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Original article

Synthesis and biological activity of novel organoselenium derivatives targeting multiple kinases and capable of inhibiting cancer progression to metastases

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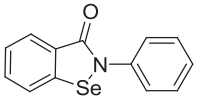
ABSTRACT

The present study reports synthesis and biological activity of novel benzoisoselenazolone compounds derived from ebselen and conjugated to a sugar molecule. Cell proliferation assay using cancer cells combined with *in vitro* biochemical assays revealed that benzoisoselenazolone **2d**, **5a**, and **6a** exerted anti-proliferative activity, which correlated with selective *in vitro* inhibition of focal adhesion kinase, AKT-1, and protein kinase C-α. Active molecules were able to significantly inhibit cell migration and invasion *in vitro* compared to cells treated with the vehicle alone or ebselen. Moreover, *in vivo* anticancer activity focusing on lead compound **2d** and using an invasive human breast cancer orthotopic mouse model revealed a potent anti-metastatic activity at well-tolerated doses. In summary, these novel benzoisoselenazolones we report herein target multiple kinases with established roles in cancer progression and possess anti-invasive and anti-metastatic activity in preclinical models supporting a potential for therapeutic application for human disease.

1. Introduction

Discovery of novel therapeutic agents for advanced invasive cancers is at the forefront of preclinical and clinical research. A wide range of small molecules belonging to both synthetic and naturally derived molecules and targeting a wide range of extracellular receptors, intracellular cell signalling molecules, cell cytoskeleton, as well as tissue microenvironment are under development. Of interest to this study, selenium (Se)-containing molecules are emerging as exciting candidate therapeutic agents due to their newfound ability to modulate multiple physiological functions implicated in cancer development. For instance, Se-containing molecules have been shown to exert anti-proliferative and proapoptotic effects on a wide range of cancer cell types, as well as endothelial and immune cells, and to inhibit the activity of drug resistance mechanisms to potentiate chemotherapy/radiotherapy efficacy [1–3]. Moreover, inhibition of angiogenic factors by seleno-compounds has been reported to result in inhibition of neo-vessel formation "angiogenesis", a key process essential for cancer progression and dissemination [4–6]. Several selenium-containing derivatives have also been reported to efficiently target key cancer cell signaling mechanisms such as calcium-insensitive nitric oxide synthase (iNOS), Akt3 kinase, and the mitogen-activated protein kinase (MAPK) signaling [2]; histone deacetylases [7]; and melanin biosynthesis by melanocytes [8]. The impact of Se-containing molecules on multiple targets is not surprising since several kinases are regulated by selenium [9].

Using high-throughput screening of chemical libraries against kinases associated with cancer cell invasion, we initially identified the Se-containing molecule ebselen (**1**) as a potential inhibitor.



Ebselen (1)

Abbreviations: BSZ, benzisoselenazolone fragment; FA, focal adhesion; FAK, focal adhesion kinase; PKC-α, protein kinase C.

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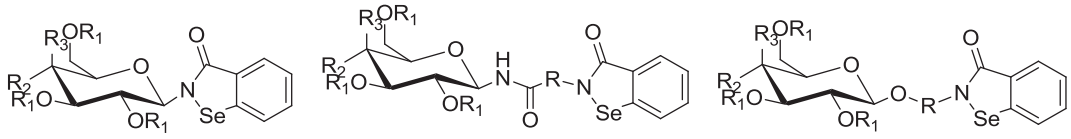
Ebselen, 2-phenylbenzo[*d*] [1,2]selenazol-3(*2H*)-one, a synthetic compound identified in 1984, was found to exhibit GSH-peroxidase-like activity *in vitro* [*d*](*2H*) [10]. Ebselen exerts a wide spectrum of biological activities, ranging from anti-oxidant, cyto-protective, neuroprotective, and anti-inflammatory. Ebselen functions in part as a glutathione peroxidase mimicking agent and free radical/peroxynitrite scavenging agent. Also, ebselen inhibits other enzymes such as cyclooxygenases, lipoxygenases, and indoleamine 2,3-dioxygenase, which play a broad functional role in cancer signalling, as well as the regulation of the immune response [11–16]. This broad target specificity could explain why clinical applications of ebselen have been limited to neurological diseases such as for the treatment of ischemic stroke [17,18]. Due to its modest anticancer activity which is in part due to its lower uptake by cancer cells and limited biodistribution [19], we undertook additional modifications to the parent ebselen molecule to improve its intracellular uptake and efficacy. In prior studies, we have observed that conjugation to sugar greatly improves uptake and biodistribution of benzoisoselenazolone compounds in cancer cells, as compared to unconjugated molecules, which is in agreement with other studies [20–22]. In this report, we describe the synthesis of novel benzoisoselenazolone-fragment containing (BSZ) compounds derived from ebselen, which demonstrate anticancer properties, including inhibition of focal adhesion kinase (FAK), AKT-1, and protein kinase C-α (PKC-α), which have been shown to play a critical role in cell survival and invasive signalling [23–30].

A series of organoselenium analogues derived from ebselen and conjugated to sugar molecules were synthesized in multistep reactions as described in the Experimental section. Three series of sugar-modified derivatives of benzoisoselenazol-3(*2H*)-ones were designed and synthesized in the current study. They are benzoisoselenazolones connected with carbohydrate directly (I), benzoisoselenazolones connected with carbohydrate by amide (II) and benzoisoselenazolones connected with carbohydrate by oxygen glycoside (III).



I II III

2. Chemistry

The general method for the synthesis of compounds of series I is shown in Scheme 1. We started with different kinds of sugars such as glucose, galactose, lactose and maltose to prepare their 1-aminosubstitute derivatives following the respective literature procedures [31,32], which were then coupled with 2-(chloroseleno) benzoyl chloride followed by deprotection of –OH group to generate **3a**–**3d** in a good yield of 48–57.9%.

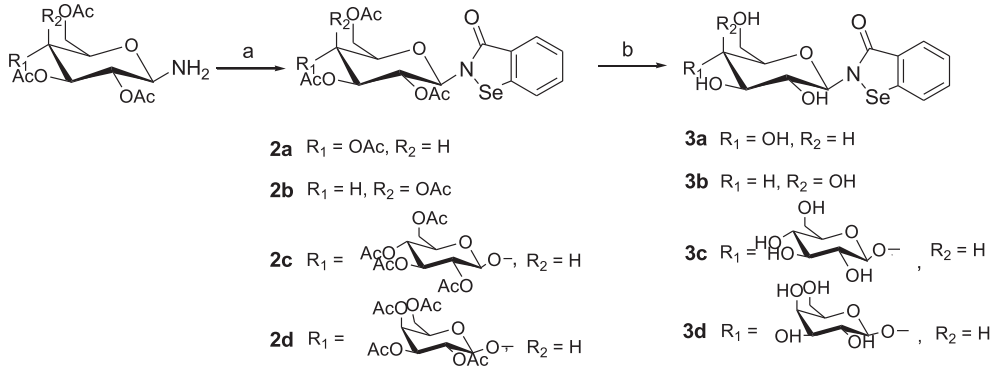
The general method for the synthesis of compounds of series II is shown in Scheme 2. The synthesis of compounds **6a** and **6b** were obtained by an efficient cyclization reaction starting from 2-(chloroseleno)benzoyl chloride and the OH-protected amino-saccharide-butyramide derivatives such as 3-amino-*N*-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-butyramide (**4a**), and 3-amino-*N*-(hepta-O-acetyl-β-D-lactopyranose)-butyramide (**4c**) in a good yield, which was carried out at 0 °C to r.t. and catalyzed by DCC and DMC. In this method, compound 10 can be obtained in high yield by the reaction between the OH-protected amino-saccharide and 4-(benzyloxycarbonylamino)butanoic acid followed by hydrolysis to remove the benzyloxycarbonyl protection group from the amino-group and were then directly used in the next step without any further purification (see experimental section).

The general method for the synthesis of compounds of series III is shown in Scheme 3. Ring closure reaction was accomplished by the treatment of 2-(chloroseleno)benzoyl chloride with **7** to afford 2-glucopyranosyl-oxyethyl-benzo*[d]* [1,2]selenazol-3*(2H)*-one (22) in a yield of 27% through a step of deprotection by methanol sodium. In this method, compound **7** can be obtained in high yield by the reaction of penta-acetylglucose and benzyl 2-hydroxyethylcarbamate followed by hydrolysis to remove the benzyloxycarbonyl protection group from the amino-group in the yields of 62% for step a and 97% for step b.

3. Results and discussion

3.1. Effect on cell proliferation and in vitro targets

As shown in Table 1, the inhibitory activity (IC_{50}) of the various compounds tested on the proliferation of the breast cancer cell line MDA-231 revealed that molecules **2b**–**d**, **5a**, **5b** and **6a** are the most active with IC_{50} in the range of 9.8–25 μM while **3b**–**d** and **9** are inactive ($IC_{50} > 100$ μM). Further screening of these molecules using our *in vitro* kinase assays revealed that compounds showing significant anti-proliferative activity, e.g. **2d**, **5a** and **6a,** were also



Scheme 1. Reagents and conditions: (a) 2-(chloroseleno)benzoyl chloride, Et_3N, $CHCl_3$, 0 °C to rt, 4 h, 50–65%; (b) MeONa/CH_3OH, rt, 1 h, 94–98%.