

# N-Terpenyl Benzisosenazolones – Evaluation of the Particular Structure-Bioactivity Relationship <sup>†</sup>

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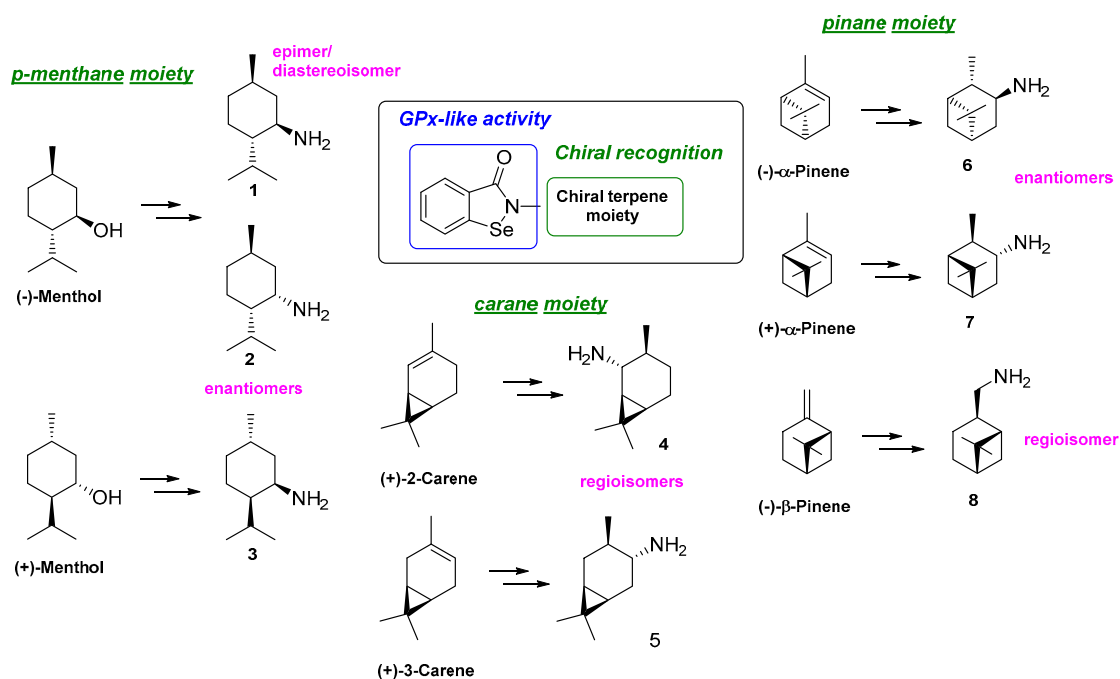
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**Abstract:** Different activity of compounds with the same molecular formula but possessing alternative bonding arrangement or orientation of atoms in space is a very important issue when constructing molecules that can selectively interact with certain domains of the biological target. Herein, we present the synthesis of the new chiral benzisosenazol-3(2H)-ones substituted on the nitrogen atom with three monoterpene moieties: *p*-menthane, pinane and carane. All derivatives were obtained by the reaction of an appropriate amine with 2-(chloroseleno)benzoyl chloride. The applied terpenyl amines were first synthesized by a multistep procedure starting from the corresponding alcohol (*p*-menthane system) or alkene (pinene and carene systems). Finally, all compounds were tested as antioxidants and antiproliferative agents on breast cancer MCF-7 and human promyelocytic leukemia cell line HL-60. The correlation between the structure of the obtained organoselenium compounds, representing examples of various isomers including enantiomers, diastereoisomers and regioisomers, and their bio-activity was thoroughly evaluated.

**Keywords:** benzisosenazol-3(2H)-ones; terpenes; antioxidant activity; antiproliferative activity

## 1. Introduction

When selecting new drug candidates several characteristics have to be considered e.g., therapeutic potential, toxicity, bioavailability, metabolism and selectivity towards specific targets. The binding affinity between the molecule and the active site of the particular protein/enzyme is determined by their structural compatibility. As biochemical reactions are often highly stereoselective, a properly fitted 3-dimensional structure of the drug candidate is an important feature for the selective molecular interaction [1,2]. The goal of this research was to evaluate the influence of a chiral substituent on the bio-activity of new organoselenium derivatives and recognize the structural motifs that increase the selective drug-target bonding. The designed compounds are a combination of two building blocks: (1) a benzisosenazol-3(2H)-one core, that is essential for the GPx-like activity and oxidative stress prevention and (2) an optically active monoterpene substituent that was constructed using terpenyl amines obtained from three different monoterpene systems: *p*-menthane **1–3**, carane **4, 5** and pinane **6–8** [3]. The selected enantiomerically pure substrates enabled to obtain derivatives that represent examples of different enantiomers from (–)- and (+)-menthol **2, 3**, (–)- and (+)- $\alpha$ -pinene **6, 7**, epimer of menthyl amine **1** and regioisomers **4, 5** and **8** (Scheme 1).

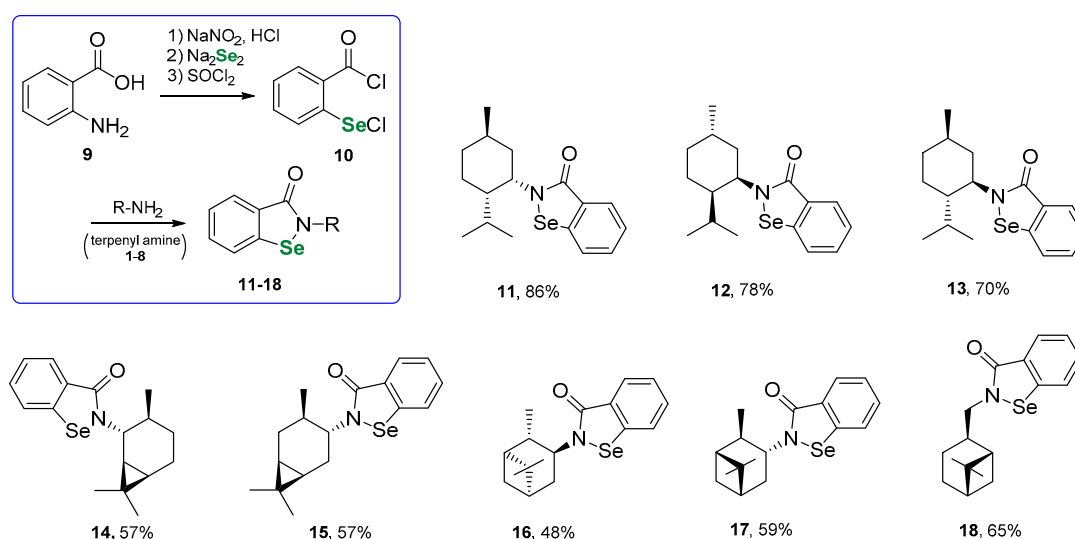


**Scheme 1.** General structures of the synthesized compounds.

All synthesized compounds were tested as antioxidants and anticancer agents. The activity-structure correlation was evaluated and several proactive features were selected.

## 2. Results and Discussion

First, a series of the new *N*-substituted benzisoselenazol-3(2H)-ones was synthesized by our previously reported multistep procedure starting from anthranilic acid **9** which was transformed to 2-(chloroseleno)benzoyl chloride **10** and further converted to the final product through the reaction with an appropriate terpenyl amine **1–8** (Scheme 2) [4].



**Scheme 2.** Synthesis of benzisoselenazol-3(2H)-ones **11–18**.

Next, the ability to reduce peroxides was tested by an NMR assay where dithiol (DTT<sup>red</sup>) is transformed to a disulphide (DTT<sup>ox</sup>) by a selenocatalyst first oxidized in the presence of hydrogen peroxide (Figure 1) [5]. The rate of the reaction was measured based on changes in the <sup>1</sup>H NMR spectra (Table 1).

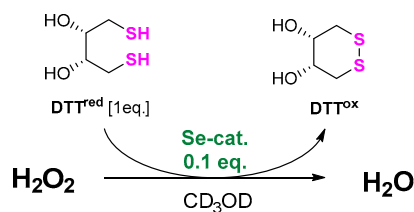


Figure 1. Antioxidant activity test.

Table 1. Results of the antioxidant activity measurements.

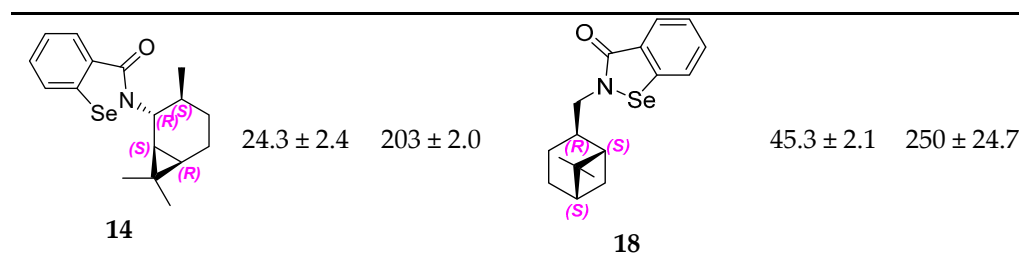
Catalyst [0.1 eq.]	Remaining DTT <sup>red</sup> [%]				
	3 min	5 min	15 min	30 min	60 min
<b>Benzisoselenazolones</b>					
<b>11</b>	91	85	75	63	49
<b>12</b>	91	85	75	63	49
<b>13</b>	80	77	74	70	67
<b>14</b>	92	89	83	74	58
<b>15</b>	69	61	43	40	39
<b>16</b>	71	39	5	0	0
<b>17</b>	71	39	5	0	0
<b>18</b>	74	61	28	6	0
<b>Ebselen</b>	84	75	64	58	52

The highest antioxidant activity was observed for all derivatives from the pinane system: enantiomers **16/17** and regioisomer **18**, the total substrate conversion was observed after 30 and 60 min, respectively. These results correlate with our previous observation that the bulky bicyclic bornane skeleton also enhances the antioxidant capacity of benzisoselenazol-3(2H)-one core and reduces the DTT<sup>red</sup> oxidation time to 5 min [4].

Next, the antiproliferative capacity was measured by a cell viability assay (MTT) on breast cancer MCF-7 and human promyelocytic leukemia cell line HL-60 (Table 2) [6].

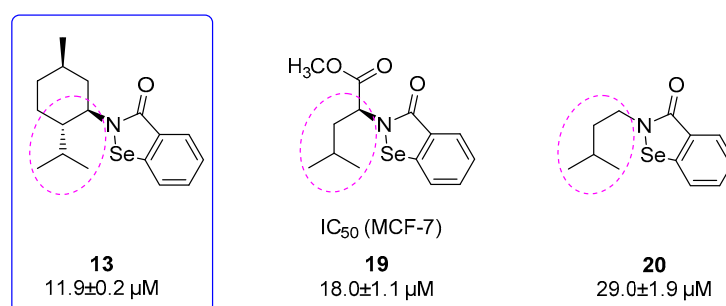
Table 2. Results of the antiproliferative activity measurements.

Structure	IC <sub>50</sub> [μM]		Structure	IC <sub>50</sub> [μM]	
	MCF-7	HL-60		MCF-7	HL-60
	12.4 ± 0.4	12.4 ± 0.9		45.2 ± 2.1	210 ± 14.1
<b>11</b>			<b>15</b>		
	85.5 ± 4.0	61.3 ± 3.2		19.9 ± 0.4	7.1 ± 0.4
<b>12</b>			<b>16</b>		
	11.9 ± 0.2	62.1 ± 2.0		13.3 ± 1.1	20.6 ± 1.0
<b>13</b>			<b>17</b>		



The *N*-isopinocampheyl-1,2-benzisoselenazol-3(2H)-one **16** exhibited the highest antiproliferative potential with  $IC_{50}$  of  $7.1 \pm 0.4 \mu\text{M}$  (HL-60 cell line). Attachment of the isoselenazolone ring to the C10 carbon decreased the activity, what was highly emphasized in the results obtained for HL-60 cells with the increase of  $IC_{50}$  from  $7.1 \pm 0.4$  to  $250 \pm 24.7 \mu\text{M}$ .

For MCF-7 cell line the lowest  $IC_{50}$  was measured for *N*-menthyl-1,2-benzisoselenazol-3(2H)-one **13** with the value of  $11.9 \pm 0.2 \mu\text{M}$ . We have also observed a different activity for both enantiomeric pairs derived from *p*-menthane and pinane. The diversity was more apparent in the case of compounds **11** and **12**, where one enantiomer was far more active with  $IC_{50}$  values  $12.4 \pm 0.4 \mu\text{M}$  and  $85.5 \pm 4.0 \mu\text{M}$ , respectively. Compounds **11** and **13**, with good antiproliferative capacity, have the same configurations on C1 and C4 carbon atoms, on the contrary to less active enantiomer **12**. The stereochemistry of C2, attached to the isoselenazolone ring, seems not to influence the reactivity. Moreover, the molecule **13** possesses the same structural motif that was present in the structure of other active *N*-alkyl benzisoselenazolones **19** and **20**—an incorporated 2-methylbutyl chain (Scheme 3) [7].



**Scheme 3.** Repetitive carbon chain in the structure of derivatives **13**, **19** and **20**.

In the case of carane regioisomers **14** and **15** the  $IC_{50}$  was lower when the  $N$ -C<sub>terpene</sub> bond was more hindered by the methyl group and the cyclopropane bridge. The overall activity decreased when the compounds were tested on HL-60 cell line.

### 3. Conclusions

In this paper we have synthesized a series of *N*-terpenyl benzisoselenazol-3(2H)-ones derived from three monoterpene systems: *p*-menthane, carane and pinane. The procedure was based on the reaction of 2-(chloroseleno)benzoyl chloride with an appropriate terpene monocyclic or bicyclic amine. The obtained 8 chiral organoselenium compounds included enantiomers, epimers and regioisomers were further tested as antioxidants and anticancer agents. The obtained results and deducted structure-activity evaluation enabled to propose that: (1) the more bulky the terpenyl substituent the higher the antioxidant potential; (2) the antiproliferative activity differs significantly for both enantiomeric pairs derived from *p*-menthane and pinane; (3) the configuration of C1 and C4 carbon in the *p*-menthane skeleton can be essential for the improvement of the bio-activity, (4) the presence of an incorporated 2-methylbutyl chain can increase the anticancer capacity; (5) the more hindered the  $N$ -C<sub>terpene</sub> bond the higher the antiproliferative potential.

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## References

1. Singh, K.; Shakya, P.; Kumar, A.; Alok, S.; Kamal, K.; Singh, S.P. Stereochemistry and its role in drug design. *IJPSR* **2014**, *5*, 4644–4659.
2. McConathy, J.; Owens, M.J. Stereochemistry in Drug Action. *Prim Care Companion J. Clin. Psychiatry* **2003**, *5*, 70–73.
3. Pacuła, A.J.; Mangiavacchi, F.; Sancineto, L.; Lenardao, E.J.; Ścianowski, J.; Santi, C. An Update on “Selenium Containing Compounds from Poison to Drug Candidates: A Review on the GPx-like Activity”. *Curr. Chem. Biol.* **2015**, *9*, 97–112.
4. Pacuła, A.J.; Kaczor, K.B.; Antosiewicz, J.; Janecka, A.; Długosz, A.; Janecki, T.; Wojtczak, A.; Ścianowski, J. New Chiral Ebselen Analogues with Antioxidant and Cytotoxic Potential. *Molecules* **2017**, *22*, 492–506.
5. Kumakura, F.; Mishra, B.; Priyadarsini, K.I.; Iwaoka, M. A Water-Soluble Cyclic Selenide with Enhanced Glutathione Peroxidase-Like Catalytic Activities. *Eur. J. Org. Chem.* **2010**, *3*, 440–444.
6. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63.
7. Pacuła, A.J.; Kaczor, K.B.; Wojtowicz, A.; Antosiewicz, J.; Janecka, A.; Długosz, A.; Janecki, T.; Ścianowski, J. New glutathione peroxidase mimetics—Insights into antioxidant and cytotoxic activity. *Bioorg. Med. Chem.* **2017**, *25*, 126–131.



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