Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 2 pp. 185–189, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG BIOCHEMISTRY

ANTIOXIDANT ACTIVITY OF β-CARBOLINE DERIVATIVES

RENATA FRANCIK[1]*, GRZEGORZ KAZEK[2], MAREK CEGŁA[1] and MAREK STĘPNIEWSKI[2]

[1]Department of Bioorganic Chemistry, [2]Department of Radioligands; Jagiellonian University, Collegium Medicum, 9 Medyczna St., Kraków, Poland

Abstract: The investigated β-carboline derivatives were synthesized to elucidate their activity as 5-HT_{1A} and 5-HT_{2A} receptor ligands. Compounds containing a carboline ring system belong to a large family of biological active indoles, which are very important for the function of the central nervous system (1). The research was carried out to determine antioxidative or oxidative properties of these derivatives. Analysis of antioxidative capacity as indication of oxidative stress was based on ability to scavenge free radicals by DPPH (free radical scavenging activity test*)* and FRAP test (2, 3). The results were compared to those of standard substances like vitamin C, trolox, quercetin and curcumin. The research of derivatives of β-carboline shows antioxidative activity comparable to vitamin C. Compounds **1**, **5** and **6**, but only in low concentration, have antioxidative activity. Substance **10** was classified as that with prooxidative activity.

Keywords: β-carboline derivatives, oxidative stress, DPPH test, FRAP test

Formation of free radicals and reactive oxygen species (ROS) is an integral part of human metabolism. The balance between the rate of ROS formation and activity of antioxidant systems is one of the conditions of homeostasis. Complete antioxidant ability is a result of the activity of low-molecule antioxidants (such as α-tocopherol, L-ascorbic acid, coumarin, quercetin) and proteins (ceruloplasmin, ferritin, albumin, transferrin). The increase of the rate of ROS formation and/or inhibition of the antioxidant mechanisms lead to oxidative stress, which is an important element of the pathogenesis of many diseases, including neurodegenerative and mental diseases. The conducted research concerns chosen individual low-molecule components, the activity or concentration of which may correlate with the course of free radical reactions (4, 5).

β-Carboline derivatives (Tab. 1 and 2) are potential ligands of 5-HT_{1A} and 5-HT_{2A} receptors. Pro- or antioxidant properties of this group of compounds may influence the antioxidant balance. It is therefore necessary to determine if the β-carboline derivatives influence the number of free radicals or ROS. The study evaluating the complete antioxidant activity of β-carboline derivatives may provide information on antioxidant effect of the non-enzymatic systems, that has the ability to counteract the ROS malfunction (formation).

MATERIALS AND METHODS

β-Carboline derivatives were synthesized in the Department of Organic Chemistry, Jagiellonian University College of Medicine, Kraków, Poland. Their structures were confirmed by ^{1}H NMR and MS spectra. Synthesis and properties of β-carboline derivatives will be the subject of separate publication. Among the investigated compounds, a group of optically active derivatives, which were coded as **3**–**6**, was present. Antioxidant properties of β-carboline derivatives were determined spectrophotometrically in two tests.

The first was DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate) test (2). Radical scavenging DPPH has a violet coloring, the intensity of which decreases in the presence of antioxidants proportionally to the ability to "sweep off" free radicals by the tested compound.

The second, the FRAP (ferric reducing ability of power) test, which uses the modification of Benzie and Strein's method, was also applied to measure the ability of β-carboline derivatives to reduce Fe^{3+} ions (3, 6). At low pH, Fe^{3+} ions undergo reduction to Fe^{2+} ions, which in the presence of 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) causes the formation of Fe^{2+}-TPTZ complex. The FRAP method, primarily used for blood plasma, was mod-

* Corresponding author: e-mail: refr@esculap.pl

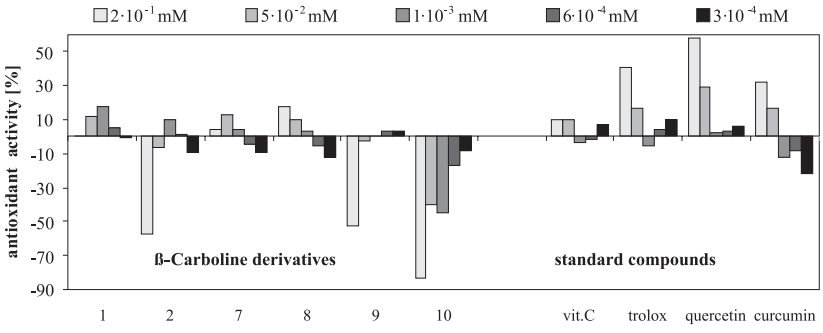


Figure 1. β-Carboline derivatives in comparison with the standard substances in the DPPH test [%]

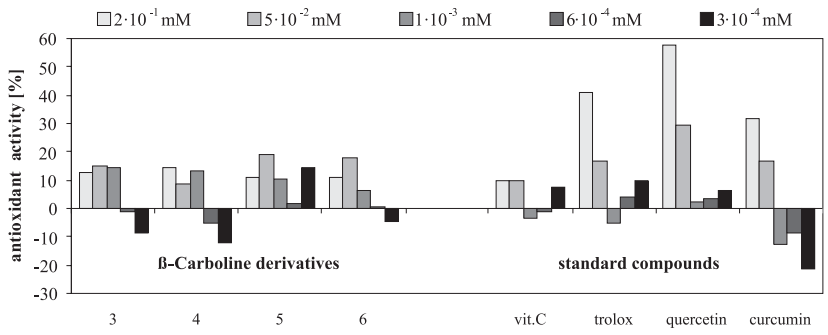


Figure 2. β-Carboline derivatives – isomeric compounds in comparison with the standard substances in the DPPH test [%]

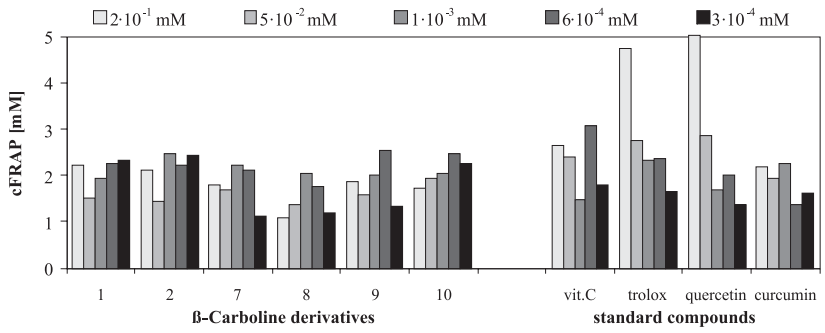
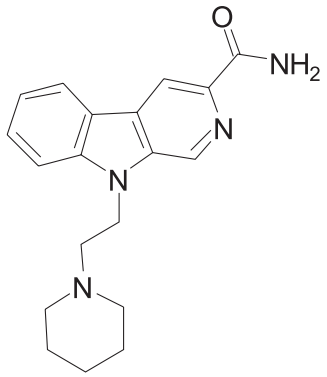
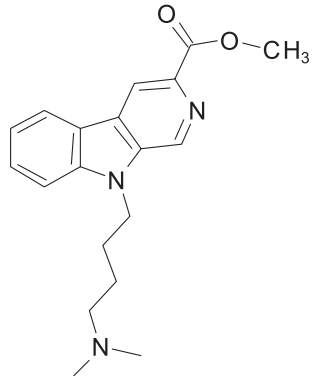
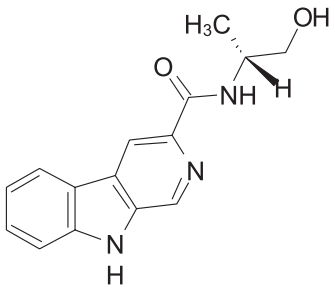
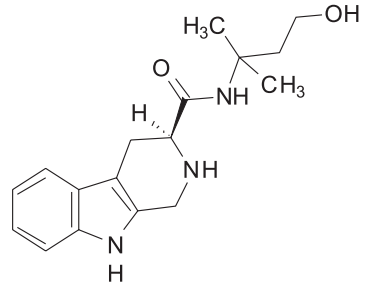
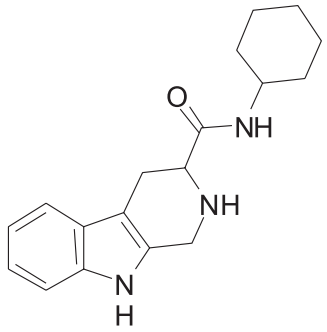
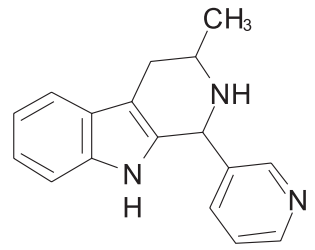


Figure 3. FRAP [mM] concentration values (mM) for the standard and tested substances β-carboline derivatives

Table 1. β-Carboline derivatives – tested compounds.

9-(2-(Piperidin-1-yl)ethyl)-9*H*-β-carboline-3-carboxamide (**1**)	Methyl 9-[4-(dimethylamino)butyl]-9*H*-β-carboline-3-carboxylate (**2**)
	
(*2'S*)-*N*-(1-hydroxy-2-methylpropan-2-yl)-9*H*-β-carboline-3-carboxamide (**7**)	(*3S*)-*N*-(4-hydroxy-2-methylbutan-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxamide (**8**)
	
(*R,S*)-*N*-cyclohexyl-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxamide (**9**)	(*R,S*)-3-methyl-1-(pyridin-3-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (**10**)
	

ified in order to establish the influence of the β-carboline derivatives on the oxidative stress of DMSO samples of antioxidant and natural compounds.

In the study, as comparative substances were used: L-ascorbic acid (vitamin C), trolox (analogous with vitamin E), quercetin and curcumin (polyphenols). For the tested and standard compounds, solutions in DMSO of the following concentrations were prepared: 3×10^{-4}, 6×10^{-4}, 1×10^{-3}, 5×10^{-2} and 2×10^{-1} mM. The ability of β-carboline derivatives to "sweep off" free radicals was expressed as a percentage of activity (test DPPH, Fig. 1 and Fig. 2). In case of the FRAP method, the Fe^{2+} content in the tested samples of β-carboline derivatives was calculated (test FRAP, Fig. 3 and Fig 4).

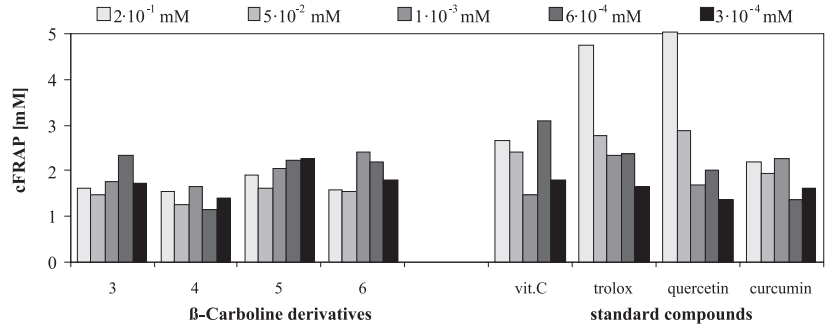
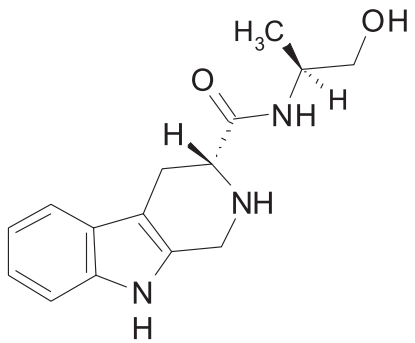
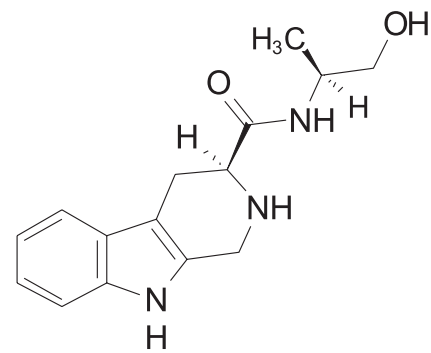
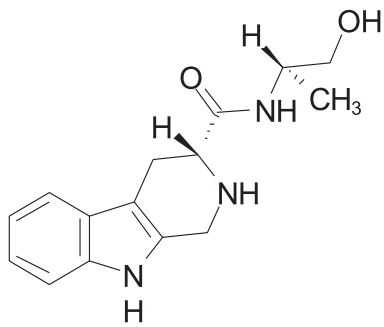
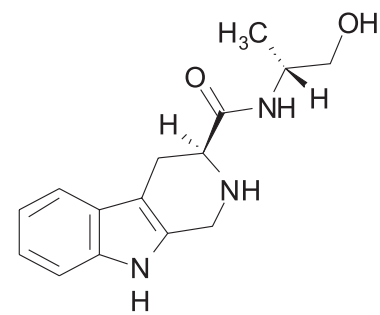


Figure 4. FRAP [mM] concentration values (mM) for the standard and stereoisomeric β-carboline derivatives

Table 2. Stereoisomeric β-carboline derivatives.

(*2',3R*)-*N*-(1-hydroxypropan-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxamide (**3**)	(*2'S,3S*)-*N*-(1-hydroxypropan-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxamide (**4**)
	
(*2'R,3R*)-*N*-(1-hydroxypropan-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxamide (**5**)	(*2'R,3S*)-*N*-(1-hydroxypropan-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxamide (**6**)
	

RESULTS AND CONCLUSIONS

It was found that β-carboline derivatives, excluding compounds **9** and **10**, in low concentrations showed antioxidant properties comparable with vitamin C, which had the weakest antioxidant properties (Fig.1 and 2). Compound **1** at $1 \cdot 10^{-3}$ mM concentration showed the activity similar to trolox at $5 \cdot 10^{-2}$ mM concentration. Compound **2** at $6 \cdot 10^{-4}$ mM concentration showed 86% of the vitamin C activity. Compound **9** showed similar activity. At the maximum concentration, the tested compounds were strongly pro-oxidant (Fig. 1). In the DPPH method, compound **7** showed the weakest antioxidant properties among the tested compounds – only 26% of the trolox activity.

Compound **8** showed the antioxidant activity at high concentration, which decreases with diluting, losing it in the most diluted solutions. The most intensive activity was observed at $2 \cdot 10^{-1}$ mM concentration, which is an equivalent of 50% of the curcumin activity.

Compound **10** showed pro-oxidant properties at each concentration tested. Based on the DPPH test results, that substance was identified as a compound with a potential pro-oxidative activity.

Isomeric compounds: **3**, **4**, **5** and **6** (Fig. 2) showed a similar antioxidant activity at the $2 \cdot 10^{-1}$ mM concentration. It is comparable with the activity of vitamin C at the same concentration. The activity of the isomeric derivatives at the concentration $5 \cdot 10^{-2}$ mM was stronger than that of vitamin C by 40% on average, except for the compound **4,** which showed the activity comparable with vitamin C. At the lowest concentration, only compound **5** showed antioxidant properties while compounds **3**, **4** and **6** at low concentrations showed weaker prooxidant properties, lower than curcumin at the concentration $3 \cdot 10^{-4}$ mM (Fig. 2).

Compounds: **1**, **5**, **6** and **8** (β-carboline derivatives) showed the strongest antioxidant properties. Compound **10** (3-methyl-1-(pyridin-3-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline hydrochloride) was found to have prooxidant properties.

FRAP method has been used in antioxidant properties measurements. In acidic environment, Fe^{3+} present in FRAP is reduced to Fe^{2+}, possessing intensive blue color, with maximum absorbance at 593 nm. This reaction undergoes with any substance, which exhibits reductive properties. It was observed that addition of DMSO solution to FRAP solution caused a change of color. Probably DMSO solution and present in FRAP solution Fe^{2+} ions created redox system, causing development of ions, which created colored complexes with TPTZ. It may be assumed that the amount of Fe^{2+} ions raised during measurement is influenced not only by β-carboline derivatives but also by DMSO, so in such cases it's not possible to determine antioxidant properties unambiguously.

β-Carboline derivatives reduce the amount of cAMP, thus affecting, among others, reduced permeability of cell membranes and reduced (decelerated) cell oxidation and ATP synthesis. Human cells have an antioxidant pool sufficient to counteract the normal physiological production of ROS and other free radicals; however, the naturally present antioxidant pool is not capable of counteracting an increase in generation of ROS; in these cases, the so-called "oxidative stress" occurs. From the above it can be seen that the insurgence of "oxidative stress" can be caused by two phenomena: the first is the lack of antioxidant molecules, and the second is the uncontrolled increase of ROS and free radicals, which are able to cause irreversible oxidation. Oxidative stress is present to a varied extent in a number of serious diseases in man. While this does not mean that it is the cause of these diseases, it does testify, as confirmed by a number of studies, that oxidative stress can have a negative influence on the progress of some diseases, causing further damage to the cells of an organism that is already sick. The most popular model of a such situation is an "oxidative burst" occurring after revescularization of an infarcted coronary heart.

In many pathological conditions, the development of which is connected with intensified ROS formation and activity, a decrease of the Q_{10} coenzyme concentration in the human body occurs. In these cases, treatment involves pharmaceutical supplementation. or increased consumption of coenzyme Q_{10} with meals as well as treatment with suitable chemical compounds, which significantly increase ubiquinone biosynthesis in the organism. β-Carboline derivatives as potential therapeutic compounds may, perhaps, influence the synthesis of ubiquinone and, in consequence, the generation or inhibition of the oxidative stress.

REFERENCES

1. Saxton J.E.: The Chemistry of Heterocyclic Compounds. Wiley-Interscience, New York 1994.
2. Schlesier K., Harwat M., Bohm V., Bitsch. R.: Free Radic. Res. 177, 36 (2002).
3. Benzie I.F., Strain J.J.: Anal. Biochem. 70, 239 (1996).
4. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J.: Int. J. Biochem. Cell. Biol. 44, 39 (2007).
5. Son Y, Lee K, Kook S, Lee J, Kim J, Jeon Y, et al.: Eur. J. Pharmacol. 195, 502 (2004).
6. Ernster L., Forsmark P., Nordenbrand K.: BioFactors 3, 241 (1992).

Received: 30. 03. 2010