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Original article

Antioxydant activity of β-carboline derivatives in the LDL oxidation model

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ABSTRACT

A series of β-carboline compounds were synthesized, starting from compound GWC22, their antioxidant activity was determined by inhibition of lipid peroxidation. The oxidation of LDL was induced in the presence of $CuSO_4$ or 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH). The protective actions of these compounds against the cytotoxicity were evaluated with lactate dehydrogenase (LDH) activity in bovine aortic endothelial cells (BAECs) and cellular vitality by measuring mitochondrial activity in the presence of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Most of compounds showed an higher antioxidant activity than GWC22 derivative ($R = 1.6$ for 5 μM $CuSO_4$). The best antioxidant activities are phenolic and benzyloxy derivatives with ratio $R = 1.9$ to 2.8 for 1 μM $CuSO_4$. These substances have protective actions and increase significantly the cell viability.

1. Introduction

Various reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and oxygen-derived free radicals, are very important mediators of cell injury; they act on biological macromolecules [1–3]. Under normal conditions, cells and tissues are protected against ROS by an array of nonenzymatic and enzymatic defense systems, such as superoxide dismutase, catalase, and glutathione peroxidase or free radical scavengers [4]. Oxidative stress, however, occurring when antioxidant systems are inadequate and/or active oxygen species are overproduced, can damage the tissues and DNA, thus resulting in a progression of a number of human diseases. It has been proposed that ROS are, directly or indirectly, involved in a variety of pathology events such as myocardial and cerebral ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation, cancer-initiation, Alzheimer's and other neurodegenerative diseases as well as in the ageing process [5–9]. Hence, free radical scavengers are expected to be promising drugs for treatment of these diseases by scavenging free radicals and therefore removing oxidative stress. The role of antioxidants in human health and disease has been reviewed [10].

It is well known that lipoprotein oxidation is a key early stage in the development of atherosclerosis. Modification of Low-Density Lipoprotein (LDL) in the arterial wall, is crucial to the cellular uptake of LDL in the first stages of atherosclerotic plaque development [11].

Once initiated, oxidation of LDL is a free-radical-driven lipid peroxidation chain reaction. Lipid peroxidation is initiated by free radical attack on a double bond associated with a polyunsaturated fatty acid (PUFA). These results in the removal of a hydrogen atom from a methylene (CH_2) group, the rate of which determines the rate of initiation, a key step. Molecular rearrangement of the resulting unstable carbon radical results in a more stable configuration, a conjugated diene. The conjugated diene reacts very quickly with molecular oxygen, and the peroxyl radical thus formed is a crucial intermediate [12]. A PUFA peroxyl radical in LDL may abstract a hydrogen atom from an adjacent PUFA to form a hydroperoxide and another lipid radical, a reaction which results in chain propagation. Removal of hydrogen atoms by the peroxyl radical from other lipids, including cholesterol, eventually yields oxysterols. Lipid hydroperoxides fragment to shorter-chain aldehydes, including malondialdehyde and 4-hydroxynonenal. These reactive aldehydes in turn may bind to e-amino groups of apo B-100, giving the protein an increased net negative charge. The classical LDL receptor recognizes a specific domain of positive charges from lysine, arginine and histidine residues on apo B. Alteration of this domain results in failure of binding by the apo B/E receptor, and an

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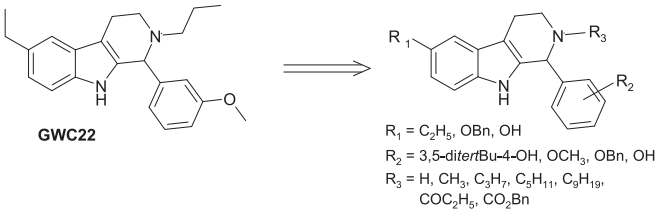


Fig. 1. Design of β-carboline derivatives starting from GWC22.

increase in negative surface charge on apo B-100 results in increased recognition by the scavenger receptor (mainly expressed in the macrophage). It's the first step of the atheroma formation.

A variety of compounds have been found to inhibit LDL oxidation, including vitamin E, β-carotene [13,14], the drugs probucol [15], indapamide [16], lovastatin [17], propranolol [18], pindolol [19], carvedilol [20], red wine polyphenols [21], and the hormone melatonin [22–27].

Investigations revealed that pineal indoles can have similar or more potent antioxydant effects than those observed with melatonin [28,29]. Indeed, pinoline (6-methoxy-1,2,3,4-tetrahydro-β-carboline) a minor cyclic metabolite of melatonin [30] has been shown to inhibit lipid peroxidation in different model systems [31,32]. In our laboratory, previous research reported that a series of 1-aryl-1,2,3,4-tetrahydro-β-carboline display antioxidant properties [33]. In this study, the substitution of the methoxy group at the 6 position of Pinoline, by an ethyl group reinforced the antioxidant activity of the series. In particular, compound GWC22 has been shown to display better antioxidant properties than melatonin towards peroxyl-induced peroxidation of a linoleate model system [34,35]. Compound GWC22, used as reference, allowed to described a series of benzazolone derivatives with antioxidant activity [36].

In this paper, we report the synthesis of novel antioxidants, which have been rationally designed to present high reactivity and efficacy against LDL oxidation. Starting from compound GWC22, we developed and synthesized a series with three modulations a) at the 6 position of the β-carboline with ethyl, hydroxy or benzoxy groups (R_1) b) various substituents (R_2) on the phenyl ring at the 1 position of the β-carboline c) at the nitrogen atom (R_3) of the β-carboline (Fig. 1). Most of the synthetised and tested compounds have an ethyl group (R_1) at the 6 position of the β-carboline, we have also introduced a benzyloxy and hydroxy groups to explore the antioxidant activities of these compounds. On the phenyl ring, with R_2 group, we have modulated the position and the number of the methoxy group and introduced other groups such as 3,5-di*tert*Bu-4–OH, benzoxy and hydroxy. It is well known that phenolic compounds exert their antioxidant activity by acting primarily as hydrogen atom donators, thereby inhibiting the propagation of radical chain reactions. The antioxidant potential of the phenolic derivatives depends on the number and the arrangement of hydroxyl groups, as well as the nature of the other substituents on the aromatic rings. For the third modulation with R_3 we have studied the length chain with methyl, propyl, pentyl, nonyl, which could influence both the lipophilic and steric parameters. We have also introduced propionyl and benzyl carbamate groups to study the importance of the basic nitrogen.

Therefore, we synthesized various novel compounds and performed a wide range of screening tests for the purpose of developing an antioxidant that inhibits lipid peroxidation.

2. Results and discussion

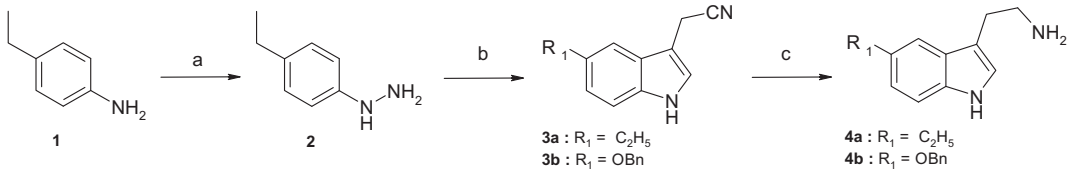
2.1. Chemistry

Final compounds **6a**–**s** were synthesized starting from key intermediate products (**4a**–**b**) [33,37] (Scheme 1). The first step was a diazotation reaction [38] in concentrated hydrochloric acid with 4-ethylaniline (**1**), as commercial material, and sodium nitrite, followed by a reduction of the diazonium intermediate salt with tin chloride to afford 4-ethylphenyl hydrazine **2**. Compound **2** was cyclised, by a fischer reaction [39], in a diluted aqueous solution of sulfuric acid (4%) with 4,4-diethyloxy butyronitrile to give (5-ethyl-*1H*-indol-3-yl)acetonitrile (**3a**). Reduction of compounds **3a** and **3b**, as commercial product, in ethyl ether with lithium aluminium hydride furnished the intermediate derivatives **4a**,**b**.

Compound **4a** or **4b** was engaged in a Pictet–Spengler reaction [40] in CH_2Cl_2, with TFA and the desired benzaldehyde to afford β-carboline derivatives **5a**–**j**,**m**. Debenzylation reaction of derivatives **5i**,**j** in methanol with Pd/C under hydrogen atmospheric pressure, afforded derivatives **5k**,**l**. Compounds **5a**–**j**,**m** were then alkylated in CH_3CN with the desired halogeno reagent and potassium carbonate to furnish alkyl products (Scheme 2). Other tested derivatives **6e**,**n**,**o** were transformed in amide or carbamate, starting from compounds **5a**,**i**,**j** in ethyl acetate with the corresponding chloride reagents. Protected benzyl derivatives **6l**,**m**,**r** were placed in methanol with Pd/C, under hydrogen atmospheric pressure to give the corresponding phenols **6p**,**q**,**s**. Compound **6s** was the 6-hydroxy analogue of GWC22 derivative.

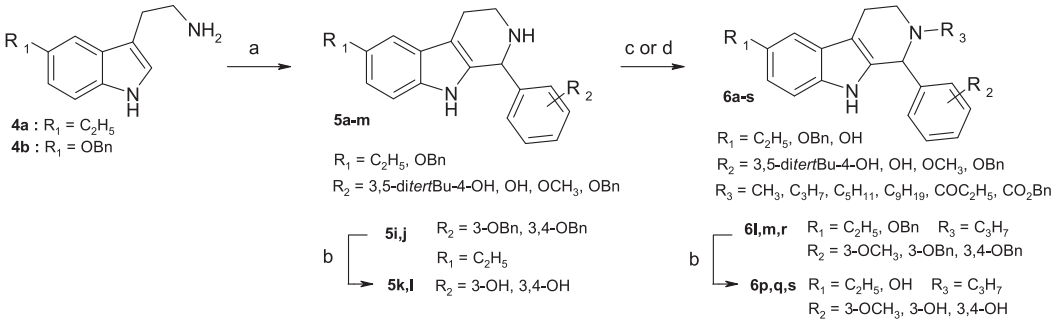
2.2. Antioxidant activity

Products can exert their antioxidant activity through various mechanisms, such as i) scavenging radicals which initiate lipid peroxidation and lipid peroxide radicals; ii) binding metal ions; iii) inhibiting enzymatic systems responsible for free radical generation. To eliminate the possible role of Cu-chelation, oxidation was induced by water- soluble 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), which generates free radical, by its spontaneous thermal decomposition. For some selected samples we have compared the capacity of compounds to inhibit LDL oxidation when Cu^{2+} or AAPH are used as pro-oxidants. The oxidation of LDL was followed for 480min by measuring conjugated diene formation at optical density (OD) 234 nm. LDL samples were subjected to oxidation immediately after isolation and each experiment consisted of 3 samples, which included 1 control**s** and 1 test.



Reagents and conditions: (a) : i: $NaNO_2$, HCl 6N ii: $SnCl_2$, HCl 6N (b): 4,4-diethyloxy butyronitrile, H_2SO_4 4% (c): $LiAlH_4$, ethyl ether.

Scheme 1. Preparation of the intermediate derivatives **4a**,**b**.



Reagents and conditions: (a) : substituted benzaldehyde, TFA, CH_2Cl_2 (b): H_2, Pd/C, MeOH (c): IR_3, K_2CO_3, CH_3CN (d): $ClCOC_2H_5$ or $ClCO_2Bn$, K_2CO_3, H_2O, CH_3CO_2Et.

Scheme 2. Preparation of β-carboline derivatives **5a**–**m** and **6a**–**s**.

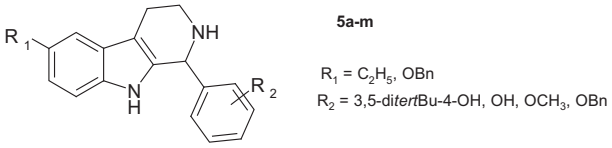
In the presence of $CuSO_4$ or AAPH, the OD_{234} curve vs. time corresponds to the typical one described by Esterbauer [41], including the lag, propagation, and decomposition phases. With our experimental conditions, the lag phase for the initiation of LDL oxidation in the presence of copper or AAPH was about 100 min ± 7min. Without Cu^{2+} or AAPH, the kinetics of conjugated diene formation was slower, and the final concentration was dramatically lower after 24 h of oxidation. However, when concentrations of derivatives from 0.1 to 10 μM were added to the incubations, there was an increase in lag time, suggesting an apparent antioxidant effect. The capacity of derivatives to reduce or inhibit copper or AAPH-initiated LDL oxidation was tested and the kinetics of LDL oxidation led us to calculate the **ED_{50}** or the ratio **R** (Tables 1 and 2).

There is no significant decrease in the maximal rate of propagation phase or in maximal amount of conjugated diene formation.

The most antioxidant products in a copper system were tested in AAPH oxidation system. In the presence of the tested molecules there was a strong correlation in the lag phase duration between these two oxidative procedures of LDL ($R = 0.9$ $p < 0.05$). Therefore, the free radical scavenging mechanism contributes towards the inhibitory action of these compounds.

Excepted for compounds **5h**, **5k and 5l**, the other derivatives **5a**–**m** showed less potent antioxidant activities than reference GWC22 ($R = 1.6$ for 5 μM $CuSO_4$). The di*tert*butylphenol group of compound **5h**, well described in the literature for its antioxidant activities [42], and the presence of hydroxy groups on the phenyl ring of compound **5k** and **5l** induced excellent antioxidant activities ($R = 2.7$ to 2.8 for 1 μM $CuSO_4$). The cyclisation of the catechol ring of compound **5l** to dihydrobenzodioxine derivative **5g** induced a downfall of the antioxidant activities to $R = 1.1$ for 5 μM $CuSO_4$ compared with GWC22. These results show the importance of phenolic groups for antioxidant activity. The variation of the position of the methoxy groups (**5a**–**c**) or the introduction of other methoxy groups (**5d**–**f**) was followed by a reduction of the antioxidant activity with $R = 1.1$ to 1.4 for 5 μM $CuSO_4$.

Table 1
Antioxidant activity and LDH release of derivatives **5a**–**m**.



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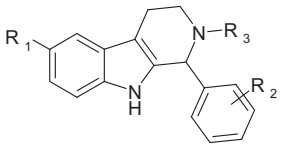
Cpd	R_1	R_2	ED_{50}(μM)	5 μM (Cu^{2+})	Activity (R) 1 μM (Cu^{2+})	1 μM (AAPH)	LDH release (%)
GWC22	–	–	**4**	**1.6**	**1.2**		**15.32 ± 1.56**
5a	C_2H_5	3–OCH_3	9	1.2	1.1	–	–
5b	C_2H_5	2–OCH_3	7	1.4	1.2	–	–
5c	C_2H_5	4–OCH_3	9	1.2	1	–	–
5d	C_2H_5	3,4–OCH_3	10	1.1	1	–	–
5e	C_2H_5	2,3,4–OCH_3	9	1.2	1	–	–
5f	C_2H_5	3,4,5–OCH_3	10	1.1	1	–	–
5g	C_2H_5	3,4- 	10	1.1	1	–	–
5h	C_2H_5	3,5-^{t}Bu-4–OH	<0.1	>5	2.8	2.7	13.26 ± 1.32
5i	C_2H_5	3-OBn	7	1.4	1	1	13.29 ± 1.35
5j	C_2H_5	3,4-OBn	7	1.4	1	1	–
5k	C_2H_5	3–OH	<0.1	>5	2.7	2.6	13.31 ± 1.29
5l	C_2H_5	3,4–OH	<0.1	>5	2.8	2.6	13.30 ± 1.30
5m	OBn	3–OCH_3	5	1.5	1.1	–	–

ED_{50}: Concentration in μM of compounds that increased the control lag phase by 1.5 times. The control lag phase is obtained in an oxidative procedure without tested compounds.
R: Ratio between the length of the lag phase of the oxidative procedure of LDL with different compounds and the length of the one without compound (control).
LDH activity was expressed as the percentage of total LDH cellular release following the addition of Triton X-100 (final concentration of 0.1% v/v in ethanol). Cells were pretreated for 24 h with 10 μM tested compounds, then for 16h with the same concentration of compounds and 100 μg/mL of air oxidized-LDL. The LDH release in presence of air oxidized-LDL alone is: 22.76 ± 1.53%.

Table 2
Antioxidant activity and LDH release of derivatives **6a**–**s**.



6a-s

R_1 = C_2H_5, OBn, OH
R_2 = 3,5-di*tert*Bu-4-OH, OH, OCH_3, OBn
R_3 = CH_3, C_3H_7, C_5H_{11}, C_9H_{19}, COC_2H_5, CO_2Bn

.

Cpd	R_1	R_2	R_3	ED_{50} (μM)	5 μM (Cu^{2+})	Activity (R) 1 μM (Cu^{2+})	1 μM (AAPH)	LDH release (%)
GWC22 (**6a**)	**C_2H_5**	**3-OCH_3**	**C_3H_7**	**4**	**1.6**	**1.2**	–	**15.32 ± 1.56**
6b	C_2H_5	3–OCH_3	CH_3	1	1.9	1	–	–
6c	C_2H_5	3–OCH_3	C_5H_{11}	9	1.2	1	–	–
6d	C_2H_5	3–OCH_3	C_9H_{19}	9	1.2	1	–	–
6e	C_2H_5	3–OCH_3	COC_2H_5	9	1.2	1	–	–
6f	C_2H_5	2–OCH_3	C_3H_7	4	1.6	1	–	–
6g	C_2H_5	4–OCH_3	C_3H_7	3	1.7	1	–	–
6h	C_2H_5	3,4–OCH_3	C_3H_7	2	1.8	1.1	–	–
6i	C_2H_5	3,4,5–OCH_3	C_3H_7	2	1.8	1.2	–	–
6j	C_2H_5	3,4- 	C_3H_7	9	1.2	1	–	–
6k	C_2H_5	3,5-^{t}Bu-4–OH	C_3H_7	<0.1	>5	2.7	2.6	13.21 ± 1.22
6l	C_2H_5	3-OBn	C_3H_7	0.1	4.2	2.2	2.3	13.42 ± 1.29
6m	C_2H_5	3,4-OBn	C_3H_7	3	1.8	1.3	–	–
6n	C_2H_5	3-OBn	CO_2Bn	10	1	1	–	–
6o	C_2H_5	3,4-OBn	CO_2Bn	8	1.3	1.1	–	–
6p	C_2H_5	3–OH	C_3H_7	<0.1	>5	2.8	2.7	13.20 ± 1.25
6q	C_2H_5	3,4–OH	C_3H_7	<0.1	>5	2.7	2.7	13.19 ± 1.52
6r	OBn	3–OCH_3	C_3H_7	0.1	4.4	2.4	2.4	13.22 ± 1.26
6s	OH	3–OCH_3	C_3H_7	0.2	3.2	1.9	2.1	14.02 ± 1.56

ED_{50}: Concentration in μM of compounds that increased the control lag phase by 1.5 times. The control lag phase is obtained in an oxidative procedure without tested compounds.
R: Ratio between the length of the lag phase of the oxidative procedure of LDL with different compounds and the length of the one without compound (control).
LDH activity was expressed as the percentage of total LDH cellular release following the addition of Triton X-100 (final concentration of 0.1% v/v in ethanol). Cells were pretreated for 24 h with 10 μM tested compounds, then for 16h with the same concentration of compounds and 100 μg/mL of air oxidized-LDL. The LDH release in presence of air oxidized-LDL alone is: 22.76 ± 1.53%.

Starting from compound GWC22 (**6a**), we have modulated the propyl chain by various length chain (R_2) with CH_3 (**6b**), which induced higher antioxidant activitycompared with (**6a**). If R_2 is C_5H_{11} (**6c**) or C_9H_{19} (**6d**) we observed a diminution of the antioxidant activity ($R = 1.2$ for 5 μM $CuSO_4$). Identically, the substitution of the *N*-propyl group of derivative GWC22 by propionyl group of compound **6e** lead to a diminution of the antioxidant activity ($R = 1.2$ for 5 μM $CuSO_4$), which emphazized that the nitrogen atom at the position 2 of the β-carboline was important for the antioxidant activity.

In a second time, we have modulated the position and the number of methoxy groups (**6f**,**g**,**h**,**i**) to enhance the antioxidant activity, however we observed similar antioxidant activity ($R = 1.6$ to 1.8 for 5 μM $CuSO_4$) compared to compound GWC22 ($R = 1.6$ for 5 μM $CuSO_4$). The benzoxy group on the 3 position of the phenyl ring of compound **6l** gave excellent antioxidant activity with $R = 4.2$ for 5 μM and $R = 2.2$ for 1 μM, however the disubstitution with 3,4-benzoxy groups of derivative **6m** induced a reduction of the antioxidant activity with $R = 1.8$ for 5 μM $CuSO_4$. Some authors [43], showed that benzyl carbamate group enhances the antioxidant activity for described derivatives, so we have synthesized compounds **6n**,**o** which showed disappointing results with a lower antioxidant activity ($R = 1$ and 1.3 for 5 μM $CuSO_4$) compared to their propyl analogs **6l**,**m** ($R = 4.2$ and 1.8 for 5 μM $CuSO_4$).

The debenzylation reaction of compounds **6l**,**m** gave phenolic derivatives **6p**,**q** with higher antioxidant activity, the ratio R was superior to 5 for 5 μM and respectively 2.8 and 2.7 for 1 μM. However, the cyclisation of the phenolic groups of derivatives **6q** to compounds **6j** indiced a downfall of the antioxidant activity with $R = 1.2$ for 5 μM. These protections of phenolic compounds **6p**,**q** with derivatives **6l**,**m** indicated that benzyl group allowed to enhance the antioxidant activity compared to dihydrobenzodioxine derivative **6j**. The best antioxidant activities of the series were observed with phenolic compounds **6k**,**p**,**q** and **5h**,**k**,**l** with $R = 2.7$ to 2.8 for 1 μM $CuSO_4$.

Considering the importance of phenolic group, we replaced the ethyl group of derivative GWC22 by benzoxy and hydroxy group to afford the analogs **6r**,**s**. The N-propyl derivative **6r**, with a benzoxy group at the 6 position, gave the best antioxidant activity with $R = 2.4$ for 1 μM $CuSO_4$. The debenzylation reaction with compound **6r** lead to the phenolic derivative **6s**, which induced a diminution of the antioxidant activity with $R = 1.9$ for 1 μM $CuSO_4$, contrary to the other series. Substitution with hydroxy or benzoxy groups induced interesting antioxidant activities in the series with compounds **5h**, **5k**, **5l**, **6k**, **6l**, **6p**–**s**.

It has been proposed that ROS are, directly or indirectly, involved in a variety of pathology events such as myocardial and cerebral ischemia, arteriosclerosis, Alzheimer's and other neurodegenerative diseases etc… Hence, free radical scavengers are expected to be promising drugs for treatment of these diseases by scavenging free radicals and therefore removing oxidative stress. In a previous paper we discussed about the relationship between melatonin derivatives and atherosclerosis [36]. The biological properties look important in term of prevention of cardiovascular diseases but the capacity of molecules to inhibit atherogenic lipoprotein oxidation in vitro offers no prediction of their capacity to inhibit in vivo atherosclerosis development. In addition to its protective effects as free radical scavenger and antioxidant, abundant studies showed that melatonin or derivatives has multifaceted properties acting

against cerebral hypoxia/ischemia and its protection could be long-lasting over months. Indeed melatonin crosses the blood–brain barrier. Contrary to experiments made with mice to study regression of atherosclerotic plaque [44,45] administering melatonin significantly reduces infract volume, oedema and oxidative damage, in animal stroke models [46].

Hence, β-carboline derivatives show free radical scavengers properties. They are expected to be promising drugs for treatment of these diseases by scavenging free radicals and therefore removing oxidative stress.

2.3. Cytotoxicity assays

In vivo low density protein (LDL) oxidation is a progressive phenomenon leading to the presence of minimally and highly oxidized LDLs in the subendothelial arterial space. Oxidized LDLs have been reported to be cytotoxic against endothelial cells. Furman [47] showed the differential toxicities of air (minimally) or copper-oxidized-LDL toward endothelial cells. Both the morphological aspect of the cells themselves and LDH test revealed that air oxidized-LDLs had cytotoxicity with up to 8 h of oxidation.

The protective effect of derivatives against cytotoxics oxidized-LDL was studied in cultured bovine aortic endothelial cells (BAECs). This model of primary culture cells is commonly used in our laboratory.

When the medium is cytotoxic, the initial visual change consisted in cell contraction with the appearance of cytoplasmic ramifications, then some cells rounded up and detached from the bottom of the wells. The attached cells showed marked alterations, such as the disappearance of the characteristic double refraction of intact membranes and nuclear condensation. When nonoxidized LDL (100 μg protein/ml) is added in cellular medium, minor cellular shape changes are induced. After 16 h of incubation most cells remained attached to the bottom of culture wells. If 100 μg proteins/ml air oxidized-LDL are added in cellular medium for 16 h there are major morphological cellular changes:

In spite of oxygen is essential to life, it damages the cells and organelles, this phenomenon is named “the oxygen paradox”. We have pointed compounds that have both antioxidant activities but also cytoprotective effects.

Cytotoxicity was quantified using LDH release. LDH release increased when cells were treated with air oxidized-LDL in comparison with non oxidized- LDL (22.76 ± 1.53% versus 11.07 ± 2.8 respectively). In presence of non oxidized- LDL the β-carboline derivatives didn’t involve any significant effect on cellular LDH release. In presence of air oxidized-LDL, the addition of GWC 22 or other β-carboline derivatives significantly reduced LDH activity in the extra cellular medium. Only compounds with the best antioxidant activities were tested.

Our results show that the most antioxidant products are also the less cytotoxics. For exemple, for compounds GWC22 (R = 1.6 for 5 μM Cu^{++}), LDH release reaches 15.32 ± 1.56%. Compounds **6p** and **6p**, with hydroxy group, with the best antioxidant activities (R = 2.8 and 2.7 respectively for 1 μM Cu^{++}) shows a lower LDH release 13.20 ± 1.25% and 13.19 ± 1.52% respectively. Derivative **6r** (R = 2.4 for 1 μM Cu^{++}), with benzyloxy group, also shows good LDH release 13.22 ± 1.26%.

In parallel, the MTT assay was performed in order to test cellular vitality by measuring mitochondrial activity in the presence of MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After 16 h of incubation with air oxidized-LDL alone MTT reactivity is about 60%. In presence of anti oxidant β-carboline derivatives the activities reached nearly 85 ± 4% without significant differences between them Both of the tested derivatives increased ($p < 0.05$) the cell viability as indicated by MTT assay.

3. Conclusion

All synthetized compounds displayed antioxidant activities superior to those of the widly studied melatonin. Some of them (**5h**, **5k**, **5l**, **6k**, **6p**–**r**) presented very strong antioxidant activities compared to GWC22 derivatives. In this paper we showed the importance of phenolic groups. The benzyloxy group (**6l**,**r**) induced also a good antioxidant activity. For all compounds, the presence of the propyl group in the N-2 position of the β-carbolines is essential for antioxidant activities, particularly for compounds **5i** versus **6l**. The series, with benzyloxy and hydroxy groups at the 6 position of the β-carboline (**6r**,**s**), will be interesting to modulate on the phenyl ring.

The oxidative modification of LDL has been alleged to play an important role in the development of human pathologies Thus, protecting LDL from oxidation by these compounds can delay or prevent the progression of these diseases. LDL resistance to oxidation is one of the models used by many researchers to investigate the efficacy of new drugs as antioxidants against radicals generated in the lipophilic phase.

Our results show that most antioxidant products are also the less cytotoxic while they increase the vital capacity of the cell. They exert their antioxidant effect on lipid peroxidation primarily by scavenging free radicals.

However these very encouraging results do not prove a beneficial effect in Vivo. Specific experiments in animals and humans are essential.

4. Experimental

Compounds were purified on a glass column using Merck silica gel 60 (230–400 mesh). Melting points were determined by a Büchi 510 capillary apparatus and are uncorrected. ^{1}H and ^{13}C NMR proton spectra were recorded on a Bruker AVANCE 300 spectrometer and chemical shifts are in ppm with TMS as internal standard. Mass spectra were recorded on a quadripolar Finnigan Mat SSQ 710 instrument. All compounds were found to be >96% pure by HPLC analysis.

4.1. 2-(5-Benzoxy-1H-indol-3-yl) ethylamine (*4b*)

In diethyl ether (100 mL), (5-benzoxy-1*H*-indol-3-yl)acetonitrile (**3b**) (4 g, 15.3 mmol), as commercial product, and lithium aluminium hydride (2.9g, 76.5 mmol) were added. The reaction mixture was refluxed for 1 day and hydrolysed in cold water (50 mL). The solution was extracted with ethyl ether. The organic layer was dried with magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane/methanol (97/3, v/v) as eluent to afford compound **4b**, as an oily product. Yield 87%; ^{1}H NMR (300 MHz, $CDCl_3$), δ = 1.35 (br s, 2H, NH_2), 2.90 (t, 2H, CH_2, J = 6.4 Hz), 3.10 (t, 2H, CH_2, J = 6.4 Hz), 5.15 (s, 2H, OCH_2), 6.96 (dd, 1H, H_6, J = 8.8 Hz, J = 2.1 Hz), 7.02 (s, 1H, H_2), 7.15 (d, 1H, H_4, J = 2.1 Hz), 7.27 (d, 1H, H_7, J = 8.8 Hz), 7.40 (m, 3H, H_{Ar}), 7.40 (m, 3H, H_{Ar}), 7.52 (d, 2H, H_{Ar}, J = 7.3 Hz), 8.35 (br s, 1H, NH); ^{13}C NMR (300 MHz, $CDCl_3$), δ = 20.1, 26.4, 69.4, 109.5, 111.1, 113.6, 114.2, 116.7, 122.1, 125.4, 133.5, 133.9, 134.6, 135.1. SM (APCI+) m/z 267.5 $[M + H]^+$.

4.2. General procedure for the synthesis of β-carboline derivatives (*5a*–*j*,*m*)

In dichloromethane (50 mL), 2-(5-ethyl-1*H*-indol-3-yl) ethylamine (**4a**) or 2-(5-benzoxy-*1H*-indol-3-yl) ethylamine (**4b**) (2.5 g, 13.3 mmol), trifluoroacetic acid (1.35 mL, 20.0 mmol) and the desired benzaldehyde (20.0 mmol) were added. The reaction

mixture was stirred for 1 day at room temperature and then evaporated. Ethyle acetate (80 mL) and 10% K_2CO_3 aqueous solution (50 mL) were added. The organic layer was dried with magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane/methanol (94/6, v/v) as eluent to afford compounds (**5a**–**j**). Solid products were recrystallized with the appropriate solvent, while the oily products were solubilized in dry ethyl acetate and treated with gazeous hydrochloric acid in diethyl ether (20 mL). The obtained precipitate was filtered and washed with ethyl acetate and the residue was recrystallized with the appropriate solvent.

4.3. 6-Ethyl-1-(3-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (**5a**)

Yield 85% (ethanol 95°); mp 152–153 °C; ^{1}H NMR (300 MHz, DMSO-d_6), δ = 1.20 (t, 3H, CH_3, J = 7.6 Hz), 2.70 (q, 2H, CH_2, J = 7.6 Hz), 3.00 (m, 1H, CH_2), 3.10 (m, 1H, CH_2), 3.35 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 5.90 (s, 1H, CH), 6.92 (d, 1H, H_{Ar}, J = 7.6 Hz), 6.95 (dd, 1H, H_7, J = 8.2 Hz, J = 1.7 Hz), 7.08 (m, 2H, H_5,et H_{Ar}), 7.20 (d, 1H, H_8, J = 8.2 Hz), 7.33 (s, 1H, H_{Ar}), 7.40 (t, 1H, $H_{5'}$, J = 7.6 Hz, J = 7.2 Hz), 9.50 (br s, 1H, NH), 10.80 (br s, 1H, NH); ^{13}C NMR (300 MHz, $CDCl_3$), δ = 16.8, 22.7, 29.3, 43.1, 50.8, 55.3, 109.5, 110.2, 110.8, 115.3, 119.8, 121.5, 127.5, 129, 129.7, 130.1, 133.9, 134.3, 135.3, 158.1; SM (APCI+) m/z 307.3 $[M + H]^+$.

4.4. 6-Ethyl-1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (**5b**)

Yield 80% (absolute ethanol); mp 120–121 °C; ^{1}H NMR (300 MHz, CDCl3), δ = 1.20 (t, 3H, CH3, J = 7.6 Hz), 2.70 (m, 4H, CH2), 2.90 (m, 2H, CH2 and NH), 3.60 (m, 1H, CH2), 3.90 (s, 3H, OCH3), 5.50 (s, 1H, CH), 6.79 (m, 2H, HAr), 6.85 (dd, 1H, H7, J = 8.2 Hz, J = 1.5 Hz), 7.05 (d, 1H, HAr, J = 8.1 Hz), 7.12 (d, 1H, HAr, J = 8.1 Hz), 7.20 (s, 1H, H5), 7.26 (m, 1H, HAr), 10.30 (br s, 1H, NH); ^{13}C NMR (300 MHz, CDCl3), δ = 16.7, 22.5, 29.1, 41.7, 50.8, 55.5, 109.9, 110.5, 110.6, 116.6, 120.6, 121.9, 127.6, 129, 129.3, 129.9, 134.3, 134.4, 135.1, 157.2; SM (APCI+) m/z 307.2 $[M + H]^+$.

4.5. 6-Ethyl-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (**5c**)

Yield 87% (absolute ethanol); mp 78–79 °C; ^{1}H NMR (300 MHz, CDCl3), δ = 1.30 (t, 3H, CH3, J = 7.6 Hz), 2.70 (m, 4H, CH2), 2.90 (m, 1H, CH2), 3.20 (m, 1H, CH2), 3.80 (s, 3H, OCH3), 5.60 (s, 1H, CH), 6.97 (d, 2H, HAr, J = 8.8 Hz), 7.01 (dd, 1H, H7, J = 8.3 Hz, J = 1.7 Hz), 7.17 (d, 1H, H8, J = 8.3 Hz), 7.27 (m, 3H, HAr, NH), 7.36 (s, 1H, H5), 10.60 (br s, 1H, NH); ^{13}C NMR (300 MHz, CDCl3), δ = 16.7, 22.5, 29.1, 42.7, 55.3, 57.3, 109.7, 110.7, 113.3, 114.1, 116.7, 122.1, 127.6, 129.7, 130.1, 133.9, 134.4, 134.9, 135.3, 159.4; SM (APCI+) m/z 307.1 $[M + H]^+$.

4.6. 6-Ethyl-1-(3,4-dimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (**5d**)

Yield 82% (acetonitrile); mp 172–173 °C; ^{1}H NMR (300 MHz, CDCl3), δ = 1.40 (t, 3H, CH3, J = 7.6 Hz), 2.00 (br s, 1H, NH), 2.80 (m, 3H, CH2), 2.95 (m, 1H, CH2), 3.25 (m, 1H, CH2), 3.41 (m, 1H, CH2), 3.80 (s, 3H, OCH3), 3.90 (s, 3H, OCH3), 5.10 (s, 1H, CH), 6.80 (m, 3H, HAr), 7.10 (d, 1H, H7, J = 8.2 Hz), 7.18 (d, 1H, H8, J = 8.2 Hz), 7.40 (s, 1H, H5), 7.60 (br s, 1H, NH); ^{13}C NMR (300 MHz, CDCl3), δ = 16.6, 22.5, 29.1, 43.3, 55.7, 55.9, 58.2, 109.7, 110.9, 111.3, 113.1, 114.9, 116.7, 120.7, 122.1, 127.6, 134.3, 134.9, 135.4, 149.3, 152.6; SM (APCI+) m/z 337.3 $[M + H]^+$.

4.7. 6-Ethyl-1-(2,3,4-trimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (**5e**)

Yield 78% (ethanol 95°); mp 100–102 °C; ^{1}H NMR (300 MHz, CDCl3), δ = 1.35 (t, 3H, CH3, J = 7.6 Hz), 1.80 (br s, 1H, NH), 2.75 (m, 3H, CH2), 2.90 (m, 1H, CH2), 3.15 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.70 (s, 3H, OCH3), 3.91 (s, 3H, OCH3), 3.94 (s, 3H, OCH3), 5.60 (s, 1H, CH), 6.60 (s, 1H, HAr), 6.75 (s, 1H, HAr), 7.00 (d, 1H, H7, J = 8.5 Hz), 7.18 (d, 1H, H8, J = 8.5 Hz), 7.38 (s, 1H, H5), 7.90 (br s, 1H, NH); ^{13}C NMR (300 MHz, CDCl3), δ = 16.1, 22.1, 29.7, 41.5, 55.1, 55.3, 55.6, 57.8, 109.3, 110.4, 112.7, 114.8, 117.3, 122.8, 129.2, 130.7, 133.5, 134.8, 135.8, 158.6, 158.9, 159.4; SM (APCI+) m/z 367.5 $[M + H]^+$.

4.8. 6-Ethyl-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**5f**)

Yield 90% (methanol); mp 142–144 °C; ^{1}H NMR (300 MHz, DMSO-d6), δ = 1.20 (t, 3H, CH3, J = 7.6 Hz), 2.70 (q, 2H, CH2, J = 7.6 Hz), 3.00 (m, 1H, CH2), 3.10 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.60 (m, 1H, CH2), 3.75 (s, 3H, OCH3), 3.80 (s, 6H, OCH3), 5.95 (s, 1H, CH), 6.70 (s, 2H, HAr), 7.00 (dd, 1H, H7, J = 8.2 Hz, J = 1.5 Hz), 7.20 (d, 1H, H8, J = 8.2 Hz), 7.40 (s, 1H, H5), 9.70 (br s, 2H, $NH2^+$), 10.80 (br s, 1H, NH); ^{13}C NMR (300 MHz, DMSO-d6), δ = 17.1, 18.6, 28.9, 56.1, 56.4, 56.7, 60.4, 107.3, 107.5, 111.8, 117.1, 122.9, 126.3, 128.9, 130.5, 134.9, 135.5, 138.9, 153.5, 158.9, 160.2; SM (APCI+) m/z 367.3 $[M + H]^+$.

4.9. 1-(2,3-Dihydrobenzo [1,4]dioxin-6-yl)-6-ethyl-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**5g**)

Yield 80% (methanol); mp 240–241 °C; ^{1}H NMR (300 MHz, DMSO-d6), δ = 1.20 (t, 3H, CH3, J = 7.6 Hz), 2.70 (q, 2H, CH2, J = 7.6 Hz), 2.95 (m, 1H, CH2), 3.15 (m, 1H, CH2), 3.40 (m, 2H, CH2), 4.25 (s, 4H, OCH2CH2O), 5.80 (s, 1H, CH), 6.90 (m, 4H, H7, HAr), 7.20 (d, 1H, H8, J = 8.2 Hz), 7.30 (s, 1H, H5), 9.40 (br s, 1H, NH), 10.30 (br s, 1H, NH^+), 10.70 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-d6), δ = 17.1, 21.7, 28.9, 55.4, 64.6, 64.8, 107.4, 111.7, 114.4, 117, 117.7, 119.1, 126.3, 128, 129.1, 134.3, 134.8, 135.4, 143.7, 144.9; SM (APCI+) m/z 335.1 $[M + H]^+$

4.10. 6-Ethyl-1-(3,5-di-tert-butyl-4-hydroxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (**5h**)

Yield 60% (ethanol 95°); mp 180–181 °C; ^{1}H NMR (300 MHz, CDCl3), δ = 1.30 (t, 3H, CH3, J = 7.6 Hz), 1.40 (s, 18H, CH3), 2.00 (br s, 1H, NH), 2.80 (q, 2H, CH2, J = 7.6 Hz), 2.80 (m, 1H, CH2), 2.95 (m, 1H, CH2), 3.15 (m, 1H, CH2), 3.50 (m, 1H, CH2), 5.10 (br s, 1H, OH), 5.30 (s, 1H, CH), 7.10 (dd, 1H, H7, J = 8.2 Hz, J = 1.5 Hz), 7.12 (s, 2H, HAr), 7.15 (d, 1H, H8, J = 8.2 Hz), 7.40 (s, 1H, H5), 7.50 (br s, 1H, NH); ^{13}CNMR (300 MHz, CDCl3), δ = 16.6, 22.7, 29.1, 30.3, 34.3, 43.8, 58.9, 109.6, 110.6, 116.6, 121.9, 125.1, 127.5, 127.8, 132.4, 134.3, 135.2, 135.5, 135.9, 136.2, 153.7; SM (APCI+) m/z 405.3 $[M + H]^+$.

4.11. 6-Ethyl-1-(3-benzoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**5i**)

Yield 88% (acetonitrile); mp 130–131 °C; ^{1}H NMR (300 MHz, DMSO-d6), δ = 1.30 (t, 3H, CH3, J = 7.6 Hz), 2.80 (q, 2H, CH2, J = 7.6 Hz), 2.90 (m, 2H, CH2), 3.15 (m, 1H, CH2), 3.40 (m, 1H, CH2), 5.00 (s, 2H, OCH2), 5.10(s, 1H, CH), 6.90 (m, 3H, HAr), 7.05 (dd, 1H, H7, J = 8.2 Hz, J = 1.8 Hz), 7.12 (d, 1H, H8, J = 8.2 Hz), 7.30 (m, 7H, H5, HAr), 9.80 (br s, 1H, NH), 10.20 (br s, 1H, NH^+), 10.50 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-d6), δ = 17.1, 21.1, 28.9, 55.8, 69.5, 107.4, 111.8, 116.1, 117.1, 122.9, 126.2, 128.2, 128.4, 128.8, 128.9,

129.2, 129.5, 130.4, 130.7, 134.9, 135.5, 136.7, 137.2, 158.9; SM (APCI+) m/z 383.2 $[M + H]^+$.

4.12. 6-Ethyl-1-[3,4-bis(benzoxy)phenyl]-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**5j**)

Yield 85% (methanol); mp > 260 °C; ^{1}H NMR (300 MHz, DMSO-$d6$), δ = 1.35 (t, 3H, CH3, J = 7.6 Hz), 2.70 (q, 2H, CH2, J = 7.6 Hz), 3.00 (m, 1H, CH2), 3.15 (m, 1H, CH2), 3.40 (m, 2H, CH2), 4.90 (s, 2H, OCH2), 5.20 (s, 2H, OCH2), 5.80 (s, 1H, CH), 6.90(dd, 1H, HAr, J = 8.2 Hz, J = 1.8 Hz), 7.00 (dd, 1H, H7, J = 8.2 Hz, J = 1.2 Hz), 7.15 (d, 1H, H8, J = 8.2 Hz), 7.20 (d, 1H, HAr, J = 8.3 Hz), 7.40 (m, 12H, H5, HAr), 9.70 (br s, 1H, NH), 10.30 (br s, 1H, NH^+), 10.70 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-$d6$), δ = 17.1, 21.1, 28.9, 55.8, 68.3, 70.3, 107.4, 109.2, 111.8, 114.2, 116.2, 116.4, 117, 122.8, 123.4, 126.4, 127.7, 127.9, 128.1, 128.3, 128.7, 128.9, 129.2, 134.8, 135.5, 137.4, 137.5, 137.7, 148.6, 149.6; SM (APCI+) m/z 489.5 $[M + H]^+$.

4.13. 6-Benzyloxy-1-(3-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**5m**)

Yield 73% (absolute ethanol), mp 191–192 °C; ^{1}H NMR (300 MHz, DMSO-d_6), δ = 2.95 (m, 1H, CH_2), 3.13 (m, 1H, CH_2), 3.35 (m, 2H, CH_2), 3.75 (s, 3H, OCH_3), 5.10 (s, 2H, OCH_2), 5.83 (s, 1H, CH), 6.84 (d, 1H, H_7, J = 8.7 Hz), 6.95 (d, 1H, H_{Ar}, J = 7.4 Hz), 7.05 (d, 1H, H_{Ar}, J = 8.0 Hz), 7.13 (m, 2H, H), 7.22 (d, 1H, H_8, J = 8.7 Hz), 7.38 (m, 4H, H_5 H_{Ar}), 7.50 (d, 2H, H_{Ar}, J = 8.5 Hz), 9.75 (br s, 1H, NH), 10.60 (br s, 1H, NH^+), 10.70 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-d_6), δ = 18.6, 55.7, 56.1, 70.3, 102.2, 107.5, 112.7, 112.9, 115.6, 116, 122.4, 126.5, 128, 128.2, 128.3, 128.8, 129.7, 130.3, 132.2, 136.6, 138.2, 152.9, 159.8; SM (APCI+) m/z 385.1 $[M + H]^+$.

4.14. General procedure for the synthesis of phenolic derivatives (**5k**,**l**)

In 20 mL of methanol, compound **5i** or **5j** (2.5 mmol), and Palladium/C were added. The reaction mixture was placed under hydrogen atmosphere and stirred for 1 day. Palladium/C was filtered and the solvent was removed *in vacuo*. The oily product was dissolved in EtOAc and ether saturated with gaseous HCl was added. The corresponding hydrochloride product (**5k** or **5l**) was filtered and recrystallized from acetonitrile.

4.15. 6-Ethyl-1-(3-hydroxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**5k**)

Yield 85%; mp > 260 °C; ^{1}H NMR (300 MHz, DMSO-d_6), δ = 1.30 (t, 3H, CH_3, J = 7.6 Hz), 2.70 (q, 2H, CH_2, J = 7.6 Hz), 3.10 (m, 2H, CH_2), 3.40 (m, 2H, CH_2), 5.70 (s, 1H, CH), 6.70 (s, 1H, H_{Ar}), 6.90 (m, 2H, H_{Ar}), 7.10 (dd, 1H, H_7, J = 8.5 Hz, J = 1.5 Hz), 7.30 (m, 3H, H_5, H_8, H_{Ar}), 9.40 (br s, 1H, NH), 9.80 (br s, 1H, OH), 10.40 (br s, 1H, NH^+), 10.70 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-d_6), δ = 17.8, 19.9, 28.9, 64.7, 107.4, 111.5, 118.2, 120.4, 122.2, 126.3, 127.3, 128.1, 129.1, 134.8, 135.4, 143.7, 144.9, 146.3; SM (APCI+) m/z 293.4 $[M + H]^+$.

4.16. 6-Ethyl-1-(3,4-dihydroxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**5l**)

Yield 80%; mp > 260 °C; ^{1}H NMR (300 MHz, DMSO-d_6), δ = 1.30 (t, 3H, CH_3, J = 7.6 Hz), 2.70 (q, 2H, CH_2, J = 7.6 Hz), 3.10 (m, 2H, CH_2), 3.40 (m, 2H, CH_2), 5.70 (s, 1H, CH), 6.70 (m, 2H, H_{Ar}), 6.85 (d, 1H, H_{Ar}, J = 7.8 Hz), 7.10 (dd, 1H, H_7, J = 8.5 Hz, J = 1.5 Hz), 7.20 (d, 1H, H_8, J = 8.5 Hz), 7.30 (s, 1H, H_5), 8.10 (br s, 1H, NH), 9.15 (br s, 1H, OH), 9.35 (br s, 1H, OH), 9.70 (br s, 1H, NH^+), 10.70 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-d_6), δ = 17.1, 19.4, 28.9, 55.5, 107.4, 111.7, 114.4, 117, 117.7, 119.1, 126.3, 128, 129.1, 134.3, 134.8, 135.5, 143.7, 144.9; SM (APCI+) m/z 309.3 $[M + H]^+$.

4.17. General procedure for the synthesis of derivatives **6a**–**d**,**f**–**m**,**r**

In CH_3CN (80 mL), compounds **5a**–**j**,**m** (10 mmol), potassium carbonate (4.2 g, 30 mmol) and the desired alkyle iodide (15 mmol) were added. The reaction mixture was refluxed for 1 day and then evaporated. Ethyle acetate (80 mL) and water (50 mL) were added. The organic layer was dried with magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane/methanol (96/4, v/v) as eluent to afford compounds **6a**–**d**,**f**–**m**,**r**. Solid products were recrystallized with the appropriate solvant, while the oily products were solubilized in dry ethyl acetate and treated with gazeous hydrochloric acid in diethyl ether (20 mL). The obtained precipitates were filtered and washed with ethyl acetate and the residues were recrystallized with the appropriate solvant.

4.18. 6-Ethyl-1-(3-methoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6a**)

Yield 60% (absolute ethanol); mp 115–116 °C; ^{1}H NMR (300 MHz, DMSO-d_6), δ = 0.80 (t, 3H, CH_3, J = 7.5 Hz), 1.20 (t, 3H, CH_3, J = 7.6 Hz), 1.50 (m, 2H, CH_2), 2.40 (m, 1H, CH_2), 2.60 (m, 3H, CH_2), 2.80 (m, 2H, CH_2), 3.10 (m, 1H, CH_2), 3.40 (m, 1H, CH_2), 3.70 (s, 3H, OCH_3), 4.50 (s, 1H, CH), 6.85 (m, 4H, H_7, H_{Ar}), 7.10 (d, 1H, H_8, J = 8.2 Hz), 7.20 (s, 1H, H_5), 7.21 (m, 1H, H_{Ar}), 10.00 (br s, 1H, NH); ^{13}CNMR (300 MHz, $CDCl_3$), δ = 11.5, 16.8, 17.4, 29.4, 46.4, 54.8, 55.7, 56.5, 62.9, 106.9, 110.7, 117.5, 118.3, 122.6, 126, 126.3, 127.5, 131, 132.4, 134.9, 135.6, 135.9, 158.6; SM (APCI+) m/z 349.2 $[M + H]^+$.

4.19. 6-Ethyl-1-(3-methoxyphenyl)-2-methyl-2,3,4,9-tetrahydro-1H-β-carboline (**6b**)

Yield 20% (absolute ethanol); mp 100–102 °C; ^{1}H NMR (300 MHz, DMSO-$d6$), δ = 1.30 (t, 3H, CH3, J = 7.6 Hz), 2.40 (s, 3H, NCH3), 2.60 (m, 3H, CH2), 2.75 (m, 1H, CH2), 3.20 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.75 (s, 3H, OCH3), 4.35 (s, 1H, CH), 6.90 (m, 4H, H8 HAr), 7.10 (d, 1H, H8, J = 8.2 Hz), 7.20 (s, 1H, H5), 7.40 (m, 1H, HAr), 10.00 (br s, 1H, NH); ^{13}CNMR (300 MHz, CDCl3), δ = 11.7, 16.8, 29.8, 48.7, 56.1, 57.2, 62.9, 106.9, 112.5, 117.1, 117.5, 122.1, 126, 126.4, 127.4, 131.4, 132, 135, 135.3, 135.9, 158.2; SM (APCI+) m/z 322.2 $[M + H]^+$.

4.20. 6-Ethyl-1-(3-methoxyphenyl)-2-pentyl-2,3,4,9-tetrahydro-1H-β-carboline (**6c**)

Yield 65% (ethanol 95°); mp 78–80 °C; ^{1}H NMR (300 MHz, CDCl3), δ = 0.80 (t, 3H, CH3, J = 7.5 Hz), 1.34 (m, 7H, CH3, CH2), 1.50 (m, 1H, CH2), 2.40 (m, 1H, CH2), 2.60 (m, 3H, CH2), 2.90 (m, 2H, CH2), 3.10 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.70 (s, 3H, OCH3), 4.50 (s, 1H, CH), 6.90 (dd, 1H, HAr, J = 8.2 Hz, J = 2.6 Hz), 7.00 (m, 3H, H7, HAr), 7.10 (d, 1H, H8, J = 8.2 Hz), 7.20 (br s, 1H, NH), 7.30 (m, 1H, HAr), 7.40 (s, 1H, H5); ^{13}CNMR (300 MHz, CDCl3), δ = 14, 16, 21.3, 22.6, 26.7, 29.1, 29.57, 48.5, 54.1, 55.1, 65, 108.5, 111, 113.5, 114, 117.1, 122, 123.1, 127.4, 128.7, 134.7, 135.2, 135.3, 143.4, 159.9; SM (APCI+) m/z 377.3 $[M + H]^+$.

4.21. 6-Ethyl-1-(3-methoxyphenyl)-2-nonyl-2,3,4,9-tetrahydro-1H-β-carboline (**6d**)

Yield 60% (isopropanol); mp 50–52 °C; ^{1}H NMR (300 MHz, CDCl3), δ = 0.80 (t, 3H, CH3, J = 7.5 Hz), 1.30 (m, 15H, CH3, CH2), 1.50 (m, 2H, CH2), 2.40 (m, 1H, CH2), 2.60 (m, 5H, CH2), 3.10 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.80 (s, 3H, OCH3), 4.50 (s, 1H, CH), 6.85

(dd, 1H, HAr, $J = 8.2$ Hz, $J = 2.6$ Hz), 7.00 (m, 3H, H7, HAr), 7.10 (d, 1H, H8, $J = 8.2$ Hz), 7.20 (br s, 1H, NH), 7.30 (m, 1H, HAr, $J = 8.2$ Hz), 7.40 (s, 1H, H5); ^{13}CNMR (300 MHz, CDCl3), $\delta = 14.1$, 16.6, 21.3, 22.7, 27, 27.3, 29.1, 29.3, 29.6, 29.8, 31.9, 48.6, 54.1, 55.2, 64.9, 108.5, 110.5, 113.6, 114, 116.7, 121.3, 121.8, 127.4, 129.5, 134.7, 135.1, 135.3, 143.4, 159.9; SM (APCI+) m/z 433.5 $[M + H]^+$.

4.22. 6-Ethyl-1-(2-methoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**6f**)

Yield 40% (methanol); mp 215–216 °C; ^{1}H NMR (300 MHz, DMSO-*d*6), $\delta = 0.90$ (t, 3H, CH3, $J = 7.5$ Hz), 1.30 (t, 3H, CH3, $J = 7.5$ Hz), 1.90 (m, 2H, CH2), 2.70 (q, 2H, CH2, $J = 7.5$ Hz), 3.10 (m, 4H, CH2), 3.40 (m, 1H, CH2), 3.60 (s, 1H, CH2), 4.00 (s, 3H, OCH3), 6.20 (s, 1H, CH), 6.85 (dd, 1H, HAr, $J = 7.5$ Hz, $J = 1.5$ Hz), 6.90 (m, 1H, HAr), 6.99 (dd, 1H, H7, $J = 8.4$ Hz, $J = 1.6$ Hz), 7.20 (m, 2H, H8, HAr), 7.40 (s, 1H, H5), 7.50 (m, 1H, HAr), 9.70 (br s, 1H, NH^+), 10.50 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-*d*6), $\delta = 11.3$, 16.5, 17.1, 28.9, 46.9, 54, 56, 56.3, 62.7, 106.7, 111.8, 117, 117.2, 122.3, 126, 126.4, 127, 131, 132.1, 134.9, 135.3, 135.7, 157.9; SM (APCI+) m/z 307.2 $[M + H]^+$.

4.23. 6-Ethyl-1-(4-methoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6g**)

Yield 48% (absolute ethanol); mp 145–146 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 0.80$ (t, 3H, CH3, $J = 7.5$ Hz), 1.30 (t, 3H, CH3, $J = 7.5$ Hz), 1.40 (m, 2H, CH2), 2.30 (m, 1H, CH2), 2.60 (m, 1H, CH2), 2.70 (m, 4H, CH2), 3.00 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.80 (s, 3H, OCH3), 4.50 (s, 1H, CH), 6.80 (d, 2H, HAr, $J = 8.3$ Hz), 7.00 (dd, 1H, H7, $J = 8.2$ Hz, $J = 1.5$ Hz), 7.10 (d, 1H, H8, $J = 8.2$ Hz), 7.10 (br s, 1H, NH), 7.20 (d, 2H, HAr, $J = 8.3$ Hz), 7.30 (s, 1H, H5); ^{13}CNMR (300 MHz, CDCl3), $\delta = 11.6$, 16.6, 20.1, 21.2, 29, 48.3, 55.3, 55.8, 63.9, 108.5, 113.5, 113.8, 116.8, 121.8, 127.6, 130.2, 130.5, 133, 133.5, 134.3, 134.6, 135, 159.2; SM (APCI+) m/z 349.3 $[M + H]^+$.

4.24. 6-Ethyl-1-(3,4-dimethoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6h**)

Yield 52% (absolute ethanol); mp 140–142 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 0.90$ (t, 3H, CH3, $J = 7.5$ Hz), 1.40 (t, 3H, CH3, $J = 7.6$ Hz), 1.60 (m, 2H, CH2), 2.30 (m, 1H, CH2), 2.80 (m, 4H, CH2), 2.90 (m, 1H, CH2), 3.10 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.80 (s, 3H, OCH3), 3.90 (s, 3H, OCH3), 4.50 (s, 1H, CH), 6.80 (d, 1H, HAr, $J = 7.9$ Hz), 6.90 (m, 2H, HAr), 7.00 (dd, 1H, H7, $J = 8.2$ Hz, $J = 1.4$ Hz), 7.10 (d, 1H, H8, $J = 8.2$ Hz), 7.30 (s, 1H, H5), 7.40 (br s, 1H, NH); ^{13}CNMR (300 MHz, CDCl3), $\delta = 11.9$, 16.7, 18.7, 20.1, 21.4, 29.1, 48.8, 55.8, 55.9, 64.9, 108.3, 110.5, 110.6, 111.3, 116.7, 121.2, 121.8, 127.4, 134, 134.7, 135.3, 135.6, 148.7, 149.4; SM (APCI+) m/z 379.5 $[M + H]^+$.

4.25. 6-Ethyl-1-(3,4,5-trimethoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6i**)

Yield 60% (absolute ethanol); mp 145–147 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 0.90$ (t, 3H, CH3, $J = 7.5$ Hz), 1.40 (t, 3H, CH3, $J = 7.6$ Hz), 1.60 (m, 2H, CH2), 2.30 (m, 1H, CH2), 2.60 (m, 2H, CH2), 2.80 (m, 3H, CH2), 3.10 (m, 1H, CH2), 3.40 (m, 1H, CH2), 3.80 (s, 6H, OCH3), 3.90 (s, 3H, OCH3), 4.50 (s, 1H, CH), 6.70 (m, 2H, HAr), 7.00 (d, 1H, H7, $J = 8.2$ Hz), 7.20 (d, 1H, H8, $J = 8.2$ Hz), 7.35 (s, 1H, H5), 7.40 (br s, 1H, NH); ^{13}CNMR (300 MHz, CDCl3), $\delta = 11.9$, 16.6, 20.1, 21.5, 29.1, 49, 55, 55.9, 56.1, 60.8, 65.7, 105.4, 106, 108.2, 110.6, 116.7, 121.8, 127.4, 134.7, 135.3, 135.5, 137.2, 137.5, 153.3; SM (APCI+) m/z 409.3 $[M + H]^+$.

4.26. 1-(2,3-Dihydrobenzo [1,4]dioxin-6-yl)-6-ethyl-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6j**)

Yield 61% (ethanol 95°); mp 62–64 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 0.90$ (t, 3H, CH3, $J = 7.4$ Hz), 1.20 (t, 3H, CH3, $J = 7.6$ Hz), 1.60 (m, 2H, CH2), 2.40 (m, 1H, CH2), 2.60 (m, 1H, CH2), 2.80 (m, 4H, CH2), 300 (m, 1H, CH2), 3.40 (m, 1H, CH2), 4.25 (s, 4H, OCH2CH2O), 4.50 (s, 1H, CH), 6.85 (d, 1H, HAr, $J = 1.3$ Hz), 6.90 (m, 2H, HAr), 7.00 (dd, 1H, H7, $J = 8.5$ Hz, $J = 1.6$ Hz), 7.12 (d, 1H, H8, $J = 8.5$ Hz), 7.30 (s, 1H, NH), 7.40 (s, 1H, H5); ^{13}CNMR (300 MHz, CDCl3), $\delta = 11.9$, 16.7, 20.2, 21.2, 29.1, 48.2, 55.9, 63.9, 64.3, 108.5, 110.5, 116.8, 117.2, 117.7, 121.8, 121.5, 127.4, 134.7, 134.9, 135.3, 135.4, 143.1, 143.5; SM (APCI+) m/z 377.2 $[M + H]^+$.

4.27. 6-Ethyl-1-(3,5-di-tert-butyl-4-hydroxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6k**)

Yield 60% (absolute ethanol); mp 55–56 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 0.80$ (t, 3H, CH3, $J = 7.4$ Hz), 1.30 (t, 3H, CH3, $J = 7.6$ Hz), 1.40 (s, 18H, CH3), 1.60 (m, 2H, CH2), 2.30 (m, 1H, CH2), 2.50 (m, 1H, CH2), 2.70 (m, 4H, CH2), 300 (m, 1H, CH2), 3.40 (m, 1H, CH2), 4.50 (br s, 1H, OH), 5.20 (s, 1H, CH), 7.10 (dd, 1H, H7, $J = 82$ Hz, $J = 1.5$ Hz), 7.13 (s, 3H, H8, HAr), 7.20 (br s, 1H, NH), 7.35 (s, 1H, H5); ^{13}CNMR (300 MHz, CDCl3), $\delta = 11.5$, 16.6, 20.3, 22.5, 29.3, 30.1, 34.1, 43.4, 48.1, 58.7, 109.76, 110.7, 116.5, 121.7, 125.3, 127.6, 128.3, 132.4, 134.3, 135.3, 135.7, 135.9, 136.5, 153.9; SM (APCI+) m/z 450.1 $[M + H]^+$.

4.28. 6-Ethyl-1-(3-benzoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**6l**)

Yield 60% (methanol); mp 241–242 °C; ^{1}H NMR (300 MHz, DMSO-*d*6), $\delta = 0.90$ (t, 3H, CH3, $J = 7.5$ Hz), 1.30 (t, 3H, CH3, $J = 7.6$ Hz), 1.50 (m, 2H, CH2), 1.60 (br s, 1H, NH^+), 2.35 (m, 1H, CH2), 2.60 (m, 1H, CH2), 2.80 (m, 4H, CH2), 3.00 (m, 1H, CH2), 3.40 (m, 1H, CH2), 4.50 (s, 1H, CH), 500 (s, 2H, OCH2), 7.00 (m, 4H, H7, HAr), 7.10 (d, 1H, H8, $J = 8.4$ Hz), 7.30 (m, 1H, HAr), 7.40 (m, 6H, H5, HAr), 9.80 (br s, 1H, NH^+), 10.60 (br s, 1H, NH); ^{13}CNMR (300 MHz, DMSO-*d*6), $\delta = 11.2$, 17, 18.1, 28.8, 49.4, 64.7, 69.8, 106.4, 111.8, 117.2, 123, 123.1, 123.2, 125.9, 128.2, 128.5, 128.6, 128.9, 131.3, 134.5, 134.9, 135.2, 135.6, 136.1, 136.9, 158.9; SM (APCI+) m/z 462.3 $[M + H]^+$.

4.29. 6-Ethyl-1-[3,4-bis(benzoxy)phenyl]-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6m**)

Yield 65% (absolute ethanol); mp 112–114 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 0.90$ (t, 3H, CH3, $J = 7.5$ Hz), 1.35 (t, 3H, CH3, $J = 7.6$ Hz), 1.50 (m, 2H, CH2), 2.30 (m, 1H, CH2), 2.60 (m, 1H, CH2), 2.80 (m, 4H, CH2), 3.00 (m, 1H, CH2), 3.30 (m, 1H, CH2), 4.40 (s, 1H, CH), 5.10 (s, 2H, OCH2), 5.20 (s, 2H, OCH2), 6.90 (dd, 1H, H7, $J = 8.1$ Hz, $J = 1.5$ Hz), 6.94 (d, 1H, HAr, $J = 8.1$ Hz), 7.00 (dd, 2H, $J = 8.7$ Hz, $J = 1.5$ Hz), 7.07 (br s, 1H, NH), 7.12 (d, 1H, H8, $J = 8.3$ Hz), 7.35 (m, 9H, H8 HAr), 7.50 (d, 2H, HAr, $J = 7.9$ Hz); ^{13}CNMR (300 MHz, CDCl3), $\delta = 11.9$, 16.7, 20.2, 21.3, 29.1, 48.5, 55.9, 64.4, 70.9, 71.2, 108.4, 110.6, 114.5, 115.2, 116.7, 116.8, 121.8, 121.9, 127.3, 127.4, 127.5, 127.7, 127.8, 128.4, 128.5, 134.7, 134.8, 135.3, 135.5, 137.2, 137.4, 148.6, 148.9; SM (APCI+) m/z 531.2 $[M + H]^+$.

4.30. 6-Benzoxy-1-(3-methoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**6r**)

Yield 63% (absolute ethanol); mp 132–132.8 °C; ^{1}H NMR (300 MHz, DMSO-d_6), $\delta = 0.80$ (t, 3H, CH_3, $J = 7.3$ Hz), 1.84 (m, 2H, CH_2), 3.10 (m, 3H, CH_2), 3.35 (m, 2H, CH_2), 3.65 (m, 1H, CH_2), 3.75 (s, 3H, OCH_3), 5.10 (s, 2H, OCH_2), 5.85 (s, 1H, CH), 6.83 (dd, 1H, H_7, $J = 8.7$ Hz, $J = 2.5$ Hz), 7.08 (m, 1H, H_{Ar}), 7.17 (m, 2H, H_8, H_{Ar}), 7.25 (s,

1H, H_{Ar}), 7.38 (m, 5H, H_5, H_{Ar}), 7.47 (d, 2H, H_{Ar}, $J = 8.3$ Hz),; ^{13}CNMR (300 MHz, DMSO-d_6), $\delta = 11.4, 16.8, 18.1, 20.3, 48.4, 54.4, 55.8, 64.1, 70.3, 102.3, 106.6, 112.8, 113, 116.3, 123.1, 126.2, 127.9, 128, 128.2, 128.5, 128.9, 129.6, 130.5, 132.5, 133.7, 135.2, 138.2, 152.9, 159.9$; SM (APCI+) m/z 427.2 $[M + H]^+$.

4.31. General procedure for the synthesis of derivatives **6e**,**n**,**o**

To a solution of 10% K_2CO_3 aqueous (30 mL) and ethyl acetate (50 mL), β-carboline derivative (**5a**,**i**,**j**) (10 mmol) was solubilised. The mixture was cooled to 0 °C, and benzyl chloroformate (2.1 mL, 15 mmol) or propionyl chloride (0.9 mL, 15 mmol) was added dropwise. The reaction mixture was stirred for 3 h at room temperature. The organic layer was washed with HCl 1M (50 mL), dried with magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography using dichloromethane/petroleum ether (90/10, v/v) as eluent to afford solid compounds **6e**,**n**,**o**.

4.32. 6-Ethyl-1-(3-methoxyphenyl)-2-propionyl-2,3,4,9-tetrahydro-1H-β-carboline (**6e**)

Yield 75% (absolute ethanol); mp 216–218 °C; ^{1}H NMR (300 MHz, $CDCl_3$), $\delta = 1.20$ (t, 3H, CH_3, $J = 7.3$ Hz), 1.40 (t, 3H, CH_3, $J = 7.6$ Hz), 2.50 (q, 2H, CH_2, $J = 7.3$ Hz), 2.80 (q, 2H, CH_2, $J = 7.6$ Hz), 3.00 (m, 2H, CH_2), 3.50 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.00 (s, 1H, CH), 6.80 (m, 2H, H_{Ar}), 7.00 (s, 1H, H_{Ar}), 7.08 (dd, 1H, H_7, $J = 8.2$ Hz, $J = 1.5$ Hz), 7.18 (m, 1H, H_{Ar}), 7.25 (d, 1H, H_8, $J = 8.2$ Hz), 7.35 (s, 1H, H_5), 8.10 (br s, 1H, NH); ^{13}CNMR (300 MHz, $CDCl_3$), $\delta = 9.6, 16.6, 26.8, 29.1, 39.7, 51.8, 55.2, 64.2, 110.9, 113.3, 114.3, 116.5, 118.7, 121.1, 121.9, 122.4, 126.7, 131.9, 134.8, 135.4, 141.9, 159.6, 172.41$; SM (APCI+) m/z 363.3 $[M + H]^+$.

4.33. 6-Ethyl-1-(3-benzoxyphenyl)-2-benzylcarbamate-2,3,4,9-tetrahydro-1H-β-carboline (**6n**)

Yield 71% (absolute ethanol); mp 65–66 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 1.45$ (t, 3H, CH3, $J = 7.6$ Hz), 2.80 (m, 4H, CH2), 3.20 (m, 1H, CH2), 4.50 (m, 1H, CH2), 5.10 (m, 2H, OCH2), 5.25 (m, 2H, OCH2), 6.40 (s, 1H, CH), 6.80 (s, 1H, HAr), 7.05 (dd, 1H, H7, $J = 8.1$ Hz, $J = 1.7$ Hz), 7.17 (d, 1H, H8, $J = 8.1$ Hz), 7.40 (m, 14H, H5 HAr), 8.40 (br s, 1H, NH); ^{13}CNMR (300 MHz, CDCl3), $\delta = 16.8, 21.6, 30.2, 37.8, 55.9, 70.5, 71.8, 108.9, 111.2, 114.6, 115.1, 115.5, 116.5, 121, 121.4, 121.8, 122.6, 127.3, 127.5, 127.8, 128.4, 128.6, 134.3, 135.5, 135.8, 137, 137.3, 137.8, 148.4$; SM (APCI+) m/z 517.8 $[M + H]^+$.

4.34. 6-Ethyl-1-[3,4-bis(benzoxy)phenyl]-2-benzylcarbamate-2,3,4,9-tetrahydro-1H-β-carboline (**6o**)

Yield 75% (absolute ethanol); mp 54–56 °C; ^{1}H NMR (300 MHz, $CDCl_3$), $\delta = 1.40$ (t, 3H, CH_3, $J = 7.6$ Hz), 2.90 (m, 4H, CH_2), 3.10 (m, 1H, CH_2), 4.40 (m, 1H, CH_2), 5.00 (m, 2H, OCH_2), 5.20 (m, 4H, OCH_2), 6.30 (s, 1H, CH), 6.75 (s, 2H, H_{Ar}), 7.05 (dd, 1H, H_7, $J = 8.2$ Hz, $J = 1.8$ Hz), 7.17 (d, 1H, H_8, $J = 8.2$ Hz), 7.40 (m, 17H, H_5 H_{Ar}), 8.00 (br s, 1H, NH); ^{13}CNMR (300 MHz, $CDCl_3$), $\delta = 16.6, 21.8, 29.1, 38.2, 55.7, 70.3, 71.1, 72.9, 108.6, 110.8, 114.3, 115.3, 115.7, 116.3, 121, 121.2, 121.4, 121.6, 122.4, 122.6, 127.2, 127.5, 127.8, 128.4, 128.5, 128.6, 134.3, 134.5, 135.5, 135.7, 135.8, 137, 137.2, 137.4, 148.6, 148.8$; SM (APCI+) m/z 623.1 $[M + H]^+$.

4.35. General procedure for the synthesis of phenolic derivatives (**6p**,**q**,**s**)

In 20 mL of methanol, compound **6l**,**m**,**r** (2.5 mmol), and Palladium/C were added. The reaction mixture was placed under hydrogen atmosphere and stirred for 1 day. Palladium/C was filtered and the solvent was removed *in vacuo*. The residue was purified by column chromatography using dichloromethane/methanol (96/4, v/v) as eluent to afford solid compounds **6p**,**q**,**s**.

4.36. 6-Ethyl-1-(3-hydroxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6p**)

Yield 75% (ethanol 95°); mp 80–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$), $\delta = 0.80$ (t, 3H, CH_3, $J = 7.4$ Hz), 1.30 (t, 3H, CH_3, $J = 7.6$ Hz), 1.60 (m, 2H, CH_2), 2.40 (m, 1H, CH_2), 2.60 (m, 1H, CH_2), 2.80 (m, 4H, CH_2), 300 (m, 1H, CH_2), 3.40 (m, 1H, CH_2), 4.50 (s, 1H, CH), 6.40 (br s, 1H, OH), 6.70 (m, 2H, H_{Ar}), 6.80 (d, 1H, H_{Ar}, $J = 7.3$ Hz), 7.10 (dd, 1H, H_7, $J = 8.5$ Hz, $J = 1.5$ Hz), 7.10 (m, 2H, H_8, H_{Ar}), 7.40 (m, 2H, H_5, NH); ^{13}CNMR (300 MHz, $CDCl_3$), $\delta = 11.9, 16.6, 19.7, 29.1, 33.9, 47.7, 55.8, 63, 108.4, 110.7, 111.4, 115.5, 116.2, 121.2, 122.1, 123.3, 127.1, 129.6, 134.7, 135.4, 136.6, 156.3$; SM (APCI+) m/z 335.2 $[M + H]^+$.

4.37. 6-Ethyl-1-(3,4-dihydroxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline (**6q**)

Yield 80% (ethanol 95°); mp 123–124 °C; ^{1}H NMR (300 MHz, CDCl3), $\delta = 0.80$ (m, 3H, CH3), 1.50 (m, 5H, CH3, CH2), 2.50 (m, 2H, CH2), 2.80 (m, 5H, CH2), 3.20 (m, 1H, CH2), 4.50 (s, 1H, CH), 6.50 (m, 3H, HAr), 700 (m, 2H, H8, H7), 7.40 (s, 1H, H5), 8.50 (br s, 3H, OH, NH); ^{13}CNMR (300 MHz, CDCl3), $\delta = 11.9, 16.6, 19.7, 29.1, 33.9, 47.7, 55.8, 63, 110.7, 114.7, 115.5, 116.1, 121.2, 122.1, 123.4, 127.1, 129.6, 134.7, 135.4, 136, 156, 157$; SM (APCI+) m/z 351.2 $[M + H]^+$.

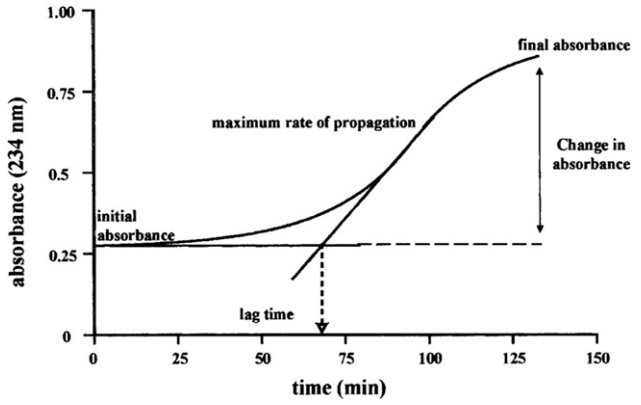
4.38. 6-Hydroxy-1-(3-methoxyphenyl)-2-propyl-2,3,4,9-tetrahydro-1H-β-carboline hydrochloride (**6s**)

Yield 87% (absolute ethanol); mp 222–223 °C; ^{1}H NMR (300 MHz, DMSO-d_6), $\delta = 0.85$ (t, 3H, CH_3, $J = 7.0$ Hz), 1.80 (m, 2H, CH_2), 3.00 (m, 3H, CH_2), 3.35 (m, 1H, CH_2), 3.70 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 5.86 (s, 1H, CH), 6.65 (dd, 1H, H_7, $J = 8.5$ Hz, $J = 2.2$ Hz), 6.83 (d, 1H, H_5, $J = 2.2$ Hz), 7.07 (m, 3H, H_8, H_{Ar}), 7.23 (s, 1H, H_{Ar}), 7.38 (m, 1H, H_{Ar}), 8.80 (br s, 1H, OH), 10.30 (br s, 1H, NH), 11.10 (br s, 1H, NH^+); ^{13}CNMR (300 MHz, DMSO-d_6), $\delta = 11.4, 16.9, 18, 48.2, 54.3, 55.8, 64.1, 102.6, 105.8, 112.4, 112.6, 116.1, 116.3, 123, 126.6, 129.3, 130.4, 131.7, 135.2, 151.3, 159.9$; SM (APCI+) m/z 337.2 $[M + H]^+$.

4.39. Antioxydant activity

4.39.1. Low Density Lipoprotein (LDL) oxidation inhibition test

Human LDLs were isolated from freshly drawn blood from healthy, normolipidemic, fasting volunteers. LDLs were isolated by sequential density gradient ultracentrifugation in sodium bromide density solutions in the density range of 1.019–1.063 g/mL as previously reported [48]. The protein concentration was determined by Peterson's method [49] using bovine serum albumin as the standard. Before oxidation, LDLs were extensively dialyzed against 0.01 M phosphate-buffered-saline (PBS) pH 7.4 under N_2 at 4 °C. Then oxidation was induced at 30 °C by adding 20 μL of 16.6 μM $CuSO_4$ or 2 mM AAPH to 160 μL of LDL (125 μg of protein/mL) in the presence or absence of 20 μl derivatives from 0.1 μM to 10 μM or PBS for control. A mixture containing LDL and the tested molecules without Cu^{2+} or AAPH was also used as control. During copper or AAPH-induced LDL oxidation, diene-conjugated formation was followed by the measurement of optical density at 234 nm every 10 min for 8 h at 30 °C with a thermostated Spectra Max Plus Molecular Devices spectrophotometer (96 wells, Molecular Devices Corporation Sunnyvale, California 94089) [50].



Three phases could be distinguished from the **O**ptical **D**ensity (**OD**) change pattern as illustrated below: (a) a lag phase, during which OD did not increase significantly, indicating that LDL are resistant to copper-catalysed modification; (b) a propagation phase, during which **OD** increases rapidly; and (c) a degradation phase, starting from the point at which the increase in **OD** reaches its plateau.

The lag phase is determined by drawing tangents to the linear segments of the curve during the lag phase and the propagation phase. The perpendicular projection on the horizontal axis (time) of the intersection point of the two tangents is defined as the lag time, expressed in minutes. The propagation rate is calculated from the slope of the tangent to the curve during the propagation phase, by using a molecular extinction coefficient for conjugated dienes of $\varepsilon = 2.95 \times 104\ M^{-1}cm^{-1}$.

In vitro studies of LDL oxidation have verified the existence of a 'lag phase', during which significant oxidation of LDL cannot be detected, prior to the onset of the 'propagation phase', presumably after the endogenous antioxidants have been consumed. There follows a steady increase in the detectable by-products of oxidation, until the substrate, i.e. the PUFAs, has been depleted, and a plateau phase is reached. Assessment of resistance of LDL to oxidation has generally involved measurement of the duration of the lag phase, although other variables, such as the rate of propagation, can also be noted.

Addition of 0.1–10 μM of tested compounds to the LDL solution induced a dose-dependent increase in the lag phase of conjugated diene formation compared with LDL with $CuSO_4$ alone. The control lag phase is obtained in an oxidative procedure without tested compounds.

Drug activities were expressed in $\mathbf{ED_{50}}$ or **R**; the ratio between the length of the lag phase of the oxidative procedure of LDL in the presence of different compounds and the length of the control lag phase.

We considered that compounds had 100% activity ($\mathbf{ED_{100}}$) when the control lag phase duration doubled and we defined $\mathbf{ED_{50}}$ as the concentration of the drug that increased this control lag phase by 1.5 times.

4.39.2. Cellular cytotoxicity and cellular vitality

Bovine aortic endothelial cells (BAECs) were isolated as described by Gospodarowicz [51]. Cells were cultured in Dubelco's-modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum, 600 ng/mL glutamine, and 100 U/mL penicillin. BAECs were used under passage 10. BAECs were then subcultured in multi well plates.

The lactate dehydrogenase assay is a means of measuring membrane integrity as a function of the amount of cytoplasmic LDH release in the medium. Cell free aliquots of the medium from cultures given different treatments are assayed, then the amount of LDH activity can be used as an indicator of relative cell viability as well as a function of membrane integrity.10 μL drugs in methanol solution (1.10^{-3} M) were added to the cell culture medium during 24h. Then cells were incubated at 37 °C in the presence of LDL or air- oxidized LDL at 100 μg/mL with an addition of 10 μL methanol or drugs in methanol solution (final concentration 10 μM). After 16h of incubation, Lactate DesHydrogenase (LDH) activity in the medium was measured by the spectrometric analysis of NAD reduction using the LDH kit (sigma, Saint Quentin Fallavier, France). LDH activity was expressed as the percentage of total LDH cellular release following the addition of Triton X-100 (final concentration of 0.1% v/v in ethanol). Results are the mean (+/-SD) of 2 different incubations each performed in triplicate.

Cellular vitality was determined by measuring mitochondrial activity in the presence of MTT:(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). MTT is reduced to purple formazan in the mitochondria of living cells. 100 μL of a 5 mg solution of MTT in PBS were added to each culture well and incubated for 5 h. Following this period, the medium was removed and the crystals were dissolved in 4 mL of dimethylformamide. Absorbance was read at 590 nm, and the results were expressed as the percentage of the value of control wells [52].

4.39.3. Data analysis

The results were expressed as mean ± SD when a minimal number of three independent experiments were performed in triplicate. Differences between the experimental results were tested using the Mann Whitney test.

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