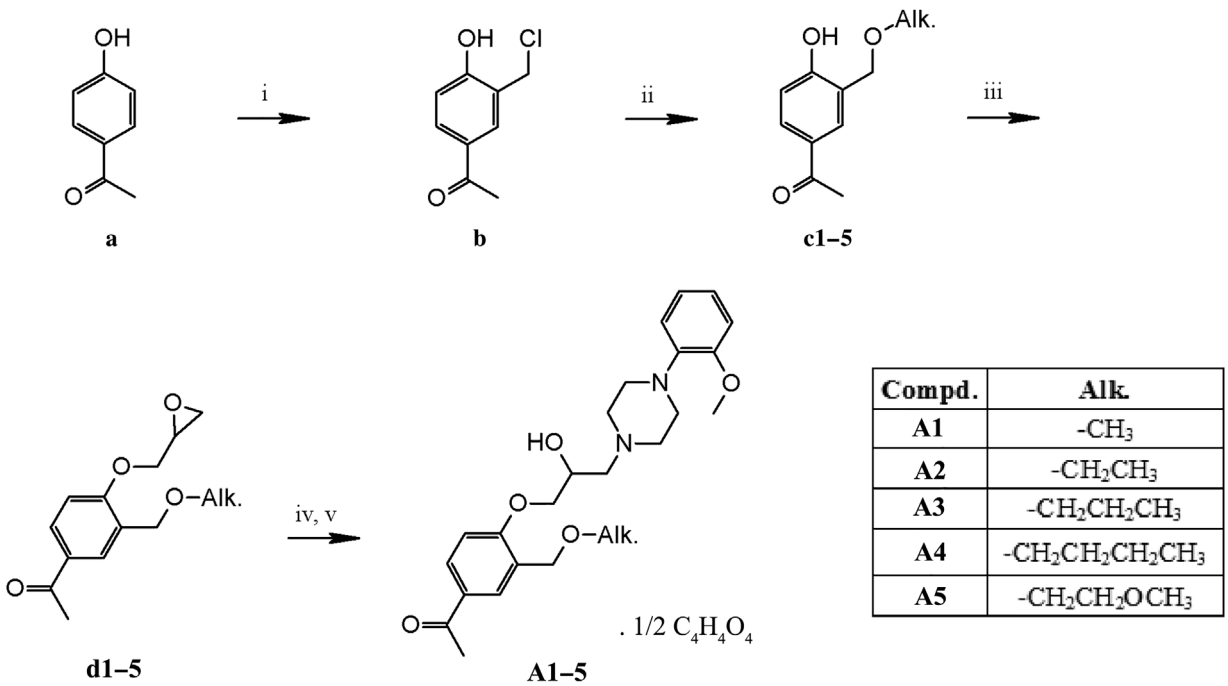
Chemistry

The target substances were synthesized (as racemates) by a four-step synthesis (Scheme 1). In the first step, the (4-hydroxyphenyl)ethanone (**a**) was chloromethylated in the second position on the aromatic ring by using paraformaldehyde and concentrated HCl to give (3-chloromethyl-4-



Naftopidil A1–5

Figure 1. Structures of naftopidil and the synthetized compounds.



Compd.	Alk.
A1	-CH_3
A2	-CH_2CH_3
A3	-$CH_2CH_2CH_3$
A4	-$CH_2CH_2CH_2CH_3$
A5	-$CH_2CH_2OCH_3$

Scheme 1. Synthetic route for title compounds **A1–5**. Reagents and conditions: (i) paraformaldehyde, conc. HCl, 4.5 h, 45–50°C; (ii) Alk-OH (1: methanol; 2: ethanol; 3: propan-1-ol; 4: butan-1-ol; 5: 2-methoxyethanol), $NaHCO_3$, 6 h, 40–50°C; (iii) chloromethyloxirane, KOH, 4 h, 50–55°C; (iv) 2-methoxyphenylpiperazine, EtOH, 2 h, 30°C, 4 h reflux; (v) diethylether, fumaric acid.

hydroxyphenyl)ethanone (**b**). The chloromethyl derivative (**b**) was treated with appropriate alcohols to form (3-alkoxymethyl-4-hydroxyphenyl)ethanones (**c1–5**) according to the published method [20]. Subsequently the prepared intermediates reacted with chloromethyloxirane to form 1-[3-(alkoxymethyl)-4-(oxirane-2-ylmethoxy)phenyl]ethanones (**d1–5**) [21]. Oxirane derivatives were treated with 2-methoxyphenylpiperazine in ethanol to give bases of the final compounds, which were subsequently transformed into salts with fumaric acid (**A1–5**). The fumarates were crystallized from ethyl acetate or propan-2-ol.

The structure of the prepared substances was determined by the interpretation of FTIR, ^{1}H NMR, ^{13}C NMR spectra, and elemental analysis. The purity of the products was verified by TLC. The FTIR spectra of prepared compounds showed characteristic functional groups, such as R_3NH^+, C=O, C=C aryl, COO^-, Ar–O–C, C–O–C. In the NMR spectral data, the chemical shifts are consistent with the proposed structures.

Pharmacology

α-Adrenolytic activity

In order to assess the α-adrenolytic activity of the tested compounds, we performed experiments on aortic rings and constructed dose–response curves for phenylephrine in the presence and absence of the tested compounds. The tested substances displayed α-adrenolytic activity of varying potency and efficacy (Fig. 2 and Table 1). The α-adrenolytic effect was shown by a rightward shift in the dose–response curves for agonist phenylephrine in the presence of the tested substances. The values of pD_2 (−log of EC_{50}) calculated from the agonist dose–response curve for phenylephrine in the presence of all tested substances were significantly lower than the values of pD_2 for phenylephrine in the presence of the vehicle (DMSO).

The compounds **A2**, **A3**, **A4**, and **A5** displayed similar potency to naftopidil, while the potency of compound A1 was lower than that of naftopidil, **A2**, **A3**, **A4**, and **A5**. The pD_2 values for phenylephrine in the presence of **A2**, **A3**, **A4**, and **A5** were not significantly different from the values for naftopidil, while the pD_2 values for **A1** were significantly higher than the values for naftopidil, **A2**, **A3**, **A4**, and **A5**.

When comparing the efficacy of the tested compounds, we observed that **A3** and **A4** had higher efficacy than naftopidil. This was shown by the maximal response of aortic rings to phenylephrine (E_{max}) in the presence of **A3** or **A4**, which was significantly lower than the E_{max} of rings in the presence of naftopidil. Furthermore, compounds **A1–A4** lowered E_{max} significantly more than did the vehicle-treated aortic rings, while E_{max} values in the presence of compound **A5** and naftopidil were not significantly different from E_{max} values obtained for phenylephrine in the presence of vehicle (DMSO).

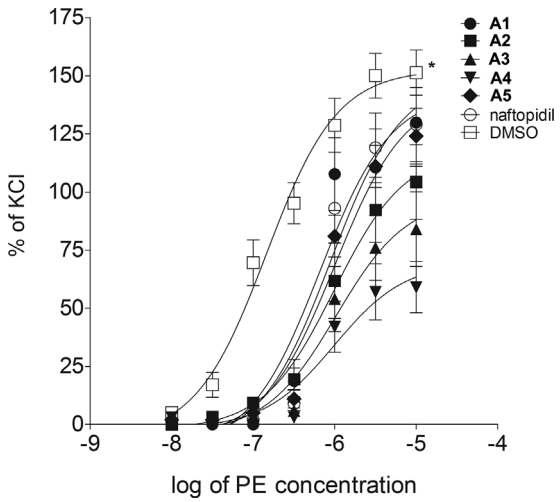


Figure 2. Dose–response curves of aortic rings for phenylephrine (PE) in the presence of tested substances (**A1**, **A2** **A3**, **A4**, **A5**, naftopidil) and in the absence of tested substances (vehicle = DMSO). Average values were calculated as a percentage of KCl-mediated constriction. Data are presented as mean ± SEM
$^{*}p < 0.05$.

β-Adrenolytic activity

In order to assess the β-adrenolytic properties, we performed experiments on spontaneously isolated and isoproterenol stimulated beating rat atria in the presence of the tested compounds (Fig. 3). We observed that **A1**, **A2**, and **A3** caused a significant decrease in both spontaneously and isoproterenol-induced beating of isolated rat atria. Substances **A4**, **A5**, and naftopidil had no effect on the spontaneous or isoproterenol-induced beating. Compound **A3** had the most pronounced effect among the tested substances. After incubating atrial strips with the tested substances, the spontaneous beating rate of **A3** was reduced by 19.1 ± 0.6% ($p < 0.05$ vs. **A1**, **A2**), which was significant compared to **A1** (7.2 ± 0.9%) and **A2** (12.9 ± 4.3%). The inhibition of isoproterenol-induced beating was comparable across the substances investigated, with no significant effect among **A1**, **A2** and **A3** (9.0 ± 2.4% for **A1**, 11.3 ± 0.9 for **A2** and 5.5 ± 1.5 for **A3**).

Antihypertensive activity

In order to investigate the antihypertensive effect of substance **A3**, which had the most pronounced effect on isolated atria, we experimented on SHRs by treating them with either the **A3** compound or naftopidil, administered in once daily doses of 30 mg/kg by oral gavage (Fig. 4). Systolic blood pressure was found to be significantly lower in SHRs subjected to the treatment for 2 weeks than in the untreated SHRs (172 ± 8 vs. 195 ± 10, $p < 0.05$). The systolic blood pressure of naftopidil-treated SHRs was not significantly different from either the vehicle-treated or **A3**-treated rats (195 ± 10).

Discussion

This work describes the synthesis, characterization, and pharmacological evaluation of new hybrid α/β-adrenoceptor antagonists. The structure of newly synthesized compounds contains the aryloxyaminopropanol structure of β-blockers and a 2-methoxyphenylpiperazine moiety that provides the α_1-antagonization. For the *in vitro* pharmacological evaluation, isolated aorta and atria from normotensive Wistar rats were used.

α-Adrenolytic activity

We observed that substances **A2**, **A3**, and **A4** decreased the maximal response of aortic rings to α-adrenergic receptor stimulation by phenylephrine (E_{max}). E_{max} reduction is a typical feature of non-competitive antagonism where even maximal concentration of the agonist cannot displace the antagonist from the receptor, thus causing a decrease in the maximal response. Unfortunately, there are insufficient data to demonstrate whether naftopidil is a competitive or non-competitive antagonist. In our study, however, naftopidil did not cause a change in E_{max}, and so the decrease of E_{max} in the

Table 1. The effect of tested substances expressed as pD_2 ($-\log EC_{50}$) and E_{max} obtained from individual dose–response curves for phenylephrine in the presence (A1, A2, A3, A4, A5, naftopidil) or absence (vehicle–DMSO) of tested substances.

pD_2	Mean	$p < 0.05$ vs.	E_{max}	Mean	$p < 0.05$ vs.
A4	5.95 ± 0.07	**A1**, DMSO	**A4**	59 ± 11	**A1**, **A5**, NAFT, DMSO
A3	5.96 ± 0.11	**A1**, DMSO	**A3**	84 ± 16	NAFT, **A5**, DMSO
A5	5.99 ± 0.06	**A1**, DMSO	**A2**	92 ± 14	DMSO
NAFT	6.02 ± 0.08	**A1**, DMSO	**A1**	111 ± 17	**A4**, DMSO
A2	6.08 ± 0.08	**A1**, DMSO	**A5**	124 ± 12	**A3**, **A4**
A1	6.26 ± 0.05	**A2**, **A3**, **A4**, **A5**, NAFT, DMSO	NAFT	129 ± 16	**A3**, **A4**
DMSO	6.80 ± 0.12	versus all	DMSO	151 ± 10	**A1**, **A2**, **A3**, **A4**

Data are presented as mean ± SEM. Differences with $p < 0.05$ were considered significantly different.

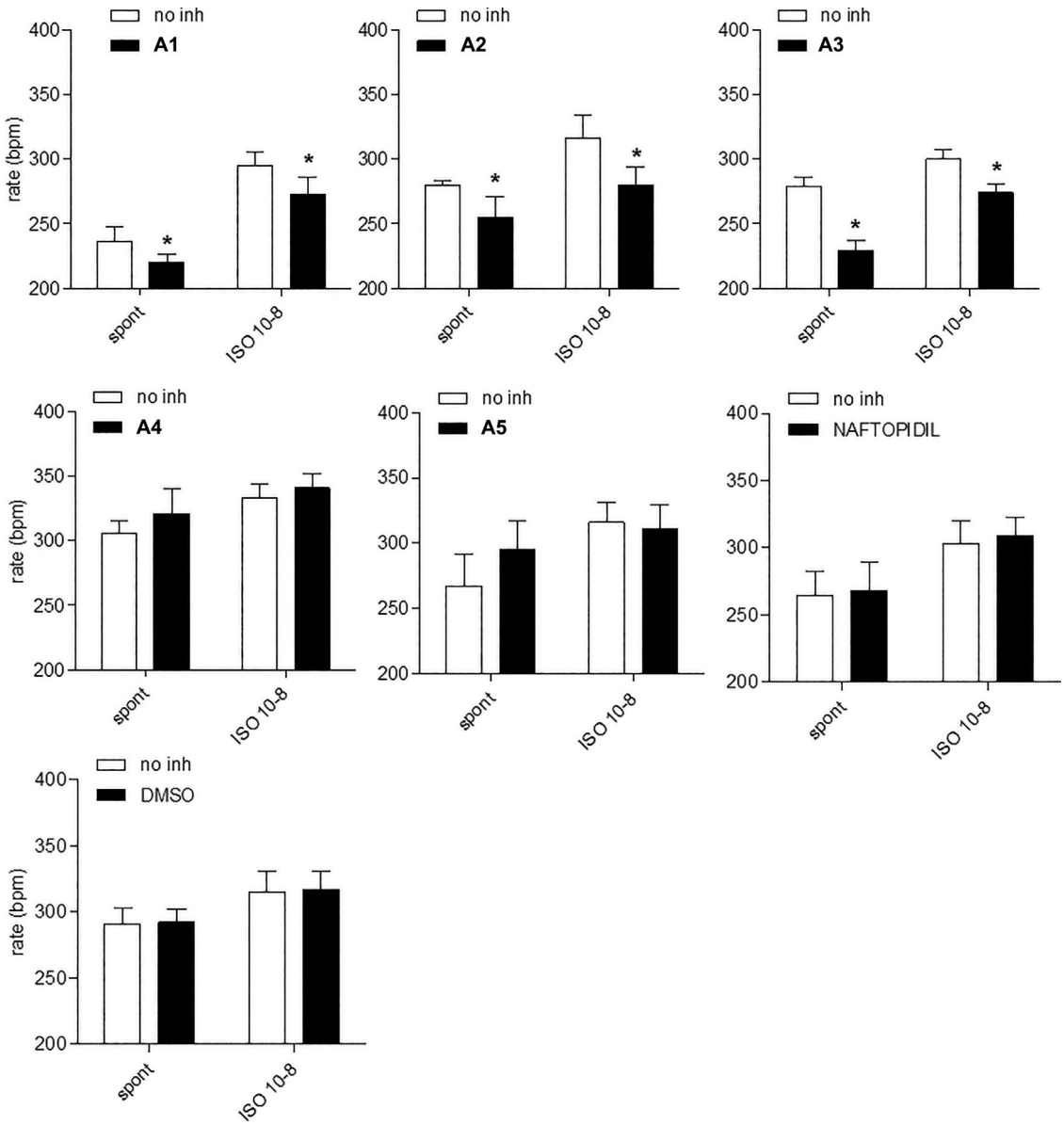


Figure 3. The effect of the tested substances (**A1**, **A2**, **A3**, **A4**, **A5**, and naftopidil) or DMSO (vehicle) on spontaneously beating isolated rat atria (spont) and after stimulation with isoproterenol in concentration 10^{-8} M (ISO 10^{-8}). Data are presented as mean ± SEM. Differences with $p < 0.05$ were considered significantly different.

case of **A2**, **A3**, and **A4** (derived from naftopidil) must have been caused by modification in the side carbon chain of the phenylethanone moiety. In the case of **A1** and **A5**, we did not observe a decrease in E_{max}, meaning that in the structure of substance **A1**, the single carbon substituent is probably too small to ensure a stronger binding to the α-adrenoceptor. Structure **A5**, on the other hand, having a side chain length of four atoms, contains oxygen in the side chain, making it more flexible in comparison with the more rigid four-carbon side chain of **A4**, which could cause different behavior in the environment of the α-adrenoceptor.

β-Adrenolytic activity

Compounds **A1**, **A2**, and **A3** caused a significant decrease in both spontaneously and isoproterenol-induced beating of isolated rat atria and thus their supposed β-adrenolytic properties could be manifested. Substances **A4**, **A5**, and naftopidil had no effect on spontaneous or isoproterenol-induced beating. Compound

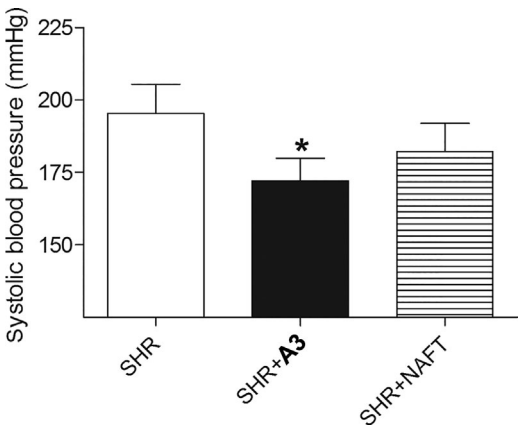


Figure 4. Effect of tested substances on systolic blood pressure of SHRs treated with **A3** or naftopidil or vehicle (DMSO), with the SBP measured using a tail cuff. Data are presented as average ± SEM; $^{*}p < 0.05$.

A3 had the most pronounced effect among the tested substances, and its antihypertensive efficacy was investigated *in vivo* on spontaneously hypertensive Wistar rats. The structural difference between molecules of naftopidil and the compound **A3** is in the aromatic moiety. If 1-naphthol in the aromatic part is combined with 2-methoxyphenylpiperazine in the basic part of its molecule, naftopidil loses its ability to antagonize the β-receptor. Replacing naphthol with substituted phenylethanone provides the β-lytic efficacy, albeit with methoxyphenylpiperazine in the basic part.

Systolic blood pressure (SBP)

It is apparent from our study that replacing naphthol with substituted phenylethanone in **A3** had an effect on blood pressure, because naftopidil had no effect on the SBP of treated SHRs. Naftopidil and **A3** were also shown to have α-adrenolytic properties *in vitro*, but with no effect on blood pressure. Unfortunately, there are no data showing whether naftopidil can decrease blood pressure in SHRs, and so we cannot compare our results with other studies. It has, however, been shown in other studies that β-blockers can decrease blood pressure of SHRs in experimental settings [22, 23], which is in line with our results. We, therefore, conclude that the substituted phenylethanone in the aromatic moiety was the prominent one in blood pressure reduction in SHRs.

Conclusion

This study describes the synthesis, characterization, and pharmacological evaluation of new hybrid α/β-adrenoceptor antagonists. Compared to naftopidil, compounds **A2**, **A3**, **A4**, and **A5** displayed similar potency to antagonize the α_1-adrenergic receptors, while compound **A1** showed lower potency.

The evaluation of β-antagonistic activity showed that compounds **A1**, **A2**, and **A3** caused a significant decrease in both spontaneously and isoproterenol-induced beating of isolated rat atria.

Compound **A3** had the most pronounced effect among tested substances. Systolic blood pressure was found to be significantly lower in SHRs subjected to the treatment for 2 weeks than in untreated SHRs. Naftopidil had no significant effect.

Experimental

Chemistry

General

The melting point was determined using a Kofler hot stage microscope and was quoted uncorrected. The purity of the newly prepared compounds was assessed using TLC silica gel plates Silufol® UV 254 (Merck). The solvent system used was ethyl acetate/diethylamine (9.5:0.5 v/v). FTIR spectra were recorded using Nicolet 6700 (Thermo Scientific). NMR spectra were recorded with the Varian Gemini 2000 spectrometer operating at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR, using $Si(CH_3)_4$ as the reference. Chemical shifts are reported in ppm (δ). In reporting the NMR multiplicities, we used the following abbreviations: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet. Elemental analysis was performed using FLASH 2000 Organic Elemental Analyzer (Thermo Scientific). All reactions were carried out using commercial grade reagents and solvents. Diethylether was dried by refluxing over potassium hydroxide and sodium followed by distillation.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*General procedure for the preparation of (3-chloromethyl-4-hydroxyphenyl)ethanone (**b**)*

The mixture of (4-hydroxyphenyl)ethanone (20.41 g; 0.15 mol) and 90 mL of concentrated HCl was heated to 45–50°C and paraformaldehyde (7.5 g; 0.25 mol) was gradually added with stirring. The reaction was allowed to proceed for 4.5 h. The solid product was filtered, washed with water, and crystallized from toluene. Yield: 71%; m.p. 155–157°C; ^{1}H NMR (CD_3OD): δ 2.53 (s, 3H, CH_3); 4.68 (s, 2H, CH_2); 6.86–6.89 (d, $J = 8.7$ Hz, 1H, Ar^5H); 7.83–7.86 (dd, $J = 2.4$, $J = 11.1$ Hz, 1H, Ar^6H); 7.98 (s, 1H, Ar^2H).

*General procedure for the preparation of (3-alkoxymethyl-4-hydroxyphenyl)-ethanones (**c1–5**)*

The solution of (3-chloromethyl-4-hydroxyphenyl)ethanone (**b**) (25.84 g; 0.14 mol) in 60 mL of appropriate alcohol (**1**: methanol; **2**: ethanol; **3**: propan-1-ol; **4**: butan-1-ol; **5**: 2-methoxyethanol) was heated to 50°C while stirring. Sodium bicarbonate (23.52 g; 0.28 mol) was added gradually over 1 h and the reaction was allowed to proceed for 6 h at a constant

temperature 50°C. After removal of redundant $NaHCO_3$ by filtration, alcohol was distilled off and the product was crystallized from cyclohexane. Yields: 65–73%; **c3**: ^{1}H NMR (CD_3OD): δ 0.93–0.98 (t, $J = 7.5$ Hz, 3H, $CH_2C\underline{H}_3$); 1.58–1.68 (m, 2H, $C\underline{H}_2CH_3$); 2.52 (s, 3H, $COCH_3$); 3.48–3.52 (t, $J = 6.6$ Hz, 2H, $OC\underline{H}_2CH_2$); 4.54 (s, 2H, Ar-CH_2); 6.83–6.85 (d, $J = 8.4$ Hz, 1H, Ar^5H); 7.79–7.83 (dd, $J = 2.2$, $J = 10.8$ Hz, 1H, Ar^6H); 7.96 (s, 1H, Ar^2H).

*General procedure for the preparation of 1-[3-(alkoxymethyl)-4-(oxirane-2-ylmethoxy)phenyl]ethanones (**d1–5**)*

To a stirred solution of (3-alkoxymethyl-4-hydroxyphenyl)-ethanones (**c1–5**) (0.15 mol; **c1**: 27.03 g; **c2**: 29.13 g; **c3**: 31.24 g; **c4**: 33.34 g; **c5**: 33.64 g) in 3 mol (235 mL) of (±)-chloromethyl-oxirane, 0.17 mol of 85% KOH (9.54 g; water: 1.7 mL) was added. The mixture was stirred at 50–55°C under nitrogen for 4 h. The inorganic salts were filtered off and the (±)-chlor-omethyloxirane was distilled off under reduced pressure. Distilled water (50 mL) was added and the residue was extracted three times with diethylether (3 × 100 mL). The organic phase was separated and dried with anhydrous Na_2SO_4. The filtered solution was concentrated under reduced pressure and the remaining oil was used without previous purification in the next reaction step. Yields: 64–84%. **d1:** ^{1}H NMR ($CDCl_3$): δ 2.52 (s, 3H, $COCH_3$); 2.75–2.78 (d, $J = 9$ Hz, 1H, $CH_2^{oxirane}$); 2.93–2.97 (m, 1H, $CH_2^{oxirane}$); 3.02–3.07 (m, 1H, $CH^{oxirane}$); 3.28 (s, 3H, OCH_3); 3.96–3.99 (d, $J = 9$ Hz, 1H, $C\underline{H}_2CH$); 4.06–4.09 (d, $J = 9$ Hz, 1H, $C\underline{H}_2CH$); 4.41 (s, 2H, $ArCH_2$); 6.84–6.86 (d, $J = 8.4$ Hz, 1H, Ar^5H); 7.78–7.83 (m, 1H, Ar^6H); 7.95 (s, 1H, Ar^2H).

*General procedure for the preparation of (2RS)-bis-1-[3-(4-acetyl-2-alkoxymethyl)phenoxy-2-hydroxypropyl]-4-(2-methoxyphenyl)piperazinium fumarates (**A1–5**)*

The solution of the oxirane derivative (**d1–5**) (80 mmol; **d1**: 18.90 g; **d2**: 20.02 g; **d3**: 21.14 g; **d4**: 22.27 g; **d5**: 22.43 g) and 1-(2-methoxyphenyl)piperazine (16.34 g; 85 mmol) in ethanol (150 mL) was kept at 30°C for 2 h and was then heated for an additional 5 h at reflux temperature. The solvent was distilled off. Distilled water (50 mL) was added to the residue, which was then washed three times with diethylether (3 × 100 mL). The combined organic layers were washed with water, separated, and dried with K_2CO_3. The filtered solution was concentrated under reduced pressure and the remaining oil was crystallized from hexane to afford the final base. The salts were prepared by quantitative reaction of anhydrous ether solution of base and anhydrous ether solution of fumaric acid. The crystals were collected by filtration and recrystallized from ethyl acetate or propan-2-ol to give fumarate salt as a white solid.

*(2RS)-bis-1-[3-(4-Acetyl-2-methoxymethyl)phenoxy-2-hydroxypropyl]-4-(2-methoxyphenyl)piperazinium fumarate (**A1**)*

Yield: 61%, m.p. 156–158°C, FTIR cm^{-1}: 2583 (R_3NH^+), 1669 (C=O), 1598 (C=C), 1354 (COO^-), 1242 (Ar–O–C), 1024 (C–O–C); ^{1}H NMR (DMSO-d_6) δ 2.50 (s, 3H, $COCH_3$); 2.54–2.98 (m, 10H, CH_2N, $pip^{2,3,5,6}$); 3.37 (s, 3H, $CH_2O\underline{CH}_3$); 3.77 (s, 3H, $ArOCH_3$); 4.01–4.07 (m, 2H, $ArOCH_2$); 4.11–4.13 (m, 1H, $CH_2\underline{CH}OH$); 4.48 (s, 2H, $ArCH_2$); 6.60 (s, 2H, fumar); 6.86–6.94 (m, 4H, pip-Ar); 7.10–7.13 (d, $J = 9.3$ Hz, 1H, Ar^5H); 7.9–7.93 (m, 2H, $Ar^{2,6}H$); ^{13}C NMR (DMSO-d_6) δ 26.39, 49.88, 53.64, 55.30, 58.00, 60.76, 66.16, 68.23, 71.31, 111.11, 111.88, 117.89, 120.82, 122.40, 126.74, 128.07, 129.40, 129.86, 134.19, 141.13, 151.95, 159.81, 166.33, 196.37. Anal. calcd. for $C_{48}H_{64}N_4O_{10}.C_4H_4O_4$, Mr 973.12; % C 64.12, % H 6.99, % N 5.75, found % C 63.84, % H 6.71, % N 5.45.

*(2RS)-bis-1-[3-(4-Acetyl-2-ethoxymethyl)phenoxy-2-hydroxypropyl]-4-(2-methoxyphenyl)piperazinium fumarate (**A2**)*

Yield: 65%, m.p. 138–140°C, FTIR cm^{-1}: 2586 (R_3NH^+), 1672 (C=O), 1599 (C=C), 1355 (COO^-), 1242 (Ar–O–C), 1022 (C–O–C); ^{1}H NMR (DMSO-d_6) δ 1.17–1.22 (t, $J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$); 2.50 (s, 3H, $COCH_3$); 2.60–2.98 (m, 10H, CH_2N, $pip^{2,3,5,6}$); 3.53–3.60 (q, $J = 7.0$ Hz, 2H, $C\underline{H}_2CH_3$); 3.77 (s, 3H, $ArOCH_3$); 4.02–4.13 (m, 3H, $C\underline{H}_2C\underline{H}OH$); 4.52 (s, 2H, $ArCH_2$); 6.60 (s, 2H, fumar); 6.85–6.95 (m, 4H, pip-Ar); 7.09–7.12 (d, $J = 9.3$ Hz, 1H, Ar^5H); 7.89–7.92 (m, 2H, $Ar^{2,6}H$); ^{13}C NMR (DMSO-d_6) δ 15.16, 26.38, 49.81, 53.60, 55.30, 60.71, 65.47, 66.11, 66.22, 71.26, 111.06, 111.89, 117.90, 120.82, 122.42, 127.12, 128.01, 129.42, 129.84, 134.24, 141.10, 151.95, 159.79, 166.43, 196.38. Anal. calcd. for $C_{50}H_{68}N_4O_{10}.C_4H_4O_4$, Mr 1001.17; % C 64.72, % H 7.19, % N 5.59, found % C 64.34, % H 7.12, % N 5.46.

*(2RS)-bis-1-[3-(4-Acetyl-2-propoxymethyl)phenoxy-2-hydroxypropyl]-4-(2-methoxyphenyl)piperazinium fumarate (**A3**)*

Yield: 63%, m.p. 148–150°C, FTIR cm^{-1}: 2582 (R_3NH^+), 1674 (C=O), 1599 (C=C), 1355 (COO^-), 1241 (Ar–O–C), 1024 (C–O–C); ^{1}H NMR (DMSO-d_6) δ 0.89–0.94 (t, $J = 7.3$ Hz, 3H, $CH_2C\underline{H}_3$); 1.58–1.60 (m, 2H, $C\underline{H}_2CH_3$); 2.50 (s, 3H, $COCH_3$); 2.60–2.95 (m, 10H, CH_2N, $pip^{2,3,5,6}$); 3.44–3.49 (t, $J = 7.5$ Hz, 2H, $OC\underline{H}_2CH_2$); 3.78 (s, 3H, $ArOCH_3$); 4.01–4,12 (m, 3H, $C\underline{H}_2C\underline{H}OH$); 4.52 (s, 2H, $ArCH_2$); 6.50 (s, 2H, fumar); 6.86–6.97 (m, 4H, pip-Ar); 7.09–7.12 (d, $J = 9.0$ Hz, 1H, Ar^5H); 7.89–7.92 (m, 2H, $Ar^{2,6}H$); ^{13}C NMR (DMSO-d_6) δ 10.63, 22.50, 26.37, 43.48, 48.00, 50.09, 55.29, 55.35, 66.36, 71,36, 71.76, 111.08, 111.89, 117.87, 120.82, 123.12, 127.14, 127.98, 129.39, 129.81, 141.24, 151.96, 159.84, 167.26, 196.37. Anal. calcd. for $C_{52}H_{72}N_4O_{10}.C_4H_4O_4$, Mr 1029.22; % C 65.29, % H 7.38, % N 5.44, found % C 65.02, % H 7.19, % N 5.14.

*(2RS)-bis-1-[3-(4-Acetyl-2-butoxymethyl)phenoxy-2-hydroxypropyl]-4-(2-methoxyphenyl)piperazinium fumarate (**A4**)*

Yield: 59%, m.p. 129–131°C, FTIR cm^{-1}: 2578 (R_3NH^+), 1674 (C=O), 1599 (C=C), 1355 (COO^-), 1242 (Ar–O–C), 1021 (C–O–C); ^{1}H NMR (DMSO-d_6) δ 0.87–0.92 (t, $J = 7.3$ Hz, 3H, $CH_2C\underline{H}_3$); 1.33–1.41 (m, 2H, $C\underline{H}_2CH_3$); 1.54–1.59 (m, 2H, $C\underline{H}_2CH_2CH_3$); 2.50 (s, 3H, $COCH_3$); 2.58–2.98 (m, 10H, CH_2N, $pip^{2,3,5,6}$); 3.48–3.53 (t, $J = 6.5$ Hz, 2H, $OC\underline{H}_2CH_2$); 3.77 (s, 3H, $ArOCH_3$); 4.02–4.12 (m,

3H, $C\underline{H}_2C\underline{H}OH$); 4.52 (s, 2H, $ArCH_2$); 6.61 (s, 2H, $ArCH_2$); 6.85–6.94 (m, 4H, pip-Ar); 7.09–7.12 (d, $J = 9.6$ Hz, 1H, Ar^5H); 7.90–7.92 (m, 2H, $Ar^{2,6}H$); ^{13}C NMR (DMSO-d_6) δ 13.79, 18.93, 26.36, 31.35, 49.86, 53.63, 55.29, 60.76, 66.15, 66.42, 69.84, 71.27, 111.07, 111.89, 117.89, 120.82, 122.41, 127.15, 127.99, 129.41, 129.81, 134.10, 141.11, 151.95, 159.80, 166.18, 196.36. Anal. calcd. for $C_{54}H_{76}N_4O_{10}.C_4H_4O_4$, Mr 1057.27; % C 65.82, % H 7.56, % N 5.29, found % C 65.56, % H 7.42, % N 5.23.

*(2RS)-bis-1-[3-(4-Acetyl-2-methoxyethoxymethyl)-phenoxy-2-hydroxypropyl]-4-(2-methoxyphenyl)-piperazinium fumarate (**A5**)*
Yield: 61%, m.p. 136–138°C, FTIR cm^{-1}: 2577 (R_3NH^+), 1678 (C=O), 1599 (C=C), 1360 (COO^-), 1240 (Ar–O–C), 1025 (C–O–C); ^{1}H NMR (DMSO-d_6) δ 2.52 (s, 3H, $COCH_3$); 2.68–3,01 (m, 10H, CH_2N, pip^{2,3,5,6}); 3.28 (s, 3H, $CH_2O\underline{CH}_3$); 3.51–3.54 (m, 2H, $CH_2\underline{CH}_2OCH_3$); 3.63–3.66 (m, 2H, $C\underline{H}_2CH_2OCH_3$); 3.771 (s, 3H, $ArOCH_3$); 4.04–4.10 (m, 3H, $C\underline{H}_2C\underline{H}OH$); 4.56 (s, 2H, $ArCH_2$); 6.61 (s, 2H, fumar.); 6.86–6.95 m (m, 4H, pip-Ar); 7.10–7.12 (d, $J = 8.7$ Hz, 1H, Ar^5H); 7.90–7.95 (m, 2H, $Ar^{2,6}H$); ^{13}C NMR (DMSO-d_6) δ 26.37, 49.50, 53.42, 55.31, 58.15, 60.43, 65.83, 66.71, 69.49, 71.16, 71.30, 111.09, 111.90, 117.94, 120.83, 122.53, 126.94, 128.16, 129.47, 129.83, 134.15, 140.93, 151.95, 159.75, 166.28, 196.39. Anal. calcd. for $C_{52}H_{72}N_4O_{12}.C_4H_4O_4$, Mr 1061.22; % C 63.32, % H 7.16, % N 5.27, found % C 63.05, % H 6.98, % N 5.08.

Pharmacological evaluation

Animals
All animal care and experimental procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the local Committee for Animals. Experiments were performed on 67 male Wistar rats (12–14 weeks old, 280–320 g) and 21 spontaneously hypertensive rats. The animals were housed under standard conditions of temperature (21–24°C), humidity (40–60%), and light (12 h/12 h light/dark cycles) at the animal facilities of Comenius University in Bratislava, Slovakia. All animals had free access to food and drinking water throughout the study.

Tissue preparation and contraction studies in rat aortic rings
Under brief anesthesia with 2.5% isoflurane in O_2, the thoracic aorta was quickly removed, cleared of fat and connective tissue, and cut into segments (rings of approximately 2 mm). The rings were mounted in a 10 mL organ baths with Krebs solution (pH 7.5) containing (in mmol/L): NaCl (120.4), KCl (5.9), $CaCl_2$ (2.5), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), glucose (11.5), $NaHCO_3$ (25.0). The solution was kept at 37°C and continuously bubbled with 95% O_2 and 5% CO_2. Prior to isotonic measurements of vascular contractility, arteries were allowed to equilibrate for 40 min. To test for viability of smooth muscle cells, arteries were twice pre-constricted with KCl (60 mM). After washout, the aortic rings were examined for full characterization of the antagonist properties of the tested substances on aorta contractions mediated by phenylephrine.

After washout and another 30 min of stabilization, the aortic rings were incubated either with the tested substances (10^{-7} M final concentration) or dimethyl sulphoxide (DMSO, 0.5% final concentration; vehicle). Subsequently, the contractility of the aortic rings was measured as the contractile response to cumulative concentrations of phenylephrine (1×10^{-8}M – 1×10^{-5}M) [24].

Preparation of atrial strips and organ baths; measurements
Under brief anesthesia with 2.5 % isoflurane in O_2, the rat heart was quickly removed. The atria were isolated and mounted in an organ bath, were attached to a fixed point at the loop end and to the tension-transducer, and were submerged in oxygenated (1.5 L/min 95% O_2 and 5% CO_2) modified Krebs–Henseleit solution (Na^+ 137 mM, K^+ 6 mM, Mg^{2+} 1.3 mM, Ca^{2+} 2.2 mM, Cl^- 134 mM, HCO_3^- 15,4 mM, $H_2PO_4^-$ 1.2 mM, glucose$^-$ 5.5 mM) at 30°C [25].

After 30 min of stabilization, a response for isoproterenol (1×10^{-8}M) was obtained on spontaneously beating atria. After washout, the tested substances were added to the organ bath in the concentration 10^{-7} M and the atria were incubated for 30 min. The response for isoproterenol (1×10^{-8}M) was then obtained in the presence of tested substances or DMSO as a vehicle.

Antihypertensive effect of tested substances—in vivo experiment
SHRs were randomized into three groups. First group comprised untreated SHRs (SHR, $n = 8$), the second group comprised SHRs treated with **A3** at doses of 30 mg/kg of body weight (SHR+**A3**, $n = 8$), and the third comprised SHRs treated with naftopidil at doses of 30 mg/kg (SHR+NAFT, $n = 5$).

After a period of acclimatization, the rats were given tested substances by oral gavage, once daily. The drugs were suspended in methylcellulose. After 2 weeks of treatment, the rats' blood pressure was measured using a tail cuff. Thereafter rats were sacrificed in isoflurane anesthesia by exsanguination.

Statistical analysis
The contractility of aortic rings was calculated as a percentage of KCl constriction. The results are expressed as mean ± SEM. Differences between the experimental groups were calculated using paired *t*-test (atrial strips), ANOVA with LSD post-hoc test (aortic rings), ANOVA, and LSD post-hoc test (SHR experiment). Differences were considered significant at $p < 0.05$.

This publication utilizes research results of the CEBV project, ITMS: 26240120034. This work was supported by the Slovak Research and Development Agency under the contract nos. APVV-0516-12, VEGA 1/0223/15 and VEGA 1/0247/17.

The authors have declared no conflicts of interest.

References

[1] R. Čižmáriková, J. Valentová, A. Némethy, I. Pechová, *Antihypertensive Drugs: Pharmacology, Medical Uses and Potential Side Effects*, (Ed.: S. Caldwell), Nova Science Publishers, New York **2015**, p. 1–54
[2] J. M. Bishop, *Curr. Top. Med. Chem*. **2007**, *7*(2), 135–145.
[3] H. Marona, M. Kubacka, B. Filipek, A. Siwek, M. Dybała, E. Szneler, T. Pociecha, A. Gunia, A. M. Waszkielewicz, *Die Pharmazie* **2011**, *66*(10), 733–739.
[4] K. Gyires, Z. S. Zádori, T. Török, P. Mátyus, *Neurochem. Int*. **2009**, *55*(7), 447–453.
[5] M. Johnson, *J. Allergy Clin. Immun*. **2006**, *117*(1), 18–24.
[6] K. S. Jain, J. B. Bariwal, M. K. Kathiravan, M. S. Phoujdar, R. S. Sahne, B. S. Chauhan, A. K. Shah, M. R. Yadav, *Bioorg. Med. Chem*. **2008**, *16*(9), 4759–4800.
[7] H. Yokoo, H. Kobayashi, S. Minami, S. Shiraishi, R. Yamamoto, T. Yanagita, K. Tsuchiya, M. Mohri, A. Wada, *Brain Res*. **2000**, *878*(1–2), 183–187.
[8] B. N. Prichard, *Drugs* **1984**, *28*(Suppl 2), 51–68.
[9] P. A. van Zwieten, *Drugs* **1993**, *45*(4), 509–517.
[10] T. B. Gottlieb, F. H. Katz, C. A. Chidsey, *Circulation* **1972**, *45*(3), 571–582.
[11] J. A. M. Christiaans, H. Timmerman, *Eur. J. Pharm. Sci*. **1996**, *4*(1), 1–22.
[12] G. Niebch, M. Locher, G. Peter, H. O. Borbe, *Arzneimittelforschung* **1991**, *41*(10), 1027–1032.
[13] J. J. Huang, Y. J. Huang, L. Zhu, M. Yuan, L. Huang, *Die Pharmazie* **2014**, *69*(8), 578–584.
[14] G. Leclerc, N. Decker, J. Schwartz, *Arzneimittelforschung* **1985**, *35*(9), 1357–1367.
[15] V. Brizzi, M. Brufani, L. Filocamo, G. Bruni, A. I. Fiaschi, *Farmaco* **1992**, *47*(6), 953–966.
[16] A. Brizzi, V. Brizzi, M. Valoti., *Farmaco* **2005**, *60*(2), 105–111.
[17] V. Cecchetti, F. Schiaffella, O. Tabarrini, A. Fravolini, *Bioorg. Med. Chem. Lett*. **2000**, *10*(5), 465–468.
[18] J. C. Liang, J. L. Yeh, C. S. Wang, S. F. Liou, C. H. Tsai, I. J. Chen, *Bioorg. Med. Chem*. **2002**, *10*(3), 719–730.
[19] S. Acharjee, K. G. Bothara, S. V. Bhandari, T. K. Maity, *Med. Chem. Res*. **2010**, *20*(6), 705–713.
[20] R. Čižmáriková, M. Polakovičová, E. Mišíková, *Chem. Paper* **2002**, *56*(4), 256–260.
[21] R. Čižmáriková, E. Račanská, K. Hroboňová, J. Lehotay, Z. Ághová, D. Halešová, *Pharmazie* **2003**, *58*(4), 237–241.
[22] K. Bruchatá, A. Némethy, R. Čižmáriková, E. Račanská, L. Habala, *Arch. Pharm*. **2016**, *349*(9), 733–740.
[23] S. O. Granstam, B. Fellström, L. Lind, *Clin. Sci*. **1998**, *95*(3), 303–309.
[24] N. Ulu, H. Gurdal, S. W. Landheer, M. Duin, M. O. Guc, H. Buikema, R. H. Henning, *Br. J. Pharmacol*. **2010**, *161*(6), 1301–1310.
[25] Y. Kubota, K. Umegaki, N. Tanaka, H. Mizuno, K. Nakamura, M. Kunitomo, K. Shinozuka, *Biol. Pharm. Bull*. **2002**, *25*(2), 197–200.