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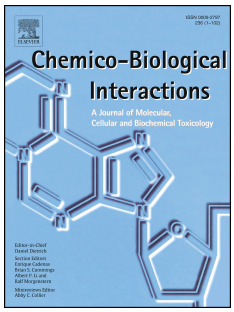

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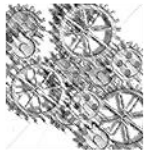
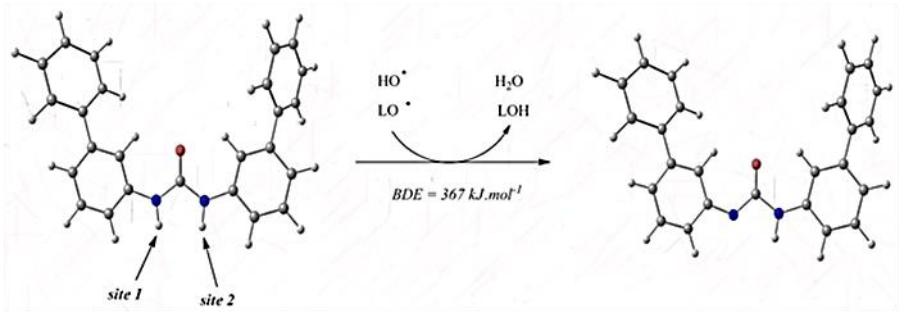
Authors contributions

J.L. and P.C. conceived and designed the study, S.C. and R.O. performed the chemical synthesis, J.L. J.Z. and A.S. performed LP experiments, D.Y. performed theoretical calculations. All the authors contributed to the paper writing.

PL90 mix
TBA





Lipid peroxidation inhibition study: a promising case of 1,3-di([1,1′-biphenyl]-3-yl)urea

Jelena Lazarević[a]*, Andrija Šmelcerović[a], Jelena Zvezdanović[b], Denitsa Yancheva[c], Silvana Casati[d], Roberta Ottria[d], Pierangela Ciuffreda[d]*

[a]Department of Chemistry, Faculty of Medicine, University of Niš, Bulevar Dr Zorana Đinđića 81, 18000 Nis, Serbia

[b]Faculty of Technology, University of Niš, Bulevar oslobođenja 124, 16000 Leskovac, Serbia

[c]Institute of Organic Chemistry and Centre of Phytochemistry, Bulgarian Academy of Sciences,Acad. G. Bonchev Str., Build. 9, 1113 Sofia, Bulgaria

[d]Dipartimento di Scienze Biomediche e Cliniche "Luigi Sacco", Università degli Studi diMilano, Via G.B. Grassi 74, 20157 Milano, Italy

***Correspondence to:**
Jelena Lazarević, e-mail: jelena217@yahoo.com; jelena.lazarevic@medfak.ni.ac.rs and Pierangela Ciuffreda, e-mail: pierangela.ciuffreda@unimi.it

Abstract

In the present study eighteen inhibitors of the hydrolytic enzymes of the endocannabinoid system were investigated for antioxidant activity using lipid peroxidation (LP) method. Among the assayed compounds ten belong to carbamates with phenyl [1,1'-biphenyl]-3-ylcarbamate (**6**), reported for the first time, and eight are retro-amide derivatives of palmitamine. Interestingly, results indicated that most of the tested compounds have good antioxidant properties. In particular, 1,3-di([1,1′-biphenyl]-3-yl)urea (**3**) shows IC_{50} = 26± 6 μM comparable toones obtained for standard antioxidants trolox and quercetin (IC_{50} = 22± 6 μM and 23 ± 6 μM, respectively). Compound **3** was investigated further by means of ab initio calculations, to clarify a possible mechanism of the antioxidant action. In order to estimate the capability of **3** to act as radical scavenger the structure was optimized at B3LYP/6-311++G⁄⁄ level and the respective bond dissociation enthalpies were calculated. The calculations in non-polar medium predicted as favorable mechanism a donation of a hydrogen atom to the free radical and formation of N-centered radical, while in polar solvents dominate mechanism of free radical scavenging by SPLET over HAT H-abstraction. The possible radical scavenging mechanisms of another compound with potent antioxidant properties (IC_{50} =53 ± 12 μM), the retro-amide derivative of palmitamine, compound **18**, was estimated computationally based on the reaction enthalpies of a

model compound (structural analogue to **18**). The computations indicated that the most favorable mechanisms are hydrogen atom transfer from the hydroxyl group in *meta*-position of the benzamide fragment in nonpolar medium, and proton transfer from the hydroxyl group in *ortho*-position of the benzamide fragment in nonpolar medium.

Keywords: antioxidant activity, lipid peroxidation inhibition, ab initio calculations, endocannabinoid modulators, urea derivatives

Abbreviations

AAPH 2,2′-Azobis(2-methylpropionamidine) dihydrochloride

B3LYP Becke's three-parameter non-local exchange in conjunction with Lee, Yang and Parr correlation potentials

BDE Bond dissociation enthalpy

$CDCl_3$ Deuterochloroform

CDI Carbonyldiimidazole

COMU 1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate

DFT Density-functional theory

DIPEA N,N-Diisopropylethylamine

DMAP 4-(Dimethylamino)pyridine

DNA Deoxyribonucleic acid

EtOAc Ethyl acetate

HAT Hydrogen atom transfer

IEFPCM Integral equation formalism polarizable continuum model

IP Ionization potential

LP Lipid peroxidation

MAGL Monoacylglycerol lipase

MDA Malondialdehyde

NAAA N-acylethanolamide acid amidase

NMR Nuclear magnetic resonance

PA Proton affinity

PL90 Phospholipon® 90

PUFA Polyunsaturated fatty acids

ROS Reactive oxygen species

SET Single electron transfer

SPLET Sequential proton loss electron transfer

TBA Thiobarbituric acid

TCA Trichloroacetic acid

TLC Thin-layer chromatography

1. Introduction

Reactive oxygen species (ROS) are produced through enzymatic and non-enzymatic mechanisms in cells and the homeostatic balance between the physiological and pathological concentration of ROS can be altered not only after radiation and pollution exposure but also in stress conditions and with incorrect diet [1]. ROS are highly reactive species that can attack various classes of biomolecules including DNA, proteins and fatty acids, especially polyunsaturated fatty acids (PUFA) [2]. Bringing to close connection with oxidative stress *in vivo*, lipid peroxidation (LP)

has received a great deal of attention. This free radical mediated chain reaction process, once initiated results in an oxidative deterioration of polyunsaturated lipids [3]. Through a proven disturbance process in membrane organization and a functional loss and modification of proteins and DNA bases, LP has been implicated in the pathogenesis of various diseases and aging, including atherosclerosis, cataract, rheumatoid arthritis and neurodegenerative disorders [4]. Moreover, correlation between the excessive production of reactive oxygen species (ROS) and their role in cancer development is well known [5,6]. Consequently, the role of antioxidants has received extensive attention and many natural and synthetic supplements and drugs with radical-scavenging capacity have been explored [7]. Beyond the beneficial effects of antioxidants, we should also recall the dark side of this class of molecules reported by recent literature. In the last decades, indeed, several research findings present antioxidants as causative risk factor in various human diseases as cardiovascular disease or diabetes [8]. Moreover, also the effects of traditional antioxidant micronutrients as cancer chemoprevention agents remain unclear revealing both benefits and detrimental effects or even resulted in harm [9,10]. On the contrary the mechanistic link between oxidative stress and carcinogenesis is well documented, prompting further researches. Additionally, special targets of LP processes are long chain fatty acids and the PUFA arachidonic acid, the principal components of the endocannabinoids' moiety, lipid signaling mediators involved in a wide range of physiological and pathological conditions, comprising cancer [11].

Starting from these considerations we decided to investigate if the monoacylglycerol lipase (MAGL) and *N*-acylethanolamide acid amidase (NAAA) inhibitors, synthesized and analyzed in our laboratories [12,13], could also have an additional antioxidant effect. The present study reports antioxidant activity of **1-18** investigated by LP method. For the most active among the studied compounds (compounds **3** and **18**), theoretical calculations were preformed to clarify a possible mechanism of an antioxidant action.

2. Materials and methods

2.1. Reagents and Materials

All of the reagents, standards and solvents used were of analytical reagent grade, obtained from commercial sources and used without further purification (unless specified otherwise, all chemicals were purchased from Merck (Darmstadt, Germany)).

Phospholipids (Phospholipon® 90 – PL90) were obtained by courtesy of Phospholipid GMBH, Cologne, Germany. According to the specification, the PL90 mixture is composed of phosphatidylcholine 98 % and lyso-phosphatidylcholine 2.1 %, where the phospholipids′ fatty acid composition is palmitic acid 12 ± 2 %, stearic acid 3 ± 1 %, oleic acid 10 ± 3 %, linoleic acid 66 ± 5 % and linolenic acid 5 ± 2 %; the peroxide value maximum is 1.3. Thiobarbituric acid (TBA), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), trichloroacetic acid (TCA), methanol, sodium hydroxyde and standards of caffeic acid, quercetin and trolox were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. General synthetic procedures

Compounds **1** [14], **2-5** and **7-10** [13], and **11-18** [12] were synthesized following procedures as described in Tarzia et al., 2003 [14], Lauria et al., 2018 [13] and Vago et al., 2017 [12], respectively (Fig 1). Melting point determination and NMR spectra confirmed the structures. Purity of all products (>98%) was verified by thin-layer chromatography and NMR measurements [12–17]. The new compound **6** was synthesized according to procedure reported by Lauria et al., 2018 [13] through Suzuki coupling reaction via the key biphenylamine intermediate, involving phenylboronic acid, 3-bromoaniline and a suitable palladium catalyst (intermediate yield 72%).

2.2.1. Synthesis of phenyl [1,1'-biphenyl]-3-ylcarbamate (**6**)

Carbonyldiimidazole (CDI) (115 mg, 7.08 mmol, 4 eq.) and [1,10-biphenyl]-3-amine (300 mg, 1.77 mmol, 1 eq.) were mixed in dry toluene (8 mL) and heated at reflux for 12 h under nitrogen atmosphere. After cooling to room temperature white precipitates were collected by filtration. The product, purified by chromatography on silica gel (petroleum ether:EtOAc, 90:10 v/v), was obtained as a white solid in 82% yield. mp: 96-97 °C. TLC (petroleum ether: EtOAc, 80:20 v/v): Rf=0.38; ^{1}H NMR ($CDCl_3$)δ 7.78 (1H, bs, 2-H), 7.63 (2H, d, J=7.3 Hz, 2', 6'-H), 7.49-7.35 (8H, m, 3', 4', 5', 4, 5, 6, 2 x *meta*-H, 8 x Ar-H), 7.29 (1H, dd, J=8.7, 1.8 Hz, *para*-H), 7.23 (2H, d, J=8.7 Hz, 2 x *orto*-H), 7.08 (1H, bs, NH); ^{13}C NMR δ: 150.5 (CO), 142.3, 140.6, 137.8, 129.5,

129.4, 128.8, 127.6, 127.2, 125.8, 122.8, 121.7 and 117.6 (*C*-Ar). Anal. Calcd. for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 78.51; H, 5.45; N, 4.71. For NMR spectra see Supplementary data.

2.3.Lipid peroxidation inhibition by thiobarbituric acid-malondialdehyde test

Lipid peroxidation (LP) and its inhibition in the presence of the tested compounds (**1-18**), was measured by thiobarbituric acid-malondialdehyde (TBA–MDA) test according to the slightly modified procedure described by Zvezdanovic *et al.* [18] and Mavrova *et al*. [19]. The modification consisted in altered volume ratio of methanol solution of PL-90, aqueous solution of hydrophilic thermal LP initiator AAPH and of methanol solution of the compounds tested (**1-18**) allowing substances to be assayed at concentrations of 500 μM in the final reaction mixture (volume ratio 2:1:2 instead of 2:2:1, respectively). Experiment was designed so that methanol concentration was set constant in all probes for which the absorbances were read.

The absorbance of TBA–MDA complex in the supernatant was read at 530 nm and used to calculate the inhibition percentage of lipid peroxidation given by the equation:

Inhibition of lipid peroxidation (%) = 100 x (Ac-As)/(Ac-Ab)

Ac - the absorbance of control (methanol solution of PL90) which is treated with the AAPH and TBA solution, As -the absorbance of sample (methanol solution of PL90/1-18) which is treated with the AAPH and TBA solution and Ab - the absorbance of blank [(methanol solution of PL90 not treated by AAPH, but treated with TBA solution (monitoring MDA level in the lipid before LP initiation by AAPH)].

Samples were assayed for LP-inhibitory activity and those showing inhibition greater than 50% at 500 μM were tested in a broader concentration range to allow calculation of IC_{50} values. The same experiments were done by using known antioxidants as standards (trolox, quercetin and caffeic acid).The standards were assayed at concentrations of 50 μM (caffeic acid) and 80 μM (quercetin and trolox) in the final reaction mixture.

2.4. DFT computations

The computational study of molecular geometry and reaction enthalpies was carried out by the use of Gaussian 09 suite of programs [20]. Hybrid functional B3LYP in conjunction with 6-311++G(d,p) basis set was used in all calculations [21,22]. The solvent effects were taken into

account by including the Integral Equation Formalism Polarizable Continuum Model (IEF-PCM) in benzene and water [23]. The optimized structures were ascertained as minima on the potential energy hypersurface based on analytic vibrational frequency computations. No imaginary frequencies were found for all structures.

Dissociation enthalpy (BDE), ionization potential (IP) and proton affinity (PA) of the most stable conformers were calculated at 298 K according to the procedure established by Klein *et al.* [24]. The enthalpy of hydrogen atom, H(H), in benzene and water were obtained using the same functional and basis set. Solvation enthalpies of proton, $H(H^+)$, and electron, $H(e^-)$, were taken from the literature [25].

3. Results and discussion

Derivatives included in our present research (Fig. 1) were part of previous studies involving either MAGL [9,11] or NAAA [10] inhibition testing, with exception of phenyl [1,1'-biphenyl]-3-ylcarbamate (**6**) which is a new compound, with spectroscopic data reported herein for the first time (for details see Fig 1S). First group includes compounds **2**-**10** synthesized based on structural modifications of the parent structure URB602 (**1**) [14], to evaluate new derivatives potentially targeting MAGL [13]. According to these results [13], compounds **8** and **10** were recognized as potent *h*MAGL inhibitors with IC_{50} 4.5 ± 0.7 and 7.9 ± 0.8 μM respectively, while a symmetric compound **3** appears to be an interesting MAGL activator. Second group, comprised of retro-amide derivatives of palmitamine (**11**-**18**), was designed, synthesized and characterized applying the coupling reaction conditions optimized for the synthesis of endogenous cannabinoids [26,27] aiming towards identification of new NAAA inhibitors. Based on these results, compounds **14**, **16** and **18** were found to represent competitive inhibitors of *h*NAAA characterized by interesting inhibitor activity (IC_{50} 20 ± 0.9 μM, IC_{50} 11 ± 1.0 μM and 38 ± 1.0 μM respectively) [12]. Both MAGL and NAAA inhibitors showed relevant anticancer activity on melanoma and bladder cancer respectively. Given the involvement of *oxidative stress* in cancer development and outcome [5,6], here we investigate the antioxidant activity of synthesized inhibitors to evaluate a possible antioxidant effect in addition to the anticancer activity of these compounds.

Lipid peroxidation inhibition effect of ten carbamates **1**-**10**, among which compound **6** reported for the first time, and eight retro-amide derivatives of palmitamine **11**-**18** was measured using the

method based on TBA-MDA assay, selecting caffeic acid, toxol and quercetin as positive controls. TBA-MDA based method is the most commonly used spectrophotometric assay to measure LP and takes advantage of a pink-colored MDA-$(TBA)_2$ formation, an adduct characterized by strong absorbance at 530 nm [2]. The assay is widely used and though having limitations (e.g. does not monitor the total kinetic aspects of LP inhibition but rather the extent of LP estimated after a fixed time, in-depth discussion is provided in excellent reviews [3,28-30]) is recognized as a reliable estimator of LP [3,28-30] having commercial kits also available [28]. LP experiments were performed, the obtained results were plotted and IC_{50} values were calculated and reported in Table 1 and in Supplementary data (Fig. S1).

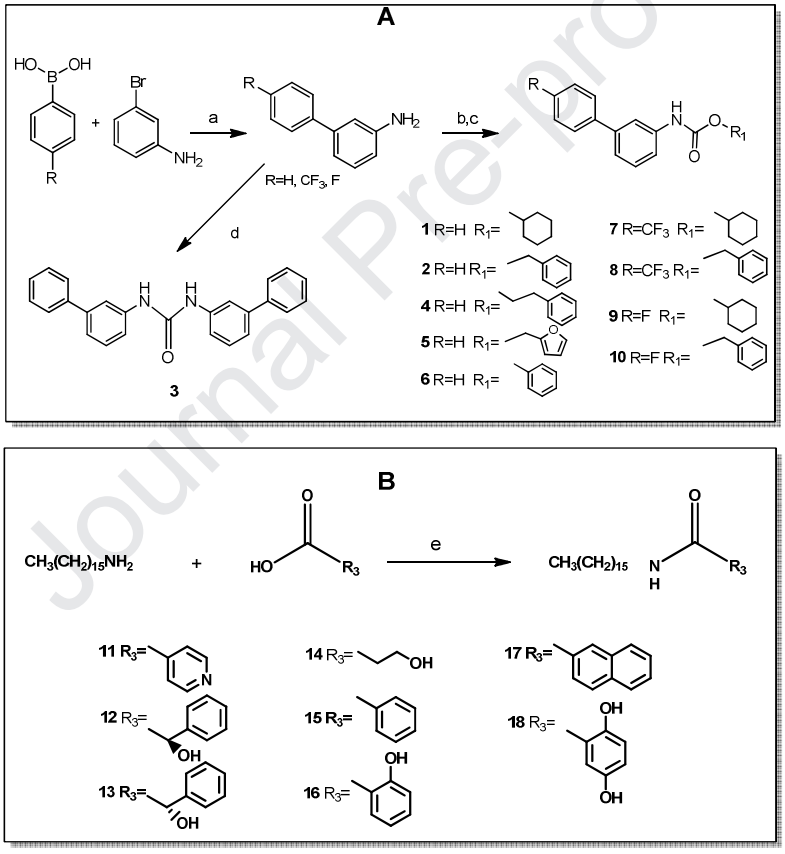


Fig. 1. Synthesis of the assayed compounds **1-18.** Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, CH_3OH, reflux, 12 h; (b) CDI, DMAP, CH_3CN, reflux, overnight; (c) R_1-OH, CH_3CN, reflux; d) CDI, DMAP,CH_3CN, reflux, overnight; e) COMU, CH_2Cl_2/CH_3CN (3/1), DIPEA, r.t., nitrogen atmosphere.

The obtained results indicated that most of the tested compounds have good antioxidant properties, except compounds **11** and **14** having $IC_{50} > 500$ μM, and **7**, **12** and **13** that have IC_{50}

comprised between 450 and 500 μM. On value range of LP inhibition displayed by compounds **1**, **2**, **4**, **6**, **8-10** and **15** (values between 133± 32 and 309± 72) a possible additive antioxidant effect cannot be considered. Three interesting compounds are **5**, **16** and **18** that showed LP inhibition with IC_{50} below 100 μM (85 ± 17, 82± 10and 53 ± 12 μM, respectively, Table 1). The most potent LP inhibition was expressed by compound **3** (IC_{50} = 26± 6 μM), which gave a result comparable to trolox and quercetin as antioxidant standards tested (Table 1) and, for this reason, was further involved in theoretical calculations aiming the clarification of the mechanism at the base of an antioxidant action.

Table 1. Lipid peroxidation inhibition effects of compounds **1-18** and of selected antioxidants (IC_{50} values given in μM)

Compound No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
LP inhibition IC_{50} (μM)	271 ± 86	133± 32	26± 6	213± 44	85 ± 17	142± 33	458 ± 88
Compound No.	**8**	**9**	**10**	**11**	**12**	**13**	**14**
LP inhibition IC_{50} (μM)	250± 27	309± 72	158 ± 38	> 500	469 ± 158	488± 164	> 500
Compound No.	**15**	**16**	**17**	**18**	**Caffeic acid**	**Trolox**	**Quercetin**
LP inhibition IC_{50} (μM)	176 ± 43	82± 10	179± 30	53 ± 12	15± 3	22± 6	23 ± 6

Being influenced by electronic effects of the neighboring groups and the overall geometry of the molecule, the conformation can be regarded as the first parameter of importance in analyzing the antioxidant capacity of any molecule [31]. In order to determine the most stable molecular geometry of compound **3** we have fully optimized the most probable conformations with different orientation of the biphenyl fragments (Fig. 2) at IEFPCM-B3LYP/6-311++G** level of theory in both, benzene and water. Based on the calculated relative energies, it was found that

the molecular structure is stabilized by the formation of short contacts between the amide O-atom and the α-phenyl C-H bonds (conformer C1 in Fig. 2). However due to the possibility of effective resonance stabilization in all the three conformers, the energy differences between them are almost neglectful - less than 0.1 kJ/mol. Therefore, it could be expected that the different conformers could interconvert freely into each other by rotation around the C-N bond in solution.

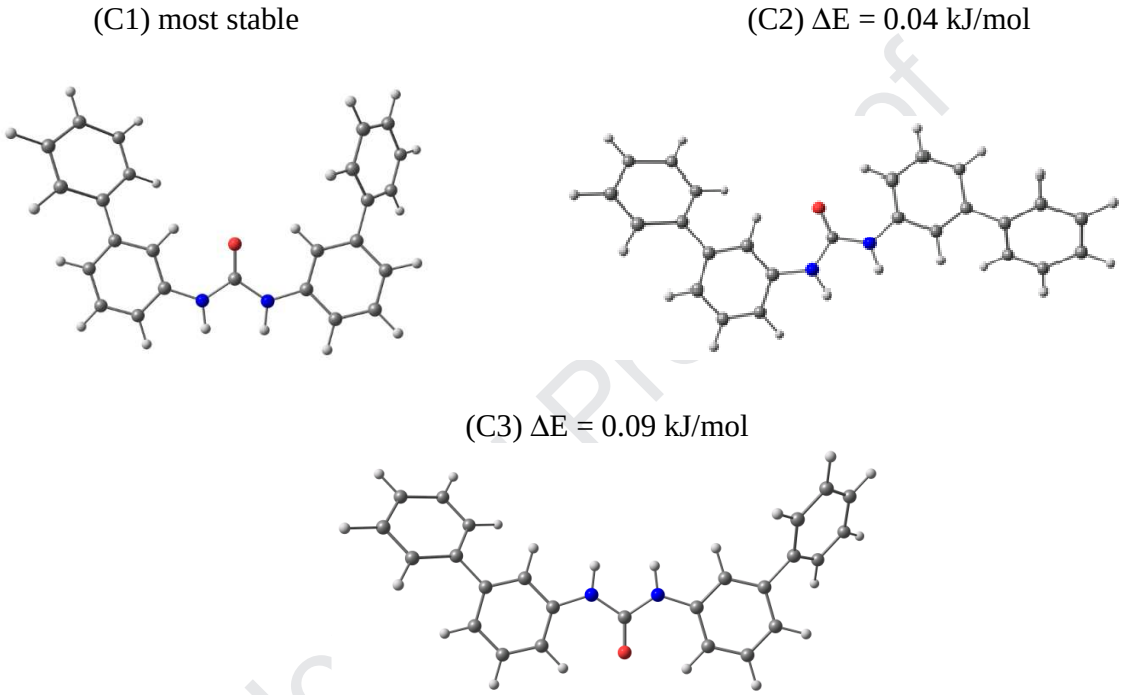


Fig. 2. DFT B3LYP/6-311++G** optimized structure of the most probable conformers of compound **3** in benzene

The radical scavenging mechanism of compound **3** was modeled in both nonpolar and polar mediaby calculating the reaction enthalpies for hydrogen atom transfer (HAT mechanism), single electron transfer (SET) and sequential proton loss electron transfer (SPLET) at the same level of theory. Nonpolar medium was represented by benzene while as a description of polar medium water was used.

In nonpolar medium where the ionization is not supported by the solvent, the radical scavenging usually proceeds by direct hydrogen atom transfer. In line with this, DFT calculations predicted that for compound **3** the most favorable would be to donate a hydrogen atom to the free radical and form a N-centered radical (Fig. 3). The corresponding N-H bond dissociation enthalpy (BDE) in benzene is 367 kJ/mol.

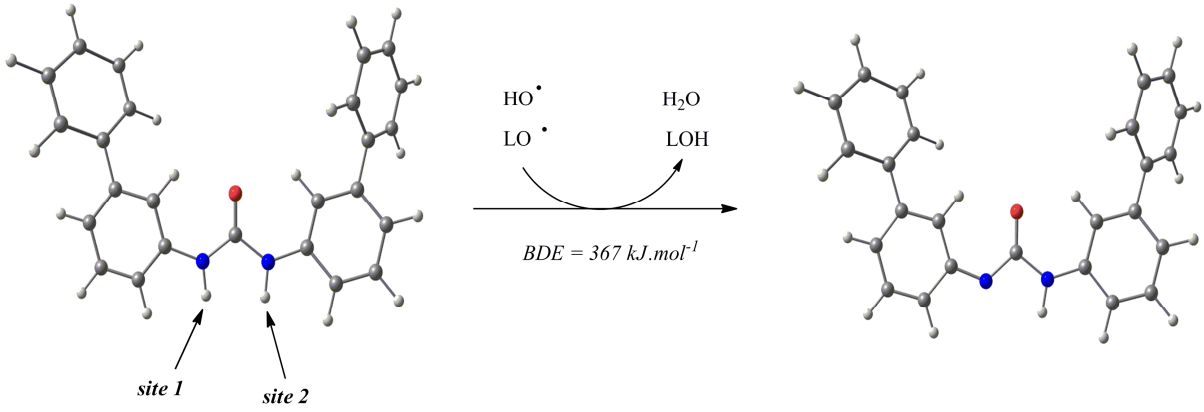


Fig. 3. Hydrogen atom transfer mechanism of compound **3** in nonpolar medium

The BDE values of free radicals in benzene were estimated by the radicals of (*Z*)-4-hydroperoxyhex-2-ene, (*Z*)-4-hydroxyhex-2-ene, methyl hydroperoxide, methanol, hydrogen peroxide, and water and used to establish the reactivity of compound **3**. The corresponding BDE values are as follows: 339 kJ/mol ((*Z*)-4-hydroperoxyhex-2-enyl radical), 344 kJ/mol ($CH_3OO^{\bullet}$), 353 kJ/mol ($HOO^{\bullet}$), 424 kJ/mol ((*Z*)-4-hydroxyhex-2-enyl radical), 418 kJ/mol ($CH_3O^{\bullet}$), 489 kJ/mol ($HO^{\bullet}$).

Comparing the BDE value of compound **3** with those of the model free radicals, it is obvious that compound **3** could efficiently scavenges the lipid alkoxyl, methoxyl and hydroxyl radicals. Therefore, despite it lacks chain-breaking capacity, compound **3** could exert protective effect by deactivating the hydroxyl radicals and thus inhibiting the initiation of lipid peroxidation as well as by deactivating the alkoxyl radicals LO formed from the reduction of lipid peroxides and thus decreasing the harmful effects of the lipid peroxidation. Moreover, there are two sites for hydrogen atom abstraction in the molecule of compound **3** with equal reactivity which enables one molecule of the antioxidant to react with two free radicals. Well-known antioxidants that also react via HAT mechanism are α-tocopherol, curcumin, epigallocatechin galate and caffeic acid [18].

In water i.e. polar medium, the calculated proton affinity PA of compound **3** is 221 kJ/mol. This value is markedly lower than the BDE value in water (364 kJ/mol) and ionization potential IP in water (460 kJ/mol),and in this way the proton transfer from compound **3** becomes more favorable than hydrogen atom donation or electron transfer. Based on that, it could be concluded that free radical scavenging by SPLET mechanism is prevailing over HAT and SET, as according to thermodynamics is the most feasible reaction route in polar medium (Fig. 4).

Similarly, to the H-atom abstraction, proton transfer is equally possible from the two N-H groups of compound **3**. It was shown that radical scavenging through homolytic or heterolytic N–H bond cleavage depends on the medium polarity, substituent effects and other structural factors [25,32,33]. On the other hand, diverse amide derivatives were shown to easily deprotonate in polar medium [34–37].

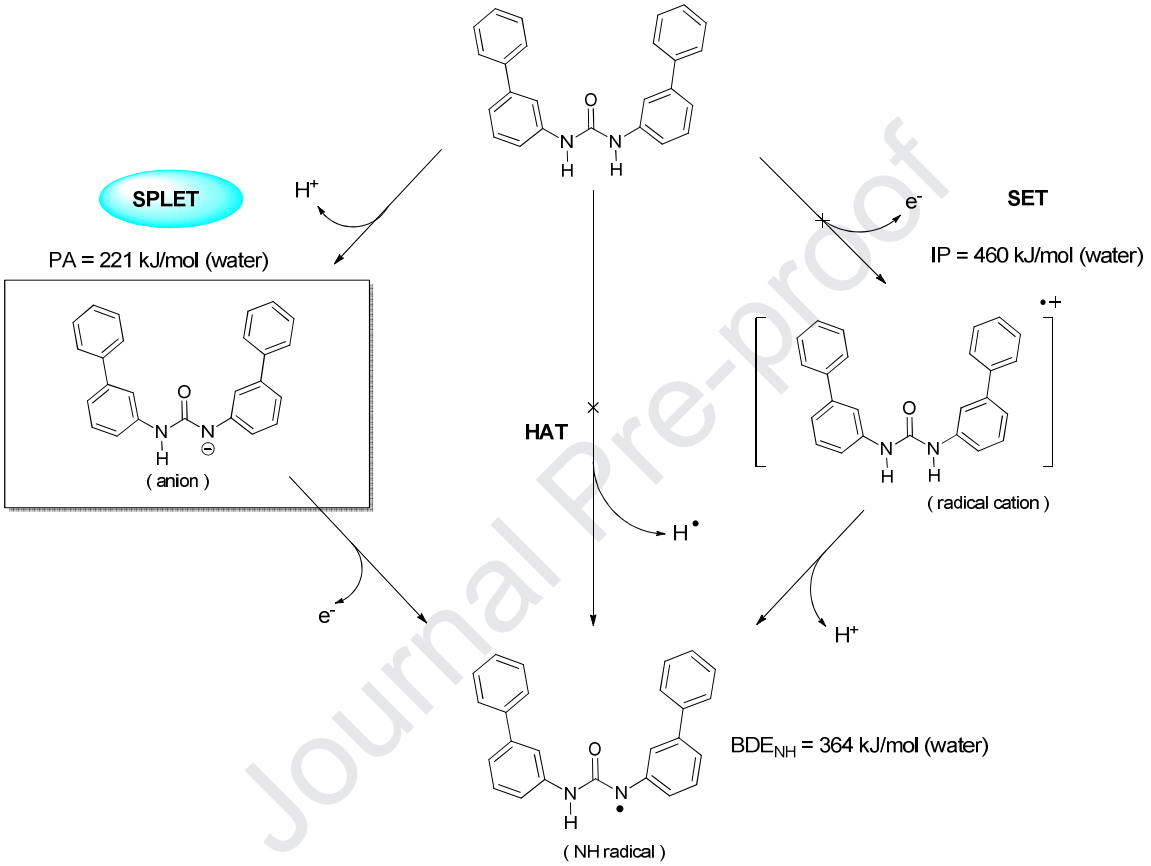


Fig. 4. The most probable mechanism of antioxidant action of compound **3** in polar medium (water)

In theoretical investigations a similar mechanism of antioxidant activity has been proposed for flavonoids [31,38-41], effective bioactive compounds found in foodstuff, which overall intake [42] is considered to be negatively correlated with the incidence of some chronic diseases including cardiovascular diseases, type II diabetes, neurodegenerative diseases, and cancers [43–46].

The radical scavenging mechanisms of compound **18** characterized by the best antioxidant properties among the second group of tested compounds,(retro-amide derivatives of

palmitamine) was characterized by a model analogue, where the C_{16} alkyl tail was replaced with a C_4 residue. The optimization of the most probable molecular structures with different possible orientation of the amide, hydroxyl and alkyl groups and respective intramolecular hydrogen bonding, at IEFPCM-B3LYP/6-311++G** level of theory in benzene and water, established the most stable geometry of the neutral compound (Fig. 5) which was further used in the study of the radical scavenging mechanisms.

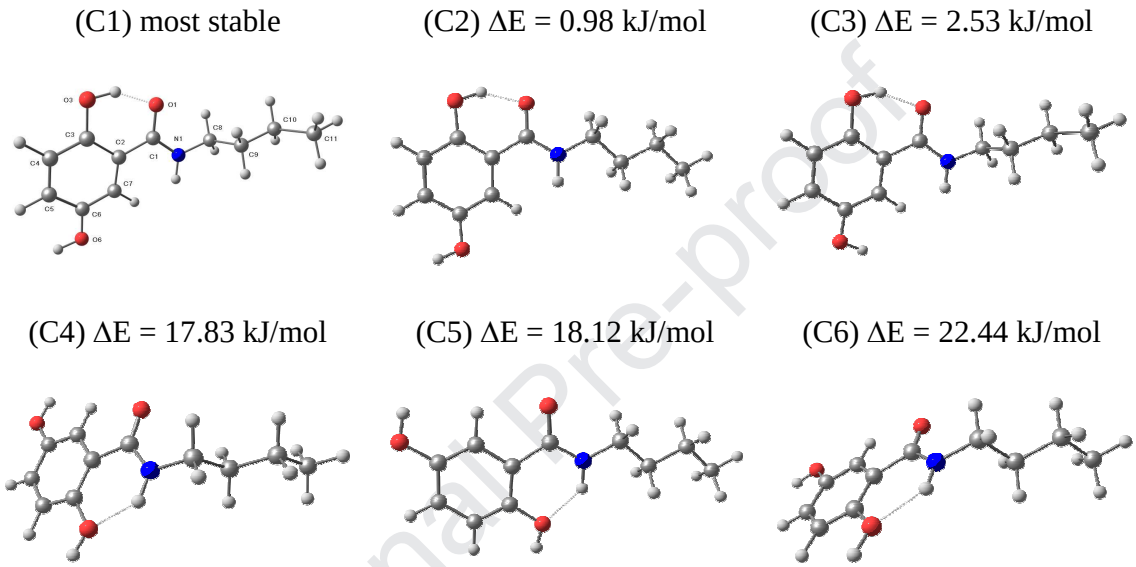


Fig. 5. DFT B3LYP/6-311++G** optimized structure of the most probable conformers of compound **18** in benzene

The total energies (E_{tot}) and relative energies (ΔE) of the radical and anionic forms of the model compound, collected in Table 2, showed that the formation of hydroxylic O6-radical is thermodynamically favored in benzene, as well as water. On the ther hand, deprotonation of the O3-H group is considerably more favorable than from O6-H and N1-H as it could be seen from the relative stability of the formed anions and the respective proton affinities in benzene and water (Table 2).

Table 2. Relative stability of the radical and anionic species, and reaction enthalpies for the model analogue of **18** (total energies E_{tot} - in Hartrees; the relative energies ΔE and reaction enthalpies BDE, IP and PA - in kJ/mol) in benzene and water

	Relative stability	**Radical scavenging mechanisms**

Species[a]	Benzene		Water		Reaction enthalpies	Benzene	Water
	E_{tot}	ΔE^b	E_{tot}	ΔE			
Molecule	-708.624405		-708.632872		***HAT mechanism***		
Radical O6$^{\cdot}$	-708.002388		-708.010972		BDE (*site 1* - O6$^{\cdot}$)	325	315
Radical O3$^{\cdot}$	-707.997215	13.58	-708.007012	10.40	BDE (*site 2* - O3$^{\cdot}$)	339	326
Radical N1$^{\cdot}$	-707.961362	107.71	-707.968441	111.67	BDE (*site 3* - N1$^{\cdot}$)	434	428
					SET mechanism		
Radical cation	-708.385589		-708.421397		IP	622	452
					SPLET mechanism		
Anion O3^{-}	-708.131270		-708.172454		PA (*site 1* - O3^{-})	407	192
Anion O6^{-}	-708.122939	21.87	-708.166598	15.37	PA (*site 2* - O6^{-})	427	207
Anion N1^{-}	-708.115111	42.43	-708.155070	45.64	PA (*site 3* - N1^{-})	448	237

[a] Numbering acc. to Fig. 5A; [b] in respect to the most stable form

Thus it could be concluded that in nonpolar medium the retro-amide derivative **18** would deactivate the free radicals preferably by hydrogen atom transfer from the hydrohyl group in *m*-position (Fig. 6). According to the calculated enthalpies the compound should be able to trap the hydroxyl, alkoxyl and peroxyl radicals.

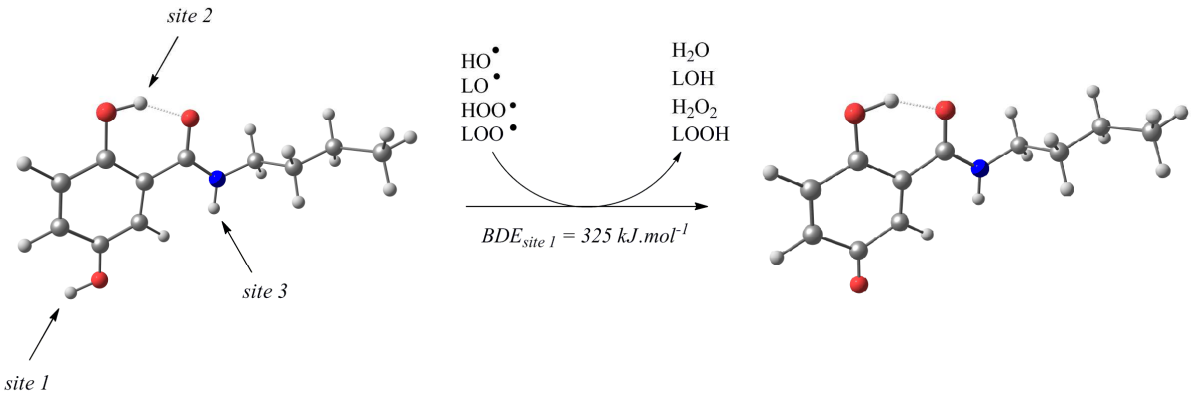


Fig. 6. Hydrogen atom transfer mechanism of compound **18** in nonpolar medium

In polar medium – water, the preferred mechanism would be SPLET by deprotonation at the *o*-hydroxyl group (Fig. 7). In water, the calculated proton affinities PA from all possible sites of compound **18** are much lower than the BDE values from any of the sites and the respective ionization potential IP (Table 2).

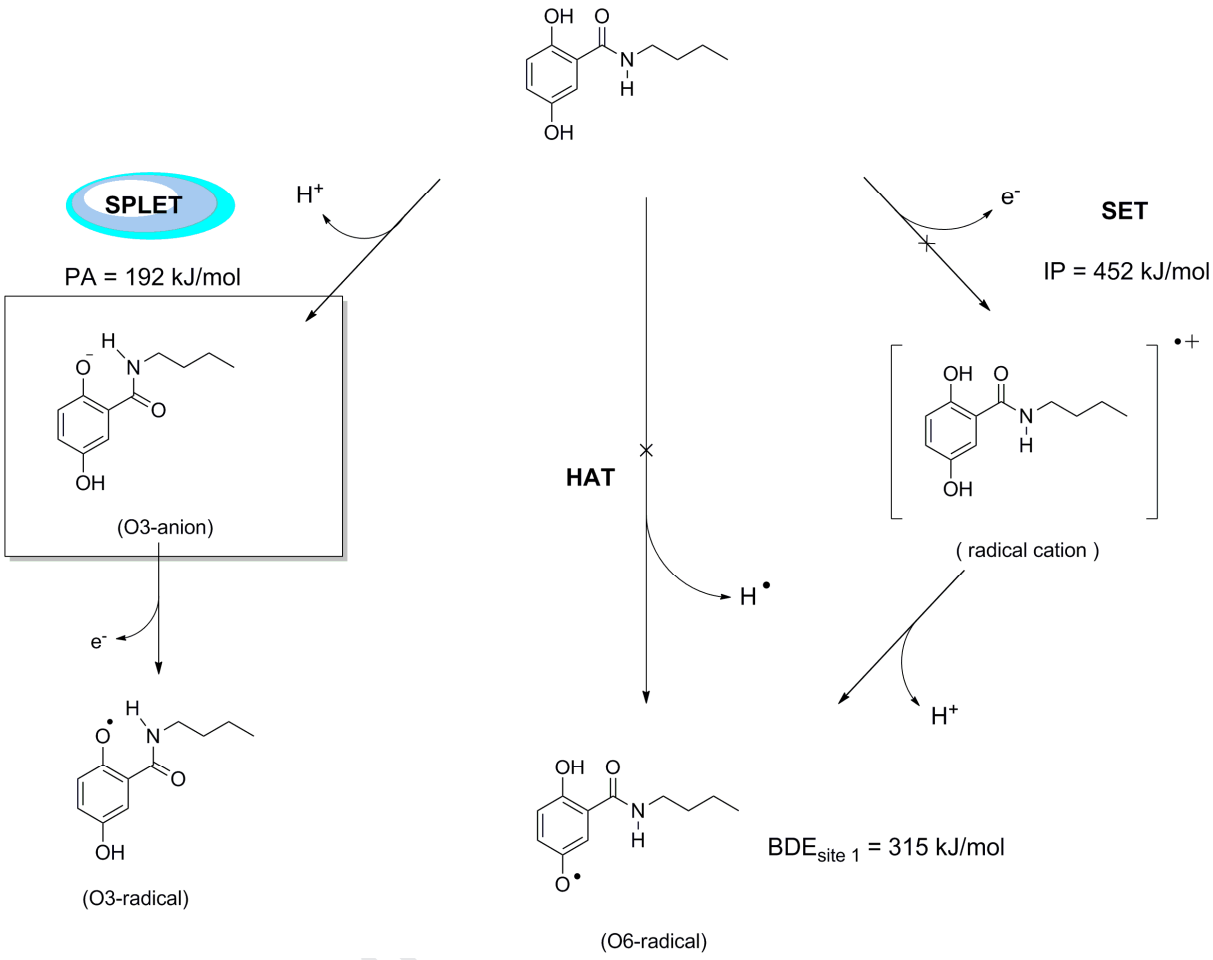


Fig. 7. The most probable mechanism of antioxidant action of compound **18** in polar medium (water)

4. Conclusion

In this study we evaluated the antioxidant activity on lipid peroxidation of eighteen inhibitors of the hydrolytic enzymes of the endocannabinoid system. Most of the tested compounds showed good antioxidant properties in our *in vitro* LP assay, with an interesting activity displayed by compounds **16** and **18**, the two retro-amide derivatives caring a phenyl and hydroxyl-phenyl group linked to the palmitate. Surprisingly, compound **3** demonstrated an antioxidant activity comparable to that of standard antioxidants, trolox and quercetin. The mechanisms based on DFT calculations for compound **3** in non-polar medium predicted a donation of a hydrogen atom to the free radical and formation of N-centered radical, while in polar solvents dominate

mechanism of free radical scavenging by SPLET over HAT H-abstraction. Compound **3**, based on comparison of the BDE value with those of the model free radicals, could efficiently scavenge the lipid alkoxyl, methoxyl and hydroxyl radicals and could exert protective effect by deactivating the hydroxyl radicals and thus inhibiting the initiation of LP, as well by deactivating the alkoxyl radicals formed from the reduction of lipid peroxides, thus decreasing the harmful effects of the LP. The computational estimation on the possible radical scavenging mechanisms of the **18**, carried out by a structural analogue where the C16-alkyl chain was replaced by C4-chain, indicated that the most favorable mechanisms are hydrogen atom transfer from the hydroxyl group in meta-position of the benzamide fragment in nonpolar medium, and proton transfer from the hydroxyl group in ortho-position of the benzamide fragment in polar medium. The computational estimations for BDE, IP and PA are not in congruence with the experimental evidences provided. Given the fact that compound **18** is a phenolic compound, in contrary to **3** which should cleave N-H bonds in order to deactivate the free radicals, it is not surprising that based on the computed values (BDE, IP and PA) **18** should be a better radical scavenger. The antioxidant capacity of the phenolic compounds is strongly reduced when the reaction medium consists of a solvent prone to the formation of hydrogen bonds with the phenolic compounds [29,47]. Possible explanation for this fact may need to be sought in reaction medium we were using (aqueous solution of methanol) or may be different lipophylicity of the samples tested. In any event, these preliminary *in vitro* results suggest an interesting antioxidant role of the tested compounds in addition to the anticancer effects.

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Conflict of interests

The authors declare no conflict of interests

Authors contributions

J.L. and P.C. conceived and designed the study, S.C. and R.O. performed the chemical synthesis, J.L. J.Z. and A.S. performed LP experiments, D.Y. performed theoretical calculations. All the authors contributed to the paper writing.

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Highlights

- antioxidant activity was evaluated using lipid peroxidation (LP) method
- activity of the most potent compound (**3**) was comparable with standard antioxidants
- theoretical calculations were involved in order to clarify a possible mechanism of action
- appropriate models for HAT, SET and SPLET-mechanisms of the antioxidant activity are proposed

Declaration of interests

XThe authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: