

In vitro Antioxidant Properties of Novel β_3 -Adrenoceptor Agonists Bearing Benzenesulfonamide Fragment

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ABSTRACT: As an extension of our research, the *in vitro* antioxidant efficiency of perspective agonists of β_3 -adrenoceptors structurally based on the aryloxypropanolamine pharmacophore, chemically 3-[4-[(alkoxycarbonyl)amino]phenoxy]-*N*-{2-[4-(aminosulfonyl)phenyl]ethyl}-2-hydroxypropan-1-ammonium chlorides, was investigated. The potential of evaluated compounds to reduce relatively stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals in the spectrophotometric tests was dependent on the length of the alkyl substituent of alkoxycarbonylamino moiety directly attached to phenyl ring. The elongation of given string was accompanied by the increase in the effectiveness. From the entire analyzed set, the compound containing butoxycarbonylamino group has been able to act most markedly as the reduction agent towards the DPPH radicals showing the percentage of the DPPH reduction (% DPPH) of 11.34±0.02. On the other hand, the tested derivative has shown approximately ninefold decrease in the efficiency compared to applied Trolox standard, a water-soluble analogue of vitamin E, with its %DPPH=95.45±0.02.

Key words: Antioxidant properties, β_3 -adrenoceptor agonists, lipophilicity

INTRODUCTION

The β_3 -adrenergic receptors (β_3 -ARs) belong to the G-protein coupled receptor family characterized by seven transmembrane domains of 22–28 amino acids, with three intracellular and three extracellular domains.¹ The β_3 -ARs have been found to mediate various pharmacological effects. For an illustration, it has been previously reported that first highly selective orally active and brain-penetrant β_3 -AR agonist, SR 58611A (amibegron; Scheme 1) has demonstrated antidepressant and anxiolytic properties in rodents.² Additionally, research by Taburella *et al.*³ has supported the idea that β_3 -ARs might be a therapeutic target of the stress-related disorders. The

β_3 -AR agonists have attracted much attention as a potential tools toward the treatment of obesity by increased mobilization of fat from white adipose tissue (lipolysis), by increased fat oxidation and by brown adipocyte tissue-mediated thermogenesis.⁴ The molecules with β_3 -AR agonistic activity might provide potential anti-diabetic agents with a perspective for the treatment of non-insulin dependent *diabetes mellitus* or type-II *diabetes*.⁵ Concerned subtype of the receptors could be suggested as a therapeutic target in gut inflammatory diseases.⁶ At present, solabegron might represent a novel therapeutic tool for the treatment of irritable bowel syndrome.^{1,6} Additionally, some of the selective β_3 -AR agonists (BRL37344, ZD7114, CGP12177A, GW427353-solabegron or YM-178-mirabegron) have been considered as potent relaxing agents of the human detrusor muscle *in vitro*.⁷⁻⁹

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Additionally, taken from recent literature,¹⁰ solabegron and mirabegron (Scheme 1) are currently in Phase I and Phase III clinical trials, respectively, for the treatment of overactive bladder. From the viewpoint of the influence on cardiovascular system in animal models, the selective β_3 -AR stimulation has led to antiarrhythmic effects and the β_3 -ARs might be regarded as a novel molecular target for antiarrhythmic therapy¹¹ or they have served a chiefly protective role in maladaptive remodeling and in the development of heart failure.^{12,13} Additionally, free radical overproduction has been observed leading to vascular complications.¹⁴ The results from the study of Rozec *et al.*¹⁵ have pointed out that nebivolol has induced relaxation of rat aorta by stimulation of endothelial β_3 -ARs which then activates the NO pathway.

Wishing to identify whether newly synthesized original compounds, perspective β_3 -AR agonists, could be effective antioxidants, as therapeutic agents, the molecules used in the present study, labelled as BL-14S2–BL-44S2 (Table 1), have been screened *in vitro* for their capability to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

MATERIALS AND METHODS

Chemicals and reagents. Currently evaluated compounds labelled as BL-14S2–BL-44S2 (Table 1), chemically 3-{4-[(alkoxycarbonyl)amino]phenoxy}-*N*-{2-[4-(aminosulfonyl)phenoxy]ethyl}-2-hydroxypropan-1-ammonium chlorides (alkoxy=methoxy to butoxy), were purchased from Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic as well as from Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. They were prepared and tested *in vitro* as the racemates, not as pure enantiomers. Synthesis and spectral data of all currently investigated molecules have been submitted for publication.

The applied standard of analytical grade, Trolox, chemically (\pm)-6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid (Fluka Chemie, Switzerland) was available commercially.

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

The free radical scavenging ability of tested compounds was determined with the DPPH assay by following the procedure described in the paper of Masteiková *et al.*¹⁶ The solution (200 μ l) of respective compound dissolved in methanol ($c=0.1$ mg/ml) was made up to 2.0 ml with methanolic solution of DPPH ($c=0.1$ mmol/l). After 5 min, the absorbance value was measured at the wavelength of 517 nm using the UV/VIS spectrophotometer HP 8453 (Hewlett Packard, USA). The decrease in the absorbance of DPPH solution has indicated an increase in DPPH radical reducing effect. The reduction of the DPPH radicals was calculated relative to the measured absorbance of the control as means \pm standard deviation of three parallel measurements (Table 1) according to the equation given below:

$$\%DPPH = \left(1 - \frac{A_{sample}}{A_{control}} \right) \times 100$$

where: %DPPH– the percentage of the DPPH reduction; A_{sample} – the absorbance at 517 nm; $A_{control}$ –the absorbance at 517 nm in control measurement.

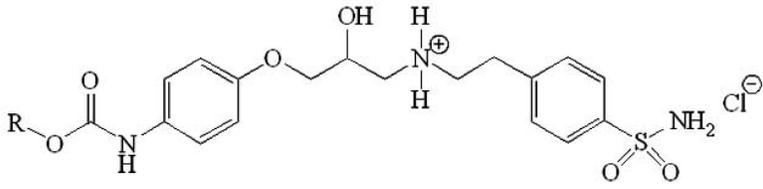
RESULTS AND DISCUSSION

The design of potent, selective human β_3 -AR agonists has been traditionally focused on the compounds in which aryloxypropanolamine or aryloethanolamine pharmacophore has been attached to substituted (hetero)aryl fragment through ethane-1,2-diyl spacer.^{17,18} Additionally, in terms of identification of the essential features for β_3 -AR agonistic activity has been previously observed, that the free secondary amino and hydroxyl groups within such molecules have been regarded as essential structural requirements.¹⁹ An insight into chemical structure of currently investigated aryloxypropanolamine-based compounds BL-14S2–BL-44S2 (Table 1) has revealed that they have met the criteria mentioned above. Furthermore, the substances used in the study contain sulfonamide group which has been present, among others, in the structure of (i) aryloxypropanolamine-based highly selective

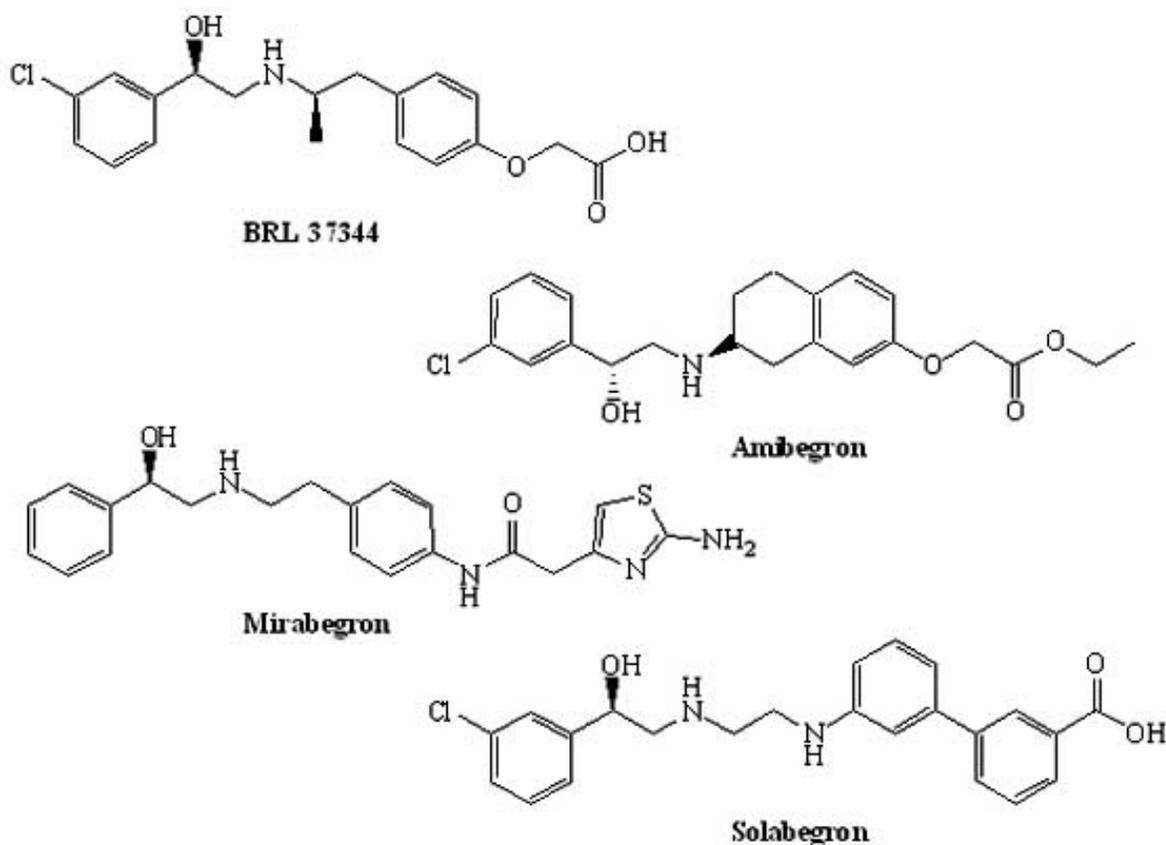
antagonists (L-748,328 and L-748,337; Scheme 2) of β_3 -ARs;²⁰ (ii) L-755,507, a highly potent subnanomolar human β_3 -ARs agonist which has also showed excellent selectivity;²¹ (iii) pyridyloxypropranolamine L-749,372, a selective partial agonist of human β_3 -ARs.²²

The presence of two aromatic rings integrated within the molecules of BL-14S2–BL-44S2 has assumed their relatively high lipophilicity. Furthermore, the elongation of alkoxy-carbonylamino fragment attached to the aromate has also enhanced its lipophilicity.

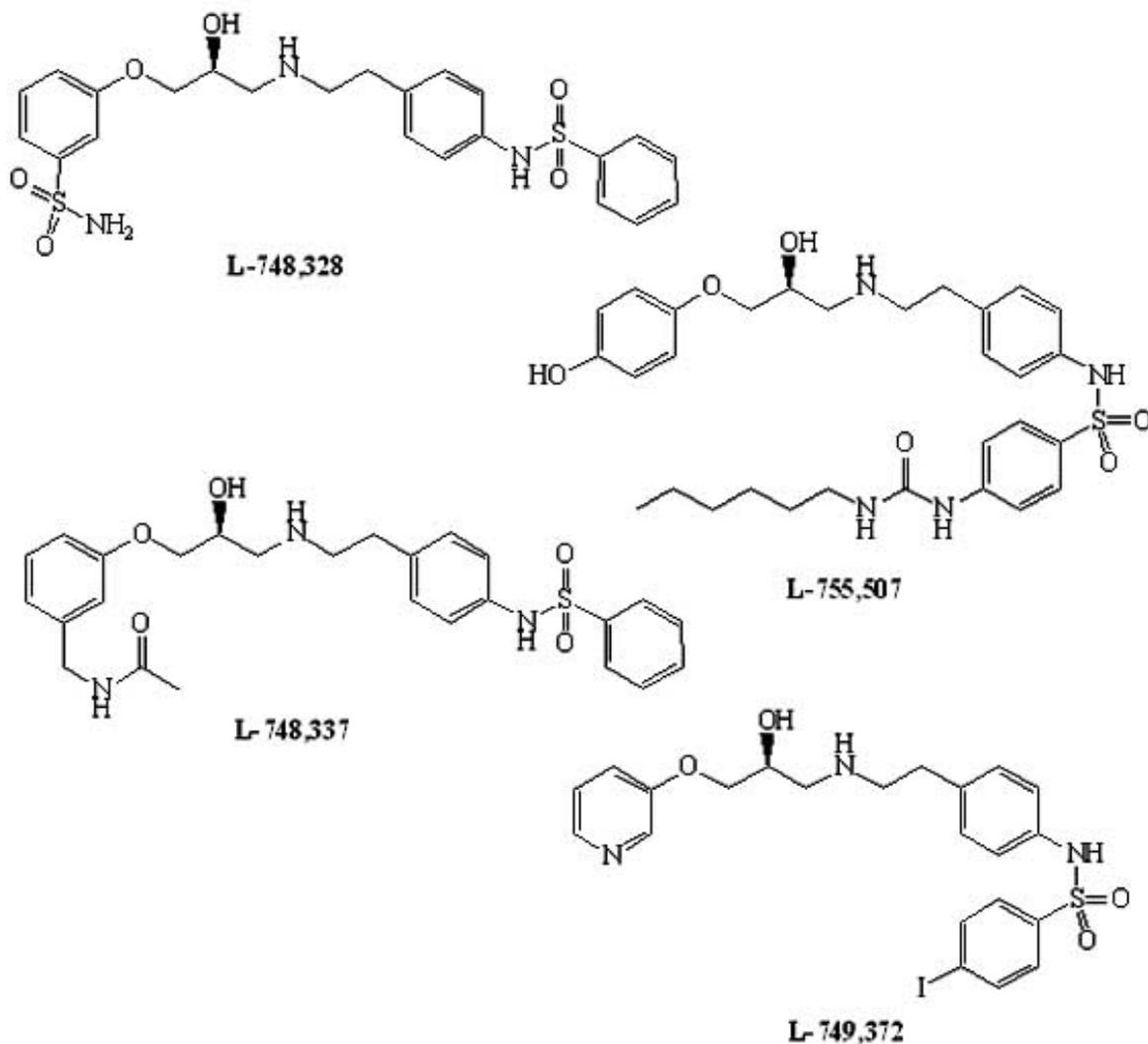
Table 1. The *in vitro* potency of evaluated compounds BL-14S2–BL-44S2 to reduce the DPPH radicals.



Entry	R	Formula	M_r	%DPPH
BL-14S2	CH ₃	C ₁₉ H ₂₆ ClN ₃ O ₆ S	459.94	1.27 ± 0.01
BL-24S2	C ₂ H ₅	C ₂₀ H ₂₈ ClN ₃ O ₆ S	473.97	2.22 ± 0.02
BL-34S2	C ₃ H ₇	C ₂₁ H ₃₀ ClN ₃ O ₆ S	488.00	4.61 ± 0.03
BL-44S2	C ₄ H ₉	C ₂₂ H ₃₂ ClN ₃ O ₆ S	502.02	11.34 ± 0.02



Scheme 1. Chemical structure of selected β_3 -adrenoceptor agonists containing aryethanolamine pharmacophore.



Scheme 2. Chemical structure of the compounds with high affinity for human β_3 -ARs which contain aryloxypropanolamine pharmacophore as well as sulfonamide (reverse sulfonamide) group.

As can be seen from Table 1, the alkyl side chain elongation has implied more pronounced ability of the tested compounds to reduce the DPPH radicals. The introduction of butoxycarbonylamino side string has led to the highest value of estimated %DPPH (11.34±0.02) which has been assigned to the molecule BL-44S2. Additionally, free DPPH radical scavenging (reducing) capability of inspected compounds was compared to Trolox, a water-soluble derivative of vitamin E.²³ As experimental results have indicated (Table 1), the most potent derivative BL-44S2 has shown approximately ninefold decrease

in the efficiency than applied standard exhibiting %DPPH=95.45±0.02.

Because of the lack of published studies dealing *in vitro* antioxidant effectiveness of structurally similar β_3 -AR agonists, there are still some ambiguous conclusions, and thus, there is still scope for further analysis. Based on the aforementioned statement, it could be taken into the consideration that the transfer of alkoxy carbonylamino group to *ortho*- or *meta*-position at phenyl ring might lead to more promising compounds in terms of their ability to reduce the DPPH radicals. Similarly, for

a systematic evaluation of structural effects in the series of such compounds, the substituent attached to aromatic moiety in basic part (i.e. free sulfonamide moiety) could be subjected to alternations. For the clarification, the SO_2 group in the benzenesulfonamide fragment is a strong hydrogen bond acceptor with the ability to show electron-withdrawing effect. On the contrary, the presence of the groups exhibiting primarily positive mesomeric effect, i.e. electron-donating impact (hydroxyl, methyl, methoxy) could appear to be favourable for antioxidant properties. Next, the influence of spacer length, i.e. extension or diminution by one methylene unit, in between the propanolamine and (substituted) aromatic unit could be also investigated. These alternations will be the subject of future investigations.

In conclusion, this study has brought to light novel original β_3 -AR agonists, containing aryloxypropanolamine moiety attached to substituted phenyl fragment through ethane-1,2-diyl chain, which have possessed relatively moderate ability to reduce the DPPH radicals. Despite limited set of evaluated compounds, such effectiveness has appeared to be dependent on the length of alkoxy-carbonylamino side string directly bonded to lipophilic aromatic ring. Among the investigated derivatives, the highest DPPH reducing potency has been related to the one containing butoxy-carbonylamino moiety. In general, such potential β_3 -AR agonists could open the field of a new therapeutic strategy reflecting their antioxidant properties as the contribution to their known clinical benefits.

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