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Divers Transformations Leading to New Potent GPx Mimetics †

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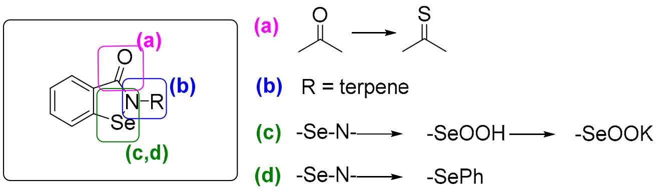
Abstract: Designing a highly active and selective Se-therapeutic that mimics the activity of the antioxidant enzyme glutathione peroxidase (GPx) still remains a challenge. Since the discovery of ebselen (*N*-phenyl-1,2-benzisoselenazol-3(2H)-one) and its ability to act as a GPx mimetic, the search for more effective peroxide scavengers has become a "hot topic" in this field of research. Herein, we present several modifications of the benzisoselenazolone core that enable improving the antioxidant and anticancer potential of the basic ebselen structure. These transformations include (a) the installation of chiral terpene skeletons, from *p*-menthane, pinane, and carane systems, on the nitrogen atom; (b) exchange of the carbonyl oxygen atom for sulfur to obtain thiocarbonyl derivatives; (c) oxidation of the selenium moiety resulting in a series of benzenoselenenic acids and their further transformation to corresponding water-soluble potassium salts; and (d) attachment of an additional phenyl group leading to variously *N*-substituted unsymmetrical phenylselenides with an *o*-amido function. All of the synthetized compounds were tested as antioxidants and antiproliferative agents. Conclusions concerning the structure–activity correlation, including the difference in the reactivity of specific Se-moieties (-Se-N-, -SeOOH, -SeOOK, -SePh), *N*-substituents (the influence of bulky aliphatic moiety and the three-dimensional orientation of atoms), and incorporated heteroatoms (-C=O, -C=S) are presented.

Keywords: ebselen; organoselenium derivatives; antioxidant activity; anticancer activity

1. Introduction

Reactive oxygen species (ROS) play an important role as mediators and regulators in the cells physiology [1,2]. However, their intense and long-lasting effect can have a destructive impact on biomolecules, such as DNA, lipids, or proteins and consequently cause a whole range of diseases, e.g., cardiovascular disorders, cancer, neurodegeneration, and aging [3–5]. Therefore, the production of ROS must be strictly controlled by the enzymatic and non-enzymatic antioxidant systems in order to avoid the disorder of homeostasis between pro- and antioxidant processes, which are defined as "oxidative stress" [6,7]. The selenoenzyme–glutathione peroxidase (GPx) is an important part of this bio-machinery. Ebselen (*N*-phenyl-1,2-benzisoselenazol-3(2H)-one) was one of the first organoselenium compounds to be discovered as a GPx mimic. Although ebselen was found to have very promising antioxidant properties, several side effects and low solubility prompted the search for specific structural modifications that could improve its bioavailability and reduce the observed negative after-effects. Until now, we have performed several transformations of the benzisoselenazolone core that enable modifying the bio-activity of the basic ebselen structure. These modifications included (a) exchanging the oxygen atom of a carbonyl group for a sulfur atom to form thiocarbonyl derivatives; (b) substitution of the nitrogen atom with chiral skeletons; (c) oxidation of the Se-N bond to form selenenic acids -SeOOH and its subsequent transformation into the water-soluble seleninic acid potassium salts; and (d) transformation of the Se-N bond into a selenide moiety (Scheme 1).



Scheme 1. Structural modification of *N*-substituted benzisozelenazol-3(2*H*)-ones.

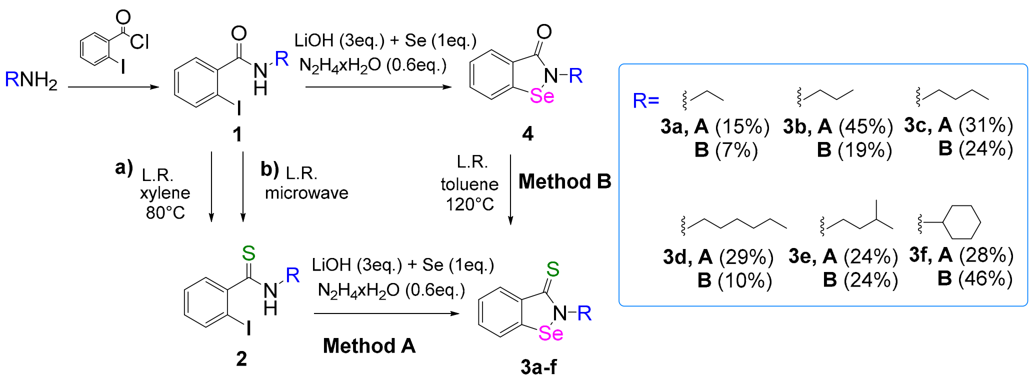
The conducted transformations enabled obtaining a variety of Se-based GPx mimics. All derivatives were tested as antioxidants and anticancer agents. The influence of specific modifications on the activity of the molecules is highlighted.

2. Results and Discussion

2.1. Synthesis of Benzisoselenazol-3-(2H)-Thiones

The exchange of the carbonyl oxygen atom with a sulfur atom, in the structure of benzisoselenazol-3-(2H)-ones, reduces the polarity of the double bond. This can influence the stability of the Se-N bond and its reactivity toward ROS (antioxidant properties) and proteins (the rate of S-Se bond formation). Based on this assumption, we have developed an efficient methodology for the preparation of benzisoselenazol-3-(2H)-thions using Lawesson's reagent [8].

N-alkylbenzisoselenazolthiones **3a–f** were obtained by two different two-step methods. The first of them (Method A) involved the reaction of N-alkyl-o-iodobenzamides **1** with Lawesson's reagent (L.R.), which was followed by the nucleophilic substitution of the obtained thioamides by Li_2Se_2. The second procedure (Method B) was based on the formation of benzisoselenazol-3-(2H)-ones **4** in the reaction of N-alkyl-o-iodobenzamides **1** with Li_2Se_2 [9] and then the reaction of ebselen derivatives **4** with Lawesson's reagent (Scheme 2).



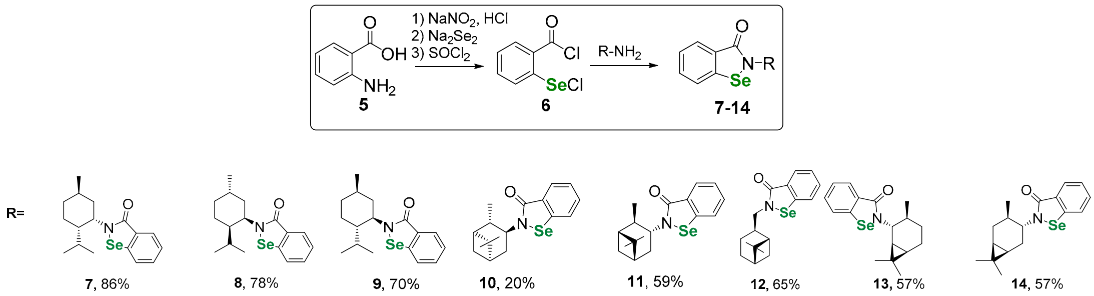
Scheme 2. Methods **A** and **B** used to obtain thio-derivatives **3a–f**.

The reaction of amides **1** with Lawesson's reagent, carried out under standard conditions (**route a**) [10], allowed obtaining the thioamides **2** in only moderate yields (reaction time: 12 h, yields: 22–61%). Performing the same reaction using microwave radiation (**route b**), under solvent-free conditions, significantly shortened the reaction time (3 min) and improved the yields of the process (44–82%).

2.2. Synthesis of N-terpenyl Benzisoselenazol-3(2H)-Ones

As chiral compounds that possess a strictly defined orientation of substituents on the asymmetric carbon can interact with specific biological targets differently, depending on their configuration and the structure of the matching receptor, we also wanted to synthetize a series of chiral N-substituted benzisoselenazolones, including different enantiomers, epimers, and regioisomers, and determine the correlation between the structure of the compound and its biological activity.

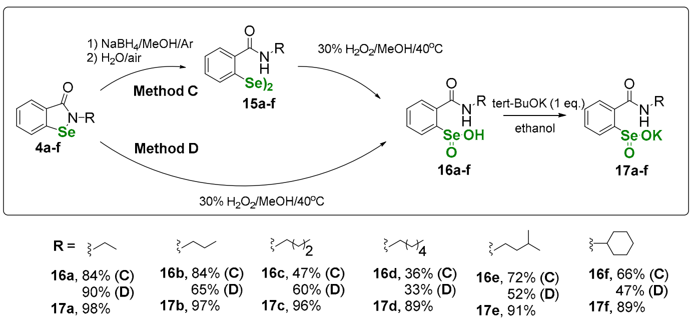
For this purpose, we have first synthetized a series of terpene amines by a multistep methodology starting from the corresponding alcohol (p-menthane system) or alkene (pinene and carene systems), which were further converted to corresponding benzisoselenazol-3(2H)-ones **7–14** by the reaction with 2-(chloroseleno)benzoyl chloride **6** (Scheme 3) [11].



Scheme 3. Synthesis of N-terpenyl benzisoselenazol-3(2H)-ones **7–14**.

2.3. Synthesis of Seleninic Acid Potassium Salts

The main way to improve the bioavailability of a chemical compound is to increase its solubility in body fluids. The moderate antioxidant activity of ebselen is mainly related to its poor solubility in water, which becomes particularly important when attempting to administer the drug intravenously. To address this issue, we have conducted the synthesis of water-soluble derivatives in the form of potassium salts of 2-(*N*-alkylcarboxamido)benzeneselenic acids **17a–f**. The first step of the research involved the synthesis of *N*-alkylbenzeneseleneinic acids **16a–f** with *o*-amide function. The acids **16a–f** were obtained using two alternative methods: by oxidation of *N*-alkylbenzisoselenazol-3(2*H*)-ones **4a–f** (Method C) or the corresponding diselenides **15a–f** (Method D) with 30% H_2O_2. *N*-alkylbenzeneseleninic acids **16a–f** in the next step were converted into the corresponding benzeneseleninic salts **17a–f** by reaction with potassium *tert*-butoxide in anhydrous ethanol (Scheme 4) [12].

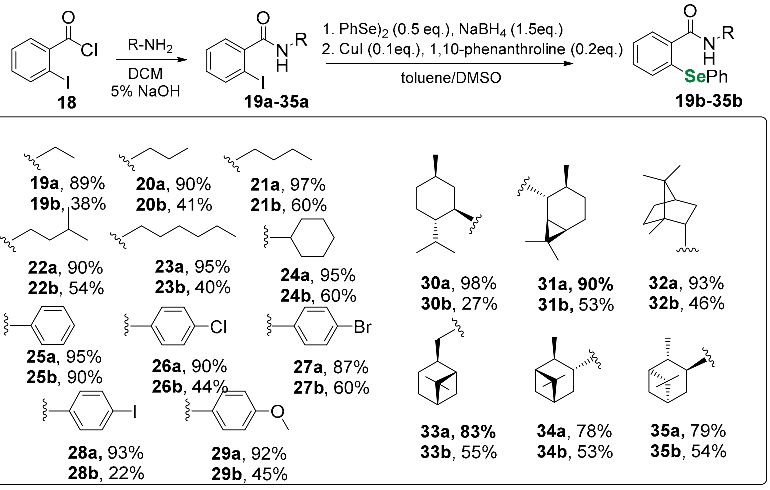


Scheme 4. Sythesis of benzeneseleninic acids **16a–f** and corresponding potassium salts **17a–f**.

2.4. Synthesis of N-substituted Unsymmetrical Phenylselenides

The simplicity of including aromatic or heteroaromatic rings in the structure of a compound and the possibility of their easy modification may turn out to be a way to increase the activity of the pharmacophore [13,14]. Taking this into account, we attempted to install an additional phenyl ring in the structure of ebselen and synthesize a series of N-substituted asymmetric phenylselenides bearing an o-amide group **19b–35b**.

The first step of the research involved the synthesis of N-substituted o-iodobenzamides **19a–35a** by the reaction of amines with o-iodobenzoic acid chloride **18**. The resulting benzamides **19a–35a** were converted into the corresponding N-aliphatic, N-aromatic, and chiral N-terpene phenylselenides **19b–35b** using a newly developed procedure involving nucleophilic copper-catalyzed substitution by Se-nucleophile generated in situ from diphenyl diselenide and sodium borohydride (Scheme 5) [15].

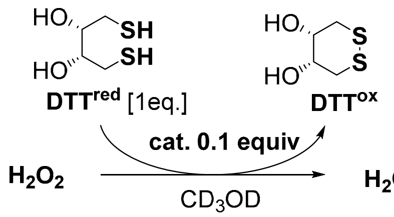


Scheme 5. Synthesis of N-substituted phenylselenides **19b–35b**.

2.5. Evaluation of the Antioxidant Activity

All obtained derivatives were tested as antioxidants using the popular NMR test developed by Iwaoka and co-workers [16]. The results with the highest antioxidant potential are presented in Table 1.

Table 1. Results of the antioxidant activity measurement.



	Remaining Dithiotreitol (%)				
Catalyst [0.1 equiv.]	**3 min**	**5 min**	**15 min**	**30 min**	**60 min**
Benzisoselenazolthiones					
3b	43	21	3	2	0
3e	40	26	18	17	15
***N*-terpenyl benzisoselenazol-3(2H)-ones**					
10/11	71	39	5	0	0
12	74	61	28	6	0
Benzeneseleninic acids					
16e	76	56	38	24	12
16f	85	64	37	18	2
Seleninic acid potassium salts					
17a–f	0	0	0	0	0
Phenylselenides					
21a	57	39	16	4	0
22a	98	97	94	88	71
Ebselen	84	75	64	58	52

Due to the fact that the change of the -SOOH group to the -SOOK group resulted in a drastic increase in activity (the reaction was completed in 3 min), all benzeneseleninic acid salts **17a–f** were evaluated by the same procedure but using 0.01 equivalent of the Se catalyst (Table 2).

Table 2. Results of the antioxidant activity measurement for salts.

	Remaining Dithiotreitol (%)				
Catalyst [0.01 equiv.]	**3 min**	**5 min**	**15 min**	**30 min**	**60 min**
Seleninic acid potassium salts					
17a	24	11	0	0	0
17e	59	16	0	0	0
Ebselen	97	96	95	94	92

The most important features improving the antioxidant activity was the presence of a bulky substituent that probably enables the facile cleavage of the Se-N bond (*N*-terpene derivatives **10–12**) and good solubility in water (benzeneseleninic acid salts **17a–f**).

2.6. Evaluation of the Cytotoxic Activity

The cytotoxic activity of the obtained derivatives was evaluated by the cell viability assay (MTT) on breast cancer MCF-7 [17] and human promyelocytic leukemia HL-60 cell lines. The IC_{50} values for compounds with the best results are presented in Table 3.

Table 3. Cytotoxic activity evaluated in vitro.

	MCF-7	**HL-60**		**MCF-7**	**HL-60**
	IC_{50}, μM			**IC_{50}, μM**	
***N*-terpenyl benzisoselenazol-3(2H)-ones**			**Seleninic acid potassium salts**		
10	19.9 ± 0.4	7.1 ± 0.4	**17f**	16.6 ± 1.1	42.1 ± 3.1
11	13.3 ± 1.1	20.6 ± 1.0	**Phenylselenides**		
7	12.4 ± 0.4	12.4 ± 0.9	**31b**	16.35 ± 0.29	16.3 ± 0.16
8	85.5 ± 4.0	61.3 ± 3.2	**Carboplatin**		
Benzeneseleninic acids				0.70 ± 0.30	3.19 ± 0.46
16a	40.1 ± 1.2	11.7 ± 1.0			

In the case of benzisoselenazolones and phenylselenides, the attachment of chiral bulky terpene substituents seemed to enhance the cytotoxic potential. Although the antiproliferative activity of all derivatives was lower than for the known drug carboplatin, the difference of reactivity of two enantiomeric pairs *N*-pinocampheyl **10** and **11** and *N*-menthyl derivatives **7** and **8** present an interesting example that the biological activity can be selectively modified by incorporating specific chiral structures on the nitrogen atom of the benzisoselenazolone core.

3. Conclusions

Herein, we have presented various modifications of the benzisoselenazolone core that enable improving the antioxidant and anticancer potential of the basic ebselen structure. The compounds with the highest antioxidant potential were the benzeneseleninic acid potassium salts **17a–f**. The best obtained antioxidant was 2-(*N*-ethylcarboxyamido)benzeneselenenic acid potassium salt **17a**, used in only 0.01 equivalent, for which the lack of substrate was observed after 15 min of reaction time. Among all tested derivatives, the highest antioxidant activity was observed for compounds with a 3-methylbutyl substituent. The highest antiproliferative potential toward the HL-60 cell line exhibited *N*-isopinocampheyl-1,2-benzisoselenazol-3(2*H*)-one 10 (IC_{50} of 7.1 ± 0.4 μM) and against MCF7 the *N*-menthyl-1,2-benzisoselenazol-3(2*H*)-one 7 (IC_{50} of 12.4 ± 0.4 μM).

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