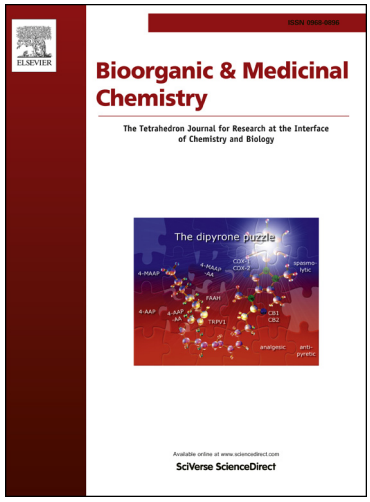

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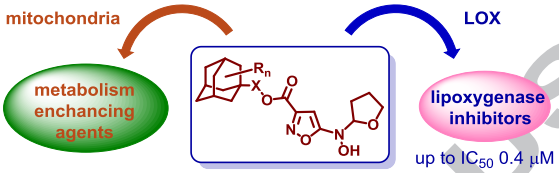

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ABSTRACT

A versatile synthesis of novel 5-hydroxylaminoisoxazoles bearing adamantane moieties has been accomplished using the heterocyclization reactions of readily available unsaturated esters by the treatment with tetranitromethane in the presence of triethylamine and subsequent reduction of resulting 5-nitroisoxazoles by $SnCl_2$ with the participation of THF. A number of obtained isoxazole derivatives were evaluated for their antioxidative activity, inhibition of lipoxygenases and impact on the rat liver mitochondria. The majority of tested compounds demonstrated moderate antiradical activity in DPPH test (up to EC_{50} 16 μM). The same compounds strongly inhibited soybean lipoxygenase (up to IC_{50} 0.4 μM) and Fe^{2+}- and Fe^{3+}-induced lipid peroxidation (LP) of rat brain cortex homogenate (up to IC_{50} 0.3 μM). All tested isoxazole derivatives promoted the phosphorylating respiratory activity simultaneously with maximal stimulated respiratory activity of mitochondria and do not reveal any toxicity towards the primary culture of rat cortex neurons.

1. Introduction

Oxidative stress induced by reactive oxygen species has been implicated in the pathogenesis of various diseases and disorders. One of the main features and biomarkers for oxidative stress is the lipid peroxidation, which may be induced through non-enzymatic or enzymatic pathways involving lipoxygenase, cyclooxygenase or cytochrome P450. Lipoxygenases (LOXs) are non-heme iron-containing dioxygenases which catalyze the conversion of unsaturated fatty acids containing one or more *cis,cis*-pentadiene fragments into hydroperoxy fatty acids.[1-4] This process is the first step in a series of reactions producing inflammatory-mediating leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs) which are associated with a variety of disease states including asthma, rheumatoid arthritis, inflammatory bowel disease, psoriasis, and allergy.[1] The human lipoxygenases are indisputably involved in human pathology not only due to their essential role in LTs and HETEs biosynthesis, but also due to their involvement in induction of lipid peroxidation. Therefore, a search for selective lipoxygenase inhibitors and multitarget antioxidants with LOX-inhibiting and antioxidant activities is of a considerable interest. Although many attempts to develop selective LOX inhibitors have been made,[5-10] Zileuton, which is an iron-binding 5-LOX inhibitor with antiasthmatic action, remains the only approved drug product.

Recently we described the first synthesis of previously unknown 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles **1** via unusual reduction of 5-nitroisoxazoles with the participation of THF used as solvent,[11] as well as the novel approach to hardly accessible functionalized 5-nitroisoxazoles based on the heterocyclization of electrophilic alkenes under the action of tetranitromethane (TNM) – triethylamine complex[12,13] (Scheme 1).

In the present work we proposed a new scaffold for structure design of LOXs inhibitors – novel *N*-hydroxylaminoisoxazoles **1**

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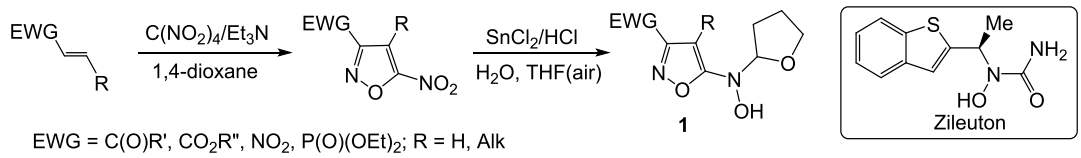


Scheme 1. A described procedure[11-13] for the synthesis of *N*-hydroxylaminoisoxazoles **1**.

possessing the fragment of THF which appear to be certain structural analogue of Zileuton.

All lipoxygenases are homologues and contain the same two domains: N-terminal "C2-like" domain and a larger C-terminal catalytic domain containing a single atom of non-heme iron.[1] Indeed, it was shown in various investigations that the molecules with bulky lipophilic fragments generally exhibit an efficient inhibition of enzymes, including LOXs.[14-19] Particularly adamantane-based compounds, due to their specific conformation, frequently demonstrated the enhanced interaction with the target enzyme's active site.[20] Moreover, the introduction of adamantane moieties increases the membrane permeability of the modified compound.[20] Therefore we introduced the adamantane framework in target heterocycles **1** employing synthetically available α,β-unsaturated esters for heterocyclization. The versatility of our synthetic methodology allowed to prepare a library of compounds **1** by varying the substituents in the 3-position of isoxazole core.

Herein, we report the design and synthesis of novel adamantane-containing 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles **1** and evaluation of their antioxidative potential, as well as LOX inhibition and mitochondria functional characteristics.

2. Results and discussion

2.1. Chemistry

For the synthesis of novel hydroxylamines **1a-h**, we proposed a three-step scheme including the acylation of adamantane-containing alcohols **2a-h** by acryloyl chloride followed by the heterocyclization of unsaturated esters **3a-h** employing our previously published procedure[12,13] (Table 1). Subsequent reduction of 5-nitroisoxazoles **4a-h** by $SnCl_2$-HCl in THF resulted in desired heterocycles **1a-h**.

The preparation of the starting alkenes **3a-h** from the alcohols bearing the adamantane moieties proceeds smoothly under reported conditions[21] using equimolar amounts of acryloyl chloride and Et_3N. Somewhat poorer yield was obtained for alkene **3g** (Table 1, entry 7) due to low solubility of corresponding alcohol in CH_2Cl_2. Dilution of the reaction mixture or the use of ether solvents (Et_2O, THF, 1,4-dioxane) did not provide any substantial increase in the alkene yield. The electrophilic alkenes **3a-h** were involved in the heterocyclization upon the treatment by *in situ* generated $C(NO_2)_4$-Et_3N complex resulting in 5-nitroisoxazoles **4a-h** with satisfactory yields. In some cases the decrease in yields of the heterocycles **4e, f** may result from the partial oxidation of alkene **3f** (Table 1, entry 6) under the reaction conditions or from the formation of small amount of 3,3,3-trinitropropanoic acid esters[12] (Table 1, entry 5). For the successful transformation of 5-nitroisoxazoles **4a-h** into desired 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles **1a-h**, THF, previously distilled from NaOH, must be bubbled by air for 20 hours. All synthesized compounds were characterized and identified using ^1H and ^{13}C NMR and HRMS.

2.2. Biological evaluation

2.2.1. Lipoxygenase inhibitory assessment

Since peroxyl radical is formed in all LOX-catalyzed reactions, LOX activation evokes lipid peroxidation. During oxidative stress, LOX can exhibit uncontrolled activity and may cause cell membrane destruction by phospholipid oxidation. Thus, lipoxygenase inhibition under pathologic conditions helps to decrease the level of total oxidative stress.[22] The LOX inhibition activity of the studied compounds was evaluated in order to determine capacity of compounds to decrease oxidative stress level. Lipoxygenase-1 from soybeans (LOX-1) has been used in the present study since it shares many important characteristics with LOXs from other sources and represents a routine model for studies on animal system.[23-25]

Compounds **1a-c, g, h** (Table 2, entries 1-3, 7, 8) demonstrate IC_{50} values lower than 50 μM and can be considered as promising lipoxygenase inhibitors. The most active compounds were **1b, c, g** (Table 2, entries 2, 3, 7), the IC_{50} values for these compounds were in the range from 0.4 to 4μM. In the presence of **1g** – one of the most active compounds – the activity of LOX was inhibited almost completely at 50 μM of **1g** (Figure 1). According to literature data[26] and our experimental results (IC_{50} >100 μM) the reference compound Zileuton does not inhibit the soybean LOX, but its inhibitory activity towards human LOX-5 (IC_{50} = 1.1 μM)[7,26] is similar to that of isoxazoles **1b, c, g** towards soybean

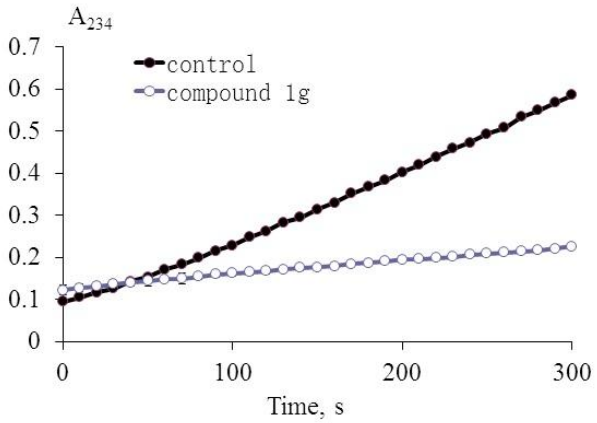
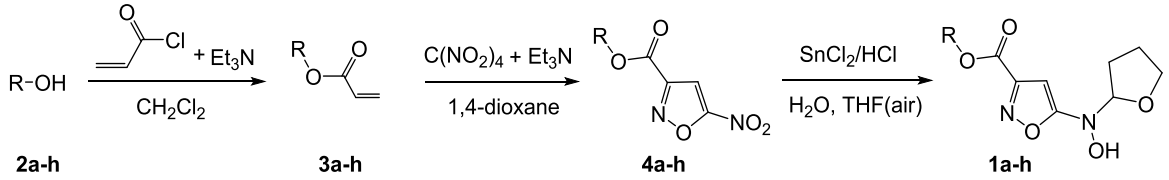


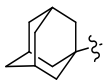
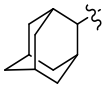
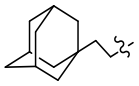
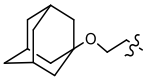
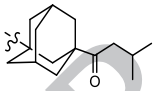
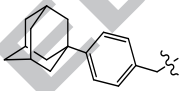
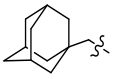
Figure 1. Kinetic curves of linolic acid hydroperoxide formation in the presence of 50μM **1g**

1.1.1. DPPH radical scavenging activity

The radical scavenging activity of compounds **1a-h** has been studied in the process of hydrogen atom transfer from the molecule to the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) producing diphenylpicrylhydrazine and radicals which can undergo further reactions such as coupling, fragmentation and addition. The reaction is accompanied by a color change from violet to yellow, which can be monitored spectrophotometrically by measuring the decrease in absorbance

Table 1. Synthesis of 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles **1a-h** from adamantane-containing alcohols.



Entry	Compound	R	Yield, %[a]		
			3	**4**	**1**
1	**a**		84	57	46
2	**b**		90	58	50
3	**c**		78	84	47
4	**d**		72	55	52
5	**e**		61	33	54
6	**f**		82	36	62
7	**g**		54	63	60
8	**h**		78	68	75

[a] Isolated yields

Table 2. Redox activity and LOX-inhibition (soybean lipoxygenase LOX-1) of 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles **1a-h**.

Entry	Compound	LP, Fe^{3+}	LP, Fe^{2+}	DPPH	LOX-1 inhibition
		IC_{50} μM	IC_{50} μM	(EC_{50} μM)	IC_{50}, μM
1	**1a**	1.37 ± 0.32	0.33 ± 0.19	16±2	25±3
2	**1b**	1.21± 0.20	10.54± 1.34	71±8	4±0.6
3	**1c**	5.25 ± 0.8	3.15 ± 0.93	21±3	0.4±0.1
4	**1d**	35 ± 2	na[a]	>100	na
5	**1e**	1.58 ± 0.07	2.74 ± 1.79	>100	77±6
6	**1f**	5.45 ± 1.52	3.41 ± 1.04	>100	na
7	**1g**	1.48 ± 0.23	5.16 ± 2.4	38±5	1±0.4
8	**1h**	22 ± 3	6.58 ± 1.44	70±5	16±2

at 517 nm.27 Antiradical activity was evaluated as the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50% (Efficient Concentration = EC_{50}). The EC_{50} values reflect the reactivity of a compound toward DPPH but give limited information on the mechanistic outcome of the reaction. The results of the DPPH test at 20 °C show that the most active compounds were **1a-c, g** (Table 2, entries 1-3, 8). The antiradical activity was the highest for compound **1a** (Table 2, entry 1) which completed the reaction with DPPH after several seconds at equimolar ratio of reagents. The compounds **1a-c, g, h** were found to demonstrate high antiradical activity. To compare the antioxidative properties of the tested compounds with the standard, the antioxidative potency of Zileuton was estimated in our experimental conditions. The obtained data clearly indicate that the antiradical activity of Zileuton (this work: EC_{50}=14 μM; for comparison, literature data: IC_{50}=43 μM[28]) is also similar to that of isoxazoles **1a-c, g, h**.

1.1.2. Influence on lipid peroxidation

Not only lipoxygenases (LOX), but also the mitochondrial respiratory chain, nitric oxide synthase, NADPH oxidase and cyclooxygenases (COX) are the main sources of reactive oxygen species in the cells. It was shown that noncompetitive LOX inhibitors may act as reducing agents or iron ligands which affect not only the LOX activity, but also the LOX-independent oxidative stress.[28] Inhibitors of LOX also possess the antioxidant activity, not only by inhibiting the enzymatic activity of LOXs, but also directly by scavenging free radicals. Zileuton, an iron-chelating inhibitor of 5-LOX, was a rather weak free radical scavenger and antioxidant with IC_{50}>50μM against Fe^{2+}-induced lipid peroxidation in rat brain homogenate[28]. However, the new 5-hydroxylaminoisoxazoles **1a-h** reveal significant antioxidant properties. We have studied the influence of these compounds on oxidative degradation of lipids in rat brain homogenate induced by ferrous and ferric ions. All synthesized compounds inhibit Fe^{2+}- and Fe^{3+}-induced lipid peroxidation (LP) of rat brain cortex homogenate. The most active was compound **1a**, but a structurally similar compound **1h** reveals lower activity and the compound **1d** is the weakest inhibitor of LP. The inhibition of ferric and ferrous ion-induced lipid peroxidation by 5-hydroxylaminoisoxazoles for all compounds except **1e** and **1f** has some correlation with their free radical scavenging capacity (Table 2).

1.1.3. Cell viability

At the next step of our work we have studied the influence of the compounds **1a-h** on the viability of the cultured tumor cells (neuroblastoma SH-SY5Y) and primary neuron culture from rat brain cortex (Figure 2). Cell viability was measured by standard MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide) assay after 24 h incubation with 30 μM of isoxazoles **1a-h.** Cell viability significantly decreased in neuroblastoma cells (to 22±1% at 30 μM of **1h** in comparison with the control). In primary cortex neurons only compound **1c** decreased the viability of cells (to 77±5%). Moreover, for compounds **1d-h** a statistically significant increase in the MTT-connected activity was observed. MTT is a tetrazolium salt reduced to purple formazan crystals mainly by mitochondrial succinate dehydrogenase. Thus the use of this assay as an indicator of metabolically active mitochondria may overestimate the number of viable cells due to increasing activity of succinate dehydrogenase or other cellular dehydrogenases in response to some compounds. Thus we have studied the direct influence of our compounds on mitochondrial respiration and other mitochondrial functions.

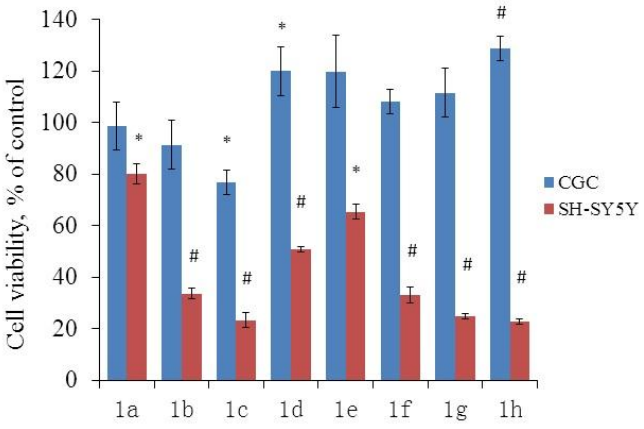


Figure 2. Influence of isoxazoles **1a-h** on the viability of rat cerebellar granule cells (CGC) and neuroblastoma SH-SY5Y. Control samples contain DMSO (≤ 1%). *indicates p<0.05, # - p<0.01 compared to the control cultures (T-test).

1.1.4. Mitochondrial function measurements

Oxygen consumption rate (OCR) by mitochondria was determined using the Seahorse extracellular flux (XF-96) analyzer (Seahorse Bioscience, Chicopee, MA, USA). Respiration of the mitochondria (0.5 mg protein/well) with or without 5-hydroxylaminoisoxazoles **1a, c-h** was sequentially measured in a coupled state with substrate of complex **II** succinate and rotenone to inhibit complex **I** of respiratory chain present (basal respiration), followed by phosphorylating respiration, in the presence of ADP and substrate, nonphosphorylating or resting respiration induced with the addition of oligomycin and then maximal uncoupler-stimulated respiration after addition of uncoupler FCCP, followed by nonrespiratory oxygen consumption in the presence of inhibitor of complex **II** antimycin A (Figure 3a).

Treatment of mitochondria with isoxazoles **1a, c-h** led to an increase in phosphorylating respiration and moreover, in maximum stimulated respiration after uncoupling of oxygen consumption (or electron transport) and ATP synthesis with FCCP treatment (Figure 3b). But there was no noted increase in OCR in the presence of olygomycin and we cannot propose the uncoupling mode of action of the compounds on mitochondrial respiratory chain.

The electron transport along the mitochondrial respiratory chain is accompanied by the formation of electrochemical gradient of hydrogen ions at the inner mitochondrial membrane. The electrical part of electrochemical potential, i.e. $\Delta\Psi$, can be measured in isolated mitochondria by changing the absorbance of the cationic dye safranine. We have shown that compounds **1a-h** do not influence $\Delta\Psi$ and calcium-induced depolarization of mitochondria regardless of the used substrates of mitochondrial respiration: succinate and rotenone or glutamate/malate (data not shown). These results are in agreement with the above mentioned absence of any change in oligomycin-dependent oxygen consumption. The increase of the phosphorylating respiration (with succinate as substrate) and of the maximal stimulated respiration caused by the studied compounds may be the reason of the overestimated MTT-dependent index of cell viability. However, more significantly, these results allow one to suggest that 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles **1a-h** may be useful as a scaffold for the development of novel metabolic enhancers.

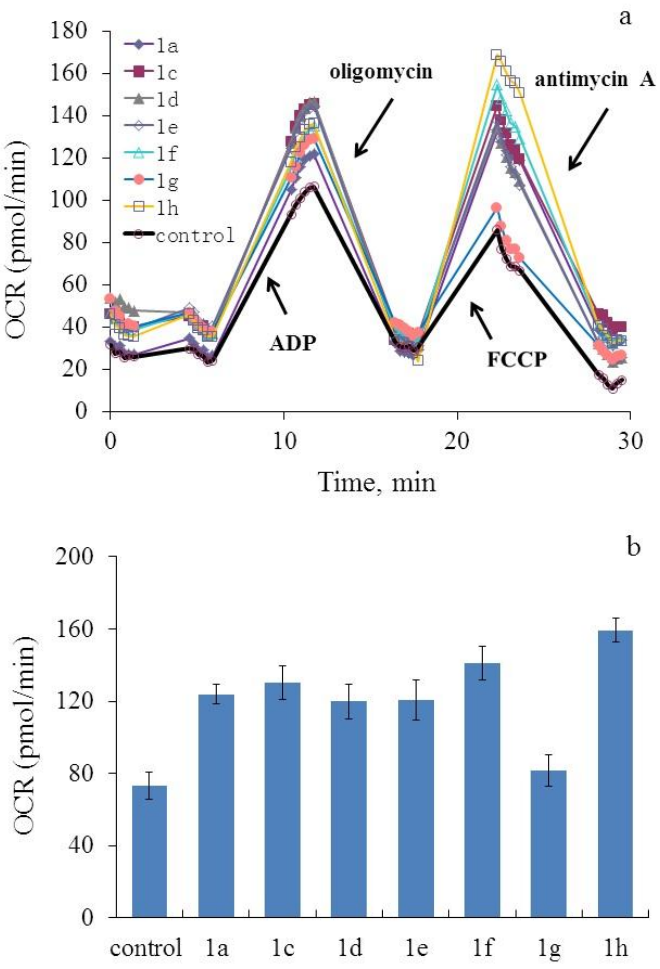


Figure 3. Influence of 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles on representative respirometric profile of mitochondria isolated from rat liver in the presence of 10 μM of compounds **1a, c-h** (**a)** and FCCP-stimulated OCR (**b**). Final concentrations of additives after port injections were 4 mM ADP; 2,5 μg/ml oligomycin; 4 μM FCCP; and 4 μM antimycin A. Calculated RCR was 7.95±0.8.

2.3. Structure-activity relationships and physico-chemical properties

Analysis of this limited series of compounds suggests that their biological activity is defined by a common structural scaffold. The elongation of the linker between adamantane and isoxazole moieties (**1d,f**) as well as the enlarging of the adamantane core (**1e**) lead to a decrease in antiradical activities and inhibition of lipoxygenase.

We have performed a preliminary computational estimation of some physicochemical and pharmacokinetic properties of the studied compounds that may be relevant in the drug development context. In particular, the lipophilicity (logP) and aqueous solubility values were predicted as well as the blood-brain barrier permeability (LogBB), human intestinal absorption and cardiotoxicity potential (hERG affinity). The predicted values are presented in the Supplementary Material (Table S1). No direct correlations between the physicochemical parameters and *in vitro* biological results can be discerned. However, for most of the compounds (except **1f**) the properties seem quite acceptable for a lead candidate. Their LogP values are about 2–2.5 and the solubility is about 10^{-4} M that is significantly higher than IC_{50}. LogBB for all compounds is close to zero that corresponds to about 50% brain penetration, intestinal absorption is about 80–100%, and hERG pK_i is about 4.5. In fact, the properties of the studied compounds are comparable or better than those of the reference compounds (Zileuton, BW-B 70C and Phenidone). In particular, the predicted blood-brain barrier permeability of the adamantane derivatives is significantly higher than that of Zileuton and BW-B 70C, which may be important in the therapy of the brain oxidative stress.

3. Conclusions

In conclusion, we have developed a universal synthetic methodology for the synthesis of isoxazole derivatives with a wide range of substituents based on the heterocyclization of readily available unsaturated esters by the treatment of TNM-TEA complex and subsequent reduction of the resulting 5-nitroisoxazoles by $SnCl_2$ with the participation of THF (solvent). A number of 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles containing a bulky adamantane framework were synthesized using this method. The obtained compounds were evaluated for their antioxidative activity, inhibition of lipoxygenase and impact on rat liver mitochondrial function. The compounds **1a-c, g, h** were found to demonstrate high antioxidative activity, as well as high inhibitory activity towards lipoxygenase. All tested compounds promote the phosphorylating respiratory activity simultaneously with maximal stimulated respiratory activity of mitochondria and do not reveal any toxicity towards the primary culture of rat cortex neurons. The attempt to correlate the *in vitro* biological results with some estimated physicochemical and pharmacokinetic properties was not quite successful. In particular, lipophilicity does not have a significant influence on the biological activity while the steric requirements are more important. However, the predicted properties are comparable or better than those of the reference compounds and seem quite acceptable for a lead candidate. Thus, novel 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazoles may be considered as promising and useful scaffold for the creation of novel drugs with antioxidant and metabolic enhancing properties.

4. Experimental

4.1. Chemistry

4.1.1. General experimental details

NMR spectra were recorded on spectrometer Agilent 400-MR (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at room temperature; chemical shifts were measured with reference to the solvent ($CDCl_3$, δ_H = 7.24 ppm, δ_C = 77.0 ppm). Chemical shifts (δ) are given in ppm; J values are given in Hz. When necessary, assignments of signals in NMR spectra were made using 2D techniques. Accurate mass measurements (HRMS) were measured on Jeol GCMate II mass spectrometer (70 eV). Analytical thin layer chromatography was carried out with Silufol silica gel plates (supported on aluminum); the detection was done by UV lamp (254 and 365 nm). Column chromatography was performed on silica gel (230–400 mesh, Merck). Compounds: **2a,**[29] **2b**,[30] **2c**,[31] **2d**,[32] **2e**,[33,34] **2f**,[35] **2g**,[36] **2h**,[37] **3h**,[21] **4h**,[11] **1h**,[11] were synthesized by known procedures. All other starting materials were commercially available. All reagents except commercial products of satisfactory quality were purified by literature procedures prior to use.

*4.1.2. General procedure for the preparation of compounds **3a-h***

To the solution of starting alcohol **2** (6 mmol) in dichloromethane (8 mL) was added Et_3N (0.84 mL, 606 mg, 6 mmol) in atmosphere of Ar. The mixture was cooled to 0…–5 °C and the acryloyl chloride (0.49 mL, 543 mg, 6 mmol) in dichloromethane (2 mL) was added dropwise. The resulting

mixture was stirred at this temperature for 3 h and then at room temperature for 24 h. The reaction mixture was poured into water (15 mL) and extracted with dichloromethane (4×20 mL). The combined organic layer was washed with brine solution (3×20 mL) and was dried over anhydrous Mg_2SO_4. The solvent was evaporated *in vacuo*; the residue was purified by preparative column chromatography on silica gel.

*2-Adamantyl acrylate (**3b**).* Obtained as colorless solid (1.11 g, 90 %); mp 64-65°C; R*f* 0.49 (petroleum ether:EtOAc 50:1); δ_H (400 MHz, $CDCl_3$): 1.52-1.59 (m, 2H, $2CH_2$ (Ad)), 1.71– 1.74 (m, 2H, $2CH_2$ (Ad)), 1.74-1.80 (m, 2H, $2CH_2$ (Ad)), 1.81-1.88 (m, 4H, 2CH+CH_2 (Ad)), 1.98 – 2.07 (m, 4H, $2CH_2$+2CH (Ad)), 4.96-5.01 (m, 1H, CHO), 5.79 (dd, ^2J = 1.6 Hz, ^3J = 10.4 Hz, 1H, =CH_2), 6.13 (dd, ^3J = 10.4 Hz, ^3J = 17.3 Hz, 1H, =CH), 6.39 (dd, ^2J = 1.6 Hz, ^3J = 17.3 Hz, 1H, =CH_2); δ_C (101 MHz, $CDCl_3$): 27.0 (CH (Ad)), 27.2 (CH (Ad)), 31.8 ($2CH_2$ (Ad)) 31.9 (2CH (Ad)), 36.3 ($2CH_2$ (Ad)), 37.4 (CH_2 (Ad)), 77.1 (CHO), 129.3 (=CH), 129.9 (=CH_2), 165.5 (C=O); HRMS (ESI, m/z): calcd for $C_{13}H_{18}O_2Na^+$ $[M+Na]^+$ 229.1199; found 229.1200.

*2-(1-Adamantyl)ethyl acrylate (**3c**).* Obtained as colorless oil (1.10 g, 78 %); R*f* 0.56 (petroleum ether:EtOAc 50:1); δ_H (400 MHz, $CDCl_3$): 1.42 (t, ^3J = 7.4 Hz , 2H, CH_2) 1.46-1.52 (m, 6H, $3CH_2$ (Ad)), 1.56-1.62 (m, 3H, $3CH_2$ (Ad)), 1.63-1.70 (m, 3H, $3CH_2$ (Ad)), 1.88-1.94 (m, 3H, 3CH (Ad)), 4.17 (t, ^3J = 7.4 Hz , 2H, OCH_2), 5.75 (dd, ^2J = 1.5 Hz, ^3J = 10.4 Hz, 1H, =CH_2), 6.06 (dd, ^3J = 10.4 Hz, ^3J = 17.3 Hz, 1H, =CH), 6.33 (dd, ^2J = 1.5 Hz, ^3J = 17.3 Hz, 1H, =CH_2); δ_C (101 MHz, $CDCl_3$): 28.5 (3CH (Ad)), 31.7 (C), 37.0 ($3CH_2$ (Ad)), 42.4 (CH_2); 42.5 ($3CH_2$ (Ad)), 60.9 (CH_2O), 128.7 (=CH), 130.2 (=CH_2), 166.2 (C=O); HRMS (ESI, m/z): calcd for $C_{15}H_{22}O_2Na^+$ $[M+Na]^+$ 257.1512; found 257.1524.

*2-(1-Adamantyloxy)ethyl acrylate (**3d**).* Obtained as colorless oil (1.08 g, 72%); R*f* 0.60 (petroleum ether:EtOAc 10:1); δ_H (400 MHz, $CDCl_3$): 1.53-1.65 (m, 6H, CH_2 (Ad)), 1.69 – 1.74 (m, 6H, CH_2 (Ad)), 2.09-2.15 (m, 3H, 3CH (Ad)), 3.63 (m, 2H, CH_2), 4.23 (m, 2H, CH_2), 5.79 (dd, ^2J = 1.5 Hz, ^3J = 10.4 Hz, 1H, =CH_2), 6.12 (dd, ^3J = 10.4 Hz, ^3J = 17.3 Hz, 1H, =CH), 6.38 (dd, ^2J = 1.5 Hz, ^3J = 17.3 Hz, 1H, =CH_2).; δ_C (101 MHz, $CDCl_3$): 30.5 (3CH (Ad)), 36.4 ($3CH_2$ (Ad)), 41.4 ($3CH_2$ (Ad)), 58.0 (CH_2), 64.5 (CH_2), 72.5 (C-O), 128.5 (=CH), 130.7 (=CH_2), 166.2 (C=O); HRMS (ESI, m/z): calcd for $C_{15}H_{22}O_3Na^+$ $[M+Na]^+$ 273.1461; found 273.1458.

*i-Propyl 3-(acryloyloxy)adamantane-1-carboxylate (**3e**).* Obtained as colorless solid (1.07 g, 61 %); mp 66-67°C; R*f* 0.42 (petroleum ether:EtOAc 20:1); δ_H (400 MHz, $CDCl_3$): 1.21 (d, ^3J = 6.3 Hz, 6H, $2CH_3$), 1.58-1.71 (m, 2H, CH_2), 1.78-1.88 (m, 4H, $2CH_2$), 2.08 - 2.14 (m, 2H, $2CH_2$), 2.16-2.22 (m, 2H, $2CH_2$), 2.24-2.26 (m, 2H, CH_2), 2.27 – 2.32 (m, 2H, 2CH), 4.98 (sept, ^3J = 6.3 Hz, 1H, OCH), 5.74 (dd, ^2J = 1.6 Hz, ^3J = 10.4 Hz, 1H, CH_2), 6.03 (dd, ^3J = 10.4 Hz, ^3J = 17.3 Hz, 1H, CHCO), 6.30 (dd, ^2J = 1.6 Hz, ^3J = 17.3 Hz, 1H, CH_2); δ_C (101 MHz, $CDCl_3$): 21.7 ($2CH_3$), 30.3 (2CH), 35.2 (CH_2), 37.8 ($2CH_2$), 40.4 ($2CH_2$), 42.3 (CH_2), 43.9 (C), 67.4 (CHO), 80.2 (C), 129.5 (=CH_2), 130.2 (=CH), 165.1 (C=O), 175.5 (C=O); HRMS (ESI, m/z): calcd for $C_{17}H_{24}O_4Na^+$ $[M+Na]^+$ 315.1567; found 315.1564.

*4-(1-Adamantyl)benzyl acrylate (**3f**).* Obtained as colorless solid (1.46 g, 82 %); mp 57-58°C; R*f* 0.81 (petroleum ether:EtOAc 10:1); δ_H (400 MHz, $CDCl_3$): 1.72-1.83 (m, 6H, CH_2 (Ad)), 1.86-1.97 (m, 6H, CH_2 (Ad)), 2.07-2.13 (m, 3H, 3CH (Ad)), 5.18 (c, 2H,CH_2O), 5.83 (dd, ^2J = 1.5 Hz, ^3J = 10.4 Hz, 1H, CH_2), 6.16 (dd, ^3J = 10.4 Hz, ^3J = 17.3 Hz, 1H, CHCO), 6.44 (dd, ^2J = 1.5 Hz, ^3J = 17.3 Hz, 1H, CH_2), 7.31-7.39 (m, 4H, 4CH(Ar)); δ_C (101 MHz, $CDCl_3$): 28.9 (3CH (Ad)), 36.1 (C (Ad)), 36.8 ($3CH_2$ (Ad)), 43.1 ($3CH_2$ (Ad)), 66.2 (CH_2O), 125.1 (2CH (Ar)), 128.2 (2CH (Ar)), 128.4 (=CH), 130.9 (=CH_2), 132.9 (C), 151.6 (C), 166.1 (C=O); HRMS (ESI, m/z): calcd for $C_{20}H_{24}O_2Na^+$ $[M+Na]^+$ 319.1669; found 319.1674.

*3,5-Dimethyl-1-adamantyl acrylate (**3g**).* Obtained as colorless oil (0.76 g, 54 %); R*f* 0.71 (petroleum ether:EtOAc 50:1); δ_H (400 MHz, $CDCl_3$): 0.85 (s, 6H, $2CH_3$), 1.09-1.14 (m, 1H, CH_2), 1.15-1.21 (m, 1H, CH_2), 1.23-1.29 (m, 2H, $2CH_2$), 1.34-1.40 (m, 2H, $2CH_2$), 1.72-1.83 (m, 4H, $2CH_2$), 1.96-1.98 (m, 2H, CH_2), 2.20 (sept, ^3J = 3.1 Hz, 1H, CH), 5.68 (dd, ^2J = 1.6 Hz, ^3J = 10.4 Hz, 1H, =CH_2), 5.99 (dd, ^3J = 10.4 Hz, ^3J = 17.3 Hz, 1H, =CH), 6.26 (dd, ^2J = 1.6 Hz, ^3J = 17.3 Hz, 1H, =CH_2); δ_C (101 MHz, $CDCl_3$): 29.9 ($2CH_3$), 31.1 (CH (Ad)), 33.8 (2C (Ad)), 39.7 (CH_2 (Ad)), 42.5 ($2CH_2$ (Ad)), 47.1 ($2CH_2$ (Ad)), 50.5 (CH_2 (Ad)), 81.9 (C-O), 129.2 (=CH_2), 130.4 (=CH), 165.3 (C=O); HRMS (ESI, m/z): calcd for $C_{15}H_{22}O_2Na^+$ $[M+Na]^+$ 257.1512; found 257.1514

*4.1.3. General procedure for the preparation of compounds **4a-h***

To the solution of $C(NO_2)_4$ (0.75 mL, 1.225 g, 6.25 mmol) in 1,4-dioxane (7 mL) was added Et_3N (0.70 mL, 505 mg, 5 mmol) in atmosphere of Ar for 30 min at –10…–5 °C. The resulting mixture was stirred at this temperature for 10 min and then the alkene **3** (2.5 mmol) in 1,4-dioxane (3 mL) was added dropwise. The reaction mixture was stirred at 80°C for 2 h and then the solvent was evaporated *in vacuo*; the residue was purified by preparative column chromatography on silica gel.

*1-Adamantyl 5-nitroisoxazole-3-carboxylate (**4a**).* Obtained as colorless solid (0.42 g, 57 %); mp 127-128°C; R*f* 0.33 (petroleum ether:EtOAc 40:1); δ_H (400 MHz, $CDCl_3$): 1.65-1.75 (m, 6H, $3CH_2$ (Ad)), 2.24 (br.s, 9H, $3CH_2$ (Ad) + 3CH (Ad)), 7.30 (s, 1H, CH); δ_C (101 MHz, $CDCl_3$): 31.0 (3CH (Ad)), 35.9 ($3CH_2$ (Ad)), 41.2 ($3CH_2$ (Ad)), 85.7 (C (Ad)), 102.2 (CH), 156.1 (C=O), 159.4 (C), 165.4 (br.s., CNO_2); HRMS (ESI, m/z): calcd for $C_{14}H_{16}N_2O_5Na^+$ $[M+Na]^+$ 315.0951; found 315.0955.

*2-Adamantyl 5-nitroisoxazole-3-carboxylate (**4b**).* Obtained as colorless solid 0.43 g, 59 %); mp 117-118°C; R*f* 0.27 (petroleum ether:EtOAc 40:1); δ_H (400 MHz, $CDCl_3$): 1.60 – 1.67 (m, 2H, $2CH_2$ (Ad)), 1.75 – 1.78 (m, 2H, $2CH_2$ (Ad)), 1.78 – 1.85 (m, 2H, $2CH_2$ (Ad)), 1.86-1.95 (m, 4H, 2CH+CH_2 (Ad)), 2.05-2.12 (m, 2H, $2CH_2$ (Ad)), 2.13 – 2.18 (m, 2H, 2CH (Ad)), 5.24 – 5.27 (m, 1H, CHO), 7.37 (c, 1H, CH); δ_C (101 MHz, $CDCl_3$): 26.7 (CH (Ad)), 27.0 (CH (Ad)), 31.7($2CH_2$ (Ad)), 31.8 (2CH (Ad)), 36.2 ($2CH_2$ (Ad)), 37.1 (CH_2 (Ad)), 80.8 (CH-O), 102.3 (CH), 156.9 (C=O), 158.6 (C) 165.5 (br.s., CNO_2); HRMS (ESI, m/z): calcd for $C_{14}H_{16}N_2O_5Na^+$ $[M+Na]^+$ 315.0951; found 315.0959.

*2-(1-Adamantyl)ethyl 5-nitroisoxazole-3-carboxylate (**4c**).* Obtained as colorless solid (0.67 g, 84 %); mp 101-102°C; R*f* 0.33 (petroleum ether:EtOAc 50:1); δ_H (400 MHz, $CDCl_3$): 1.52-1.56 (m, 6H, $3CH_2$ (Ad)), 1.58 (t, ^3J = 7.5 Hz, 2H, CH_2), 1.59-1.65 (m, 3H, $3CH_2$ (Ad)), 1.67-1.74 (m, 3H, $3CH_2$ (Ad)), 1.93-1.98 (m, 3H, 3CH (Ad)), 4.47 (t, ^3J = 7.5 Hz, 2H, OCH_2), 7.36 (s, 1H, CH); δ_C (101 MHz, $CDCl_3$): 28.5 (3CH(Ad)), 31.8 (C (Ad)), 36.9 ($3CH_2$ (Ad)), 42.0 (CH_2), 42.4 ($3CH_2$ (Ad)), 63.9 (CH_2O), 102.2 (CH), 157.7 (C=O), 158.3 (C), 165.6 (br.s., CNO_2); HRMS (ESI, m/z): calcd for $C_{16}H_{20}N_2O_5Na^+$ $[M+Na]^+$ 343.1264; found 343.1253.

*2-(1-Adamantyloxy)ethyl 5-nitroisoxazole-3-carboxylate (**4d**).* Obtained as colorless solid (0.46 g, 55%); mp 45-46°C; R*f* 0.11 (petroleum ether:EtOAc 20:1); δ_H (400 MHz, $CDCl_3$): 1.52-1.64 (m, 6H, $3CH_2$ (Ad)), 1.68-1.72 (br.s, m ,6H, $3CH_2$ (Ad)), 2.10-2.14 (m, 3H, 3CH (Ad)), 3.72-3.76 (m, 2H, CH_2), 4.47-4.51 (m, 2H, CH_2), 7.37 (c, 1H, CH); δ_C (101 MHz, $CDCl_3$): 30.4 (3CH (Ad)), 36.3 ($3CH_2$ (Ad)), 41.4 ($3CH_2$ (Ad)), 57.5 (C (Ad)), 66.9

(CH_2O), 72.8 (CH_2O), 102.3 (CH), 157.6 (C=O), 158.1 (C), 165.6 (br.s.,CNO_2); HRMS (ESI, m/z): calcd for $C_{16}H_{20}N_2O_6Na^+$ $[M+Na]^+$ 359.1214; found 359.1212.

*3-(i-Propoxycarbonyl)-1-adamantyl 5-nitroisoxazole-3-carboxylate (**4e**).* Obtained as colorless solid (0.31 g, 33%); mp 55-56°C; R*f* 0.29 (petroleum ether:EtOAc 15:1); δ_H (400 MHz, $CDCl_3$): 1.20 (d, ^3J = 6.2 Hz, 6H, $2CH_3$), 1.62-1.72 (m, 2H, CH_2 (Ad)), 1.83-1.85 (m, 4H, $2CH_2$ (Ad)), 2.16 - 2.21 (m, 2H, $2CH_2$ (Ad)), 2.24-2.30 (m, 2H, $2CH_2$ (Ad)), 2.32-2.36 (m, 4H, CH_2 (Ad)+2CH (Ad)), 4.96 (sept, ^3J = 6.2 Hz, 1H, OCH), 7.30 (s, 1H, CH); δ_C (101 MHz, $CDCl_3$): 21.7 ($2CH_3$), 30.4 (2CH (Ad)), 34.8 (CH_2 (Ad)), 37.5 ($2CH_2$ (Ad)), 40.2 ($2CH_2$ (Ad)), 42.1 (CH_2 (Ad)), 44.1 (C(Ad)), 67.7 (CHO), 85.1 (C-O), 102.2 (CH), 156.1 (C=O), 159.2 (C), 165.4 (br.s, .CNO_2), 175.0 (C=O); HRMS (ESI, m/z): calcd for $C_{18}H_{22}N_2O_7Na^+$ $[M+Na]^+$ 401.1319; found 401.1312.

*4-(1-Adamantyl)benzyl 5-nitroisoxazole-3-carboxylate (**4f**).* Obtained as colorless solid (0.34 g, 36 %); mp 155-156°C; R*f* 0.47 (petroleum ether:EtOAc 10:1); δ_H (400 MHz, $CDCl_3$): 1.70-1.82 (m, 6H, CH_2 (Ad)), 1.88-1.91 (m, 6H, CH_2 (Ad)), 2.06-2.12 (m, 3H, 3CH (Ad)) 5.41 (c, 2H, CH_2O), 7.37 (c, 1H, CH), 7.39 (br.s, 4H, 4CH (Ar)); δ_C (101 MHz, $CDCl_3$): 28.9 (3CH (Ad)), 36.2 (C (Ad)), 36.7 ($3CH_2$ (Ad)), 43.1 ($3CH_2$ (Ad)), 68.7 (CH_2-O), 102.3 (CH), 125.4 (2CH (Ar)), 128.8 (2CH(Ar)), 131.0 (C (Ar)), 152.5 (C (Ar)), 157.5 (C=O), 158.2 (C), 165.6 (br.s., CNO_2); HRMS (ESI, m/z): calcd for $C_{21}H_{22}N_2O_5Na^+$ $[M+Na]^+$ 405.1421; found 405.1414.

*3,5-Dimethyl-1-adamantyl 5-nitroisoxazole-3-carboxylate (**4g**).* Obtained as colorless solid (0.50 g, 63 %); mp 103-104°C; R*f* 0.37 (petroleum ether:EtOAc 40:1); δ_H (400 MHz, $CDCl_3$): 0.91 (s, 6H, $2CH_3$), 1.15-1.20 (m, 1H, CH_2 (Ad)), 1.21-1.26 (m, 1H, CH_2 (Ad)), 1.29-1.35 (m, 2H, $2CH_2$ (Ad)), 1.39-1.45 (m, 2H, $2CH_2$ (Ad)), 1.83-1.88 (m, 2H, $2CH_2$ (Ad)) 1.90-1.95 (m, 2H, $2CH_2$ (Ad)), 2.08 – 2.11 (m, 2H, CH_2 (Ad)), 2.29 (sept, ^3J = 3.2 Hz, 1H, CH (Ad)), 7.29 (s, 1H, CH); δ_C (101 MHz, $CDCl_3$): 29.7 ($2CH_3$), 31.2 (CH (Ad)), 34.3 (2C (Ad)), 39.6 (CH_2 (Ad)), 42.2 ($2CH_2$ (Ad)), 47.0 ($2CH_2$ (Ad)), 50.2 (CH_2 (Ad)), 86.9 (C (Ad)), 102.2 (CH), 156.2 (C=O), 159.4 (C), 165.4 (br.s., CNO_2); HRMS (ESI, m/z): calcd for $C_{16}H_{20}N_2O_5Na^+$ $[M+Na]^+$ 343.1264; found 343.1260.

*4.1.4. General procedure for the preparation of compounds **1a-h***

THF was distilled over NaOH. Air was bubbled through the THF during 20 h prior its use in the reaction. To the solution of nitroisoxazole **4** (0.4 mmol) in a mixture (1:1) H_2O-THF (14 mL) was added $SnCl_2$ (265 mg, 1.4 mmol) in one portion, and conc. HCl (0.3 mL) dropwise at 0°C. The resulting mixture was stirred at this temperature for 3 h, then THF was evaporated *in vacuo* and saturated aq. $NaHCO_3$ (15 mL) was added to the residue. The water phase was extracted with dichloromethane (4×20 mL). The combined organic layer was dried over anhydrous Mg_2SO_4. The solvent was evaporated *in vacuo*; the residue was purified by preparative column chromatography on silica gel.

*1-Adamantyl 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazole-3-carboxylate (**1a**).* Obtained as yellowish oil (64 mg, 46 %); R*f* 0.45 (petroleum ether:EtOAc 3:1); δ_H (400 MHz, $CDCl_3$): 1.62-1.73 (m, 6H, $3CH_2$ (Ad)), 1.86-1.96 (m, 1H, CH_2), 2.05-2.17 (m, 2H, CH_2), 2.17-2.29 (m, 10H, 3CH (Ad) + $3CH_2$ (Ad) + CH_2), 3.82-3.89 (m, 1H, CH_2O), 4.02-4.09 (m, 1H, CH_2O), 5.56 (dd, ^3J = 4.0 Hz, ^3J = 6.7 Hz, 1H, CHO), 5.93 (s, 1H, CH), 6.44 (br. s, 1H, OH); δ_C (101 MHz, $CDCl_3$): 25.3 (CH_2), 27.9 (CH_2), 30.9 (3CH (Ad)), 36.0 ($3CH_2$ (Ad)), 41.2 ($3CH_2$ (Ad)), 69.9 (CH_2O), 83.6 (C (Ad)), 87.1 (CH), 92.4 (CHO), 158.2 (C), 158.6 (C=O), 172.5 (C-NOH); HRMS (ESI, m/z): calcd for $C_{18}H_{24}N_2O_5Na^+$ $[M+Na]^+$ 371.1577; found 371.1576.

*2-Adamantyl 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazole-3-carboxylate (**1b**).* Obtained as yellowish oil (70 mg, 50 %); R*f* 0.42 (petroleum ether:EtOAc 3:1); δ_H (400 MHz, $CDCl_3$): 1.54-1.62 (m, 2H, $2CH_2$ (Ad)), 1.72-1.76 (m, 2H, $2CH_2$ (Ad)), 1.76-1.83 (m, 2H, $2CH_2$ (Ad)), 1.83-1.90 (m, 4H, 2CH+CH_2 (Ad)), 1.88-1.96 (m, 1H, CH_2), 2.06-2.17 (m, 6H, $2CH_2$ (Ad)+2CH (Ad)+CH_2), 2.20-2.29 (m, 1H, CH_2), 3.83-3.89 (m, 1H, CH_2O), 4.03 – 4.09 (m, 1H, CH_2O), 5.16-5.18 (m, 1H, CHO (Ad)), 5.58 (dd, ^3J = 4.0 Hz, ^3J = 6.8 Hz, 1H, CHO), 6.00 (s, 1H, CH), 6.48 (br. s, 1H, OH); δ_C (101 MHz, $CDCl_3$): 25.3 (CH_2), 26.9 (CH (Ad)), 27.1 (CH (Ad)), 27.9 (CH_2), 31.7 ($2CH_2$ (Ad)), 31.8 (2CH (Ad)), 36.2 ($2CH_2$ (Ad)), 37.2 (CH_2 (Ad)), 69.9 (CH_2O), 79.1 (CHO (Ad)), 87.0 (CH), 92.4 (CHO), 157.4 (C) , 159.3 (C=O), 172.7 (C-NOH); HRMS (ESI, m/z): calcd for $C_{18}H_{24}N_2O_5Na^+$ $[M+Na]^+$ 371.1577; found 371.1578.

*2-(1-Adamantyl)ethyl 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazole-3-carboxylate (**1c**).* Obtained as yellowish solid (71 mg, 47 %); mp 105-107°C; R*f* 0.14 (petroleum ether:EtOAc 5:1); δ_H (400 MHz, $CDCl_3$): 1.51-1.57 (m, 8H, $3CH_2$ (Ad)+CH_2), 1.58-1.65 (m, 3H, $3CH_2$ (Ad)), 1.66-1.73 (m, 3H, $3CH_2$ (Ad)), 1.87 – 1.97 (m, 4H, 3CH (Ad) + CH_2), 2.05-2.28 (m, 3H, $2CH_2$), 3.83-3.90 (m, 1H, CH_2O), 4.03 – 4.10 (m, 1H, CH_2O), 4.38 (t, ^3J = 7.4 Hz, 2H, CH_2O), 5.58 (dd, ^3J = 4.0 Hz, ^3J = 6.8 Hz, 1H, CHO), 5.98 (s, 1H, CH), 6.37 (s, 1H, OH); δ_C (101 MHz, $CDCl_3$): 25.3 (CH_2), 27.9 (CH_2), 28.5 (3CH (Ad)), 31.8 (C (Ad)), 36.9 ($3CH_2$ (Ad)), 42.1 (CH_2), 42.4 ($3CH_2$ (Ad)), 69.9 (CH_2O), 85.3 (CH_2O), 87.0 (CH), 92.3 (CHO), 157.0 (C), 160.1 (C=O), 172.7 (C); HRMS (ESI, m/z): calcd for $C_{20}H_{28}N_2O_5Na^+$ $[M+Na]^+$ 399.1890; found 399.1887.

*2-(1-Adamantyloxy)ethyl 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazole-3-carboxylate (**1d**).* Obtained as brownish solid (82 mg, 52 %); mp 98-101°C; R*f* 0.24 (petroleum ether:EtOAc 3:1); δ_H (400 MHz, $CDCl_3$): 1.51-1.63 (m, 6H, $3CH_2$ (Ad)), 1.69-1.72 (m, 6H, $3CH_2$ (Ad)), 1.82-1.94 (m, 1H, $2CH_2$), 2.04-2.14 (m, 5H, 3CH (Ad) + $2CH_2$), 2.16-2.27 (m, 1H, $2CH_2$), 3.69-3.72 (m, 2H, CH_2), 3.78-3.85 (m, 1H, CH_2O), 3.99 – 4.06 (m, 1H, CH_2O), 4.36-4.39 (m, 2H, CH_2), 5.54 (dd, ^3J = 4.0 Hz, ^3J = 6.7 Hz, 1H, CHO), 5.94 (s, 1H, CH), 7.46 (br.s, 1H, OH); δ_C (101 MHz, $CDCl_3$): 25.3 (CH_2), 27.9 (CH_2), 30.4 (3CH (Ad)), 36.3 ($3CH_2$ (Ad)), 41.3 ($3CH_2$ (Ad)), 57.8 (CH_2O), 65.6 (CH_2O), 69.9 (CH_2O), 73.1 (C (Ad)), 86.6 (CH), 92.1 (CHO), 156.6 (C), 160.0 (C=O), 173.0 (C-NOH); HRMS (ESI, m/z): calcd for $C_{20}H_{28}N_2O_6Na^+$ $[M+Na]^+$ 415.1840; found 415.1852.

*3-(i-Propoxycarbonyl)-1-adamantyl 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazole-3-carboxylate (**1e**).* Obtained as yellowish oil (94 mg, 54 %); R*f* 0.10 (petroleum ether:EtOAc 4:1); δ_H (400 MHz, $CDCl_3$): 1.18 (d, ^3J = 6.3 Hz, 6H, $2CH_3$), 1.57-1.71 (m, 2H, CH_2 (Ad)), 1.78-1.86 (m, 4H, $2CH_2$ (Ad)), 1.87-1.96 (m, 1H, CH_2), 2.04-2.19 (m, 4H, CH_2 (Ad)+CH_2), 2.20-2.34 (m, 7H, 2CH (Ad)+$2CH_2$ (Ad) +CH_2), 3.82-3.89 (m, 1H, OCH_2), 4.02-4.09 (m, 1H, OCH_2), 4.95 (sept, ^3J = 6.3 Hz, 1H, CHO), 5.55 (dd, ^3J = 4.0 Hz, ^3J = 6.9 Hz, 1H, CHO), 5.93 (s, 1H, CH), 6.57 (br.s., 1H, OH); δ_C (101 MHz, $CDCl_3$): 21.7 ($2CH_3$), 25.3 (CH_2), 27.9 (CH_2), 30.4 (2CH (Ad)), 35.0 (CH_2 (Ad)), 37.6 ($2CH_2$ (Ad)), 40.2 ($2CH_2$ (Ad)), 42.2 (CH_2 (Ad)), 44.0 (C (Ad)), 67.6 (CHO), 69.9 (CH_2O), 83.0 (C (Ad)), 87.0 (CH), 92.4 (CHO), 157.9 (C), 158.6 (C=O), 172.6 (C), 175.4 (C=O); HRMS (ESI, m/z): calcd for $C_{22}H_{30}N_2O_7Na^+$ $[M+Na]^+$ 457.1945; found 457.1939.

*4-(1-Adamantyl)benzyl 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazole-3-carboxylate (**1f**).* Obtained as yellowish oil

(109 mg, 62%); R*f* 0.09 (petroleum ether:EtOAc 5:1); δ_H (400 MHz, $CDCl_3$): 1.69-1.81 (m, 6H, $3CH_2$ (Ad)), 1.84-1.94 (m, 7H, $3CH_2$ (Ad)+CH_2), 2.02-2.13 (m, 5H, 3CH (Ad) + $2CH_2$), 2.14-2.25 (m, 1H, CH_2), 3.80-3.87 (m, 1H, CH_2O), 4.00-4.07 (m, 1H, CH_2O), 5.32 (s, 2H, CH_2), 5.55 (dd, ^3J = 4.0 Hz, ^3J = 6.8 Hz, 1H, CHO), 5.99 (s, 1H, CH), 6.55 (br.s), 7.34-7.35 (m, 4H, 4CH (Ar)); δ_C (101 MHz, $CDCl_3$): 25.3 (CH_2), 27.8 (CH_2), 28.9 (3CH (Ad)), 36.1 (C (Ad)), 36.7 ($3CH_2$ (Ad)), 43.1 ($3CH_2$ (Ad)), 67.5 (CH_2O), 69.9 (CH_2O), 87.0 (CH), 92.2 (CHO), 125.1 (2CH (Ar), 128.5 (2CH (Ar)), 131.9 (C (Ar)), 151.9 (C (Ar)), 156.8 (C), 159.9 (C=O), 172.8 (C); HRMS (ESI, m/z): calcd for $C_{25}H_{30}N_2O_5Na^+$ $[M+Na]^+$ 461.2047; found 461.2036.

*3,5-Dimethyl-1-adamantyl 5-[hydroxy(tetrahydrofuran-2-yl)amino]isoxazole-3-carboxylate (**1g**).* Obtained as yellowish oil (90 mg, 60 %); R*f* 0.14 (petroleum ether:EtOAc 5:1); δ_H (400 MHz, $CDCl_3$): 0.88 (6H, $2CH_3$), 1.12-1.24 (m, 2H, CH_2 (Ad)), 1.26-1.32 (m, 2H, $2CH_2$ (Ad)), 1.37-1.43 (m, 2H, $2CH_2$ (Ad)), 1.81-1.97 (m, 5H, $2CH_2$ (Ad)+CH_2), 2.05-2.09 ((m, 2H, CH_2 (Ad)), 2.09-2.28 (m, 4H, $2CH_2$+CH (Ad)), 3.83-3.90 (m, 1H, CH_2O), 4.03-4.10 (m, 1H, CH_2O), 5.57 (dd, ^3J = 4.1 Hz, ^3J = 6.9 Hz, 1H, CHO), 5.91 (s, 1H, CH), 5.94 (s, 1H, OH); δ_C (101 MHz, $CDCl_3$): 25.3 (CH_2), 27.9 (CH_2), 29.8 ($2CH_3$), 31.2 (CH (Ad)), 34.1 (2C (Ad)), 39.6 (CH_2 (Ad)), 42.4 ($2CH_2$ (Ad)), 47.1 ($2CH_2$ (Ad)), 50.4 (CH_2 (Ad)), 69.9 (CH_2O), 84.9 (C (Ad)), 86.9 (CH), 92.3 (CHO), 158.1 (C), 158.7 (C=O), 172.6 (C); HRMS (ESI, m/z): calcd for $C_{20}H_{28}N_2O_5Na^+$ $[M+Na]^+$ 399.1890; found 399.1889.

4.2. Biological studies

4.2.1. Animals

The experiments were carried out on outbreed albino male rats weighing 200-300 g. The animals had free access to food and water.

4.2.2. Brain homogenate

The animals were narcotized with carbon dioxide and decapitated using a guillotine. The procedure is in compliance with the Guidelines for Animal Experiments at IPAC RAS. Their brains were removed and homogenized in 120 mM KCl/20 mM HEPES on ice. For isolation of the subcellular fraction, the brain homogenate was centrifuged at 4000 rpm and supernatant was used in further experiments on the same day. The protein content in brain homogenate and mitochondria extracts was measured using a microbiuret method.[38]

4.2.3. Mitochondria

Mitochondria were isolated by conventional differential centrifugation from the livers of adult Wistar strain rats fasted overnight as in this work.[39] The standard mitochondrial fraction contained 130-150 mg of protein per liver. The mitochondria were stored at 4°C. Functional activity of the rat liver mitochondria remained constant for 2.5…4 h.

4.2.4. Redox activity

Lipid peroxidation intensity in brain homogenate was assayed using a modified TBA test.[40]

Fe(II)-chelating activity was evaluated using a modified method described elsewhere.[41]

The free radical scavenging activity was evaluated using the stable radical DPPH, according to the method described by Brand-Williams et al.[42] with a slight modification. All compounds were tested in 1:1 ratio expressed as moles of compound per mole of DPPH radical. A 1 mL sample of compound solution in methanol was added to 1 mL of DPPH solution in methanol so that the initial DPPH concentration in the cuvettes was 0.1 mM. The samples were incubated for 30 min at 20°C in methanol and the decrease in the absorbance values of DPPH solution was measured at λ_{max} 517 nm on the Thermo Scientific Multiscan Go microplate spectrophotometer. Results were expressed as scavenging activity, calculated as follows:

Scavenging activity, % = $[(A_0-A_1)/A_0] \times 100$

The concentration of the compound needed to decrease 50% of the initial substrate concentration (EC_{50}) is a parameter widely used to measure the antioxidant effect.[27] For determination of EC_{50}, the values of DPPH decrease after 30 min were used. The EC_{50} values were calculated graphically by plotting scavenging activity against compound concentration. Different sample concentrations (0.01, 0.02, 0.05 and 0.1 mM) were used in order to obtain kinetic curves and to calculate the EC_{50} values.

4.2.5. Lipoxygenase activity

Lipoxidase (LOX) type 1-B from *Glycine max* (soybean), boric acid, linoleic acid, NaOH, ethanol (96%) were used with no further purification. LOX inhibition activity was determined spectrophotometrically by measuring the increase in absorbance at 234 nm for the oxidation of linoleic acid[43] The reaction mixture contained (final concentration) the test compounds, dissolved in DMSO at concentrations of 1-100 μM, or the solvent (control) and linoleic acid 100 μM, in borate buffer (pH=9.0). The reaction was started by adding a lipoxygenase in amount of 500 units. Six different concentrations of each complex were used for the inhibition activity experiments. The increase in absorbance was recorded every 10 s during 10 min under controlled temperature 25 ˚C.

The degree of LOX activity (A%) in the presence of the complexes was calculated according to the following.[44]

A,% = (v_0 in the presence of inhibitor/v_0 in the absence of inhibitor) * 100, where v_0 is the initial rate.

The value of the initial rate (v_0, μM • min^{-1}) was calculated according to the formulas:

$$\mathbf{v_0 = \Delta C/\Delta t = \Delta A/\Delta t\varepsilon = tg\alpha/\Delta t\varepsilon,}$$

where C is the product concentration (hydroperoxylinoleic acid), t is the reaction time, ε is molar absorbance coefficient of hydroperoxylinoleic acid, tgα is the slope of the kinetic curve plotted as absorbance *vs* time.

IC_{50} values were obtained graphically by plotting logistic curve in coordinates inhibition activity (A, %) *vs* inhibitor concentration ([I], μM). The general curve equation is the following:

$$\mathbf{A, \% = 100 \times (1/(1+[I]/IC_{50}))}$$

4.2.6. Mitochondrial membrane potential

Mitochondrial membrane potential of isolated rat liver mitochondria was measured using potential-dependent indicator Safranine A at 25°C as in our previous work.[45]

4.2.7. Mitochondrial O_2 consumption rate (OCR) measurements

Mitochondrial O_2 consumption measurements were made using an XF96 Extracellular Flux Analyzer (Seahorse Bioscience) as described.[46] Mitochondria were added to XF96 Analyzer plate with 30 μM of compounds in the presence of succinate (5mM) and rotenone (1μM) and then the measurement of the oxygen consumption was started. Final concentrations of the additives after port injections were 4 mM ADP; 2,5 μg/ml oligomycin; 4 μM FCCP; and 4 μM antimycin A. Calculated RCR was 7.95±0.8. OCR was automatically calculated and recorded by the Seahorse XF96 software.

4.2.8. Viability of cultured neuroblastoma SH-SY5Ycells and rat cerebellar granule cells (CGC)

Cell viability was evaluated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[47] Briefly, the SH-SY5Y cells or primary culture of rat cerebellar granule cells were incubated with 30 μM of compounds or equal volume of vehicle (less than 1% of the total volume of medium under the cell layer) during 24 h. Then 20 μL of MTT (2 mg/mL in PBS) were added to each well and the cells were incubated at 37°C for 2 h. The supernatants were aspirated carefully, 200 μl of DMSO were added to each well to dissolve the precipitate, and absorbance was measured at 570 nm using a microplate reader Victor (Perkin Elmer).

4.2.9. Statistics

Data are presented as the mean ± SD. Statistical comparisons were made by Student's t-test. Values of $P<0.05$ and $P<0.01$ were considered significant.

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Supplementary Material

Supplementary data (some details of biological studies, computational estimation of some physicochemical and pharmacokinetic properties of compounds 1a–h and reference

compounds, and NMR spectra for compounds 3a–g, 4a-g, 1a–g) associated with this article can be found in the online version.