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Synthesis and biological evaluation of novel 5-hydroxylaminoisoxazole derivatives as lipoxygenase inhibitors and metabolism enhancing agents

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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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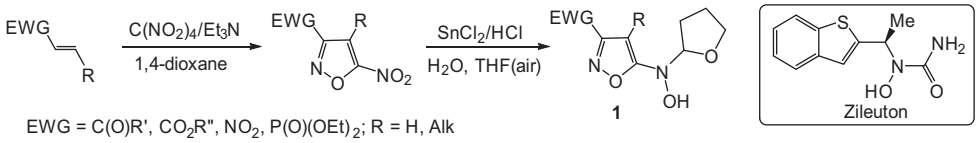
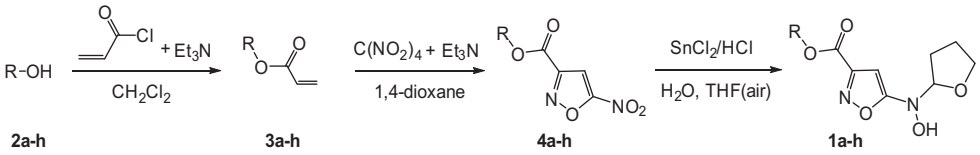
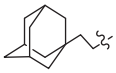
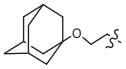
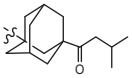
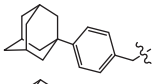


Scheme 1. A described procedure[11–13] for the synthesis of *N*-hydroxylaminoisoxazoles **1**.

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.

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