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Research Article

THE *IN VITRO* ANTIOXIDANT PROPERTIES OF 2--ALKOXYPHENYLCARBAMIC ACID DERIVATIVES CONTAINING A 4´-(SUBSTITUTED PHENYL)PIPERAZIN-1´-YL MOIETY DETERMINED BY THE 2,2´-AZINOBIS(3-ETHYL-BENZOTHIAZOLINE-6-SULFONIC ACID) DERIVED RADICAL CATION ($ABTS^{\bullet+}$) AND FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAYS

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ABSTRACT

In an effort to comprehensively characterize an antioxidant profile of 2-alkoxyphenylcarbamic acid-based compounds containing a 4´-(substituted phenyl)piperazin-1´-yl fragment, they were *in vitro* screened in the 2,2´-azino-bis(3-ethylbenzothiazoline-6--sulfonic acid) derived radical cation ($ABTS^{\bullet+}$) and ferric reducing antioxidant power (FRAP) assay using the UV/VIS spectrophotometry. The $ABTS^{\bullet+}$ scavenging (reducing) potential of 1-[3-(2-methoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(4--fluorophenyl)piperazin-1-ium chloride was found to be the most promising and it was comparable to the efficiency of the carvedilol reference drug. Moreover, that 4´-fluoro group-containing compound was regarded as more active than the atenolol standard. When testing the molecules´ power to reduce the ferric 2,4,6-tris (2-pyridyl)-*s*-triazine complex $[Fe(III)(TPTZ)_2]^{3+}$, the most prospective was 1-[3-(2-ethoxyphenylcarbamoyl)oxy-2--hydroxypropyl]-4-(4-fluorophenyl)piperazin-1-ium chloride. On the other hand, its Fe^{3+} reducing power was lower compared to both standards carvedilol and atenolol. The study discussed structure–antioxidant properties relationships considering electronic, steric and lipophilic features.

Key word: Substituted phenylcarbamates, *N*-arylpiperazines, antioxidant properties, $ABTS^{\bullet+}$, FRAP, electronic features, lipophilicity

INTRODUCTION

Cardiovascular disease (CVD) is still the most common cause of death in Europe, causing almost twice as many deaths as cancer across the continent and places a substantial burden on the health care systems and economies of Europe. Mortality and morbidity from the CVD continue to have a major social and economic impact in Europe and significant inequalities are evident between countries. There have been major improvements in recent years on many measures of the CVD, however, these improvements have not been universal and substantial inequalities persist (Nichols *et al.*, 2013; Townsend *et al.*, 2015; Townsend *et al.*, 2016).

Notable evidence-based lifestyle interventions, as non-pharmacological approaches, leading to the changes in a diet, physical activity, and functional parameters can produce modest but clinically significant weight loss, reduce the prevalence of a type 2 *diabetes mellitus* and improve cardiovascular risk factors and mortality for the people, who are not overweight (Antala *et al.*, 2008; Eguchi *et al.*, 2014; Gillet *et al.*, 2012; Kyselovičová *et al.*, 2014; Loveman *et al.*, 2011; Tibenská and Medeková, 2014).

In the viewpoint of a pharmacological therapy of the CVD, the use of the *β*-adrenoceptor antagonists (*β*-AdrAs), which have shown notable cardioprotective and antioxidant properties, could be very beneficial. One of the best examples of such a drug has been lipophilic, a non-selective third-generation carvedilol (Book, 2007; Dandona *et al.*, 2007). In addition, several metabolites of carvedilol have been considered extremely strong anti-

oxidants, being 30- to 80-fold more efficient than carvedilol and up to 1000-fold more potent than vitamin E (Feuerstein *et al.*, 1997; Kramer and Weglicki, 1996).

Presently investigated 2-alkoxy substituted phenyl carbamic acid-based compounds **1–4** have shown some common structural features, as carvedilol (Figure 1). Particularly, the molecules **1–4** have contained (i) a lipophilic (2-alkoxy substituted) aromatic ring (the fragment **A** in Figure 1), (ii) a hydrophilic (carbamoyloxy) bridge (**B**) attached to (iii) a 2-hydroxypropane-1,3-diyl connecting chain (**C**) and (iv) a basic centre of protonation (**D**).

Following physicochemical properties of the mentioned compounds, carvedilol was considered slightly more basic with potentiometrically estimated dissociation constant (pK_a) of 7.97 (Caron *et al.*, 1999) than the substances **1–4**, which pK_as were found in the interval of 5.83 (the compound **3**) to 6.73 (**1**), as published by Malík *et al.* (2005; 2006). On the other hand, the most of the *β*-AdrAs (acebutolol, alprenolol, atenolol, and carazolol, for example) has shown similar values of the pK_a of around 9.50 (Caron *et al.*, 1999). The origin of thus discrepancy in the pK_as between carvedilol and the other *β*--AdrAs was attributed to an inductive effect of a *β*-O-atom, which lowered the basicity of an amino group.

In addition, the derivatives **1–4** were regarded as highly lipophilic compounds (Malík *et al.* 2005; Malík *et al.*, 2006) with the log P_{exp} values in the range of 3.57 (**3**) to 3.90 (**2**), as listed (Table I). Their lipophilicity was comparable to those of carvedilol with log P_{exp}=3.40 estimated in the octan-1--ol/buffer partitioning system (Yue *et al.*, 1992).

Those backgrounds have motivated the current research to (i) investigate under the *in vitro* conditions, whether any of that prospective *β*-AdrAs **1–4** could be potential antioxidants in terms of their capability to scavenge the 2,2´-azinobis(3-ethylbenzotiazoline-6-sulfonic acid) derived radical cation ($ABTS^{\bullet+}$) and reduce the ferric 2,4,6-tris (2--pyridyl)-*s*-triazine complex $[Fe(III)\ (TPTZ)_2]^{3+}$ by using the UV/VIS spectrophotometry; (ii) reveal some structural, electronic and physicochemical features of those substances, which might appear to be essential for their antioxidant potential.

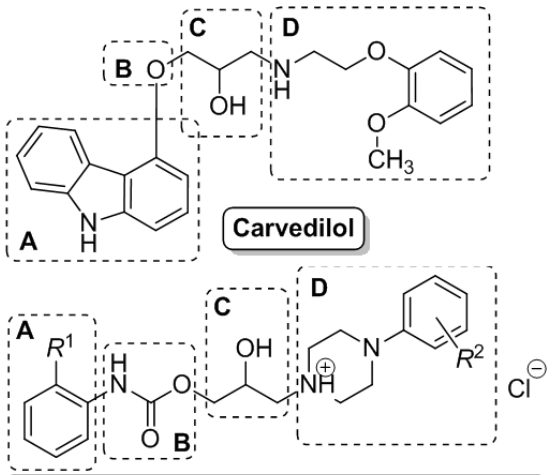

Figure 1. Chemical structure of carvedilol and currently screened 1-[3-(2-alkoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(4-fluoro-/3-trifluoromethylphenyl)piperazin-1-ium chlorides **1–4**

MATERIALS AND METHODS

The compounds under the study, reference drugs, reagents and solvents

The currently *in vitro* screened compounds **1–4** (Figure 1), chemically 1-[3-(2--methoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(4-fluorophenyl)piperazin-1-ium chloride (**1**), 1-[3-(2-ethoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(4-fluorophenyl)piperazin-1-ium chloride (**2**), 1-{2-hydroxy-3-(2-methoxyphenylcarbamoyl)oxy)propyl}-4-(3-trifluoromethylphenyl)piperazin-1-ium chloride (**3**) and 1-{2--hydroxy-3-(2-ethoxyphenylcarbamoyl)oxy)-propyl}-4-(3-trifluoromethylphenyl)piperazine--1-ium chloride (**4**), were synthesized previously (Malík *et al.*, 2004; Malík *et al.*, 2006). Their experimentally observed pK_a values, which were determined by a potentiometric titration, and the log P_{exp}s estimated by a classical shake flask method (Table I) were already published in the research papers of Malík *et al.* (2005; 2006).

Other compounds, which were used in performed *in vitro* experiments as the standard drugs, were purchased from Sigma-Aldrich (Germany) showing the purity of an analytical grade: Atenolol (ATN) and carvedilol (CRV), respectively, as the compounds, which have possessed antioxidant features (Book, 2007; Dandona *et al.*, 2007; Gomes *et al.*, 2006).

Furthermore, potassium persulfate (Sigma-Aldrich, Germany), 2,2´-azino-bis(3--ethylbenzothiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich, Germany) and ethanol (CentralChem, Slovak Republic) were used to measure the antioxidant capability of the molecules **1**–**4** and the reference drugs by the 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) derived radical cation ($ABTS^{\bullet+}$) method. The ethanol solvent was dried before use.

The following chemicals were used to estimate the antioxidant potency by the ferric reducing antioxidant power (FRAP) assay: Ferric chloride hexahydrate, sodium acetate trihydrate, glacial acetic acid, concentrated hydrochloric acid (all the reagents and solvents were purchased from CentralChem, Slovak Republic) and 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ; Sigma-Aldrich, Germany).

The *in vitro* investigation of the antioxidant potential by the 2,2´--azinobis(3-ethylbenzotiazoline-6-sulfonic acid) derived radical cation ($ABTS^{\bullet+}$) assay

The $ABTS^{\bullet+}$ decolourisation assay was carried out according to the method developed earlier by Rice-Evans *et al.* (1996) and Re *et al.* (1999) with some modifications of Iqbal *et al.* (2014).

In brief, a stock solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation ($ABTS^{\bullet+}$) was prepared by dissolving 0.038g of ABTS in 10mL of distilled water, then 0.270g of potassium persulfate was added in the solution.

The ABTS, a colorless parent compound, was used because of its high chemical stability, high water solubility and its UV/VIS absorption spectrum with a maximum at λ_{max}=340nm (Cano *et al.*, 1998). In the assay, the potassium persulfate reagent has shown the ability to oxidize ABTS to the radical cation $ABTS^{\bullet+}$, a long-living species with high extinction coefficients at 416, 650, and 734nm (Campos and Lissi, 1997).

Because ABTS and potassium persulfate reacted stoichiometrically at a ratio of 1:0.5, that would result in an incomplete oxidation of the ABTS. The oxidation of ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6h had elapsed (Re *et al.*, 1999).

Following given, the stock solution of $ABTS^{\bullet+}$ was kept in dark for 24h at room temperature in order to generate deep colored radical containing solution. After that period, the volume of 1.1mL of the concentrated radical was transferred into a 50-mL volumetric flask and made up with an anhydrous ethanol. It was proven that the radical cation $ABTS^{\bullet+}$, a blue-green chromogen (chromophore), was stable for more than 48h when stored in the dark at room temperature (Re *et al.*, 1999) from p*H*=3.0 to 6.5, however, unstable above 35°C (Cano *et al.*, 1998).

Before each analysis session, the $ABTS^{\bullet+}$ solution was diluted with an anhydrous ethanol to an absorbance of 1.00±0.02 at 734nm. Although Re *et al.* (1999) or Katalinic *et al.* (2005) recommended $ABTS^{\bullet+}$ with the absorbance of approximately 0.70±0.02, the authors of a current article found that the using a higher concentration allowed the testing of a more extended range of antioxidant concentrations.

After the addition of 2.0mL working ethanolic $ABTS^{\bullet+}$ solution to 100μL of each compound (c=1×10^{-3}mol/L), the particular mixture was stirred for 30 seconds and the absorbance was measured after additional (i) 4min and 30s (overall time was 5 minutes) and (ii) 59min and 30s (overall time was 1 hour) as well at λ=734nm using the UV/VIS spectrophotometer HP 8453 (Hewlett Packard, USA).

The extent of decolourisation as the percentage inhibition of $ABTS^{\bullet+}$ was determined relative to the measured absorbance of the control. When the antioxidant was added and mixed, the drop in the absorbance ($A_{control}$–A_{sample}) was measured after given reaction periods.

The percentages of the $ABTS^{\bullet+}$ scavenging (reduction) were calculated after 5min and 1h, respectively, according to the equation given below:

$$\%ABTS^{\bullet+} = \frac{100\times(A_{control}-A_{sample})}{A_{control}}$$

where $\%ABTS^{\bullet+}$ was the percentage of the

$ABTS^{\bullet+}$ scavenging, A_{sample} and $A_{control}$ were the absorbances at λ=734nm before ($A_{control}$) and after 5min (and 1h) adding samples to the $ABTS^{\bullet+}$ working solution, respectively.

Those *%ABTS*$^{\bullet+}$ values were presented as means ± standard deviation (SD) of at least triplicate experiments (Table II).

The *in vitro* investigation of the antioxidant potential by the ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay took an advantage of an electron transfer reaction (Benzie and Strain, 1996), in which a ferric salt $[Fe(III)(TPTZ)_2]Cl_3$ was used as an oxidant. That method depended upon the reduction of a colorless ferric 2,4,6--tris (2-pyridyl)-*s*-triazine complex $[Fe(III)(TPTZ)_2]^{3+}$ to the blue-coloured ferrous 2,4,6-tris (2--pyridyl)-*s*-triazine one $[Fe(II)(TPTZ)_2]^{2+}$.

In other words, the antioxidant potentials of the compounds were estimated as their power to reduce the $[Fe(III)(TPTZ)_2]^{3+}$ complex to the $[Fe(II)(TPTZ)_2]^{2+}$ one.

The difference between the $ABTS^{\bullet+}$ and FRAP assay was that the first one occurred at a neutral p*H* (Re *et al.*, 1999) whereas the FRAP assay needed acidic conditions, it means the p*H*=3.6 (Benzie and Strain, 1996).

A 300mmol/L acetate buffer of p*H*=3.6 (3.1g of sodium acetate trihydrate and 16mL of a glacial acetic acid made up to 1000mL with distilled water), 10mmol/L 2,4,6-tris (2-pyridyl)--*s*-triazine (TPTZ; 3.1mg/mL in 40mmol/L hydrochloric acid) and 20mmol/L ferric chloride hexahydrate (5.4mg/mL in distilled water) were mixed together in a volume ratio of 10:1:1 (25mL of the acetate buffer, 2.5mL of a TPTZ solution and 2.5mL of a ferric chloride hexahydrate solution), respectively, as required to give the working FRAP reagent. That FRAP working solution must be always freshly prepared (Benzie and Strain, 1996; Then *et al.*, 2003).

The temperature of the FRAP working reagent was raised to 37°C before use and allowed to react (3mL) with the prepared solution of each evaluated compound (100μL, $c=1\times10^{-3}$mol/L).

The absorbance value was recorded after 5min as well as 1h with the UV/VIS spectrophotometer HP 8453 (Hewlett Packard, USA). The formed $[Fe(II)(TPTZ)_2]^{2+}$ complex has shown an intensive blue color, which was monitored at λ=593nm. Increased absorbance at that wavelength indicated a stronger reducing power.

The antioxidant efficiency of the compounds **1**–**4** after 5min and 1h, respectively, was calculated as the ascorbic acid equivalent (*AAE*; the analogical amount of ascorbic acid expressed in the mg/mL units) according to the equation bellow:

$$AAE = \frac{A}{0.134}$$

where *AAE* meant ascorbic acid equivalent and the *A* parameter was the absorbance of the sample.

Those *AAE* data were presented as means ± standard deviation (SD) of at least triplicate experiments (Table II).

RESULTS AND DISCUSSION

Following the chemical structure of currently *in vitro* screened compounds **1**–**4**, their capability to scavenge a large nitrogen-centered and sterically-hindered blue-green radical cation $ABTS^{\bullet+}$ and a colourless $[Fe(III)(TPTZ)_2]^{3+}$ complex could be dependent on: (i) electronic, steric and lipohydrophilic properties of the R^2 substituent attached to the salt-forming moiety and (ii) a length of the R^1 group of a lipophilic part (Table I). Possible impacts of given aspects were discussed in next sections of the paper.

Table I. Experimentally observed values of the dissociation constants (pK_a) by the potentiometric titration and the partition coefficients (log P_{exp}) of the compounds **1**–**4** determined in the octan-1-ol/phosphate buffer (p*H*=7.4)

Entry	R^1	R^2	pK_a	log P_{exp}
1	2-OCH_3	4´-F	6.73	3.61
2	2-OC_2H_5	4´-F	6.58	3.90
3	2-OCH_3	3´-CF_3	5.83	3.57
4	2-OC_2H_5	3´-CF_3	6.00	3.60

Electronic properties of substituents might be described by the Hammett substituent constant σ; a more positive value of σ means a stronger electron-withdrawing influence of the substituent (Kubinyi, 1993). The σ output for

the 4´-F substituent ($\sigma_{4'\text{-F}}$) was 0.06, the σ value related to the 3´-CF_3 moiety ($\sigma_{3'\text{-}CF_3}$) was set to 0.43 and that parameter for a hydrogen atom (σ_H) was 0.00.

The electronic features of all the *in vitro* studied 2-alkoxyphenylcarbamates/*N*-arylpiperazines **1–4** could be also characterized by the values of their dissociation constants pK_a (Table I). It was found that the presence of the 4´-F substituent provided higher values of the compounds' pK_a, which were observed in the range of 6.58 (**2**) to 6.73 (**1**). The introduction of a 3´-CF_3 moiety led to lower pK_as, namely the pK_a=5.83 was estimated for the substance **3** and the pK_a=6.00 was found for the derivative **4** (Malík *et al.*, 2005; Malík *et al.*, 2006).

Furthermore, the values of representative steric descriptors *L* and B_i–B_{iv} (Kubinyi, 1993) proved that the 3´-CF_3 group was sterically almost twice as bulky than the 4´-F one.

The presence of a 4´-F substituent (compounds **1** and **2**) provided only a very slight advantage for those screened derivatives in their capability to scavenge (reduce) the radical cation ABTS$^{\bullet+}$ after 5min as well as 1h compared to the potential of the 3´-CF_3 substituted compounds (**3** and **4**; Table II).

Table II. The *in vitro* capability of the compounds **1–4** and the reference drugs atenolol (ATN) and carvedilol (CRV) to scavenge (reduce) the radical cation ABTS$^{\bullet+}$ (*%ABTS*$^{\bullet+}$) and reduce the $[Fe(III)(TPTZ)_2]^{3+}$ complex (the ascorbic acid equivalent; *AAE*)

Entry	*%ABTS*$^{\bullet+}$ (%)		*AAE* (mg/mL)	
	5min	1h	5min	1h
1	16.82 ±0.62	35.22 ±1.03	1.28 ±0.02	2.62 ±0.05
2	6.67 ±0.10	21.98 ±0.44	1.50 ±0.02	3.02 ±0.07
3	14.49 ±0.21	32.90 ±0.72	1.26 ±0.06	2.32 ±0.03
4	8.78 ±0.25	19.46 ±0.33	1.51 ±0.04	2.26 ±0.04
ATN	9.89 ±0.12	32.66 ±1.05	1.82 ±0.12	3.88 ±0.05
CRV	18.36 ±0.72	45.84 ±1.35	2.03 ±0.14	4.08 ±0.09

The most promising potential to scavenge the radical cation ABTS$^{\bullet+}$ after 5min was found for both 1-[3-(2-methoxyphenylcarbamoyl)oxy-2-hydroxypropyl]-4-(4-fluorophenyl)piperazin-1-ium chloride (**1**) with the *%ABTS*$^{\bullet+}$=16.82±0.62% and 1-{2-hydroxy-3--(2-methoxyphenylcarbamoyl)oxy)propyl}-4-(3-trifluoromethylphenyl)piperazin-1-ium chloride (**3**) with the *%ABTS*$^{\bullet+}$=14.49±0.21%. Their efficiency was comparable to the antioxidant action of the carvedilol (CRV) reference drug, which has shown the *%ABTS*$^{\bullet+}$=18.36±0.72%. Both derivatives **1** and **3** were considered more prospective than the atenolol (ATN) standard drug with *%ABTS*$^{\bullet+}$=9.89±0.12% (Table II).

The measurements after 1h led to higher *%ABTS*$^{\bullet+}$ data for all the investigated substances **1–4**, the ABTS$^{\bullet+}$ scavenging process was the most notable under the influence of a molecule **1** with the *%ABTS*$^{\bullet+}$=35.22±1.03% and **3**, which has shown the *%ABTS*$^{\bullet+}$=32.66±1.05%. Those derivatives were more potent than the ATN standard (Table II). On the other hand, the CRV reference substance was regarded as the most effective within an entire set of investigated compounds showing the *%ABTS*$^{\bullet+}$=45.84±1.35% (Table II).

Indeed, it was found practically no difference in the potential of the compounds **1** and **3** to scavenge (reduce) the radical cation ABTS$^{\bullet+}$ after 5min as well as 1h, regardless of very slight/strong electron-withdrawing properties or steric features of the R^2 substituent.

The 4´-F substitution on the aromatic system of the molecules **1** and **2** rendered the remaining aromatic hydrogen substituents slightly more acidic, so the capacity of that compounds to act as hydrogen bridge donors has been moderately enhanced. On the other hand, not only one free electron pair of that fluorine atom could act as the hydrogen bridge acceptor. The aromatic π electron system of the 4´-(substituted phenyl)piperazin-1´-yl fragment could serve as the electron donor site due to only a slight decrease in an electron density of a phenyl ring.

An insight into the chemical structure of the compounds **3** and **4** indicated that the ability of fluorines of the 3´-CF_3 group to act as hydrogen bridge acceptors has been enhanced due to a strong electron-withdrawing effect and, furthermore, the acidity of aromatic

hydrogens let them considered potential hydrogen bond donors.

Mistry *et al.* (2016, 2017) noticed that the antioxidant activity of the substituted *N*--arylpiperazine-based berberine compounds were considerably dependent on the substitution of the aromatic system. Different electron--withdrawing functional groups such as chloro, fluoro, nitro as well as the electron-donating one (methyl) were attached to the 4--(substituted phenyl)piperazin-1-yl moiety. The compounds containing 4-(3-chloro-/4-chloro-/2-fluoro- or 4-fluorophenyl)piperazin-1-yl were considered the most promising in terms of the ability to scavenge the $ABTS^{\bullet+}$. On the other hand, the molecules, in which structure was incorporated 4-(4-methyl-/2-nitro-/4-nitro- or 4-trifluoromethylphenyl)piperazin-1-yl, were less efficient.

It would be preliminary stated that the most prospective derivative **1** was the most basic with the pK_a=6.73. However, that output was one magnitude down compared to those of the CRV standard drug (the pK_a=7.97; Caron *et al.*, 1999).

Furthermore, the increase in lipophilicity of the inspected molecules due to the elongation of a 2-alkoxy side chain (the compound **1** *versus* **2** and **3** *versus* **4**, respectively) led to lower $\%ABTS^{\bullet+}$s (Table II). The most efficient substances **1** (log P_{exp}=3.61) and **3** (log P_{exp}=3.57) have shown the log P_{exp}s, which were very close to those of CRV (log P_{exp}=3.40; Yue *et al.*, 1992).

The FRAP assay was employed to inspect the Fe^{3+} ion reducing capabilities of the title compounds **1**–**4**. The measurements of the absorbance values after 5min revealed that the derivatives, which reduced the metal ion complexes to their lower oxidation state in that assay most markedly, were 1-[3-(2--ethoxyphenylcarbamoyl)oxy-2-hydroxypropyl]--4-(4-fluorophenyl)piperazin-1-ium chloride (**2**; AAE=1.50±0.02mg/mL) and 1-{2-hydroxy-3--(2-ethoxyphenylcarbamoyl)oxy)propyl}-4-(3--trifluoromethylphenyl)piperazin-1-ium chloride (**4**; AAE=1.51±0.04), as listed in Table II.

It could be stated that in the FRAP assay, an opposite trend of the results appeared when compared to the data, which were observed by the $ABTS^{\bullet+}$ method. It was found that the increase in lipophilicity of a whole molecule was the most essential for its ability to reduce the $[Fe(III)(TPTZ)_2]^{3+}$ complex.

In addition, the dependence between the pK_as and the AAE values would not be correct to clearly interpret due to a relatively limited set of inspected compounds.

As mentioned, both compounds **2** and **4** have shown a high lipophilicity, the log P_{exp}=3.90 was estimated for the substance **2**, the log P_{exp}=3.60 was related to the derivative **4** (Malík *et al.* 2005; Malík *et al.*, 2006). On the other hand, the ATN and CRV reference drugs were slightly more prospective with the AAE=1.82±0.12mg/mL (ATN) and 2.03±0.14mg/mL (CRV; Table II).

The potential of the compounds **1**–**4** to reduce the $[Fe(III)(TPTZ)_2]^{3+}$ complex after 1h within the process of the FRAP assay was slightly changed. It was confirmed that the most lipophilic substance **2** was the most effective, as the AAE=3.02±0.07mg/mL has shown. In general, the 4´-F substituted compounds (**1** and **2**) were more promising compared to those with a 3´-CF_3 group (**3** and **4**). From an entire investigated set of the substances, the CRV standard drug was the most efficient, that molecule has shown the AAE=4.08±0.09mg/mL (Table II).

Predominant features of a fluorine--containing substituents have been their strong electronegativity and their extremely low polarizability. Thus, depending on the interaction partner, steric interactions can be dominated either by the attractive complementary distribution of partial charges, e.g. hydrogen bonding to a fluorine or by the strong electrostatic repulsion of tightly bounded lone electron pairs in steric fluorine–fluorine interactions (Kirsch, 2004). In regard to the steric properties, a simplistic comparison between the „size" of hydrogen, fluoro and fluorocarbon substituents must be made with care.

Indeed, for a clarification of the steric impacts of the fluorine-containing substituents on the scavenging the radical cation $ABTS^{\bullet+}$ and reduction of the $[Fe(III)(TPTZ)_2]^{3+}$ complex, another *in vitro* experiments will be presented in further research papers regarding structurally very similar compounds. The only

structural difference between investigated sets of the molecules will be in the salt-forming moiety, which will be formed by the 4´-(2´-fluorophen-yl)piperazin-1´-yl fragment.

CONCLUSION

Part of beneficial cardiovascular effects shown by *β*-adrenoceptor antagonists has already been associated with the antioxidant properties that some of them seem to possess. The series of such perspective molecules, the 2--alkoxyphenylcarbamic acid esters, which contained 4´-(4´-fluoro-/3´-trifluoromethyl-phenyl)piperazin-1´-yl moiety, has been evaluated for its capability to *in vitro* scavenge the radical cation $ABTS^{\bullet+}$ and reduce the $[Fe(III)(TPTZ)_2]^{3+}$ complex.

The major novel findings of the current study have been that the ability of those screened compounds to scavenge the $ABTS^{\bullet+}$ was increased by the presence of a 4´-F substituent, which has probably shown convenient electronic, steric and lipohydrophilic properties. Moreover, the highest effectiveness of the tested molecules was related to a certain level of their lipophilicity, which could be expressed by the $\log P_{exp}$ of approximately 3.60. The further increase in lipophilicity led to the decrease in a potency.

On the other hand, the $[Fe(III)(TPTZ)_2]^{3+}$ complex reduction was most efficient by the most lipophilic substance ($\log P_{exp}$=3.90) within the investigated set of 2--alkoxyphenylcarbamic acid-based compounds. Given molecule contained sterically less bulky substituent attached to a 4´-(substituted phenyl) piperazin-1´-yl moiety with only a slight electron-withdrawing properties. Furthermore, the linearity of the salt-forming fragment was regarded as the favorable structural feature.

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