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Identification of methionine aminopeptidase 2 as a molecular target of the organoselenium drug ebselen and its derivatives/analogues: Synthesis, inhibitory activity and molecular modeling study

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ABSTRACT

A collection of twenty-six organoselenium compounds, ebselen and its structural analogues, provided a novel approach for inhibiting the activity of human methionine aminopeptidase 2 (MetAP2). This metalloprotease, being responsible for the removal of the amino-terminal methionine from newly synthesized proteins, plays a key role in angiogenesis, which is essential for the progression of diseases, including solid tumor cancers. In this work, we discovered that ebselen, a synthetic organoselenium drug molecule with anti-inflammatory, anti-oxidant and cytoprotective activity, inhibits one of the main enzymes in the tumor progression pathway. Using three-step synthesis, we obtained twenty-five ebselen derivatives/analogues, ten of which are new, and tested their inhibitory activity toward three neutral aminopeptidases (MetAP2, alanine and leucine aminopeptidases). All of the tested compounds proved to be selective, slow-binding inhibitors of MetAP2. Similarly to ebselen, most of its analogues exhibited a moderate potency (IC_{50} = 1–12 μM). Moreover, we identified three strong inhibitors that bind favorably to the enzyme with the half maximal inhibitory concentration in the submicromolar range.

Alanine (APNs), leucine (LAPs), and methionine aminopeptidases (MetAPs) are three major groups of neutral aminopeptidases containing metal ion(s) in their active site that catalyze the removal of amino acids from the N-terminus of a peptide or protein. Biomedical significance of these exopeptidases is related to therapeutic intervention against devastating human diseases. The leucine and alanine aminopeptidases represent promising targets for the development of a new generation of anti-inflammatory drugs. The alanine and methionine aminopeptidases possess a well-recognized potential for the design of anti-angiogenesis agents. Furthermore, neutral aminopeptidases are involved in the apoptosis of cancer cells, which makes them highly interesting objects of oncological research.[1–4]

Human MetAP type 2 (MetAP2) is one of the three known methionine aminopeptidases responsible for the removal of the N-terminal translation initiator methionine from newly synthesized proteins, which is a critical step in protein maturation.[3,5] Protein translation mainly begins with a methionine in eukaryotes and a formylated methionine in prokaryotes. Consequently, the removal of methionine is indispensable for post-translational amino group modifications, protein stability and proper localization.[6,7] MetAP2 possesses a characteristic bimetallic center activated by cobalt, manganese or iron, surrounded by residues that form a pita-bread-shaped fold.[8–10] MetAPs are expressed in many mammalian tissues and cell lines, but only type 2 is upregulated during cell proliferation.[11] Higher expression of MetAP2 has been observed in tumor cells compared with normal cells.[12,13] To date, increased expression of MetAP2 has been correlated with several types of cancer, for instance, mesothelioma,[14] neuroblastoma,[15] and colorectal carcinoma.[16] MetAP2 became a promising target molecule after the discovery that the potent anti-angiogenic agent fumagillin and its synthetic analogue ovalicin bind covalently and inhibit its aminopeptidase activity without affecting the ability of the enzyme to stabilize eukaryotic initiation factor-2.[17] TNP-470, one of the synthetic analogues of fumagillin that advanced to clinical trials,[18] as well as more recently published analogues with improved pharmacological profiles,[19] act similarly. Reversible inhibitors are generally considered as safer due to the decreased risk of toxicity or immunogenic responses. The non-covalent inhibitors of MetAP2 include compounds based on fumagillin,[20] bestatin,[21] 1,2,4-triazole,[22] and most recently pyrazolo[4,3-b]indole.[23] Other types of reversible ligands of MetAP2, such as anthranilic acid sulfonamides[24–26] and bengamides,[27] have also been reported.[28] In

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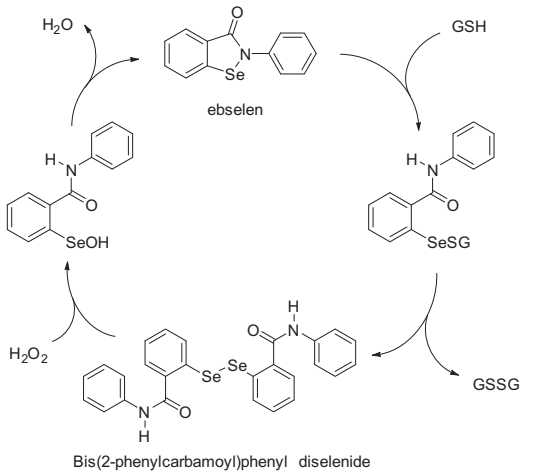
this communication, we describe the discovery and optimization of the nontoxic synthetic selenium-containing drug ebselen as an inhibitor of MetAP2.

Ebselen, IUPAC name 2-phenyl-1,2-benzisoselenazol-3(2*H*)-one, also called PZ51, is a low-molecular-weight drug with a pleiotropic mode of action and with very low toxicity in humans.[29] It was first identified as an anti-inflammatory agent with glutathione peroxidase-like activity in living cells.[30] One of the most likely modes of action of ebselen is as follows: the Se−N bond is readily cleaved by the thiol group of glutathione (GSH) to produce the corresponding selenenyl sulfides, which undergo disproportionation toward GSSG and 2,2′-dicarbamoyldiphenyl diselenide. The diselenide is oxidized to the corresponding selenenic acid in the presence of hydroperoxides, such as hydrogen peroxide (H_2O_2). After water elimination, ebselen is regenerated (Scheme 1).[31,32]

Ebselen is a well-known agent with therapeutic activity in neurological disorders, acute pancreatitis, and noise-induced hearing loss. It also exhibits antiatherosclerotic, antithrombotic, antioxidant and cytoprotective properties.[29,33–35] A recent study showed that hypoxia-induced cytotoxicity in human alveolar cells is reduced by ebselen owing to these properties.[36] An antiviral effect on hepatitis C virus nonstructural protein 3 was also recently demonstrated.[37] Ebselen and its derivatives act as antiproliferative

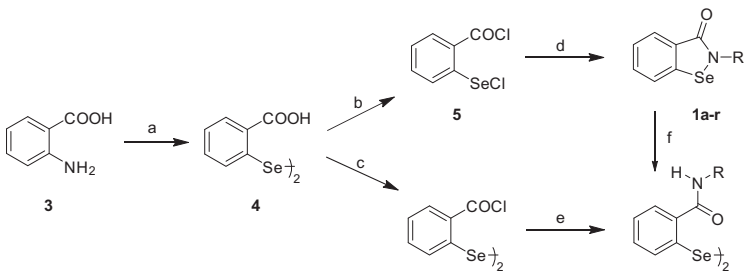


Scheme 1. Plausible mechanism for the GSH activity of ebselen.[31,32]

compounds against pancreatic and renal,[38] liver, breast,[39] and lung cancer and cervical adenocarcinoma.[40] Organoselenium compounds exhibit strong electrophilic activity and are therefore capable of forming selenenyl-sulfide bonds with the cysteine residues in proteins.[32,41,42] The anticancer activity is usually linked to the inhibition of a selenocysteine-containing enzyme, thioredoxin reductase, that is overexpressed in many types of cancer.[43] All the above examples prove not only the broad spectrum of ebselen activity, but also convenience and safety of its use. However, no reports are available that show the relationship between ebselen and aminopeptidases.

Ebselen has previously been prepared by several methods,[44–49] first by Lesser and Weiss in 1924.[49] In this work, we synthesized ebselen and its derivatives/analogues using a four-step literature procedure starting from cheap and easily available reagents, anthranilic acid and elementary selenium.[47,48] The overall procedure for obtaining ebselen, its derivatives/analogues and their acyclic dimeric forms is outlined in Scheme 2. The protonation of anthranilic acid (**3**), diazotization, and dilithium diselenide selenenylation with nitrogen elimination gave 2,2′-dicarboxydiphenyl diselenide (**4**), a stable crystalline compound. The reaction of diselenide with thionyl chloride in the presence of dimethylformamide (DMF) produced 2-(chloroseleno)benzoyl chloride (**5**) or its acyclic form **6** depending on the quantity of thionyl chloride used, i.e., 7 equiv or 3.5 equiv, respectively. Acylation or tandem selenenylation/acylation reaction with appropriate amines alone or in the presence of Et_3N base in anhydrous acetonitrile (benzisoselenazolones **1a**–**r**) or appropriate amines in the presence of Na_2CO_3 in anhydrous dichloromethane (diselenides **2a**, **2c**–**e** and **2l**–**n**) completed the reaction sequence. We used ammonia or eighteen structurally diversified primary amines (methyl, aniline and its derivatives, including heteroatom- and halogen-substituted, benzyl, *p*-*tert*-butylbenzyl, and phenylethyl, Scheme 2). In particular, benzyl diselenide **2p** was prepared from corresponding benzisoselenazolone by hydrogenation with hydrazine monohydrate. The final compounds were purified by recrystallization or standard liquid column chromatography.

Twenty-six compounds, eighteen benzisoselenazol-3(2*H*)-ones (**1a**–**r**) and eight 2,2′-dicarbamoyldiaryl diselenides (**2a**, **2c**–**e**, **2l**–**n** and **2p**), ten of which are new (**1f**, **1i**–**m**, **1q**, **1r**, **2l** and **2m**), were obtained. The products were characterized by ^{1}H, ^{13}C, and ^{77}Se NMR spectroscopy, mass spectrometry and melting point (see Supplementary data).



R = Ph (**1a, 2a**), 4-methylphenyl (**1b**), 2-hydroxyphenyl (**1c, 2c**), 2-methoxyphenyl (**1d, 2d**), 4-methoxyphenyl (**1e, 2e**), 4-*n*-butoxyphenyl (**1f**), 3-fluorophenyl (**1g**), 4-fluorophenyl (**1h**), 2-methoxy-5-methylphenyl (**1i**), 4-methoxy-2-methylphenyl (**1j**), 5-*tert*-butyl-2-methoxyphenyl (**1k**), 2-chloro-4-methylphenyl (**1l, 2l**), 5-chloro-2-methoxyphenyl (**1m, 2m**), H (**1n, 2n**), Me (**1o**), Bz (**1p, 2p**), 4-*tert*-butylbenzyl (**1q**), 2-phenylethyl (**1r**)

Scheme 2. Synthesis of the benzisoselenazol-3(2*H*)-ones **1a**–**r** and bis(2-carbamoyl)phenyl diselenides **2a**, **2c**–**e**, **2l**–**n** and **2p**. Reagents and conditions: (a) (i) HCl, (ii) $NaNO_2$, 0 °C, (iii) Li_2Se_2, 0 °C; (b) 7 equiv $SOCl_2$, DMF, benzene, reflux; (c) 3.5 equiv $SOCl_2$, DMF, benzene, reflux; (d) RNH_2, Et_3N, MeCN, or RNH_2, MeCN, (e) RNH_2, Na_2CO_3, CH_2Cl_2, (f) $H_2NNH_2 \times H_2O$, MeOH, 25 °C.