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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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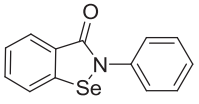
ABSTRACT

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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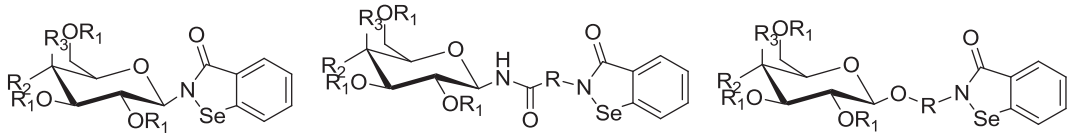
Ebselen, 2-phenylbenzo[*d*] [1,2]selenazol-3(*2H*)-one, a synthetic compound identified in 1984, was found to exhibit GSH-peroxidase-like activity *in vitro* [*d*](*2H*) [10]. Ebselen exerts a wide spectrum of biological activities, ranging from anti-oxidant, cyto-protective, neuroprotective, and anti-inflammatory. 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%.

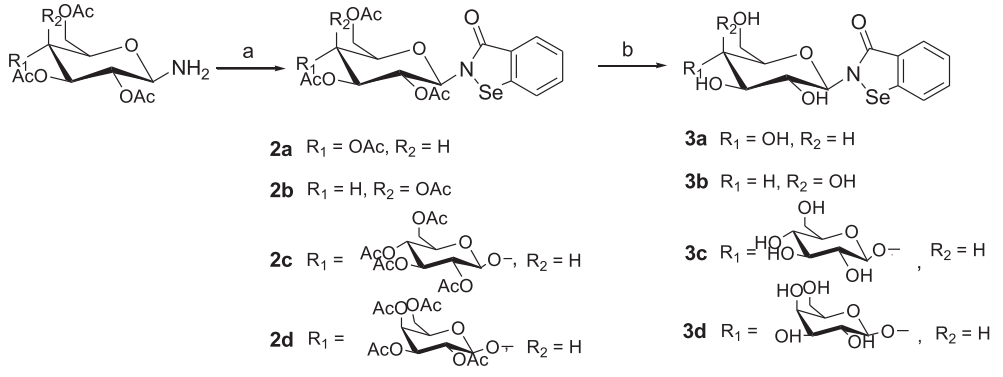
The general method for the synthesis of compounds of series II is shown in Scheme 2. The synthesis of compounds **6a** and **6b** were obtained by an efficient cyclization reaction starting from 2-(chloroseleno)benzoyl chloride and the OH-protected amino-saccharide-butyramide derivatives such as 3-amino-*N*-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-butyramide (**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.

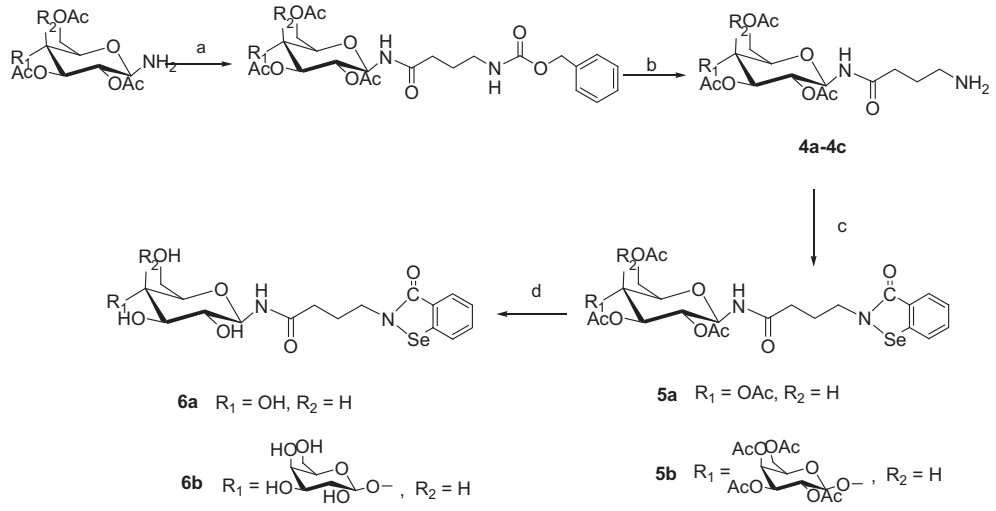
3. Results and discussion

3.1. Effect on cell proliferation and in vitro targets

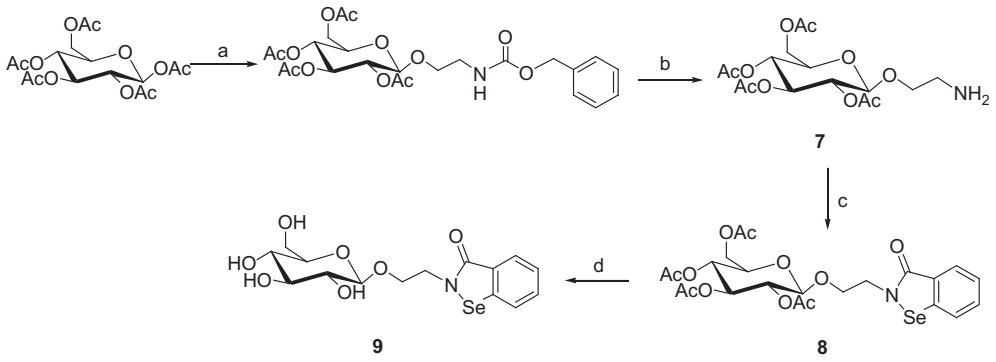
As shown in Table 1, the inhibitory activity (IC_{50}) of the various compounds tested on the proliferation of the breast cancer cell line MDA-231 revealed that molecules **2b**–**d**, **5a**, **5b** and **6a** are the most active with IC_{50} in the range of 9.8–25 μM while **3b**–**d** and **9** are 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%.



Scheme 2. Reagents and conditions: (a) 4-(benzyloxycarbonylamino)butanoic acid, DCC, DCM, 0 °C to rt, 5 h; (b) H_2, Pd/C, $CH_3OH/CHCl_3$ (v/v = 1:1), 4 h; (c) 2-(chloroseleno) benzoyl chloride, Et_3N, THF, 0 °C to rt, 4 h, 42–52%; (d) $MeONa/CH_3OH$, rt, 1 h, 92–97%.



Scheme 3. Reagents and conditions: (a) benzyl 2-hydroxyethylcarbamate, BF_3 Et_2O, DCM, 0 °C, 4 h, 62%; (b) H_2, Pd/C, $CH_3OH/CHCl_3$ (v/v = 1:1), 4 h, 97%; (c) 2-(chloroseleno)benzoyl chloride, Et_3N, THF, 0 °C to rt, 4 h, 30%; (d) $MeONa/CH_3OH$, rt, 1 h, 90%.

able to inhibit AKT-1, PKC-α and FAK kinase activity by 55–95%, as compared to ebselen (**1**) at 1 μM final concentration, while no inhibitory effect was observed on several other kinases, including Src, EGFR, Aurora B, IGF-R, VEGFR, and ErbB2/Her 2 kinases (Tables 1 and 2). The cellular inhibitory activity of our most active BSZ, namely compound **2d**, was demonstrated on FAK phosphorylation at the critical phospho-site Y397 (Fig. 1A) and FAK kinase activity in intact cells (Fig. 1B). As noted, a concentration of 10 μM was able to inhibit FAK phosphorylation (Fig. 1A), whereas inhibition of FAK kinase activity was obtained at 10–50 μM (Fig. 1B). Higher concentrations were found to be toxic to the cells. To rule-out potential "off target" effects of the BSZs, due to their structural similarities to ebselen, which have been shown to inhibit enzymes such as lipooxygenases [33] and to act as an anti-oxidant, *in vitro* studies revealed that the BSZ compounds with inhibitory activity against FAK, AKT-1 and PKC-α had no significant activity on lipooxygenase (using Lipoxygenase Screening Assay Kit, Cayman Chemical), nor do they inhibit free radical formation (using the CM-H2DCFDA-based assay for the detection of reactive oxygen species) (data not shown). Together, this data support specificity toward the triple kinases but the mechanism of this selectivity and its occurrence in *in-vivo* conditions deserves further investigations.

A crucial early event by which cancer cells acquire autonomous motile properties is driven by focal adhesion (FA) and survival signalling, which are essential for cell-substrate generating forces needed for the process of cancer cell migration and invasion. Central to FA signalling is the FAK, which is targeted by the BSZ compounds. FAK is activated by integrins as well as numerous growth factor receptors [34,35]. FAK also serves as a scaffolding protein mediating multiple protein–protein interactions critical for

Table 1
Inhibitory activity on MDA-231 cell proliferation and *in vitro* FAK kinase inhibition of BSZ compounds.

Cpd	Inhibition of cell proliferation (IC_{50} :μM))	% FAK inhibition (1 μM)
1	42.3	0
2b	21.0	69.3
2c	20.0	54.9
2d	9.8	80.1
3b	>100	7.4
3c	>100	9.9
3d	>100	1.3
5a	18.9	71.1
5b	25	55.9
6a	20.4	51.9
6b	53	13.9
8	35	51.9
9	>100	50.9

Table 2
Kinase inhibition profile of compounds **2d**, **5a**, **6a** and ebselen (**1**) at 1 μM final concentration *in vitro*.

Compound name	Kinase tested	% Inhibition; IC50 [μM]
2d	AKT-1	63; 0.83
	FAK	89; 0.42
	PKC-α	95; 0.22
	IGF1R	1
	ABL1	0
	Aurora A	0
	Aurora B	0
	CDK1/Cyclin B	0
	DDR1	0
	EGFR	0
	EPHA1	0
	FGFR1	0
	FLT3	0
	FYN	0
	HER2	0
	IKK-ε	0
	INSR	0
	LYN	0
	MET	0
	P38-γ	0
	PAK1	0
	PDGFR-β	0
	PIM1	0
	PLK4	0
	RPS6KA4	0
	SGK3	0
	SRC	0
	SYK	0
	TIE1	0
	TRKB	0
	VEGFR2	0
	YES1	0
5a	AKT-1	67; 0.80
	FAK	69; 0.92
	PKC-α	77; 0.74
	IGF1R	0
	ABL1	0
	Aurora A	0
	Aurora B	0
	CDK1/Cyclin B	0
	DDR1	0
	EGFR	0
	EPHA1	0
	FGFR1	0
	FLT3	0
	FYN	0
	HER2	0
	IKK-ε	0
	INSR	0
	LYN	0
	MET	0
	P38-γ	0
	PAK1	0
	PDGFR-β	0
	PIM1	0
	PLK4	0
	RPS6KA4	0
	SGK3	0
	SRC	0
	SYK	0
	TIE1	0
	TRKB	0
	VEGFR2	0
	YES1	0
6a	AKT-1	60; 0.88
	FAK	55; 0.96
	PKC-α	74; 0.73
	IGF1R	0
	ABL1	0
	Aurora A	0
	Aurora B	0
	CDK1/Cyclin B	0
	DDR1	0
	EGFR	0
	EPHA1	0
	FGFR1	0
	FLT3	0
	FYN	0
	HER2	0
	IKK-ε	0
	INSR	0
	LYN	0
	MET	0
	P38-γ	0
	PAK1	0
	PDGFR-β	0
	PIM1	0
	PLK4	0
	RPS6KA4	0
	SGK3	0
	SRC	0
	SYK	0
	TIE1	0
	TRKB	0
	VEGFR2	0
	YES1	0
1	AKT-1	22
	FAK	1
	PKC-α	25
	IGF1R	0
	ABL1	0
	Aurora A	0
	Aurora B	0
	CDK1/Cyclin B	0
	DDR1	0
	EGFR	0
	EPHA1	0
	FGFR1	0
	FLT3	0
	FYN	0
	HER2	0
	IKK-ε	0
	INSR	0
	LYN	0
	MET	0
	P38-γ	0
	PAK1	0
	PDGFR-β	0
	PIM1	0
	PLK4	0
	RPS6KA4	0
	SGK3	0
	SRC	0
	SYK	0
	TIE1	0
	TRKB	0
	VEGFR2	0
	YES1	0

cancer cell survival. For instance, the non-catalytic domain of FAK interacts with phosphatidylinositol 3-kinase (PI3-K) secondary to FAK autophosphorylation at Y397, which serves as a binding site for the SH2 domains of PI3-K, which can act as a second lipid messenger regulating protein kinase C (PKC) family members, as well as the phosphoinositide-dependent serine/threonine protein kinase AKT-1. These interactions are critical for cell migration and cell survival signaling [35,36]. Not surprising, FAK, PKC-α, and AKT-1 are upregulated/hyperactivated in invasive cancers [23,37–39], and inhibition of specific components of these networks, e.g. using RNA interference or small molecules, prevented cancer cell proliferation, invasion and/or metastasis formation [23–30]. However, approaches targeting these proteins individually have been proven of limited efficacy due in part to compensatory signalling loops that can overcome a single target inhibition. As such, the triple kinase inhibitory profile of these BSZs is novel and has great biological and pharmacological implications. In order to speculate on the binding

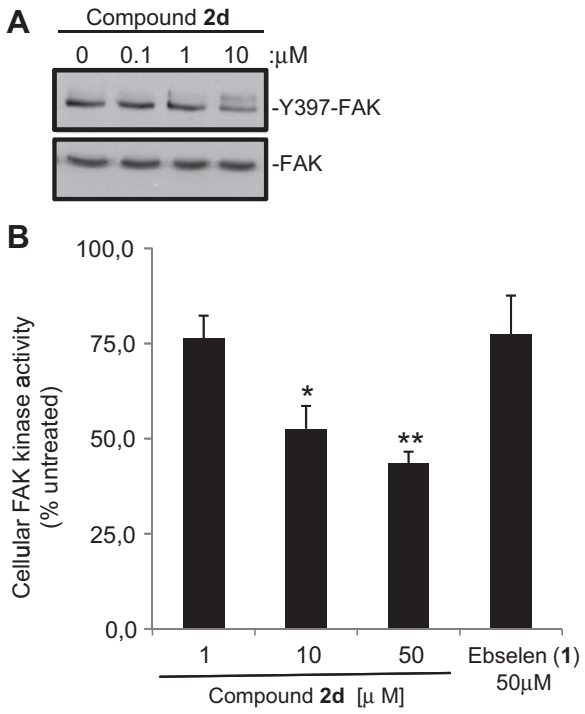


Fig. 1. Compound **2d** inhibits FAK phosphorylation and kinase activity in intact cells. A) representative western blot analysis of total cell extracts of MDA-231 cells treated with increasing concentrations of compound **2d**, as indicated. Inhibition of FAK activation, as demonstrated by FAK (Tyr-397) phosphorylation is observed at 10 μM of compound **2d**. B) FAK was immunoprecipated from lysates of MDA-231 cells treated with indicated concentration of compound **2d** and were incubated with [γ-^{32}P]-ATP in order to monitored for FAK kinase activity. Significant inhibition of FAK kinase activity was observed at 10 μM ($^{*}P < 0.05$) and 50 μM ($^{**}P < 0.01$) of compound **2d**, as compared to untreated controls ($N = 4$). Ebselen was unable to inhibit FAK kinase activity at 50 μM.

mode of these compounds to each of these kinases, through molecular modelling studies we demonstrated the predictive binding mode of compound **2d** in the ATP-binding pocket of the AKT-1, FAK and PKC-α kinase domains (Fig. 2).

3.2. Effect on cell motility and invasion

The effect of some of the compounds listed above on cell motility was investigated using the wound healing motility assay. In this study, cells were grown on coverslips and then wounded by cell scraping using a micropipette tip. Cells were allowed to migrate and heal the wound. Photomicrographs were taken at each time point in order to examine the wound healing areas, which were measured and quantified. As shown in Fig. 3A, compounds **2d**, **5a** and **6a** were able to inhibit MDA-231 cell motility by approximately 50%, as compared to vehicle (DMSO) treated control sample, thereby suggesting a possible impairment in the cell's motility machinery. This is in contrast to ebselen (**1**), which was unable to significantly inhibit MDA-231 cell motility.

Similarly, cell invasion experiments were performed using the Boyden chamber assay where serum starved MDA-231 cells were placed into the upper compartment of the Boyden chamber, treated with BSZ molecules or ebselen (**1**). Cells were then allowed to invade through a Matrigel matrix for 48 h. As shown in Fig. 3B, compounds **2d**, **5a** and **6a** were able to significantly inhibit cell invasion revealed by an approximate 50%inhibition of the number of cancer cells able to invade through the matrigel at BSZ concentrations that had no apparent direct cytotoxic effects, as compared to vehicle treated (DMSO) control samples. Ebselen (**1**) alone had no effect on MDA-231 cell invasion (Fig. 3).

3.3. Efficacy in vivo

The ability of these compounds to inhibit breast cancer cell metastasis is also investigated in a highly invasive mouse model of human breast cancer *in vivo*. MDA-231-M2 cells, a highly invasive human breast cancer cell line, were implanted into the mammary fat pad of female SCID mice. Once tumors become palpable, mice were randomised into experimental groups and then treated with vehicle, or 60 mg/kg of compound **2d** or ebselen (**1**) administered by intraperitoneal (IP) route 3 times a week for a total of 9 administrations. 60 mg/kg was very well tolerated with no body weight loss or apparent toxicity. 40 days after the initiation of treatment, mice were sacrificed and primary tumors and lungs metastases were examined as described in methods. As demonstrated in Fig. 4, compound **2d** at 60 mg/kg induced a significant inhibition of tumor growth and lung metastases, as compared to vehicle- or ebselen (**1**)-treated mice. Therapeutic activity was also observed at lower doses while higher doses were not tested. On the other hand, ebselen (**1**) treatment did not significantly differ from the vehicle treated controls. In summary, the BSZ compounds reported here represent a novel class of kinase inhibitors with biological activity against invasive breast cancer.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

Solvents were purified in a conventional manner. Thin-layer chromatography (TLC) was performed on precoated E. Merck silica-gel 60 F_{254} plates. Flash-column chromatography was performed on silica gel (200–300 mesh, Qingdao, China). Melting point is determined on a Mitamura-Riken micro-hot stage and is not corrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Jeol JNM-ECP600 spectrometer with tetramethylsilane (Me_4Si) as the internal standard, and chemical shifts were recorded in values. Mass spectra were recorded on a Q-TOF Global mass spectrometer. All reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. The intermediate compounds **4a**, **4c** [40], **7** [41] and 2-(chloroseleno)benzoyl chloride [42] were prepared according to the literature. According to the reference, 2-(chloroseleno)benzoyl chloride was prepared from the intermediate of 2,2′-bisselanyldibenzoic acid which was prepared in a good yield from the diozotization of o-aminobenzoic acid with a basic solution of Na2Se2, which was in turn obtained from the reaction of selenium and hydrazine hydrate in the basic solution under the protection of nitrogen. Then, a solution of 2,2′-bisselanyldibenzoic acid (20 g, 50 mmol) in thionyl chloride (80 mL) was refluxed for 3 h and the unreacted thionyl chloride was removed by vacuum distillation. The crude solid was extracted three times by hexane and after hexane removal, the solid product was recrystallized by vacuum distillation from diethyl ether to give a yellow solid (18 g) in the yield of 60.5%, mp: 60–62 °C.

4.1.2. *2-(2,3,4,6-Tetra-O-acetyl-1-deoxy-β-D-glucopyranosyl)-benzo[d] [1,2] selenazol-3 (2H)-one (**2a**)*

1-amino-2,3,4,6-tetra-*O*-acetyl-1-deoxy-*b*-D-glucopyranose (690 mg, 2 mmol) was dissolved in dry $CHCl_3$ (10 mL). Then solutions of 2-(chloroseleno)benzoyl chloride (500 mg, 2 mmol) in dry $CHCl_3$ (10 mL) and triethylamine (0.60 mL, 4 mmol) in dry $CHCl_3$ (10 mL) were added dropwise to the solution at 0 °C over a period of 30 min. The reaction mixture was allowed to stir for an additonal

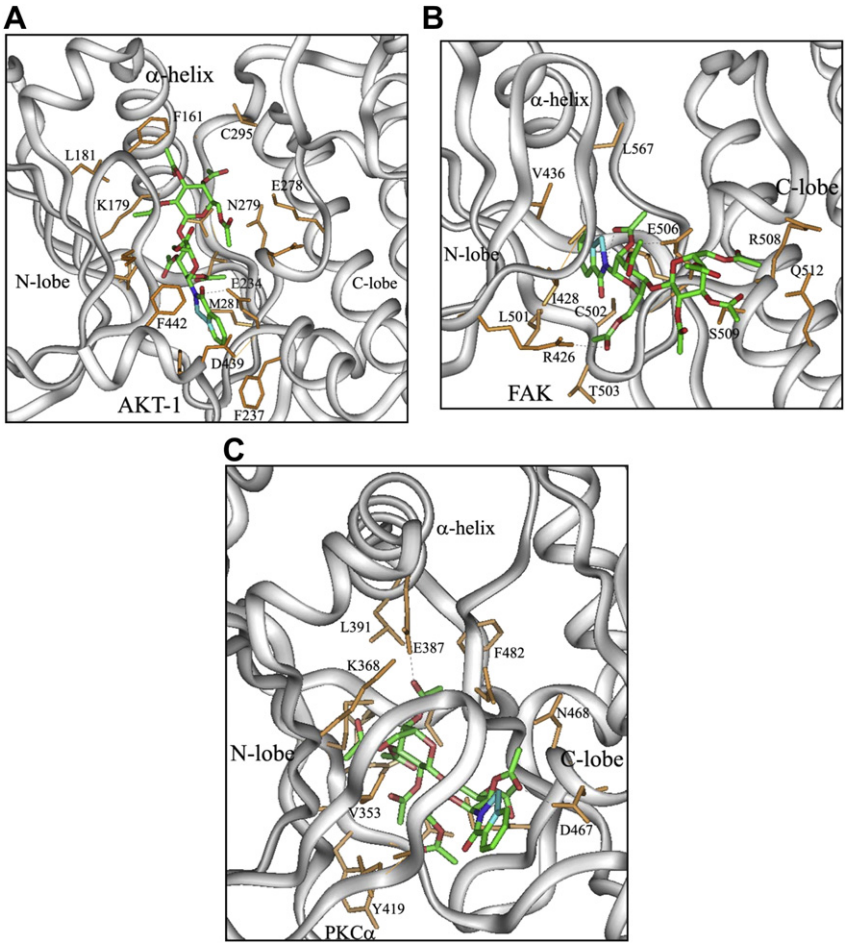


Fig. 2. Predicted binding mode of compound **2d** in the ATP-binding pocket of the AKT-1, FAK and PKC-α kinase domains (a–c). Compound **2d** was colored according to the atomic coloring scheme (C in green, O in red and N in blue). The side chains that are within 3 Å of the docked ligand are shown in orange sticks. Hydrogen bonds are indicated by dash lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4 h at rt. The organic layer was washed successively with water and aqueous $NaHCO_3$ and dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford a crude product. The purification of the residue by column chromatography gave the title compound as a yellow powder (630 mg, 60.3%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–7.40 (m, 4H, ArH), 5.94 (d, 1H, J = 9.2 Hz, C_1'–H), 5.37 (t, 1H, J = 9.1 Hz C_2'–H), 5.17 (m, 2H, C_4'-H, C_3'–H), 4.25 (dd, 1H, J = 4.7, 12.5 Hz, C_6'-H_1), 4.15 (dd, 1H, J = 2.2, 12.5 Hz, C_6'–H_2), 3.96 (m, 1H, C_5'–H), 2.09–1.92 (4s, 12H, Ac-H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.6, 169.9, 169.5, 169.4, 167.7, 138.7, 132.9, 129.1, 126.4, 126.2, 124.2, 80.7, 74.5, 73.2, 71.4, 67.9, 61.7, 20.7, 20.6, 20.5, 20.4. ESIMS m/z 530.0 $[M + H]^+$.

4.1.3. 2-(2,3,4,6-Tetra-O-acetyl-1-deoxy-β-D-galcopyranosyl)-benzo[d][1,2] selenazol-3 (2H)-one (**2b**)

Following the procedure for the preparation of compound **2a** but substituting 1-amino-2,3,4,6-tetra-*O*-acetyl-1-deoxy-*b*-D-glucopyranose with 1-amino-2,3,4,6-tetra-*O*-acetyl-1-deoxy-*b*-D-galacopyranose provided the title compound in 65% yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–7.44 (m, 4H, ArH), 5.92 (d, 1H, J = 9.1 Hz, C_1'–H), 5.50 (d, 1H, J = 3.2 Hz C_4'–H), 5.35 (dd, J = 9.2, 10.1 Hz 1H, C_2'–H), 5.22 (dd, 1H, J = 3.2, 10.1 Hz, C_3'–H), 4.20–4.11 (m, 3H, C_5'-H, C_6'–H), 2.22–1.94 (4s, 12H, Ac-H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.4, 170.0, 169.9, 169.5, 167.5, 138.8, 132.9, 129.0, 126.3, 126.2, 124.2, 81.2, 73.3, 71.3, 68.9, 67.1, 61.3, 20.7, 20.6, 20.5, 20.5. ESIMS m/z 530.0 $[M + H]^+$.

4.1.4. 2-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-1-deoxy-β-D-malcopyranosyl)-benzo[d][1,2]selenazol-3(2H)-one (**2c**)

Following the procedure for the preparation of compound **2a** but substituting 1-amino-2,3,4,6-tetra-*O*-acetyl-1-deoxy-*b*-D-glucopyranose with 1-amino-2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-deoxy-*b*-D-maltopyranose provided the title compound in 52% yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–7.44 (m, 4H, ArH), 5.96 (d, 1H, J = 9.2 Hz, C_1'–H), 5.46 (m, 2H, C_3'-H, C_1''–H), 5.39 (dd, 1H, J = 9.7, 10.5 Hz, C_3''–H), 5.09 (dd, 1H, J = 9.7, 10.1 Hz, C_4''–H), 5.06 (t, 1H, J = 9.2 Hz, C_2'–H), 4.89 (dd, 1H, J = 4.1, 10.5 Hz, C_2''–H), 4.53–4.11 (m, 5H, C_4'-H, C_6'-H, C_6''–H), 3.98 (m, 1H, C_5'-H, C_5''–H), 2.14–1.90 (7s, 21H, Ac-H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.6, 170.5, 170.4, 169.9, 169.8, 169.4, 167.5, 138.5, 132.9, 129.1, 126.4, 126.3, 124.1, 95.7, 80.4, 75.7, 74.8, 72.5, 72.1, 69.9, 69.3, 68.6, 67.9, 62.6, 61.4, 20.9, 20.8, 20.7, 20.6, 20.4. ESIMS m/z 818.1 $[M + H]^+$.

4.1.5. 2-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-1-deoxy-β-D-lactopyranose)-benzo[d][1,2]selenazol-3(2H)-one (**2d**)

Following the procedure for the preparation of compound **2a** but substituting 1-amino-2,3,4,6-tetra-*O*-acetyl-1-deoxy-*b*-D-glucopyranose with 1-amino-2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-deoxy-*b*-D-lactopyranose provided the title compound in 52% yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–7.44 (m, 4H, ArH), 5.91 (d, 1H, J = 8.7 Hz, C_1'–H), 5.39 (m, 2H, C_3'-H, C_4''–H), 5.15 (m, 2H, C_2'-H, C_2''–H), 4.99 (dd, 1H, J = 3.3, 10.6 Hz, C_3''–H), 4.53 (d, 1H, J = 8.3 Hz, C_1''–H), 4.51 (d, 1H, J = 12.4 Hz, C_6''–H), 4.17–4.08 (m,

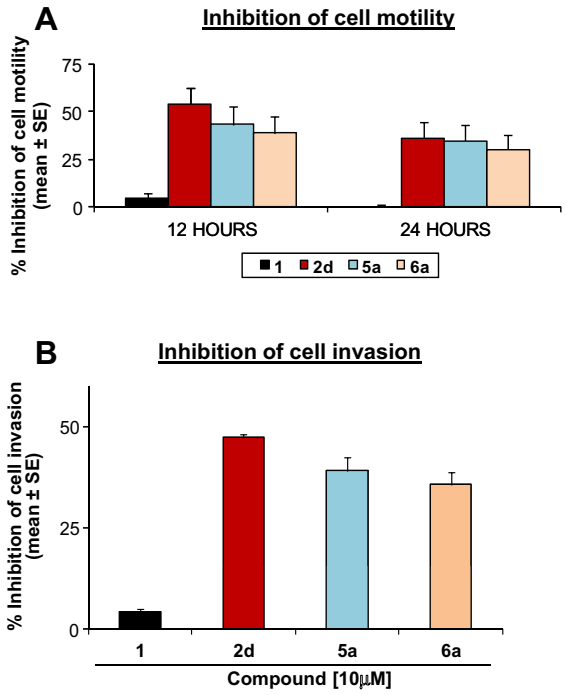


Fig. 3. EBZ compounds inhibit MDA-231 cell motility and invasion. Cell migration (A) and invasion (B) were analyzed by the wound healing and Boyden chamber assays respectively. A) MDA-231 cells treated with the indicated EBZ compounds at 10 μM, were wounded and monitored for 12 and 24 h to determine the rate of migration into the scratched area. Bar graph represents the mean ± SD of five independent experiments. B) invasion of MDA-231 cells treated with the indicated EBZ compounds at 10 μM was analyzed using the Boyden chamber assay as described in the experimental section. Bar graph represents the mean number of invaded cells. Results are expressed as the mean ± SD of five independent experiments and five fields per condition.

3H, C_6'-H, C_6''–H), 3.91 (m, 3H, C_4'-H, C_5'-H, C_5''–H), 2.17–1.92 (7s, 21H, Ac-H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 170.1, 169.7, 169.5, 169.0, 167.6, 138.6, 132.9, 129.1, 126.4, 126.3, 124.2, 101.1, 80.6, 75.9, 75.4, 73.1, 71.7, 70.9, 70.8, 69.0, 66.6, 61.8, 60.8, 20.9, 20.8, 20.7, 20.7, 20.6, 20.5. ESIMS *m/z* 818.3 $[M + H]^+$.

4.1.6. 2-(1-Deoxy-β-D-glucopyranosyl)-benzo[d][1,2]selenazol-3(2H)-one (**3a**)

Compound **2a** (270 mg, 0.5 mmol) was dissolved in methanol-chloroform (20 mL, v/v = 1:1). Then freshly prepared sodium methoxide (0.63 M, 1 mL) was added to the solution at 0 °C. After stirring at rt for 1 h, the resulting precipitate was filtrated and washed with methanol, and the solvent was removed under reduced pressure to obtain title compound as a yellow powder (170 mg, 96%). ^{1}H NMR (600 MHz, CD_3OD) : δ 7.92–7.42 (m, 4H, ArH), 5.53 (d, 1H, *J* = 8.7 Hz, C_1'–H), 3.86 (dd, 1H, *J* = 2.4, 12.4 Hz, C_6'-H_1), 3.66 (dd, 1H, *J* = 5.9, 11.9 Hz, C_6'–H_2), 3.49 (m, 3H, C_2'-H, C_3'-H, C_5'–H), 3.35 (m, 1H, C_4'–H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.1, 139.8, 131.9, 128.2, 127.6, 125.8, 82.4, 79.6, 77.4, 74.3, 70.0, 61.0. ESIMS *m/z* 362.0 $[M + H]^+$.

4.1.7. 2-(1-Deoxy-β-D-galcopyranosyl)-benzo[d][1,2]selenazol-3(2H)-one (**3b**)

Following the procedure for the preparation of compound **3a** but substituting **2a** with **2b** provided the title compound in 94% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 7.94–7.42 (m, 4H, ArH), 5.50 (d, 1H, *J* = 8.7 Hz, C1′–H), 3.95 (d, 1H, *J* = 3.3 Hz, C4′–H), 3.86 (dd, 1H, *J* = 8.7, 9.2 Hz, C2′–H), 3.78–3.72 (m, 3H, C6′-H, C5′–H), 3.65 (dd, 1H, *J* = 3.3, 9.6 Hz, C3′–H). ^{13}C NMR (150 MHz, DMSO-d6): δ 166.9, 139.9, 131.8, 128.2, 127.5, 125.9, 125.7, 82.8, 77.8, 74.1, 71.3, 68.3, 60.6. ESIMS *m/z*: 362.0 $[M + H]^+$.

4.1.8. 2-(1-Deoxy-β-D-malcopyranosyl)-benzo[d][1,2]selenazol-3(2H)-one (**3c**)

Following the procedure for the preparation of compound **3a** but substituting **2a** with **2c** provided the title compound in 98% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 7.97–7.45 (m, 4H, ArH), 5.56 (d, 1H, *J* = 8.7 Hz, C_1'–H), 5.23 (d, 1H, *J* = 4.1 Hz, C_1''–H), 3.91 (dd, 1H, *J* = 1.4, 12.4 Hz, C_6'–H), 3.84–3.79 (m, 3H, Mal-H), 3.72–3.62 (m, 5H, Mal-H), 3.60 (t, 1H, *J* = 8.7 Hz, C_2'–H), 3.46 (dd, 1H, *J* = 3.7, 9.7 Hz, C_2''–H), 3.29 (t, 1H, *J* = 9.6 Hz, C_3''–H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.1, 139.9, 131.9, 128.1, 127.6, 125.9, 125.8, 100.9, 82.3, 79.4, 77.7, 77.1, 73.8, 73.5, 73.3, 72.5, 69.9, 60.8, 60.5, 59.8. ESIMS *m/z* 524.1 $[M + H]^+$.

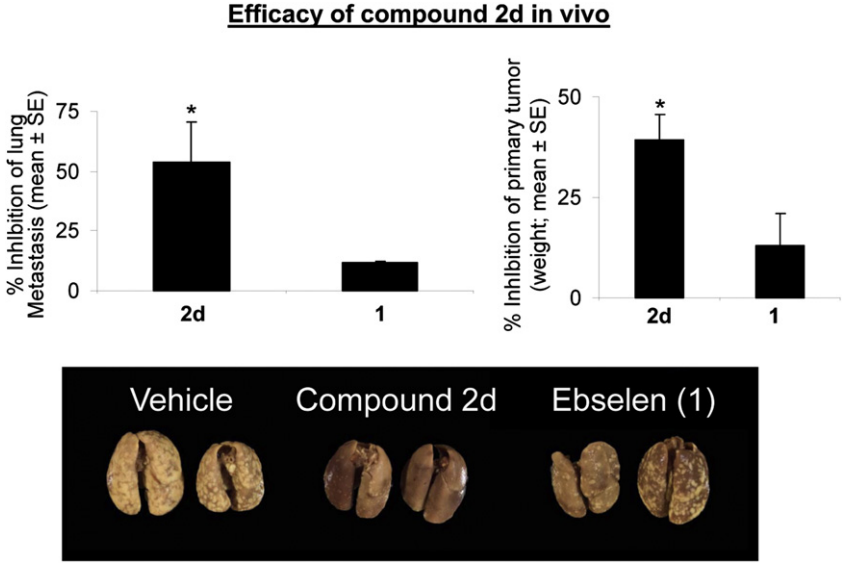


Fig. 4. Compound **2d** inhibits cancer progression in a preclinical orthotopic breast cancer model. MDA-231-M2 cells were implanted into the mammary fat pad of 6 SCID mice per condition as described in the experimental section. Once the tumor became palpable, mice were treated with vehicle, compound **2d** or ebselen (**1**) at 60 mg/kg IP for a total of 9 injections. Mice were then subjected to autopsy and lung metastases and primary tumor volume were examined. Graphs show the mean % inhibition of lung metastases (*, $P < 0.01$) (left panel), and primary tumor volume ± SEM (*, $P < 0.01$), as compared to vehicle controls. Bottom panel shows representative lungs with metastatic nodules.

*4.1.9. 2-(1-Deoxy-β-D-laccopyranosyl)-benzo[d][1,2]selenazol-3 (2H)-one (**3d**)*

Following the procedure for the preparation of compound **3a** but substituting **2a** with **2d** provided the title compound in 96% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 7.97–7.46 (m, 4H, ArH), 5.57 (d, 1H, J = 8.7 Hz, C_1'–H), 4.41 (d, 1H, J = 7.8 Hz, C_1''–H), 3.93 (dd, 1H, J = 2.3, 12.4 Hz, C_6'-H_1), 3.87 (dd, 1H, J = 4.1, 12.4 Hz, C_6'–H_2), 3.82–3.59 (m, 8H, Lac-H), 3.59 (dd, 1H, J = 7.8, 9.6 Hz, C_2''–H), 3.51 (dd, 1H, J = 3.3, 9.6 Hz, C_3''–H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 167.8, 132.6, 132.2, 131.9, 129.9, 126.1, 103.9, 80.6, 80.3, 76.7, 75.7, 75.6, 73.2, 71.6, 70.6, 68.1, 60.4, 60.3. ESIMS m/z 524.1 $[M + H]^+$.

*4.1.10. 4-(benzo[d][1,2]selenazol-3(2H)-one-2-yl)-N-(2,3,4,6-tetra-O-acetyl-1-deoxy-β-D-glucopyranosyl)-butyramide (**5a**)*

2-amino-*N*-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)- butyramide (**4a)** was prepared from hydrolysis of *N*-[2-oxo-2-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amino] butyl]-Carbamic phenylmethyl ester, with H_2/Pd-C which was in turn obtained from 4-(benzyloxycarbonylamino)butanoic acid and 1-amino-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose as starting material in 90% yield according to the method of Sawaki M [40]. Then, to a solution of compound **4a (**800 mg, 1.8 mmol)) in dry THF (30 mL) was added a solution of 2-(chloroseleno)benzoyl chloride (500 mg, 2 mmol) in dry THF (10 mL) and a solution of triethylamine (0.6 mL, 4 mmol) in dry THF (10 mL) dropwise at 0 °C over a period of 30 min. The reaction mixture was continued stiring for an additional 4 h at room temperature. When the reaction was completed as detected by TLC, the reaction mixture was filtered and the organic layer was washed with water and aqueous $NaHCO_3$ and dried over anhydrous Na_2SO_4. After removing the dryer by filtration, the solvent was removed under reduced pressure to afford a crude product. The purification of the crude product by column chromatography to afford yellow power, the title compound, 530 mg in 48% yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–7.43 (m, 4H, ArH), 6.99 (d, 1H, J = 9.2 Hz, N-H), 5.27 (m, 2H, C_3'-H, C_4'–H), 5.07 (m, 1H C_2'–H), 4.97 (m, 1H, C_1'–H), 4.27 (dd, 1H, J = 4.6, 12.4 Hz, C_6'-H_1), 4.08 (dd, 1H, J = 2.3, 12.4 Hz, C_6'–H_2), 3.92 (m, 1H, C_5'–H), 3.80 (m, 2H, α-CH_2), 2.24 (m, 2H, γ-CH_2), 2.01–2.08 (4s, 12H, Ac-H), 1.77 (m, 2H, β-CH_2). ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.7, 170.7, 170.0, 169.6, 167.7, 137.8, 132.3, 129.1, 127.1, 126.4, 124.1, 78.2, 73.6, 73.1, 70.7, 68.2, 61.7, 43.7, 33.0, 26.2, 20.8, 20.8, 20.7. ESIMS m/z 615.1 $[M + H]^+$.

*4.1.11. 4-(benzo[d][1,2]selenazol-3(2H)-one-2-yl)-N-(2,3,6,2′,3′,4′, 6′-hepta-O-acetyl-1-deoxy-β-D-laccopyranosyl)-butyramide (**5b**)*

Following the procedure for the preparation of compound **5a** but substituting **4a** with **4c** and the starting material with 1-amino-2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-deoxy-β-D-laccopyranose provided the title compound in 42% yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03–7.43 (m, 4H, ArH), 7.11 (d, 1H, J = 9.1 Hz, N-H), 5.35 (d, 1H, J = 2.3 Hz, C_4''–H), 5.27 (t, 1H, J = 9.1 Hz, C_1'–H), 5.24 (t, 1H, J = 9.1 Hz, C_1''–H), 5.10–3.72 (m, 13H, Lac-H, α-CH_2), 2.22 (m, 2H, γ-CH_2), 2.16–1.97(7s, 21H, Ac-H), 2.00 (m, 1H, β–CH_2–1), 1.26(m, 1H, β–CH_2–2). ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.7, 170.9, 170.5, 170.3, 170.2, 169.6, 169.1, 167.8, 137.9, 132.3, 129.1, 127.1, 126.5, 124.2, 101.1, 78.1, 76.1, 74.5, 72.8, 71.1, 71.0, 70.8, 69.1, 66.7, 62.0, 61.0, 43.7, 33.0, 26.3, 21.0, 20.9, 20.8, 20.7, 20.7, 20.6. ESIMS m/z 903.2 $[M + H]^+$.

*4.1.12. 4-(benzo[d][1,2] selenazol-3 (2H)-one-2-yl)-N-(1-deoxy-β-D-glucopyranosyl)-butyramide (**6a**)*

Compound **5a** (300 mg, 0.5 mmol) was dissolved in methanol-chloroform (20 mL, v/v = 1:1). Then freshly prepared sodium methoxide (0.63 M, 1 mL) was added to the solution at 0 °C. After stirring at rt for 1 h, the resulting precipitate was filtrated and washed with methanol, and the solvent was removed under reduced pressure to obtain title compound as a yellow powder (205 mg, 94%). ^{1}H NMR(600 MHz, DMSO-d_6): δ 8.39 (d, 1H, J = 9.4 Hz, N-H), 8.05–7.40 (m, 4H, ArH), 4.96 (d, 1H, J = 5.0 Hz, –OH), 4.86 (d, 1H, J = 6.0 Hz, –OH) , 4.85 (d, 1H, J = 5.4 Hz, –OH), 4.69 (dd, 1H, J = 8.8, 9.3 Hz, C_1'–H), 4.47 (dd, 1H, J = 5.4, 6.1 Hz, –OH), 3.68 (m, 2H, α-CH_2), 3.61–3.00 (m, 6H, Glu-H) , 2.12 (m, 2H, γ-CH_2), 1.80 (m, 2H, β-CH_2). ^{13}C NMR (150 MHz, DMSO-d_6): δ 171.9, 166.4, 139.2, 131.4, 128.0, 127.3, 125.8, 125.7, 79.5, 78.5, 77.5, 72.5, 69.9, 42.8, 32.3, 25.8. ESIMS m/z 447.1 $[M + H]^+$.

*4.1.13. 4-(benzo[d] [1,2] selenazol-3 (2H)-one-2-yl)-N-(1-deoxy-β-D-laccopyranosyl)-butyramide (**6b**)*

Following the procedure for the preparation of compound **6a** but substituting **5a** with **5b** provided the title compound in 97% yield. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.46 (d, 1H, J = 9.1 Hz, N-H), 8.08–7.41 (m, 4H, ArH), 5.13 (d, 1H, J = 4.6 Hz, –OH), 5.04 (d, 1H, J = 5.5 Hz, –OH), 4.81 (d, 1H, J = 5.0 Hz, –OH), 4.74 (t, 1H, J = 9.1 Hz, C_1'–H), 4.70 (d, 1H, J = 1.3 Hz, –OH), 4.66 (t, 1H, J = 5.0 Hz, –OH), 4.56 (t,1H, J = 5.9 Hz, –OH), 4.53 (d, 1H, J = 4.6 Hz, –OH),4.19 (d, 1H, J = 7.3 Hz, C_1''–H), 3.67–3.09 (m, 13H, Lac-H, α-CH_2), 2.12 (m, 2H, γ-CH_2), 1.80 (m, 2H, β-CH_2). ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.0, 166.4, 139.2, 131.5, 128.0, 127.3, 125.9, 125.8, 80.6, 79.2, 76.4, 75.6, 75.5, 73.2, 72.1, 70.5, 68.2, 60.4, 60.3, 42.8, 32.4, 25.9. ESIMS m/z 609.1 $[M + H]^+$.

*4.1.14. 2-(2-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) ethyl)-benzo[d][1,2] selenazol-3 (2H)-one (**8**)*

Compound **7** can be obtained in high yield by reaction of penta-acetylglucose and benzyl 2-hydroxyethylcarbamate followed by hydrolysis to remove benzyloxycarbonyl protection group for amino-group according to the literature [41]. Then compound **7** (0.76 g, 1.9 mmol) was dissolved in dry THF (20 mL). A solution of 2-(chloroseleno)benzoyl chloride (0.52 g, 2 mmol) in dry THF (10 mL) and a solution of triethylamine 0.6 mL in dry THF (10 mL) were added dropwise at 0 °C over a period of 30 min. And the reaction was continued for an additional 2 h at room temperature. When the reaction was completed, purification of the crude reaction product by column chromatography to afford yellow power (540 mg, 50%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–7.43 (m, 4H, ArH), 5.21 (t, 1H, J = 9.1 Hz, C4′–H), 5.12 (m, 2H, C2′-H, C3′–H), 4.57 (d, 1H, J = 7.8 Hz, C1′–H), 4.26 (m, 1H, β-CH2), 4.15 (m, 1H, α-CH2), 3.99 (m, 1H, C6′-H1), 3.84 (m, 1H, C6′-H2), 3.73 (m, 1H, C5′–H), 2.08–1.95 (4s, 12H, Ac-H). ^{13}C NMR (150 MHz, CDCl3): δ 170.6, 170.2, 169.4, 167.5, 139.0, 132.1, 128.7, 126.5, 126.1, 123.7, 100.5, 72.8, 71.9, 71.2, 68.9, 68.3, 61.8, 44.5, 20.7, 20.6. ESIMS m/z 574.1 $[M + H]^+$.

*4.1.15. 2-(2-(1-Deoxy-β-D-glucopyranosyl) ethyl)-benzo [d] [1,2] selenazol-3 (2H)-one (**9**)*

Compound **8** (286 mg, 0.5 mmol) was dissolved in methanol-chloroform (20 mL, v/v = 1:1). Then freshly prepared sodium methoxide (0.63 M, 1 mL) was added to the solution at 0 °C. After stirring at rt for 1 h, the resulting precipitate was filtrated and washed with methanol, and the solvent was removed under reduced pressure to obtain title compound as a yellow powder (182 mg, 90%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.93–7.45 (m, 4H, ArH), 4.35 (d, 1H, J = 7.8 Hz, C1′–H), 4.15 (m, 3H, C6′-H1, β-CH2), 3.86 (m, 1H, α-CH2), 3.65 (m, 1H, C6′-H2), 3.36–3.22 (m, 4H, C2′-H, C3′-H, C4′-H, C5′–H). ^{13}C NMR (150 MHz, DMSO-d6): δ 166.6, 140.1, 131.4, 127.6, 127.2, 125.7, 125.6, 103.2, 77.1, 76.7, 73.5, 70.1, 67.9, 61.1, 39.8. ESIMS m/z 406.0 $[M + H]^+$.

4.2. Biological studies

4.2.1. Cell culture

The metastatic cell variant MDA231-M was established from metastatic lung nodules induced *in vivo* by the parental breast carcinoma cells MDA-231 engineered to overexpress ErbB-2 and

implanted into the mammary fat pad of Scid mice [34]. These cells were cultured in DMEM (Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 1% (v/v) non-essential amino acids, 100 M 2-mercaptoethanol, and penicillin/streptomycin. Cells were maintained in culture at 37 °C in an atmosphere of 5% CO_2.

4.2.2. Cell proliferation

Exponentially growing cells (1×10^3) were seeded in 96 well plates and incubated for 24, 48 and 72 h. Cell survival was evaluated using the metabolic MTT assay by removing culture media and replacing with fresh media containing 1 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dissolved in PBS. Following 3–4 h incubation at 37 °C, the medium containing MTT was removed and 200 μL dimethyl sulfoxide (DMSO) was added to dissolve the insoluble reduced MTT formazan precipitate into a colored solution, followed by 25 μL of glycine/NaCl buffer (0.1 M glycine, 0.1 M NaCl pH 10.5). Once the formazan crystals dissolved, the absorbance was determined at 570 nm using a microplate reader. As yellow MTT is reduced to purple formazan in the mitochondria of living cells, the assay allows discrimination between viable and non-viable cells. The concentration that inhibits 50% of cell growth (IC_{50}) for each compound was determined graphically [34].

4.2.3. In vitro kinase activity

Human recombinant full-length FAK was incubated in kinase buffer containing ATP and substrate (Poly Glu:Tyr) for 4 h at room temperature with or without the presence of the compounds at various concentrations. Remaining ATP in solution was then quantified utilizing the Kinase-Glo-luminescence kit (Promega). Moreover, the inhibitory activity of these compounds against a multitude of related kinases was also analyzed through Invitrogen's SelectScreen® kinase profiling service [43].

4.2.4. Cellular FAK kinase assay

MDA231 cells were serum starved overnight, treated with each compound for 2 h and then stimulated with 20 ng/mL EGF. Control without EGF and no treatment was included to confirm FAK activation. After 30min, cells were washed with cold PBS and then lysed in a lysis buffer (50 mM HEPES; 150 mM NaCL; 10 mM $MgCl_2$; 0.5 mM EGTA; 0.1% Triton X-100; 10% glycerol; 0.5 mM DTT; 1 mM Na_3VO_4; 1 mM PMSF; 5 mg/mL aprotinin; and 5 mg/mL leupeptin) and immunoprecipitated with a polyclonal anti-FAK antibody (C-20; Santa Cruz Biotechnologies). The resulting immunocomplex was used for FAK kinase assay using a kinase buffer (10% glycerol, 20 mM Hepes, 10 mM $MgCl_2$, 10 mM $MnCl_2$, and 100 mM NaCl) containing 3 μCi/nmol [γ-^{32}P]-ATP (4500 Ci/mmol) as previously described [23,34]. Reactions were stopped with SDS-PAGE sample buffer and boiling for 5 min. Samples were resolved by SDS-PAGE electrophoresis and excised bands were used to quantify the radioactivity using scintillation counting. The data shown represents 2 duplicate experiments for each condition.

4.2.5. Cell motility and invasion

The effect of each compound on cell motility was investigated using the wound healing motility assay. In this study, cells were grown on sterile cover slips for 24 h and were then wounded by cell scraping using a micropipette tip. Cultures were washed and then incubated with fresh culture media at 37 °C with or without the presence of EBZ compounds (10 μM final concentration) for the indicated time periods. Cells were allowed to migrate and heal the wound. Photomicrographs were taken at each time point in order to examine the wound healing areas. Remaining areas were measured and displayed as a bar graph [34].

Cell invasion experiments were performed with 8-μm porous chambers coated with Matrigel (Becton Dickinson). Briefly, serum starved cells were placed into the upper compartment (30,000 cells) of the Boyden chamber with or without indicated compounds and the chambers were then placed into 24-well culture dishes containing 400 μL of DMEM 0.2% BSA with 10% serum (lower compartment). Cells were allowed to invade through the Matrigel membrane for 48 h. The invasive cells underneath the membrane were fixed and stained. Filters were viewed under bright-field 40× objective and the counting was performed for at least three fields in each sample [34]. In all cases, DMSO treated samples were normalized to 0% inhibition.

4.2.6. In vivo tumorigenic and invasion studies in mice

The ability of compound **2d** to inhibit breast cancer cell metastasis was further investigated in a highly invasive mouse model of human breast cancer *in vivo*. *In vivo* studies were approved by the McGill Animal Care Committee (Protocol number 4101) and were conducted in accordance with institutional and Canadian Federal Guidelines. Female Scid mice were obtained from Charles River Laboratories, St. Zotique, QC, Canada. Briefly, 1.5 million MDA-231-M2 cells, a highly invasive human breast cancer cell line, were injected into the mammary fat pad of female SCID mice. Once a palpable tumor was observed, animals were treated with vehicle, compound **2d** or ebselen (**1**) 3 times a week (60 mg/kg IP, 9 total injections). Animals were sacrificed on day 40, lungs were fixed in 10% Bouin's fixative, and lung surface metastases were counted using a stereomicroscope. 6 mice were used per condition. For data analysis, an unpaired Student *t* test was used to compare tumor growth between control and chemical-treated groups. ANOVA was used to compare groups, and significance was set at $P < 0.05$ level [34].

4.2.7. Docking studies

Crystal structures of AKT1, FAK and PKC-α in complex with their inhibitors (PDB entries: 3MVH, 2JK0 and 3IW4, respectively) were utilized in the docking study after the bound inhibitors were taken out. Compound **2d** was docked into the ATP-binding pocket of AKT1, FAK and PKC-α, respectively, using software Gold v3.2 (The Cambridge Crystallographic Data Centre, Cambridge, U.K.) by Goldscore function and standard default parameter settings [44]. The ligand is treated as fully flexible, whereas the proteins were kept rigid except that each Ser, Thr and Tyr hydroxyl group was allowed to rotate to optimize hydrogen-bonding to the ligand, whereas other parts of the protein were kept rigid.

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