

Article

The Influence of O/S Exchange on the Biocatalytical Activity of Benzisosenazol-3(2*H*)-ones

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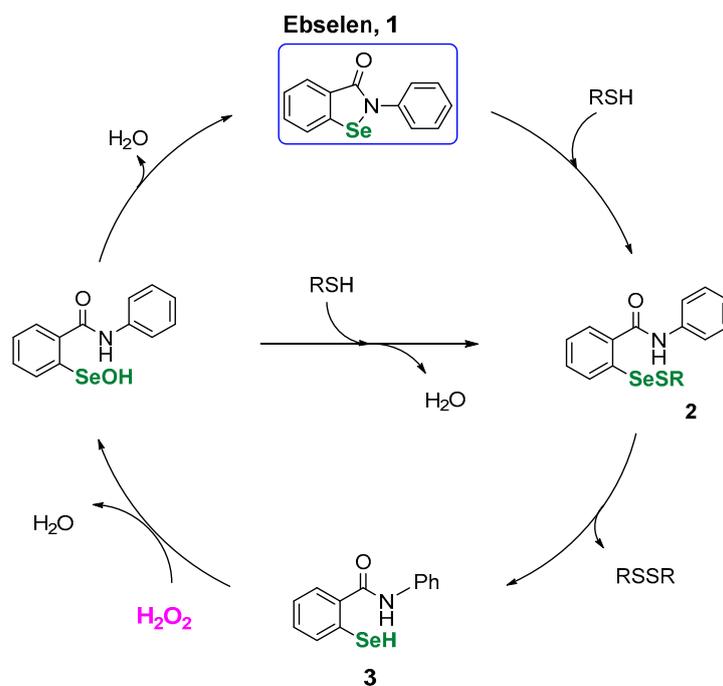
Abstract: The crucial feature of organoselenium compounds, when considering them as promising drug candidates in cancer therapy, is their unique ability to alter the cellular redox regulations. Organic Se-molecules continue to demonstrate a positive therapeutic effect both in cancer prevention—as antioxidants, and treatment—as prooxidants. The growing interest in this field of research highlights the need to search for particular pharmacophore motifs, which could enhance the efficiency and selectivity, and decrease the toxicity of potential anticancer agents. Herein, a series of redox-active organoselenium derivatives—*N*-functionalized benzisosenazol-3(2*H*)-thiones, has been designed and synthesized. A new synthetic pathway, with the application of Lawesson’s reagent, has been developed and efficiently applied. The key steps involving microwave irradiation facilitated performing the reaction in solvent-free conditions, shortening the reaction time and significantly improving the overall yield of the process. Six *N*-alkyl derivatives have been obtained and tested as antioxidant catalysts and anti-proliferative agents. The *N*-propyl benzisosenazol-3(2*H*)-thione was the best peroxide scavenger and the *N*-cyclohexyl derivative exhibited the best cytotoxic activity towards prostate cancer cell line DU145.

Keywords: selenium; benzisosenazol-3(2*H*)-thiones; redox catalysis; antioxidants; anti-proliferative activity

1. Introduction

Preservation of redox homeostasis, provided by powerful enzymatic and nonenzymatic antioxidant systems, is essential for cell viability and proper organ function. Environmental factors, UV light, air pollution and failure of the cellular antioxidant defense tools can induce redox imbalance, trigger subsequent overproduction of reactive oxygen species (ROS) and lead to oxidative stress. As physiological oxidative stress (eustress) is essential for redox regulations and signaling, the overoxidation (distress) can cause cell damage and contribute to various diseases including neurodegeneration, diabetes, hypertension and asthma. However, generation of ROS, in some cases like tissue regeneration and cancer, has a therapeutic influence [1]. Depending on the type of the pathophysiological process and also on the stage of the disease, the ability of molecules to influence the redox regulations, decreasing or increasing oxidative stress, can serve a positive impact. The “two-face” character of Selenium, able to act as an antioxidant and prooxidant, creates a good background to design new redox-active compounds with multifunctional pharmacological potential [2].

The unique properties of selenium, facile introduction, transformation and elimination from the structure of various organic species along with further numerous synthetic and biological application routes, build-up a constantly developing field of research [3–7]. Among all redox-active organoselenium molecules, ebselen (*N*-phenyl-benzisoselesazol-3(2*H*)-one) is still the only organoselenide admitted to clinical trial. Its mechanism of action is associated with the ability to mimic the catalytic activity of glutathione peroxidase (GPx), a selenoenzyme that protects from oxidative damage [8–11]. Ebselen 1 can directly reduce H_2O_2 after transformation to the corresponding selenol 3 [12,13] (Scheme 1).



Scheme 1. Mechanism of peroxide reduction by ebselen.

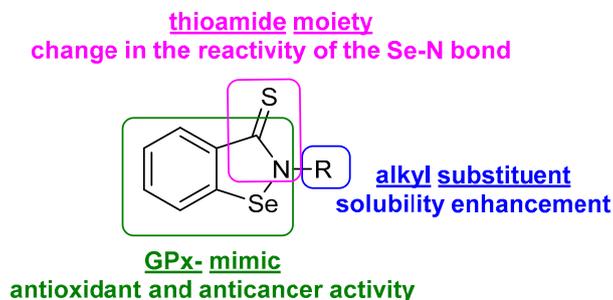
According to the most probable cycle of action, the essential step is the ability of compound 1 to form a Se-S bond in compound 2 with glutathione or other endogenous thiols. This way the molecule can act as an enzyme inhibitor, forming a seleno-sulfide conjugation with a cysteine residue, and thus providing other interesting pharmacological properties. Additionally, in higher concentrations, it can be used as a prooxidant and induce apoptosis of cancer cells [14].

To increase the activity and selectivity of ebselen, various modifications of its structure were efficiently conducted by several research groups. Promising results exposed the significant potential in this field of organoselenium chemistry [15–19]. However, there are only few examples of derivatives bearing a sulfur atom in the place of oxygen [20]. Until now, the influence of the thiocarbonyl group on the biological activity of benzisoselesazolones has not been thoroughly evaluated. Designing a new efficient method for the synthesis of benzisoselesazolthiones would make this group of organoselenium derivatives more accessible and as a consequence enable us to thoroughly investigate the influence of this modification on the activity of benzisoselesazolones. We assumed that the exchange of the carbonyl to thiocarbonyl group, leading to the decrease of the polarity of the double bond, could influence the stability of the Se-N bond and the speed of the formation of S-Se bond.

2. Results and Discussion

All obtained derivatives are a combination of 3 structural motifs: (1) the ebselen core, essential for the GPx-like activity, ability to catalytically eliminate reactive oxygen species and influence the physiological function of proteins by forming a seleno-sulfide bond with their thiol groups; (2) a thioamide moiety, similar to the thiourea structure, an important pharmacophore in medicinal

chemistry, broadly incorporated in the structure of various drugs e.g., anti-cancer, anti-bacterial and anti-viral [21]; (3) and an *N*-alkyl substituent that could potentially enhance the solubility of the compounds and influence the interaction of benzoselenazolthiones with different cell structures (Scheme 2).

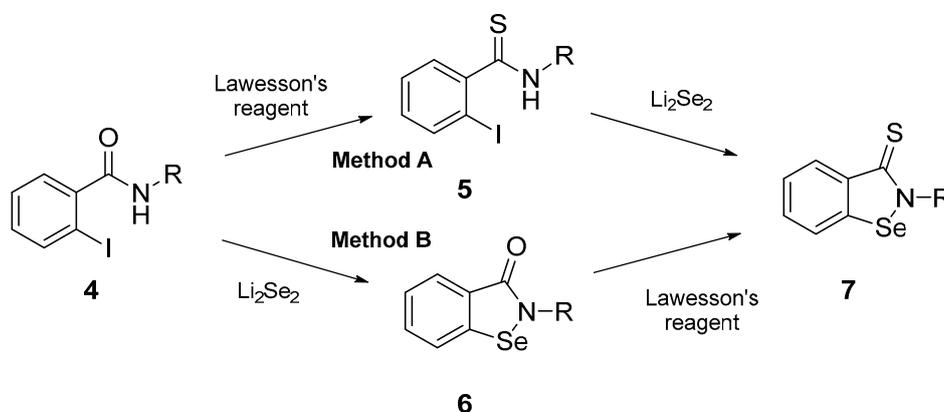


Scheme 2. General structure of the designed molecules.

Previously, we had observed that the stability of the Se-N bond influences the reactivity of benzoselenazolones. Derivatives that could be easily transformed to diselenides were the most efficient antioxidants. In contrast, compounds possessing a stable Se-N bond were weak peroxide scavengers but good anticancer agents [22]. The exchange of the carbonyl to thiocarbonyl group, presented in this paper, could influence the strength of the Se-N bond and the reactivity of the synthesized compounds. Additionally, the selection of different *N*-alkyl substituents, short, long and more sterically hindered carbon chains can enable us to choose the best chain length for achieving the highest biological activity.

2.1. Chemistry

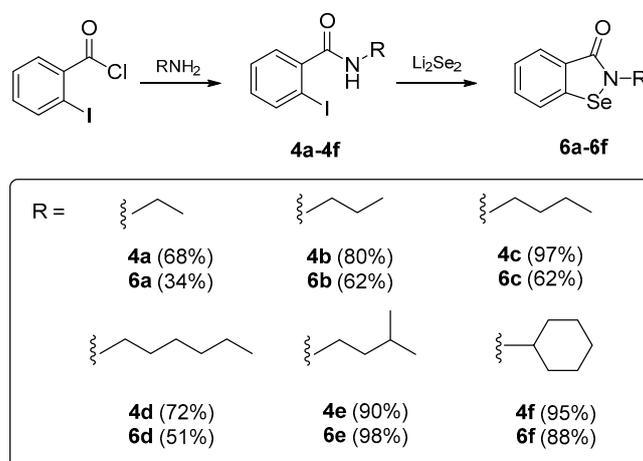
The main synthetical goal of the study was to develop an efficient procedure, using the commonly applied Lawesson's reagent [23] for the introduction of the sulfur atom in the structure of *N*-alkylbenzoselenazolones. We have planned to obtain *N*-alkylbenzoselenazolthiones by different two-step procedures: (Method A) involving the reaction of *N*-alkyl-*o*-iodobenzamides **4** with Lawesson's reagent to obtain corresponding thioamides **5**, followed by nucleophilic substitution with Li_2Se_2 leading to the final Se-N bond formation, and (Method B) also starting from *N*-alkyl-*o*-iodobenzamides **4**, their next reaction with Li_2Se_2 to obtain benzoselenazolones **6**, which are then directly converted to their sulfur analogues **7** (Scheme 3).



Scheme 3. Methods A and B used to obtain thio-derivatives 7.

The first step included the synthesis of carbonyl substrates for the transformation of oxygen-based functional groups to their thio-analogues. Using our previously designed procedures we have obtained

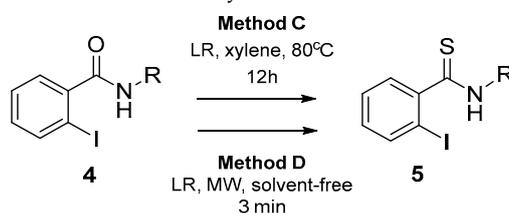
a series of *N*-alky-*o*-iodobenzamides **4a–f** that were further transformed, by the reaction with dilithium diselenide, into corresponding *N*-alkylbenzisoselenazolones **6a–f** [24,25] (Scheme 4).



Scheme 4. Synthesis of carbonyl substrates.

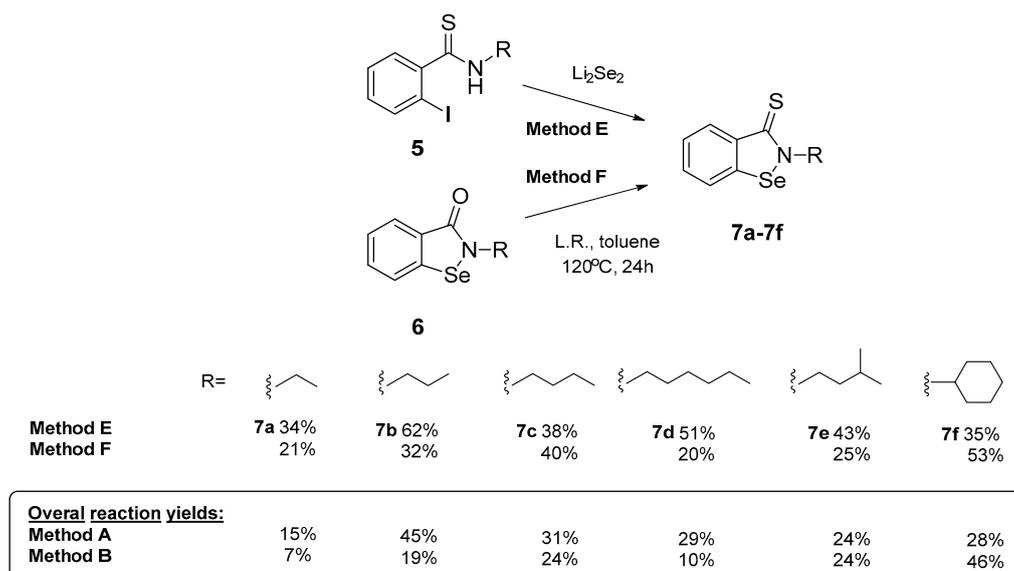
The treatment of amides **4a–f** with Lawesson's reagent (LR) carried out in standard conditions [26] (Method C) resulted in only moderate yields of the thioamides **5a–f**. Performing the reaction with microwave irradiation in solvent-free conditions (Method D) enabled us to significantly reduce the reaction time (3 min) and obtain the final product in good yield (44–81%) (Table 1).

Table 1. Thioamide synthesis—Method C and D.



<i>N</i> -Substituted <i>o</i> -Iodobenzothioamides	Yield (%)	
	Method C	Method D
5a	38	44
5b	61	73
5c	58	82
5d	48	58
5e	26	57
5f	22	81

Obtained thioamides **5a–f** were further transformed to benzisoselenazolthiones **7a–f**, by the procedure using dilithium diselenide (Method E) [24]. The same series of sulfur derivatives **7a–f** was synthesized by the reaction of previously obtained benzisoselenazolones **5a–f** with Lawesson's reagent (Method F). In contrast to method D, the reaction could not be performed under microwave irradiation. Decomposition of the substrate was observed and only traces of the product were isolated. High temperature and longer reaction time needed to be applied giving low to moderate reaction yields. The results of methods E and F, and overall yields of both two-step procedures A and B are presented in Scheme 5.



Scheme 5. Synthesis of *N*-alkylbenziselenazolthiones.

For all compounds besides the *N*-cyclohexyl derivative **7f**, method **A** was more efficient. The newly developed procedure involving microwave irradiation improved the methodology by shortening the time and increasing the yield of the reaction.

2.2. Catalytical and Biological Activity Evaluation

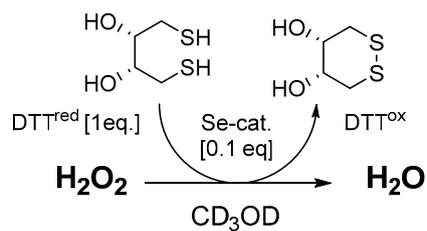
The final goal of the project was to evaluate the catalytical and biological potential of benziselenazolthiones **7a–f**. First, all derivatives were tested as antioxidants using a conventionally applied nuclear magnetic resonance spectroscopy (NMR) assay proposed by Iwaoka and co-workers [27]. The efficiency of the hydroperoxide reduction by the Se-catalyst was equal to the rate of oxidation of DTT^{red} to DTT^{ox}. The amount of the unreacted dithiol DTT^{red} was measured by ¹H NMR spectra in specific time intervals. The results for both carbonyl **6a–f** and thiocarbonyl **7a–f** derivatives are collected in Table 2.

The highest reactivity was observed for the *N*-propyl **7b** and *N*-3-methylbutyl benziselenazolthione **7e**. After 1 h for compound **7e** the amount of the remaining dithiol was only 15% and in case of derivative **7b** no substrate was present in the reaction mixture. Similar results were collected for corresponding oxo-derivatives **6b** and **6e**. In case of *N*-propyl benziselenazolthione **7b** and benziselenazolone **6b** the presence of sulfur atom instead of oxygen significantly increased the antioxidant potential. Interestingly, the high activity of compound **7e** fits to our previously observed trend that derivatives possessing a 3-methylbutyl carbon chain in their structure, like *N*-3-methylbutylbenziselenazolone **6e** [22] and *N*-leucine methyl ester derivative **8** [28], exhibit significantly better antioxidant potential than ebselen (Figure 1).

It can be concluded, that the presence of *N*-propyl and 3-methylbutyl carbon chain in the structure of *N*-alkyl benziselenazolones and benziselenazolthiones improves the peroxide scavenging properties. We can assume that the selected carbon chains provide an optimal combination of steric effect, Se–N bond stability and solubility enhancement.

Next, cytotoxic activity of the obtained derivatives was evaluated by the SRB viability assay on prostate cancer cell line DU-145 and also on non-cancerous cell line PNT1A. The IC₅₀ values for the tested benziselenazolthiones **7a–f**, in comparison to the activity of the corresponding oxo-analogues **6a–f** are presented in Table 3.

Table 2. Results of the antioxidant activity assay.



Catalyst [0.1 equiv.]	Remaining Dithiotreitol (%)				
	3 min	5 min	15 min	30 min	60 min
Benzisoselenazolones					
6a	87	80	65	56	46
6b	77	57	38	26	15
6c	81	59	41	32	29
6d	92	84	81	78	75
6e	77	58	42	28	13
6f	75	69	62	55	44
Benzisoselenazolthiones					
7a	99	97	96	95	93
7b	43	21	3	2	0
7c	97	97	96	96	95
7d	99	98	98	98	97
7e	40	26	18	17	15
7f	90	84	74	65	47
Ebselen (1)	84	75	64	58	52

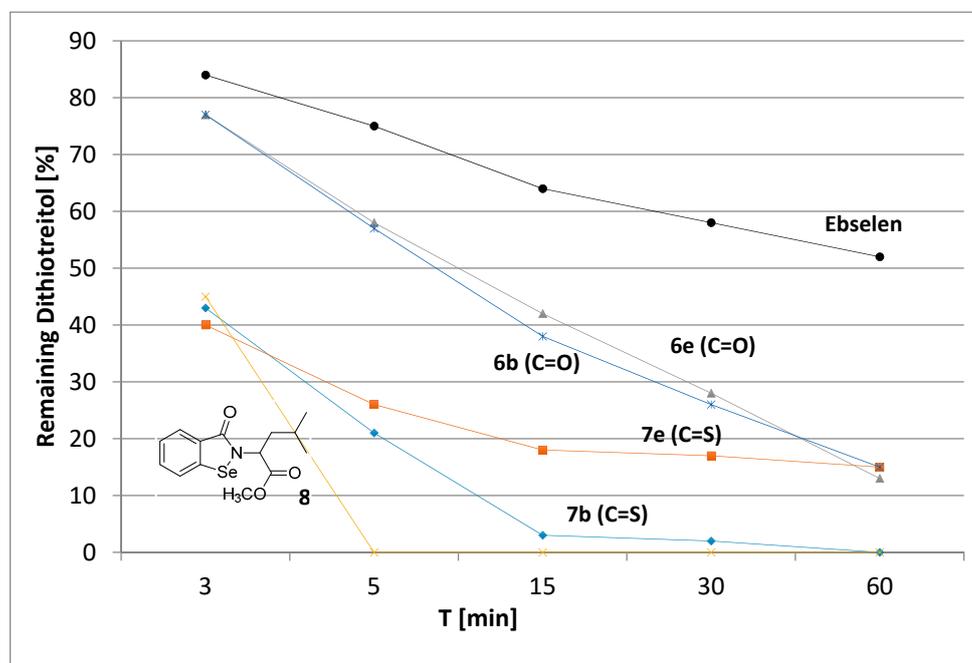


Figure 1. Graphical comparison of antioxidant activities.

Table 3. Cytotoxic activity evaluated in vitro.

Compound	DU-145	PNT1A
	IC ₅₀ , μ M	
6a	30.06	20.68
6b	30.21	>60
6c	20.76	>40
6e	30.39	>40
6f	5.71	>40
7a	15.33	20.29
7b	20.36	20.77
7f	10.7	5.63
40 μ M (Cell Viability (%))		
6d	51.429	95.669
7c	52.161	58.060
7d	56.829	65.027
7e	61.177	60.74

The best antiproliferative activity was observed for *N*-cyclohexylbenzisoselenazolthione **7f**. The corresponding *N*-cyclohexylbenzisoselenazolone **6f** was also the most efficient cytotoxic agent among the previously tested *N*-alkyl benzisoselenazolones [22]. However, in contrary to thio-analogue **7f**, the carbonyl derivative selectively inhibited the proliferation of cancer cell line DU-145 and was not toxic to non-cancerous cell line PNT1A.

3. Materials and Methods

3.1. Chemistry

3.1.1. General

¹H NMR spectra were obtained at 400 or 700 MHz and chemical shifts were recorded relative to SiMe₄ (δ 0.00) or solvent resonance (CDCl₃ δ 7.26, CD₃OD δ 3.31). Multiplicities were given as: s (singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet) and m (multiplet). The number of protons (n) for a given resonance was indicated by nH. Coupling constants were reported as a *J* value in Hz. ¹³C NMR spectra were acquired at 100.6 MHz and chemical shifts were recorded relative to solvent resonance (CDCl₃ δ 77.25). NMR spectra were carried out using ACD/NMR Processor Academic Edition (please see the Supplementary Materials). Commercially available solvents DMF, DCM and MeOH (Sigma Aldrich, St. Louis, MO, USA) and chemicals were used without further purification. Column chromatography was performed using Merck 40-63D 60 Å silica gel (Sigma Aldrich, St. Louis, MO, USA).

3.1.2. Synthesis of *o*-iodobenzamides **4a**, **4b** and **4d**

2% NaOH (4.4 mL) was added to a solution of an amine (1.0 mmol) in DCM (2 mL). The mixture was cooled to 0 °C and *o*-iodobenzoic acid chloride (1.1 mmol) dissolved in DCM (3 mL) was added dropwise. The reaction mixture was stirred in room temperature for 20 h and the product was extracted with DCM. Combined organic layers were washed with saturated NaHCO₃ and dried over magnesium sulfate. The solvent was removed under reduced pressure and the product was obtained as white solid. Synthesis and characterization of compounds **4c**, **4e** and **4f** are described in our previous paper [24]

N-ethyl-*o*-iodobenzamide **4a**

Yield: 68%; m.p. 107–109 °C; ¹H NMR (400 MHz, CDCl₃) δ = 1.29 (t, *J* = 7.2 Hz, 3H, CH₃), 3.48–3.56 (m, 2H, N-CH₂), 5.73 (bs, 1H, NH), 7.10 (ddd, *J* = 8.0, 6.8, 2.4 Hz, 1H, 1H_{ar}), 7.36–7.43 (m, 2H, 2H_{ar}), 7.86 (dd, *J* = 7.6, 0.8 Hz, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 14.68 (CH₃), 35.01

(CH₂-N), 92.42 (C_{ar}), 128.16 (CH_{ar}), 128.24 (CH_{ar}), 131.00 (CH_{ar}), 139.80 (CH_{ar}), 142.47 (C_{ar}), 169.26 (C=O) ppm [29].

N-propyl-*o*-iodobenzamide **4b**

Yield: 80%; m.p. 105–107 °C; ¹H NMR (400 MHz, CDCl₃) δ = 1.04 (t, *J* = 7.2 Hz, 3H, CH₃), 1.66–1.73 (m, 2H, CH₂), 3.42–3.47 (m, 2H, N-CH₂), 5.78 (bs, 1H, NH), 7.10 (ddd, *J* = 8.0, 6.8, 2.4 Hz, 1H, 1H_{ar}), 7.36–7.42 (m, 2H, 2H_{ar}), 7.87 (dd, *J* = 7.6, 0.8 Hz, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 11.53 (CH₃), 22.72 (CH₂), 41.83 (CH₂-N), 92.38 (C_{ar}), 128.16 (CH_{ar}), 128.27 (CH_{ar}), 131.00 (CH_{ar}), 139.83 (CH_{ar}), 142.60 (C_{ar}), 169.37 (C=S) ppm [30].

N-hexyl-*o*-iodobenzamide **4d**

Yield: 72%; m.p. 98–100 °C; ¹H NMR (700 MHz, CDCl₃) δ = 0.93 (t, *J* = 7.0 Hz, 3H, CH₃), 1.30–1.35 (m, 4H, 2×CH₂), 1.39–1.43 (m, 2H, CH₂), 1.61–1.65 (m, 2H, CH₂), 3.43–3.46 (m, 2H, N-CH₂), 5.73 (bs, 1H, NH), 7.10 (ddd, *J* = 7.7, 7.0, 2.1 Hz, 1H, 1H_{ar}), 7.35–7.40 (m, 2H, 2H_{ar}), 7.85 (dd, *J* = 7.7, 0.7 Hz, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 14.03 (CH₃), 22.57 (CH₂), 26.68 (CH₂), 29.36 (CH₂), 31.47 (CH₂), 40.13 (CH₂-N), 92.44 (C_{ar}), 128.12 (CH_{ar}), 128.25 (CH_{ar}), 130.93 (CH_{ar}), 139.78 (CH_{ar}), 142.58 (C_{ar}), 169.32 (C=O) ppm [31].

3.1.3. Synthesis of *o*-iodobenzthioamides **5a–5f**

Method C: To a solution of amide (1.0 mmol) in xylene (10 mL) Lawesson's reagent (0.70 mmol) was added portionwise and the mixture was stirred overnight at 80 °C. Solvent was removed under vacuum and the resulting crude product was purified by silica gel column chromatography (solvent: Dichloromethane).

Method D: Amide (1.0 mmol) and Lawesson's reagent (0.70 mmol) was manually mixed in a beaker and placed in a household microwave oven for 3 min (700 W). The crude product was dissolved in dichloromethane, directly placed on silica and purified by silica gel column chromatography (solvent: Dichloromethane).

N-ethyl-*o*-iodobenzthioamide **5a**

Yield: 38, 44%; m.p. 155–157 °C; ¹H NMR (400 MHz, CDCl₃) δ = 1.41 (t, *J* = 7.6 Hz, 3H, CH₃), 3.83–3.90 (m, 2H, N-CH₂), 7.05 (ddd, *J* = 8.0, 8.0, 1.6 Hz, 1H, 1H_{ar}), 7.25 (bs, 1H, NH), 7.37 (dt, *J* = 7.6, 1.2 Hz, 1H, 1H_{ar}), 7.45 (dd, *J* = 7.6, 1.2 Hz, 1H, 1H_{ar}), 7.83 (dd, *J* = 8.0, 1.2 Hz, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 12.97 (CH₃), 41.21 (CH₂-N), 92.04 (C_{ar}), 128.25 (CH_{ar}), 128.35 (CH_{ar}), 130.24 (CH_{ar}), 139.60 (CH_{ar}), 148.29 (C_{ar}), 200.88 (C=S); IR: 3158, 3042, 2973, 2930, 1548, 1449, 1398, 1336, 1298, 1259, 1231, 1053, 1017 cm⁻¹. Elemental Anal. Calcd for C₉H₁₀INS (291.15): C, 37.13; H, 3.46. Found: C, 37.02; H, 3.41.

N-propyl-*o*-iodobenzthioamide **5b**

Yield: 61, 73%; m.p. 128–130 °C; ¹H NMR (400 MHz, CDCl₃) δ = 1.09 (t, *J* = 7.2 Hz, 3H, CH₃), 1.79–1.88 (m, 2H, CH₂), 3.77–3.82 (m, 2H, N-CH₂), 7.05 (ddd, *J* = 8.0, 7.6, 2.0 Hz, 1H, 1H_{ar}), 7.25 (bs, 1H, NH), 7.37 (dt, *J* = 7.6, 1.2 Hz, 1H, 1H_{ar}), 7.45 (dd, *J* = 7.6, 1.6 Hz, 1H, 1H_{ar}), 7.83 (dd, *J* = 8.4, 1.2 Hz, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 11.73 (CH₃), 21.20 (CH₂), 48.04 (CH₂-N), 91.92 (C_{ar}), 128.27 (CH_{ar}), 128.35 (CH_{ar}), 130.22 (CH_{ar}), 139.63 (CH_{ar}), 148.48 (C_{ar}), 201.19 (C=S); IR: 3151, 3025, 2987, 2963, 2926, 2869, 1538, 1454, 1441, 1429, 1413, 1394, 1372, 1353, 1296, 1282, 1252, 1224, 1179, 1153, 1115, 1059, 1034, 1011 cm⁻¹. Elemental Anal. Calcd for C₁₀H₁₂INS (305.177): C, 39.36; H, 3.96. Found: C, 39.45; H, 3.92.

N-butyl-*o*-iodobenzthioamide 5c

Yield: 58, 62%; m.p. 73–75 °C; $^1\text{H NMR}$ (700 MHz, CDCl_3) δ = 1.02 (t, J = 7.7 Hz, 3H, CH_3), 1.50–1.54 (m, 2H, CH_2), 1.77–1.81 (m, 2H, CH_2), 3.82–3.85 (m, 2H, N- CH_2), 7.06 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H, 1H_{ar}), 7.28 (bs, 1H, NH), 7.39 (ddd, J = 7.7, 7.0, 1.4 Hz, 1H, 1H_{ar}), 7.45 (dd, J = 7.7, 1.4 Hz, 1H, 1H_{ar}), 7.84 (dd, J = 8.4, 1.4 Hz, 1H, 1H_{ar}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3) δ = 13.76 (CH_3), 20.38 (CH_2), 29.85 (CH_2), 46.10 ($\text{CH}_2\text{-N}$), 91.96 (C_{ar}), 128.26 (CH_{ar}), 128.35 (CH_{ar}), 130.20 (CH_{ar}), 139.62 (CH_{ar}), 148.45 (C_{ar}), 201.05 (C=S); IR: 3158, 3029, 2989, 2961, 2927, 2869, 1540, 1455, 1428, 1392, 1354, 1339, 1303, 1280, 1261, 1217, 1154, 1115, 1060, 1044, 1013 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{11}\text{H}_{14}\text{INS}$ (319.204): C, 41.39; H, 4.42. Found: C, 41.34; H, 4.49.

N-hexyl-*o*-iodobenzthioamide 5d

Yield: 48, 58%; yellow oil; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ = 0.93 (t, J = 7.2 Hz, 3H, CH_3), 1.35–1.37 (m, 4H, $2\times\text{CH}_2$), 1.44–1.51 (m, 2H, CH_2), 1.76–1.83 (m, 2H, CH_2), 3.79–3.84 (m, 2H, N- CH_2), 7.04 (ddd, J = 9.2, 7.6, 2.0 Hz, 1H, 1H_{ar}), 7.25 (bs, 1H, NH), 7.37 (dt, J = 7.2, 0.8 Hz, 1H, 1H_{ar}), 7.44 (dd, J = 7.6, 1.6 Hz, 1H, 1H_{ar}), 7.83 (dd, J = 8.0, 1.2 Hz, 1H, 1H_{ar}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3) δ = 14.01 (CH_3), 22.54 (CH_2), 26.84 (CH_2), 27.78 (CH_2), 31.42 (CH_2), 46.39 ($\text{CH}_2\text{-N}$), 91.92 (C_{ar}), 128.28 (CH_{ar}), 128.35 (CH_{ar}), 130.20 (CH_{ar}), 139.62 (CH_{ar}), 148.49 (C_{ar}), 201.05 (C=S); IR: 3193, 3045, 2953, 2925, 2854, 1581, 1521, 1459, 1429, 1391, 1337, 1306, 1281, 1257, 1239, 1220, 1195, 1159, 1114, 1100, 1070, 1015 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{13}\text{H}_{18}\text{INS}$ (347.258): C, 44.96; H, 5.22. Found: C, 44.60; H, 5.30.

N-(3-methylbutyl)-*o*-iodobenzthioamide 5e

Yield: 26, 57%; m.p. 69–71 °C; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ = 1.00 (d, J = 6.4 Hz, 6H, $2\times\text{CH}_3$), 1.64–1.69 (m, 2H, CH_2), 1.73–1.80 (m, 1H, CH), 3.79–3.84 (m, 2H, N- CH_2), 7.04 (ddd, J = 8.0, 7.2, 2.0 Hz, 1H, 1H_{ar}), 7.30 (bs, 1H, NH), 7.36 (dt, J = 7.2, 1.2 Hz, 1H, 1H_{ar}), 7.40 (dd, J = 8.0, 2.0 Hz, 1H, 1H_{ar}), 7.81 (dd, J = 8.0, 1.2 Hz, 1H, 1H_{ar}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3) δ = 22.48 ($2\times\text{CH}_3$), 26.14 (CH), 36.56 (CH_2), 44.72 (N- CH_2), 92.04 (C_{ar}), 128.21 (CH_{ar}), 128.35 (CH_{ar}), 130.20 (CH_{ar}), 139.60 (CH_{ar}), 148.39 (C_{ar}), 200.90 (C=S); IR: 3197, 3050, 2955, 2929, 2866, 1542, 1454, 1428, 1384, 1361, 1339, 1327, 1308, 1278, 1263, 1248, 1213, 1156, 1068, 1044, 1014 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{12}\text{H}_{16}\text{INS}$ (333.231): C, 43.25; H, 4.84. Found: C, 43.46; H, 4.83.

N-cyclohexyl-*o*-iodobenzthioamide 5f

Yield: 41, 52%; m.p. 138–141 °C; $^1\text{H NMR}$ (700 MHz, CDCl_3): δ = 1.25–1.31 (m, 1H, CH), 1.37–1.41 (m, 2H, CH_2), 1.47–1.54 (m, 2H, CH_2), 1.69–1.72 (m, 1H, CH), 1.81–1.84 (m, 2H, CH_2), 2.27–2.28 (m, 2H, CH_2), 7.54–7.58 (m, 1H, CH), 7.05 (dt, J = 7.0, 1.4 Hz, 1H, 1H_{ar}), 7.14 (bs, 1H, NH), 7.38 (dt, J = 7.0, 0.7 Hz, 1H, 1H_{ar}), 7.45 (dd, J = 7.7, 1.4 Hz, 1H, 1H_{ar}), 7.84 (dd, J = 9.1, 1.4 Hz, 1H, 1H_{ar}) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): δ = 25.64 ($2\times\text{CH}_2$), 26.49 (CH_2), 32.33 ($2\times\text{CH}_2$), 55.66 (CH-N), 92.84 (C_{ar}), 129.24 (CH_{ar}), 129.35 (CH_{ar}), 131.11 (CH_{ar}), 140.62 (CH_{ar}), 149.45 (C_{ar}), 200.54 (C=S) ppm; IR: 3202, 3039, 2924, 2852, 1533, 1459, 1445, 1426, 1382, 1358, 1344, 1309, 1277, 1260, 1251, 1227, 1152, 1093, 1044, 1013 cm^{-1} . Elemental Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{INS}$ (345.242): C, 45.23; H, 4.67. Found: C, 45.63; H, 4.53.

3.1.4. Synthesis of Benziselenazol-3(2H)-ones 6a, 6b and 6d

Hydrazine hydrate (8.0 mmol) was added dropwise to the mixture of selenium powder (1.2 mmol) and lithium hydroxide (3.6 mmol) in DMF (3 mL). Reaction was heated to 120 °C and stirred for 15 min under argon atmosphere. After cooling to room temperature, the amide (1.0 mmol) in DMF (2 mL) was added. The reaction mixture was heated to 120 °C and stirred for 20 h under argon atmosphere. The solution was cooled, 25 mL of brine was added and the mixture was stirred for an additional 20 h. Precipitate was filtered under vacuum, washed with water and dried in air. The crude product was purified by column chromatography (silica gel, DCM). Synthesis and characterization of compounds **6c**, **6e** and **6f** are described in our previous paper [24].

N-ethyl-1,2-benzisoselenazol-3(2H)-one 6a

Yield: 68%; m.p. 93–96 °C, (lit. [32] m.p. 92–94 °C); ^1H NMR (400 MHz, CDCl_3) δ = 1.34 (t, J = 7.2 Hz, 3H, CH_3), 3.88–3.93 (m, 2H, N- CH_2), 7.41 (ddd, J = 7.6, 7.2, 1.2 Hz, 1H, 1H_{ar}), 7.57 (ddd, J = 7.6, 7.2, 1.2 Hz, 1H, 1H_{ar}), 7.66 (dt, J = 8.0, 0.8 Hz, 1H, 1H_{ar}), 8.04 (ddd, J = 8.0, 1.2, 0.8, 1H, 1H_{ar}) ppm; ^{13}C NMR (100.6 MHz, CDCl_3) δ = 15.66 (CH_3), 39.85 ($\text{CH}_2\text{-N}$), 124.09 (CH_{ar}), 126.17 (CH_{ar}), 127.72 (C_{ar}), 128.71 (CH_{ar}), 131.83 (CH_{ar}), 137.68 (C_{ar}), 166.93 (C=O) ppm; ^{77}Se (76.3 MHz, CDCl_3), δ = 877.01 ppm.

N-propyl-1,2-benzisoselenazol-3(2H)-one 6b

Yield: 81%; m.p. 71–71 °C, (lit. [32] m.p. 70–72 °C); ^1H NMR (700 MHz, CDCl_3) δ = 1.00 (t, J = 7.7 Hz, 3H, CH_3), 1.73–1.79 (m, 2H, CH_2), 3.83 (t, J = 7.7 Hz, 2H, N- CH_2), 7.42 (ddd, J = 7.0, 7.0, 0.7 Hz, 1H, 1H_{ar}), 7.58 (ddd, J = 7.0, 7.0, 1.4 Hz, 1H, 1H_{ar}), 7.63 (dd, J = 9.1, 1.4 Hz, 1H, 1H_{ar}), 8.05 (dd, J = 7.7, 0.7 Hz, 1H, 1H_{ar}) ppm; ^{13}C NMR (100.6 MHz, CDCl_3) δ = 11.16 (CH_3), 23.89 (CH_2), 46.47 ($\text{CH}_2\text{-N}$), 123.92 (CH_{ar}), 126.17 (CH_{ar}), 127.67 (C_{ar}), 128.87 (CH_{ar}), 131.84 (CH_{ar}), 137.63 (C_{ar}), 167.20 (C=O) ppm; ^{77}Se (76.3 MHz, CDCl_3), δ = 884.45 ppm.

N-hexyl-1,2-benzisoselenazol-3(2H)-one 6d

Yield: 72%; m.p. 83–85 °C, (lit. [33] m.p. 84–85 °C); ^1H NMR (400 MHz, CDCl_3) δ = 0.90 (t, J = 7.2 Hz, 3H, CH_3), 1.32–1.43 (m, 6H, $3\times\text{CH}_2$), 1.70–1.77 (m, 2H, CH_2), 3.87 (t, J = 7.2 Hz, 2H, N- CH_2), 7.41–7.46 (m, 1H, 1H_{ar}), 7.59 (dt, J = 6.8, 1.6 Hz, 1H, 1H_{ar}), 7.65 (ddd, J = 8.0, 1.2, 0.8 Hz, 1H, 1H_{ar}), 8.05 (ddd, J = 8.0, 1.2, 0.8 Hz, 1H, 1H_{ar}) ppm; ^{13}C NMR (100.6 MHz, CDCl_3) δ = 13.99 (CH_3), 22.50 (CH_2), 26.30 (CH_2), 30.55 (CH_2), 31.45 (CH_2), 44.89 ($\text{CH}_2\text{-N}$), 123.93 (CH_{ar}), 126.16 (CH_{ar}), 127.70 (C_{ar}), 128.84 (CH_{ar}), 131.82 (CH_{ar}), 137.64 (C_{ar}), 167.13 (C=O) ppm; ^{77}Se (76.3 MHz, CDCl_3), δ = 883.31 ppm.

3.1.5. Synthesis of Benzisoselenazol-3(2H)-thiones 7a–7f

Method E: Hydrazine hydrate (8.0 mmol) was added dropwise to the mixture of selenium powder (1.2 mmol) and lithium hydroxide (3.6 mmol) in DMF (3 mL). Reaction was heated to 120 °C and stirred for 15 min under argon atmosphere. After cooling to room temperature, the thioamide (1.0 mmol) in DMF (2 mL) was added. The reaction mixture was heated to 120 °C and stirred for 20 h under argon atmosphere. The solution was cooled, 25 mL of brine was added and the mixture was stirred for additional 20 h. The crude product was extracted with DCM combined organic layers were washed with water to remove DMF, dried with magnesium sulfate and evaporated. The crude product was purified by column chromatography (silica gel, DCM/hexane, 80:20).

Method F: To a solution of benzisoselenazolone (1.0 mmol) in toluene (10 mL) Lawesson's reagent (0.70 mmol) was added portionwise and the mixture was stirred overnight at 120 °C. Solvent was removed under vacuum and the resulting crude product was purified by silica gel column chromatography (solvent: CHCl_3).

N-ethyl-1,2-benzisoselenazol-3(2H)-thione 7a

Yield: 34, 21%; yellow oil; ^1H NMR (400 MHz, CDCl_3) δ = 1.48 (t, J = 7.2 Hz, 3H, CH_3), 3.57–3.61 (m, 2H, N- CH_2), 7.35 (t, J = 7.2 Hz, 1H, 1H_{ar}), 7.48 (t, J = 6.8 Hz, 1H, 1H_{ar}), 7.55 (d, J = 8.0 Hz, 1H, 1H_{ar}), 8.08 (bs, 1H, 1H_{ar}) ppm; ^{13}C NMR (100.6 MHz, CDCl_3) δ = 16.48 (CH_3), 51.33 ($\text{CH}_2\text{-N}$), 126.79 (CH_{ar}), 126.93 (CH_{ar}), 129.45 (CH_{ar}), 132.33 (CH_{ar}), 135.30 (C_{ar}), 140.96 (C_{ar}), 164.91 (C=S) ppm; ^{77}Se (76.3 MHz, CDCl_3), δ = 527.74 ppm [20].

N-propyl-1,2-benzisoselenazol-3(2H)-thione 7b

Yield: 62, 32%; yellow oil; ^1H NMR (700 MHz, CDCl_3) δ = 1.10 (t, J = 7.0 Hz, 3H, CH_3), 1.88–1.93 (m, 2H, CH_2), 3.50 (t, J = 7.0 Hz, 2H, N- CH_2), 7.35 (t, J = 7.0 Hz, 1H, 1H_{ar}), 7.47 (t, J = 7.0 Hz, 1H, 1H_{ar}), 7.56 (d, J = 8.4 Hz, 1H, 1H_{ar}), 8.09 (bs, 1H, 1H_{ar}) ppm; ^{13}C NMR (100.6 MHz, CDCl_3) δ = 12.22 (CH_3),

23.95 (CH₂), 57.82 (CH₂-N), 125.80 (CH_{ar}), 125.85 (CH_{ar}), 128.45 (CH_{ar}), 131.20 (CH_{ar}), 134.45 (C_{ar}), 139.95 (C_{ar}), 163.81 (C=S) ppm; ⁷⁷Se (76.3 MHz, CDCl₃), δ = 525.91 ppm [20].

N-butyl-1,2-benzisoselenazol-3(2*H*)-thione **7c**

Yield: 40, 38%; yellow oil; ¹H NMR (700 MHz, CDCl₃) δ = 0.99 (t, *J* = 7.0 Hz, 3H, CH₃), 1.48–1.53 (m, 2H, CH₂), 1.80–1.84 (m, 2H, CH₂), 3.51 (t, *J* = 7.0 Hz, 2H, N-CH₂), 7.31 (dt, *J* = 7.7, 0.7 Hz, 1H, 1H_{ar}), 7.43 (td, *J* = 8.4, 1.4 Hz, 1H, 1H_{ar}), 7.51 (d, *J* = 7.7 Hz, 1H, 1H_{ar}), 8.05 (bs, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 13.97 (CH₃), 20.80 (CH₂), 32.67 (CH₂), 55.77 (CH₂-N), 125.82 (CH_{ar}), 125.87 (CH_{ar}), 128.45 (CH_{ar}), 131.23 (CH_{ar}), 134.44 (C_{ar}), 139.96 (C_{ar}), 163.95 (C=S) ppm; ⁷⁷Se (76.3 MHz, CDCl₃), δ = 526.60 ppm [20].

N-hexyl-1,2-benzisoselenazol-3(2*H*)-thione **7d**

Yield: 51, 20%; yellow oil; ¹H NMR (700 MHz, CDCl₃) δ = 0.91 (t, *J* = 7.0 Hz, 3H, CH₃), 1.33–1.38 (m, 4H, 2xCH₂), 1.45–1.50 (m, 2H, CH₂), 1.81–1.85 (m, 2H, CH₂), 3.50 (t, *J* = 7.0 Hz, 2H, N-CH₂), 7.31 (t, *J* = 7.7 Hz, 1H, 1H_{ar}), 7.44 (t, *J* = 7.0 Hz, 1H, 1H_{ar}), 7.52 (d, *J* = 7.7 Hz, 1H, 1H_{ar}), 8.02 (bs, 1H, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 14.10 (CH₃), 22.67 (CH₂), 27.34 (CH₂), 30.51 (CH₂), 31.69 (CH₂), 56.13 (CH₂-N), 125.79 (CH_{ar}), 125.88 (CH_{ar}), 128.47 (CH_{ar}), 131.24 (CH_{ar}), 134.41 (C_{ar}), 139.94 (C_{ar}), 163.67 (C=S) ppm; ⁷⁷Se (76.3 MHz, CDCl₃), δ = 525.70 ppm; IR: 3048, 3009, 2952, 2922, 2853, 1606, 1582, 1555, 1460, 1434, 1402, 1375, 1347, 1314, 1299, 1278, 1254, 1241, 1200, 1159, 1121, 1078, 1041, 1016 cm⁻¹. Elemental Anal. Calcd for C₁₃H₁₇NSSe (298.306): C, 52.34; H, 5.74. Found: C, 52.03; H, 5.65.

N-(3-methylbutyl)-1,2-benzisoselenazol-3(2*H*)-thione **7e**

Yield: 43, 25%; yellow oil; ¹H NMR (700 MHz, CDCl₃) δ = 0.99 (d, *J* = 7.0 Hz, 6H, 2xCH₃), 1.72–1.75 (m, 2H, CH₂), 1.80–821. (m, 1H, CH), 3.53 (t, *J* = 7.0 Hz, 2H, N-CH₂), 7.31 (t, *J* = 7.7 Hz, 1H, 1H_{ar}), 7.43 (dt, *J* = 7.7, 0.7 Hz, 1H, 1H_{ar}), 7.52 (d, *J* = 8.4 Hz, 1H, 1H_{ar}), 8.03 (bs, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃) δ = 22.63 (2xCH₃), 26.34 (CH), 39.34 (CH₂), 54.31 (CH₂-N), 125.79 (CH_{ar}), 125.87 (CH_{ar}), 128.46 (CH_{ar}), 131.22 (CH_{ar}), 134.44 (C_{ar}), 139.96 (C_{ar}), 163.83 (C=S) ppm; ⁷⁷Se (76.3 MHz, CDCl₃), δ = 526.38 ppm; IR: 2952, 2922, 2866, 1608, 1582, 1523, 1460, 1437, 1383, 1364, 1327, 1299, 1265, 1218, 1158, 1128, 1047, 1023, 1007 cm⁻¹. Elemental Anal. Calcd for C₁₂H₁₅NSSe (284.279): C, 50.70; H, 5.32. Found: C, 50.41; H, 5.37.

N-cyclohexyl-1,2-benzisoselenazol-3(2*H*)-thione **7f**

Yield: 35, 53%; yellow oil; ¹H NMR (400 MHz, CDCl₃): δ = 1.33–1.38 (m, 1H, CH), 1.38–1.50 (m, 2H, CH₂), 1.54–1.62 (m, 2H, CH₂), 1.69–1.72 (m, 1H, CH), 1.85–1.95 (m, 4H, 2xCH₂), 3.45–3.52 (m, 1H, N-CH), 7.31 (dt, *J* = 8.0, 1.2 Hz, 1H, 1H_{ar}), 7.44 (dt, *J* = 6.8, 1.2 Hz, 1H, 1H_{ar}), 7.51 (d, *J* = 8.0 Hz, 1H, 1H_{ar}), 8.05 (d, *J* = 7.2 Hz, 1H, 1H_{ar}) ppm; ¹³C NMR (100.6 MHz, CDCl₃): δ = 24.67 (2xCH₂), 25.79 (CH₂), 32.78 (2xCH₂), 65.60 (CH-N), 125.75 (CH_{ar}), 125.81 (CH_{ar}), 128.82 (CH_{ar}), 131.20 (CH_{ar}), 134.68 (C_{ar}), 139.77 (C_{ar}), 161.22 (C=S) ppm; ⁷⁷Se (76.3 MHz, CDCl₃), δ = 514.27 ppm; IR: 2926, 2850, 1438, 1384, 1345, 1277, 1257, 1240, 1127, 1099, 1019 cm⁻¹. Elemental Anal. Calcd for C₁₃H₁₅NSSe (296.290): C, 52.70; H, 5.10. Found: C, 52.97; H, 5.04.

3.2. Biological Activity Evaluation

3.2.1. Antioxidant Activity Assay

To a solution of compounds and **16–25** (0.015 mmol) and dithiothreitol DTT^{red} (0.15 mmol) in 1.1 mL of CD₃OD 30% H₂O₂ (0.15 mmol) was added. ¹H NMR spectra were measured right after the addition of hydrogen peroxide and then in specific time intervals. The concentration of the substrate was determined according to the changes in the integration on the ¹H NMR spectra. [27]

3.2.2. SRB viability Assay

Cell Culture

The prostate cancer cell line DU-145 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The DU-145 cells were cultured in MEME medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 2 mM glutamine and 1 mM sodium pyruvate at 37 °C. The PNT1A cells were cultured in RPMI 1640 supplemented with serum, L-glutamine and antibiotics. The cells were maintained at 37 °C in an atmosphere containing 5% CO₂. Stock solutions of *N*-substituted ebselen derivatives were prepared in (0.1%) DMSO.

SRB Assay

Cell viability was measured by Sulphorhodamine B (SRB) assay. The cells were grown to sub-confluent levels at the certain culture medium and then seeded into 96-well plates at 6.0×10^3 cells/well in the final volume of 200 μ L in the culture medium for 24 h. Then, they were treated with various concentrations (2.5, 5, 10, 20, 30, 40 μ L) of *N*-substituted ebselen derivatives for the next 24 h. After incubation, the cells were fixed in 20% trichloroacetic acid for an 1 h. The plates were washed with distilled water and 0.4% SRB (Sigma Aldrich, St. Louis, MO, USA) in 1% acetic acid solution was added to the plates for 15 min. The SRB solution was washed with 1% acetic acid. SRB was then solubilized in 10 mM Tris-base solution and the absorbance was measured at 570 nm using an automated microplate reader. The experiments were done in triplicates and the IC₅₀ values were calculated.

4. Conclusions

We have designed a new efficient two-step method to obtain a series of *N*-alkyl benzoselenazol-3(2*H*)-thiones. The first step involving the conversion of *N*-substituted benzamides to their thiocarbonyl analogues has been significantly upgraded by applying microwave irradiation. The further conversion to corresponding benzoselenazolthiones was efficiently conducted by our previously presented procedure using dilithium diselenide as the nucleophile. We have obtained a series of *N*-alkyl benzoselenazolthiones and tested them as catalysts for H₂O₂ reduction, using a commonly used DTT assay, and cytotoxic agents on prostate cancer cell line DU145 and non-cancerous cell line PNT1A. The *N*-propyl **7b** and *N*-3-methylbutyl benzoselenazolthione **7e** were the most efficient antioxidants. In comparison to the previously tested corresponding carbonyl derivatives, it can be concluded that propyl and 3-methylbutyl carbon chains, and the introduction of the sulfur atom increase the ability to catalytically reduce H₂O₂. The highest anti-proliferative activity was evaluated for *N*-cyclohexylbenzoselenazolthione **7f**. This result proves our previous observation, concerning the cytotoxic activity of *N*-alkylbenzoselenazolones, that the presence of the *N*-cyclohexyl moiety is correlated with high cytotoxic potential.

Supplementary Materials: Supplementary materials are available online.

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