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Cytoprotective and antioxidant properties of organic selenides for the myelin-forming cells, oligodendrocytes

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ABSTRACT

Here a new series of twenty-one organoselenides, of potential protective activity, were synthesized and tested for their intrinsic cytotoxicity, anti-apoptotic and antioxidant capacities in oligodendrocytes. Most of the organoselenides were able to decrease the ROS levels, revealing antioxidant properties. Compounds **5b** and **7b** showed a high glutathione peroxidase (GPx)-like activities, which were 1.5 folds more active than ebselen. Remarkably, compound **5a** diminished the formation of the oligodendrocytes SubG1 peak in a concentration-dependent manner, indicating its anti-apoptotic properties. Furthermore, based on the SwissADME web interface, we performed an *in-silico* structure-activity relationship to explore the drug-likeness of these organoselenides, predicting the pharmacokinetic parameters for compounds of interest that could cross the blood-brain barrier. Collectively, we present new organoselenide compounds with cytoprotective and antioxidant properties that can be considered as promising drug candidates for myelin diseases.

1. Introduction

In the brain, the myelin sheath plays a crucial role for neuron signal transmission and their survival [1]. Such a sheath is built by a multilayered spiral extension of oligodendrocyte cell membranes to wrap the neuron's axons [2]. Chronic erosion of this myelin sheath leads to demyelination, and disrupting the neuron integrity and the normal axonal function [3]. Demyelination is largely associated with numerous neurodegenerative diseases (e.g., Parkinson' and Alzheimer's diseases) as well as multiple sclerosis and peroxisomal leukodystrophies rare diseases [4,5]. Thus for the myelination process, oligodendrocyte integrity is central and their dysfunction was also detected in several psychiatric disorders, including autism, schizophrenia, and depression [6]. Oligodendrocytes support the long-term neurons integrity by myelinating their axons [7]. One of the hallmarks of oligodendrocyte dysfunction and apoptosis is their high susceptibility to oxidative stress (OS), which results from the excess production of reactive oxygen species (ROS; e.g., hydrogen peroxide, superoxide, and hydroxyl radicals), by cellular aerobic metabolism, particularly from mitochondrial respiration [8]. Oxidative damage, caused in a variety of neurodegenerative diseases, is mediated by excessive production of ROS, which can be linked to cell lysis or oxidative burst associated to the immune response [9]. This overproduction occurs when the generation of reactive oxygen species (ROS) exceeds the antioxidant cellular capacity, including enzymatic (catalase, glutathione peroxidase and superoxide dismutase) and non-enzymatic defense systems (glutathione and

Abbreviations: CNS, Central nervous system; MTT, 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DCF, 7-ketocholesterol (7kc), dichlorofluorescein; DHE, dihydroethidium; GPx, glutathione peroxidase; ROS, reactive oxygen species; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid

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ascorbic acid) [10]. The selenoprotein family, including glutathione peroxidases, represents one of the first-line defenses against ROS. Selenoproteins play also a crucial role for neuronal function, as demonstrated by deactivation of the tRNA [Ser] (Sec) gene in mice, resulting in extensive neurodegeneration [11]. The second line of defense is represented by cellular antioxidants, such as glutathione (GSH) and selenium (Se), the latter for its implication in the active center of several glutathione peroxidases and in the catalytic activity of thioredoxin reductase [12]. Depleted states of the reduced form of GSH and selenium were associated with the progression of neurodegenerative diseases [13–15]. This underlines the overarching importance of an essential dietary supply of Se for mammals. Interestingly, there seems to be a connection between Se and other antioxidants on the one side, and the role of ROS in the pathogenesis of neurodegenerative diseases on the other [16–18]. Yeo et al., reported that Se antioxidant activity prevented cell apoptosis in an experimental rat spinal cord injury [19]. Numerous data revealed that Se levels decline in humans with age and that Se might play different roles in the evolution of Alzheimer's disease [20,21]. Se deficiency leads to inhibition of the normal upregulation of myelin genes in differentiating oligodendrocytes and the progression from immature to mature oligodendrocytes as well [22]. Curiously, experiments using animal models reveal that organoselenium has a better bioavailability and biological activity than inorganic Se [23]. Therefore, during the last decade or two, the biological chemistry of selenium derivatives has often focused on potential antioxidant and neuroprotective activities [24], and here primarily on organic rather than inorganic selenium agents [25,26]. During our research on selenium derivatives with possible antioxidant and neuroprotective activities, we recently developed organoselenium compounds with enhanced antioxidant and GPx-like activities [27–32]. The mechanism by which these compounds exert their antioxidant effect is related to the modulation of the ROS and GSH levels in cancer cells. Interestingly, some of these compounds showed better antioxidant and GPx-like activities than classical antioxidants (e.g., vitamin C, ebselen) [27,30,32–36]. Based on these results, we decided to further direct our research to obtain evidences regarding the structural requirements underlying the potential cytoprotective/neuroprotective activities of organic selenides taking the myelin-forming cells, oligodendrocytes as a model of study.

Different reports on the synthesis of organoselenium compounds have already been published; however, they generally require the handling of hazard reagents (e.g., KSeCN), harsh reaction conditions (e.g., strongly acidic or basic), and mostly multistep procedures [37,38]. Therefore, we decided to avoid rather sophisticated synthetic procedures and employ simple, straight forward and facile routes towards the synthesis of our preliminary organoselenium-based library.

Here, we present the results obtained during extensive *in vitro* evaluations of the selenium derivatives in oligodendrocytes. Besides a rational design of synthetic organoselenium compounds, we have aimed at gaining a deeper insight into the potential use of these compounds as neuroprotectors in neurodegenerative diseases through the protective effect on oligodendrocytes, the myelinating cells. For the chemical synthesis, we adopted both multistep and one-pot synthesis as part of a diversity-oriented approach to achieve the synthesis of the twenty-one organoselenides. The *in vitro* evaluations were conducted using immortalized murine oligodendrocyte cell lines [39]. Flow cytometry was used to estimate the ROS levels employing the H2-DCFDA and the DHE assays. Furthermore, the antioxidant potential of these compounds was further investigated employing different chemical and biochemical assays and the apoptotic/anti-apoptotic properties of the compounds were detected by flow cytometry follow-up of the sub-G1 peak. Finally, to assess the structure-activity relationship of the synthesized organoselenium compounds, we determined *in silico* their physicochemical and pharmacokinetic parameters, and their drug-likeness as well through the free web tools in SwissADME.

2. Results and discussions

2.1. Design and syntheses

The last decade has witnessed significant advance in the synthesis of diverse classes of organic selenides e.g., diselenides, selenocyanates, and seleno-heterocyclic compounds. Although, these compounds have a pivotal role in human health and disease prevention; they have limited application in biological systems due to their often toxicity especially if higher concentrations were administrated. Combining selenium with better physico-chemical properties bioactive pharmacophores will interactively not only stimulate their corresponding cytotoxicity but will also foster their bioavailability. Within this context, our design strategy relies on the synthesis of agents in which selenium is combined with different functionalities such as quinone, cyclic imides, *N*-substituted maleanilic and succinanilic acids, formamide, acetanilide, acetaldehyde diethyl acetal, and/or benzyl. Most of these functionalities are commonly found in bioactive compounds, thus enabling the chemical diversity and increasing polarity.

Based on our recent reports on organoselenium agents [28–35], we envisioned the synthesis of novel selenium-based primary aromatic amines **2a-d**. The amine functionality in turn can give an access to a large group of agents either via isocyanide-based multicomponent reactions (IMCR) (e.g., Passerini, azido-Ugi Passerini, Ugi, and Groebke-Blackburn-Bienaymé reactions) or via the classical amines reactions (e.g., formylation, acetylation, etc.).

The key synthons selenoamines **2a-d** were synthesized in good yields (up to 91%) *via* reduction of the diselenide (**1**) with sodium tetrahydridoborate ($NaBH_4$) followed by subsequent nucleophilic substitution (S_N) reaction with appropriate organic halides (Scheme 1).

Chalcogen-bearing isonitriles are not commercially available and usually challenging to synthesize. In 2009, we reported the synthesis of three chalcogen-based isonitriles, including the tellurium isonitrile for the first time [32,34]. Therefore, our preliminary target was to convert the amines **2a**, **2b**, **2c**, and **2d** into the corresponding isonitriles, suitable for further applications in IMCR [29,40]. The synthesis of isocyanides is usually performed in two steps i.e. amine formylation employing acetic formic anhydride followed by subsequent dehydration using phosphoryl chloride ($POCl_3$). In accordance with our aim, we decided to apply the same methodology for the transformation of organoselenium-based amines **2a-d** into the corresponding isocyanides. Unexpectedly, acetanilides **4a-c** were invariably obtained instead of the corresponding formamides. This promoted us to use only formic acid in place of the acetic formic anhydride. Under neat conditions, the desired corresponding formamides were obtained in good yields (up to 88%).

The dehydration of formamides was thereafter investigated using $POCl_3$. A non-polar product (eluted with petroleum ether) with the characteristic isonitrile odor was detected by TLC; however, the triplet ^{13}CNMR peak ($\approx$156 ppm) corresponding to the isocyanide carbon did not appear, suggesting that the isocyanide may be unstable. Therefore, we designed a one-pot synthetic approach based on the use of the *in situ* formed isocyanide in subsequent isocyanide-based MCRs (IMCRs) such as Groebke-Blackburn-Bienaymé and Ugi reactions [40]. Unfortunately, very poor conversion ($< 5\%$) was constantly observed with subsequent disappearance of the isocyanide from the reaction mixture (monitored by TLC). Hence, we concluded, that this result is related to the high reactivity and low stability of the *in situ* formed isocyanides. Different dehydrating agents such as cyanuric chloride [41], triphenylphosphine [42], 4-toluenesulfonyl chloride [43], DABCO [44] and methyl *N*-(triethylammoniumsulfonyl)carbamate [45] were investigated; however, these reagents turned out to be either low yielding or ineffective with our seleno-formamides.

Further efforts were then directed to evaluate the reactivity of the novel selenoamines **2a-d** via their reactions with various anhydrides aiming to synthesize selenocyclic imides which we recently proved to possess enhanced antioxidant activities (e.g., GPx-mimicking and free