-Short communication

Preliminary *In vitro* Investigation of Antioxidant Potential of Ultra Short Acting Arylcarbamoyloxy-aminopropanols Containing *N*-Phenylpiperazine Moiety

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Cardiovascular disease (CVD) still determines the healthstatus of the vast majority of industrialized countries around the world.[1] In a few years, Eastern European countries have significantly higher total and CVD mortality rates when compared to the Western European ones.[2]

Evidence-based lifestyle interventions, as non-pharmacological approaches, leading to changes in diet, physical activity and functional parameters can produce modest but clinically significant weight loss, reduce the prevalence of type 2 *diabetes mellitus* and improve cardiovascular risk factors and mortality for the people who are not overweight.[3-7] In the viewpoint of pharmacological therapy of CVDs, the use of ultra short acting antagonists of beta-1-adrenoceptors, i.e. the compounds containing carboxy group (drawn by dashed line in Scheme) and 2-hydroxypropane-1,3-diyl connecting chain in their chemical structure, could be very beneficial. For example, clinical studies indicated that the efficacy of esmolol (Scheme) is equivalent to that of propranolol and verapamil for control of supraventricular tachycardia and to sodium nitroprusside for control of postoperative hypertension. Esmolol has also been shown to control heart rate and blood pressure during episodes of acute myocardial ischemia. Due to its ultrashort half-life, this substance can be administered safely in critically ill patients whose disease status makes treatment with currently available beta-blockers risky.[8-10] Moreover, esmolol has been able to modulate both free-radical-mediated reaction and arachidonic acid metabolism.[11] Free radical levels are raised in patients with myocardial infarction (MI) which may contribute to reperfusion injury. The antioxidant action of esmolol was clearly observed by significant difference in malondialdehyde A level and glutathione peroxidase sparing effect. Large scale clinical trials might establish conclusively role of (ultra short acting) beta-blockers as antioxidants as adjuvant to thrombolytic therapy in MI.[12,13]

Furhermore, non-selective beta-adrenoceptor blocking agent, carvedilol (Scheme) has been shown to possess very notable cardio protective and antioxidant properties as well, and its use has been associated with a reduction in oxidative stress.[14-17] In terms of its ability to act against lipid peroxidation, it might be concluded that carvedilol did not act as a radical-scavenging antioxidant and did not reduce hydroperoxides, but it could be regarded as an antioxidant against iron-induced lipid peroxidation by sequestering ferric ion.[17]

Outlined backgrounds have motivated current research (i) to preliminary investigate *in vitro* antioxidant potential of novel prospective ultra short acting antagonists of beta-adrenoceptors labelled as

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1-4, their ability to reduce stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), one of a few stable organic nitrogen radicals with strong visible absorption[18], applying UV/VIS spectrophotometry; (ii) to calculate their lipohydrophilic properties *in silico*; (iii) to reveal some structural, electronic and physicochemical features of these substances which might appear to be essential for their antioxidant potential.

The compounds under the study **1-4** (Scheme, Table), such as 1-(2-hydroxy-3-{4-[(methoxycarbonyl)amino]benzoyloxy}propyl)-4-phenylpiperazin-1-ium chloride (**1**), 1-(3-{4-[(ethoxycarbonyl)amino]benzoyloxy}-2-hydroxy-propyl)-4-phenylpiperazin-1-ium chloride (**2**), 1-(2-hydroxy-3-{4-[(propoxycarbonyl)amino] benzoyl-oxy}propyl)-4-phenylpiperazin-1-ium chloride (**3**) and 1-(3-{4-[(butoxycarbonyl)amino] benzoyloxy}-2-hydroxypropyl)-4-phenylpiperazin-1-ium chloride (**4**) were prepared as racemates at the Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences in Brno, Czech Republic. 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH; Sigma-Aldrich, Germany) and standard drugs carvedilol, {1-(9*H*-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl]amino]-2-propanol}, and *L*-ascorbic acid (Sigma-Aldrich, Germany) were commercially available.

Free radical reduction ability of tested compounds was determined by the DPPH assay following the procedure described by Blois and Kurin *et al.*[19,20]

The solution of DPPH (solution **A**) was prepared by thorough addition of 1 mg of DPPH in 50 mL of methanol *ex tempore*. Similarly, solution **B** was prepared by dissolving particular studied derivative in methanol medium, $c=1\times10^{-4}$ mol/L. From the solution **A**, 1.8 mL (i.e. the solution of DPPH•) was transferred into the flask containing 200µL of solution **B**. Changes in absorbance of the samples (investigated substances or standard drugs, respectively) were measured spectrophotometrically after 5 and 60 min at 517 nm using UV-1800 Shimadzu Spectrophotometer (Shimadzu, Japan) and UVProbe ver. 2.34 software (Shimadzu, Japan) to control the UV-1800 with a PC. The solution without the sample was chosen as the reference, prepared methanolic control solution contained ascorbic acid only as active substance.[19,20] After the DPPH• reduction, the colour of inspected solution was changed from the original violet to yellow.

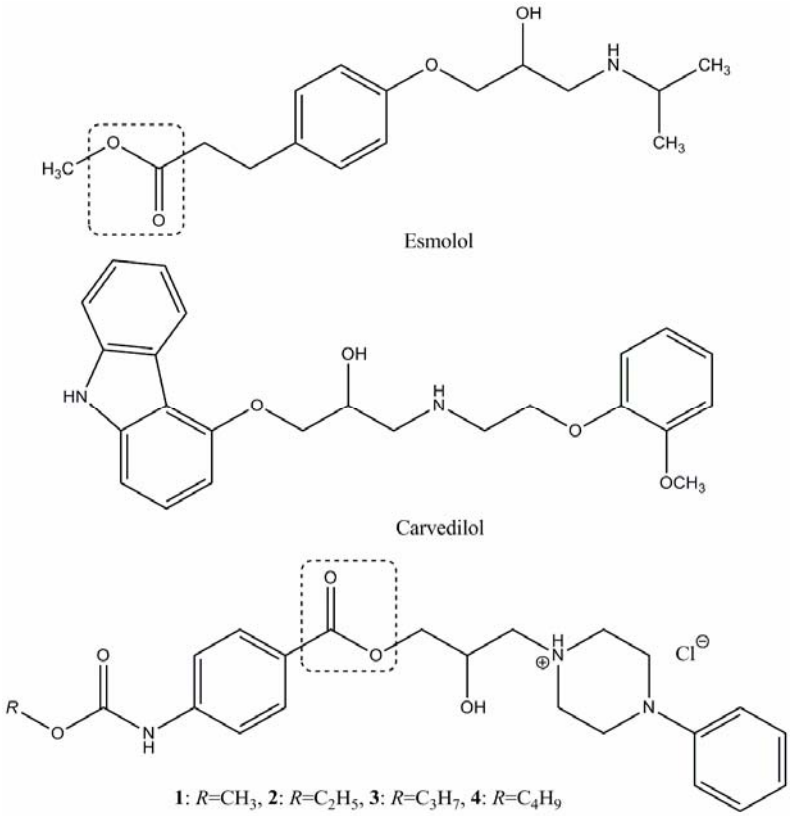
The antioxidant efficiency was expressed in percentages (*%DPPH*) as the reduction of DPPH• which was calculated relative to the measured absorbance of the control as mean ± standard deviation of three parallel measurements (Table 1), taking into account the estimations after 5- and 60-min, respectively, according to the equation given below:

$$\%DPPH = \frac{100 \times (A_{ref} - A)}{A_{ref}}$$

where, A_{ref} – the absorbance of the reference solution, A – the absorbance of the sample (inspected derivative or the standard), respectively.

The values of logarithm of partition coefficient related to derivatives (**1-4**) and to the standards were predicted *in silico* for octan-1-ol/water partitioning system (log *P*s): a) applying the ALOGPS 2.1 method, as an integral part of Virtual Computational Chemistry Laboratory Applet,[21] which was based on whole molecule approach with the application of Associative Neural Networks for the prediction[22] as well as b) by fragmental CLOGP 4.0 method[23], an interactive tool of ChemBioDraw Ultra 11 software package (CambridgeSoft, USA).The solvation forces which determine the equilibrium of a solute between water and a non-polar solvent, such as octan-1-ol, cannot be assigned on an atom-by-atom basis in the solute structure. Implemented program CLOGP 4.0 defined the hydrophobic hydrocarbon portions of any structure in such a way that the remaining polar fragments were unambiguously defined and were of a manageable size. Early versions required that each polar fragment thus defined be present in a measured solute before it could be used in calculations of log P_{oct}, but in versions 4.0 or higher, these could be calculated *ab initio*. The evaluated substances **1-4**

were calculated in non-protonated form as bases. In terms of the calculation of the log p outputs (Table 1), both interactive applets could not be able to take into account sterochemical aspects of all the molecules under the study.



Scheme. Chemical structure of esmolol, carvedilol and the compounds **1-4** evaluated for their capability to reduce DPPH radical *in vitro.*

Table. 1. Capability of the compounds under the study (1-4) and the standards to reduce DPPH radical *in vitro* and their predicted values of log *P* related to octan-1-ol/water partitioning system.

Entry	*%DPPH* (5 min)	*%DPPH* (60 min)	log *P* ALOGPS 2.1	log *P* CLOGP 4.0
1	3.2±0.2	2.1±0.2	2.10	3.20
2	2.3±0.1	3.7±0.2	2.70	3.73
3	-4.8±0.1	-4.6±0.1	3.00	4.26
4	3.5±0.2	2.5±0.2	3.25	4.79
Carvedilol	-4.8±0.2	0.4±0.1	3.05	4.04
Ascorbic acid	97.2±0.2	97.2±0.3	-1.58	-2.17

Regarding experimental details obtained after 5 min under conditions *in vitro*, butoxy derivative **4** could be able to reduce DPPH• most efficiently with estimated *% DPPH*=3.5±0.2. Methoxy substituted compound **1** has shown slightly lower value *% DPPH*=3.2±0.2, as can be seen in the Table 1. Both molecules were considered more effective than carvedilol. It could be primarily assumed that relatively high lipophilicity could be important but it was probably not an essential factor in terms of the

abilities of given substances to reduce the DPPH•. To support such a statement, for the compound **4** calculated log *P* readout was 3.25 and 4.79, respectively, applying both principally different *in silico* approaches. On the contrary, substance **1** has been regarded as more hydrophilic with the log *P*=2.10 and 3.20, respectively. The explanation of observed phenomenon could be that the linearity of studied 4-alkoxycarbonylamino substituted compounds made mesomeric effect at phenyl ring which affected electron distribution and lipohydrophilic properties. In this case, the substituents attached to 4-position acted primarily through the resonance as electron-donating groups which have been able to enhance the basicity of nitrogen atom. Given substituents could distribute negative charge towards amino moiety of carbamate group facilitating its protonation. Nevertheless, the described electron-donating resonance effect has been countered by the electron-withdrawing inductive one of these substituents. Anyway, for 4-position, positive mesomeric effect dominated.[24] This influence was enhanced in the case of methoxy derivative (**1**) due to stronger electron-donating effect. Its was also important to note that experimental determination of log *P* data by classical shake-flask method could not be possible due to insolubility of the derivatives **1**-**4** in water (buffer) medium.

Surprisingly, propoxy substituted compound **3** with log *P*=3.00 and 4.26, has shown negative output of *%DPPH* similarly to carvedilol (*%DPPH* = -4.8±0.2). Current research revealed that this highly lipophilic standard drug[25], with experimentally estimated log P_{exp}=3.40 and predicted log *P*=3.05 and 4.04, has no capability to reduce DPPH•.

The decrease in antioxidant efficiency *in vitro* was observed after 60 min for the compound **1** and **2**. On the other hand, slight increase in the potency was assigned to the derivatives **2** and **3** as well as for the standard carvedilol (Table 1).

From entire analyzed set, highly hydrophilic ascorbic acid (Table 1) was the most efficient antioxidant *in vitro* with estimated *% DPPH* = 97.2±0.2 (after 5 min) and *% DPPH* = 97.2±0.3 (60 min).

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