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Synthesis and biological evaluation of organoselenium (NSAIDs-SeCN and $SeCF_3$) derivatives as potential anticancer agents

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Abstract:

A series of organoselenium compounds based on the hybridization of nonsteroidal antiinflammatory drugs (NSAIDs) scaffolds and Se functionalities (-SeCN and -$SeCF_3$) were synthesized and characterized, and evaluated against four types of cancer cell lines, SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), MCF-7 (human breast adenocarcinoma cells). Interestingly, most of the investigated compounds showed active in reducing the viability of different cancer cell lines. The most active compound **3h** showed IC_{50} values lower than 20 μM against the four cancer cell lines, particularly to SW480 and MCF-7 with IC 50 values of 4.9 and 3.4 μM, respectively. Furthermore, NSAIDs-SeCN derivatives (**2h** and **2i)** and NSAIDs-$SeCF_3$ derivatives (**3h** and **3i**) were selected to investigate their ability to induce apoptosis in MCF-7 cells via modulation the expression of anti-apoptotic Bcl-2 protein, pro-inflammatory cytokines (IL-2) and proapoptotic caspase-3 protein. Moreover, the redox properties of the synthesized organoselenium candidates were conducted by 2, 2-didiphenyl-1-picrylhydrazyl (DPPH), bleomycin dependent DNA damage and glutathione peroxidase (GPx)-like assays. Taken together, these NSAIDs-Se candidates could provide promising new lead derivatives for further potential anticancer drug development.

Keywords: NSAIDs, selenocyanates, trifluoromethyl selenides, anticancer

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of drugs widely used clinically to treat a variety of inflammatory conditions including pain associated with arthritis in the world [1, 2]. On the other side, a growing body of studies addressed the chemo-preventive activities of NSAIDs, such as aspirin (ASA) and other NSAIDs can be used as chemo-preventive agents, especially in colorectal cancer (CRC) [3, 4]. Other studies suggest that daily dosing of ASA decreases the risk of a great variety of cancer types, including lung, breast, skin, pancreas, and ovarian cancers[5-8]. Additionally, a growing body of studies addressed the anticancer activities of NSAIDs [9, 10], although their exact molecular mechanism has remained elusive.

Selenium (Se), a unique trace element plays a crucial role in human health and disease [11]. Organic selenium compounds with diverse functional groups, including selenoesters [12], selenocyanates [13, 14], methylseleninic acid [15], isoselenocyanates [16], diselenides [17] and endocyclic selenium [18] have been reported to exhibit anticancer activity (**Fig 1)**. Among these compounds, organic selenocyanates have emerged as a promising candidate during the past two decades. The first selenocyanate described was the 1,4-phenylenebis(methylene)selenocyanate (p-XSC), which proved to be effective against prostate and oral carcinoma cells [19]. Recently, growing interest has been paid to bioactive organic trifluoromethyl sulfides (-SCF_3) because of its unique properties which were brought by the trifluoromethylthio moiety including high lipophilicity (Hansch's constant p = 1.44), metabolic stability and electron withdrawing effect [20, 21]. In contrast to trifluoromethyl sulfides group, trifluoromethyl selenides (-$SeCF_3$) group is suspected to have more lipophilic and stable group. However, the biological property of $SeCF_3$ attached molecular is hardly documented at the moment: in the past few years, particular attention has focused on the synthetic methods to obtain trifluoromethylselenylated molecules [22-26].

In this report, considering the chemo-preventive effects of NSAIDs and the anticancer activity of organic selenium compounds, along with the reports that

support the modification of NSAIDs scaffolds with Se functionalities [27, 28], several NSAIDs-SeCN and NSAIDs-$SeCF_3$ derivatives were designed with a general model consist of three essential fragments in their molecular: i) NSAIDs fragment; ii) electron donating group; iii）functional group bearing the Se atom (**Fig2**). The anticancer activity of the compounds was assessed using human cancer cell lines, SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), MCF-7 (human breast adenocarcinoma cells). Furthermore, the antioxidant potential of the compounds was investigated by employing DPPH, bleomycin-dependent DNA damage and GPx-like assays. Finally, docking studies were applied as a preliminary prediction tool to estimate the drugability of the prepared NSAIDs-Se hybrid compounds.

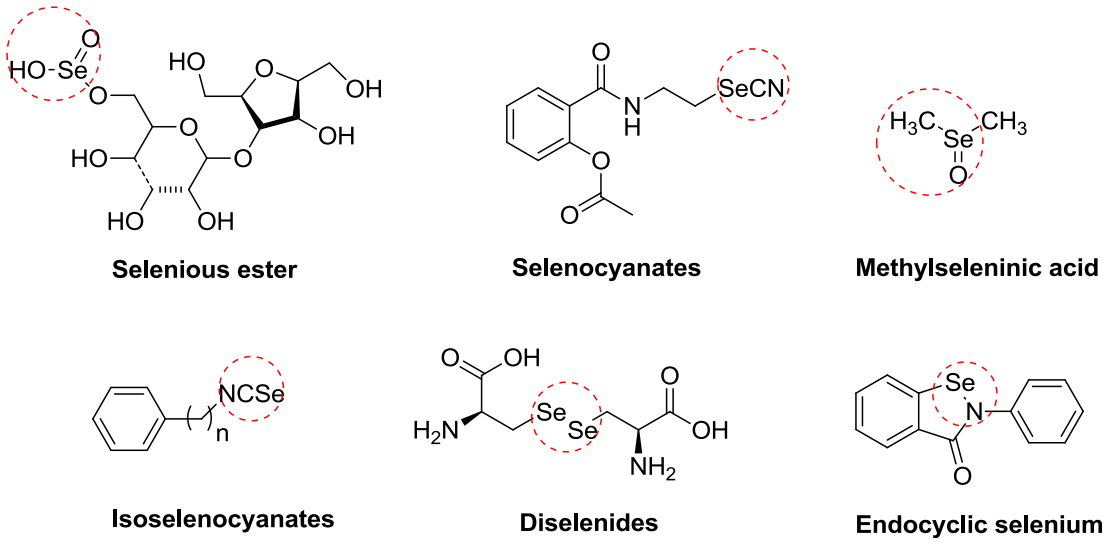


Fig. 1. Organic selenium compounds with diverse functional groups previously reported to exhibit anticancer activity

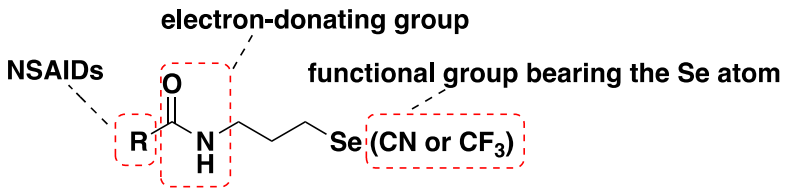


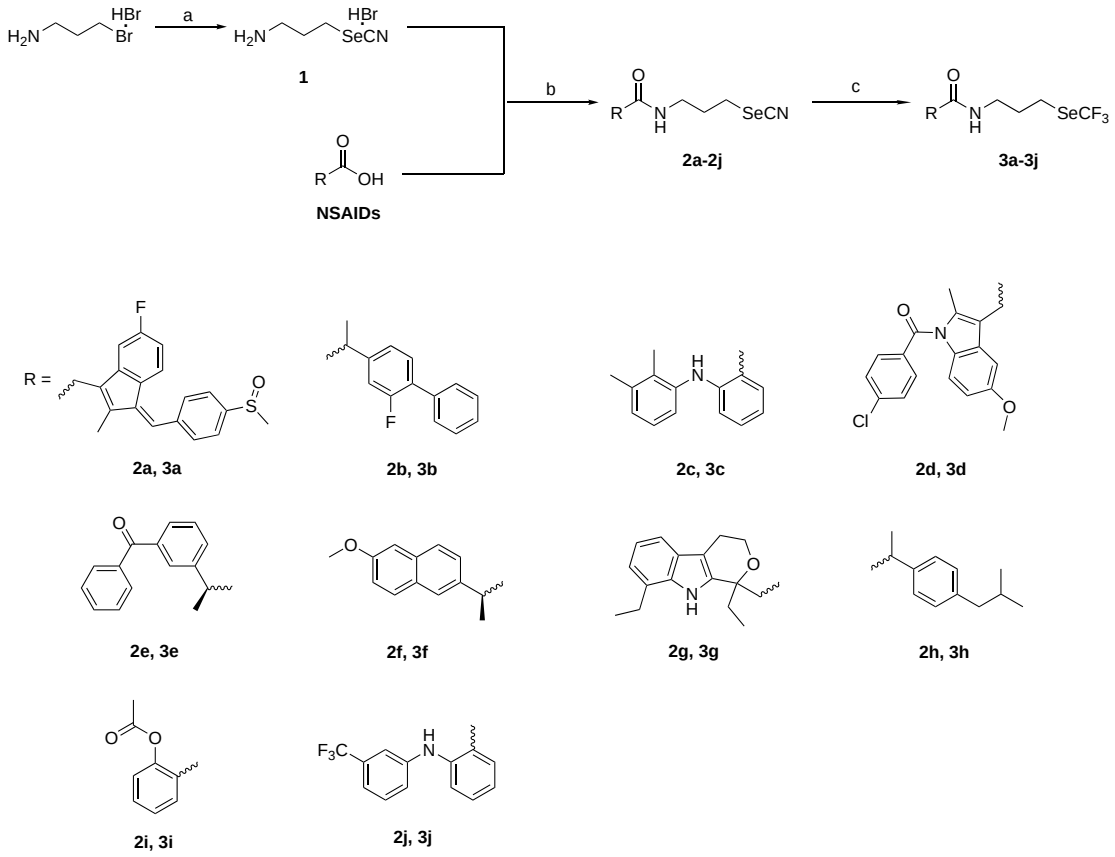
Fig. 2. Structure of NSAIDs-Se derivatives

2. Results and Discussion

2.1. Chemistry

Herein we present the synthesis of novel families of NSAIDs-based selenoderivatives as potential anticancer agents: selenocyanates, trifluoromethyl selenides.

The synthesis of the NSAIDs-SeCN derivatives (**2a-2j**) was started from commercially available NSAIDs and 3-selenocyanatopropanamine hydrobromide (**1**) in the present of EDCI and HOBT as condensation agent, in DMF as solvent and under a nitrogen atmosphere (Scheme **1**) [29].



Scheme 1. (a) KSeCN, CH_3CN, 80°C, 18 h, 90%; (b) EDCI, HOBT, TEA, CH_2Cl_2/DMF, N_2, r.t. 0.5 h, 65%-80%; (c) TBAF, $TMSCF_3$, THF, rt, 6 h, 70%-85%.

Compound **1** was obtained by the nucleophilic substitution of -Br atom in 3-bromopropionamide hydrobromide by -SeCN, using KSeCN as nucleophilic

donor, in acetonitrile as solvent and under a nitrogen atmosphere (Scheme **1**) [30]. The trifluoromethyl selenide derivatives were obtained by conducting corresponding selenocyanate derivative with trimethyl(trifluoromethyl)silane ($TMSCF_3$) in the present of etrabutylammonium fluoride (TBAF) as catalyst to afford **3a-3j** [31] in good yields (yield ≥ 85%) (Scheme **1**).

2.2. Cell viability assay

All the tested NSAIDs-Se derivatives reported in Scheme **1** were evaluated for their anticancer activity towards human tumor cell lines derived from various human cancer types: SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), MCF-7 (human breast adenocarcinoma cells). *In vitro* evaluation of anticancer activity was determined by the MTT assay at three time points (24 h, 48 h and 72 h), following previously published method of NSAID-Se hybrid compounds (selenocoxib-1 and its glutathione conjugate) with little change [32].

As reported in Table **1**, the selected patent NSAIDs (Sulindac, Indometacin and ketoprofen) had no effect on cancer cell viability even in the maximum dose of 50 μM. The IC_{50} values obtained for the hybrid Se derivatives **2a**, **2d** and **3e**, showed that introduction of the SeCN or $SeCF_3$ moiety in corresponding parent NSAIDs result in the significant effect on cancer cell line [33]. From this observation, the present study reports the synthesis of NSAIDs-Se derivatives bearing selenocyanates and trifluoromethyl selenides scaffolds and their *in vitro* anticancer activity against the same cell lines as used in Table **2**.

An overview analysis of the IC_{50} values obtained and summarized in Table **2** showed that all the compounds presented moderate effect against all four cancer cell lines, while compounds **2a**, **2e, 2h, 2i, 3a, 3b, 3d, 3e, 3g, 3h and 3i** were effective at all time points. Furthermore, compounds **2h, 2i, 3h and 3i** showed cytotoxic to SW480 cells with IC_{50} value below 10 μM. Compounds **3h** and **3i** exhibited cytotoxic to MCF-7 cell lines with IC_{50} value below 5 μM. From the current cytotoxic activity

experiment, all the compounds seem not selective to special cancer cell lines, further work will be performed to expand the scope of cancer cell lines to find selective cytotoxicity of these compounds.

Interestingly, the anticancer activity of NSAIDs-$SeCF_3$ derivatives (**3a-3j**) is better than corresponding NSAIDs-SeCN derivatives (**2a-2j**), maybe the increasing of lipophilicity increased anticancer activity for these NSAIDs-$SeCF_3$ derivatives [34]. Among the tested compounds, it was determined that compounds **3h** and **3i** showed higher promising activities than other derivatives. Compound **3h** exhibited the most potent activity against all four cancer cell lines with IC_{50} value below 20μM and with remarkable anticancer activity against MCF-7 (2.8 μM at 72 h) and SW480 (3.3 μM at 48 h).

Table 1. Cytotoxic activity expressed by IC_{50} of NSAID-Se hybrid compounds (**2a**, **2d**, and **3e**) compared to their respective parent NSAIDs on different cancer cell lines

		IC_{50}(μM)[a]					
	h	**2a**	Sulindac	**2d**	indometacin	**3e**	ketoprofen
SW480	24	15.4±0.5	>50	16.3±1.2	>50	8.2±0.3	>50
	48	12.4±0.7	>50	18.2±1.2	>50	7.4±0.1	>50
	72	12.1±0.8	>50	14.2±1.3	>50	6.5±0.2	>50
HeLa	24	28.4±2.5	>50	32.1±11	>50	19.6±7	>50
	48	16.2±1.5	>50	24.4±6	>50	17.5±4	>50
	72	21.5±2.0	>50	19.6±5	>50	28.7±5	>50
A549	24	11.4±1.8	>50	28.5±4	>50	13.1±1.2	>50
	48	15.3±2.2	>50	31.2±6	>50	18.4±2.1	>50
	72	9.4±0.3	>50	>50	>50	22.6±2.6	>50
MCF-7	24	13.2±1.3	>50	28.3±3.2	>50	8.6±0.2	>50
	48	8.4±0.8	>50	>50	>50	9.3±0.3	>50
	72	11.3±1.1	>50	>50	>50	9.5±0.3	>50

[a] IC_{50} values (±SD) of % cell viability determined by the MTT assay of three repititions.

Table 2. Cytotoxic activity expressed by IC_{50} of NSAID-Se hybrid compounds (**2a-2j** and **3a-3j**) on different cancer cell lines

Compd. No.	h	IC_{50} (μM)[a]			
		SW480	HeLa	A549	MCF-7
5-Fu [b]	24	15.3±0.6	20.6±3.5	25.3±3.6	8.5±0.5

	48	12.4±0.8	15.5±4.2	22.5±2.4	10.4±0.7
	72	13.1±1.4	12.7±3.4	17.3±1.3	12.6±0.8
2a	24	15.4±0.5	28.4±2.5	11.4±1.8	13.2±1.3
	48	12.4±0.7	16.2±1.5	15.3±2.2	8.4±0.8
	72	12.1±0.8	21.5±2.0	9.4±0.3	11.3±1.1
2b	24	23.2±1.1	36.2±8	23.5±9	22.4±3.2
	48	30.5±4	32.4±7	32.2±11	32.1±12.6
	72	21.7±5	15.9±8	>50	>50
2c	24	14.4±0.6	26.5±4	37.3±8	18.5±1.6
	48	9.7±0.4	34.3±6	>50	11.2±0.9
	72	13.2±0.5	>50	>50	7.9±0.5
2d	24	16.3±1.2	32.1±11	28.5±4	28.3±3.2
	48	18.2±1.2	24.4±6	31.2±6	>50
	72	14.2±1.3	19.6±5	>50	>50
2e	24	10.2±0.4	25.6±6	24.1±4	9.6±0.7
	48	8.4±0.2	18.5±7	28.4±5	11.3±0.9
	72	7.7±0.1	34.7±8	32.6±8	12.5±0.9
2f	24	12.0±3.1	21.5±5	32.2±2.5	28.3±5
	48	21.7±2.2	24.5±5	>50	27.1±4
	72	30.4±5.8	35.7±7	>50	>50
2g	24	19.5±1.6	26.8±3.2	28.4±6	35.2±5
	48	18.9±1.5	17.4±2.8	21.7±4	37.4±6
	72	20.6±1.2	28.5±6	36.6±9	>50
2h	24	8.9±0.4	13.5±1.2	24.4±2.6	12.1 ±0.6
	48	5.3±0.2	26.3±2.3	30.2±4	14.3±1.2
	72	6.4±0.2	29.3±2.5	28.5±3.2	16.5±1.4
2i	24	9.3±0.1	31.4±9	18.4±2.5	6.8 ±0.6
	48	7.4±0.3	28.4±5	22.6±3.3	8.6±0.8
	72	8.2±0.2	18.3±2.3	22.3±3.5	10.3±1.1
2j	24	25.2±1.4	38.2±11	26.1±5	14.2±0.4
	48	17.4±1.2	33.4±9	28.3±7	16.4±0.5
	72	24.7±2.3	>50	36.4±9	13.3±0.5
3a	24	13.4±0.4	24.3±2.3	9.4±1.5	8.2±0.2
	48	11.4±0.6	15.1±1.3	11.3±2.0	6.4±0.1
	72	10.1±1.2	19.4±2.1	7.4±0.3	10.4±0.6
3b	24	18.3±1.3	30.2±7	14.5±1.6	12.1±1.2
	48	24.4±2.4	28.4±6	23.2±2.2	17.1±2.0
	72	19.5±1.8	14.9±4	35.4±6	18.5±2.2
3c	24	12.4±0.3	24.5±2.2	27.3±2.8	8.5±0.3
	48	8.4±0.2	28.3±3.5	36.8±4	9.2±0.2
	72	10.3±0.3	>50	>50	6.9±0.1
3d	24	14.6±1.2	30.1±9	18.3±1.6	17.3±2.8
	48	16.4±1.3	21.4±5	26.5±2.9	26±3.1
	72	13.1±1.1	15.6±4	30.3±5	35±5

3e	24	8.2±0.3	19.6±7	13.1±1.2	8.6±0.2
	48	7.4±0.1	17.5±4	18.4±2.1	9.3±0.3
	72	6.5±0.2	28.7±5	22.6±2.6	9.5±0.3
3f	24	10.0±3.0	16.5±3.2	28.4±2.0	19.3±4
	48	15.7±4	22.5±4	33.5±2.4	22.1±5
	72	25.4±5	30.7±7	>50	35.5±7
3g	24	17.5±1.4	22.8±3.4	19.4±1.8	26.2±3.2
	48	14.5±1.7	14.4±2.5	17.7±1.6	28.4±3.5
	72	19.6±2.0	25.5±4	26.6±2.3	33.5±4
3h	24	4.9±0.2	11.5±1.0	9.4±1.1	3.4 ±0.1
	48	3.3±0.1	17.4±2.1	15.2±1.6	4.3±0.1
	72	4.2±0.1	19.7±2.2	18.5±2.3	2.8±0.1
3i	24	8.2±0.2	28.7±7	16.4±1.8	3.5±0.1
	48	7.2±0.1	26.3±5	18.6±3.0	4.2±0.2
	72	7.8±0.2	15.4±2.4	17.3±3.3	4.4±0.2
3j	24	22.2±2.4	33.2±9	18.1±1.5	7.2±0.4
	48	15.7±1.1	30.3±8	21.3±2.7	8.4±0.3
	72	19.7±2.2	>50	26.4±3.6	9.3±0.5

[a] IC_{50} values (±SD) of % cell viability determined by the MTT assay of three repititions. [b] Standard benchmark compound.

2.3. Evaluation of Bcl-2, IL-2 and caspase-3 molecular biomarkers in MCF-7 cells.

Previous studies showed that potential death mechanism(s) of organoselenium compounds may be due to apoptosis induction [35]. This was confirmed via the detection of various cellular alterations (e.g., cell morphology, cell cycle delay, and activation of caspase 3/7 and caspase 8) [36].

In order to explore the underlying mechanism for the reduced cell viability of the synthesized compounds, the most promising NSAIDs-Se derivatives **2h**, **2i**, **3h** and **3i** were selected and investigated their ability to induce apoptosis in MCF-7 cells via modulation the expression of anti-apoptotic Bcl-2 protein, pro-inflammatory cytokines (IL-2) and proapoptotic caspase-3 protein.

As shown in Fig **3**, all the compounds were able to downregulate the expression of Bcl-2 and upregulate the expression of IL-2 and Caspase-3 in MCF-7 cells compared with untreated cells. Interestingly, compounds **3h** and **3i** downregulate over 50% the expression levels of Bcl-2 compared to untreated cells. Furthermore, compound **2h** modulate the IL-2 level at most 1.5 fold increase in expression when

compared to the untreated control cells. Finally, compound **3i** exhibited a superior activity increased the expression level of caspase-3 by 5-fold compared to untreated cells. From the above results, it's likely that compounds **2h**, **2i**, **3h** and **3i** may induce apoptosis to inhibit tumor cells growth, and in line with the underlying mechanism of some organoselenium compounds which was reported to be effective against prostate and oral carcinoma cells via the estimation of potential biomarkers [37].

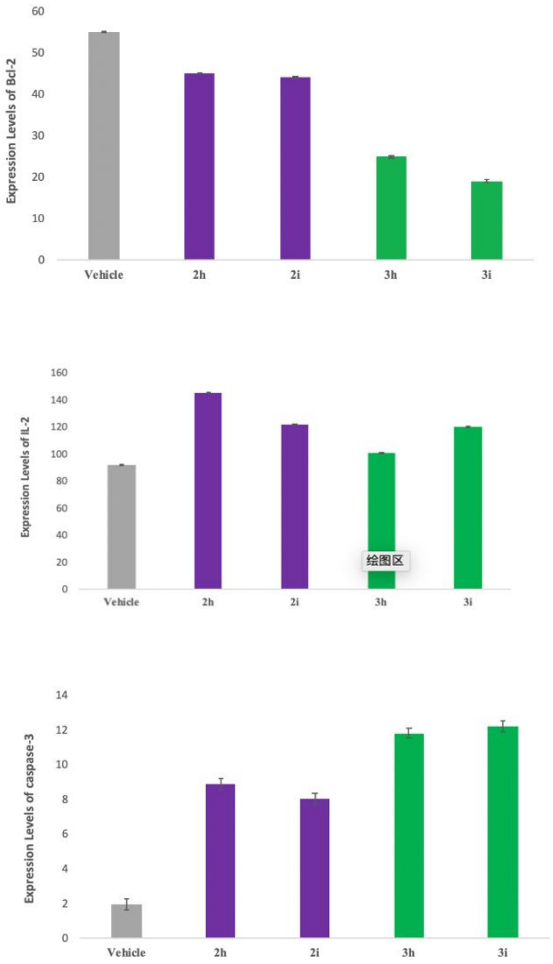


Fig. 3. Protein expression levels of Bcl-2, IL-2 and caspase-3 in MCF-7 cells after 48 h incubation with compounds **2h**, **2i**, **3h** and **3i** at their respective IC_{50}s compared to untreated cells.

2.4. Antioxidant assay

Reactive oxygen species (ROS) is a series of active oxygen clusters and are produced in all aerobic cells. ROS is considered as a signal molecule that regulates a

variety of physiological processes. Various human diseases, including different types of cancer, are associated with a disturbed intracellular redox balance and oxidative stress (OS) [38]. Redox modulators play an important role in chemotherapeutic potential antitumor agents [39].

Owing to the fact that a number of synthetic organoselenium compounds have been synthesized for their use as redox-modulators in the last few years [40-42], the antioxidant activity of the selected synthesized compounds are further estimated employing different biochemical assays such as DPPH, bleomycin-dependent DNA damage and Gpx-like assays [43, 44].

2.4.1. Radical scavenging capacity (DPPH) assay.

There are various methods which have been developed to provide fast prediction of antioxidant of natural compounds [45], however, the DPPH chemical assay is considered to be the rapid tools to evaluate the radical-scavenging activities of nutritional products and organic selenides [46]. The antioxidant activity of a compound is assessed by its ability to decolorize $DPPH^{\cdot}$ radical (purple color in methanol) to DPPHH (colorless) and the corresponding radical-scavenging activity is estimated by the decrease in the absorbance at 517 nm [47]. Vitamin C was used as a positive control (**Table 3**).

As depicted in **Table 3**, NSAIDs-$SeCF_3$ derivatives **3h** and **3i** were the most active compounds in this assay, demonstrating a good free-radical scavenging activity compared to Vitamin C. The family of NSAIDs-$SeCF_3$ derivatives is better than the corresponding NSAIDs-SeCN derivatives on this assay except for the comparison of **2d** and **3d**.

2.4.2. Bleomycin DNA damage assay.

Bleomycin (BLM) is a group of anti-neoplastic agents from Streptomyces verticillus, it is believed to oxidize DNA and induces single and double strand breaks [48]. The bleomycin-iron DNA damage assay has been routinely used as a preliminary method to test potential of drugs and organic selenium compound [49, 50].

As shown in **Table 3**, compounds **2d**, **3b**, **3g** and **3i** induced DNA degradation significantly more than other tested compounds.

Table 3. Redox modulation activity of NSAID-Se hybrid compounds.

Compd. No.	DPPH assay		Bleomycin-dependent DNA damage assay
	Inhibition %	Fold	Absorbance
Vitamin C	92.4±2.2	1	295±3.22
2a	21.2±1.8	0.2	76.5±0.65
2b	16.3±1.5	0.2	80.4±0.84
2c	26.5±2.6	0.3	62.9±0.43
2d	49.6±2.9	0.5	105.6±1.84
2e	30.4±1.4	0.3	75.5±0.62
2f	22.6±1.4	0.2	80.6±0.88
2g	24.5±1.5	0.3	92.1±0.78
2h	60.8±3.4	0.7	88.5±1.26
2i	52.1±2.3	0.6	95.6±1.44
3a	32.8±2.1	0.4	99.3±0.72
3b	40.5±2.8	0.4	104.5±2.23
3c	38.9±2.4	0.4	68.3±1.42
3d	25.8±1.4	0.3	97.6±1.63
3e	48.4±2.6	0.5	82.3±1.44
3f	37.2±2.0	0.4	90.7±1.28
3g	26.8±1.6	0.3	110.8±2.25
3h	68.7±2.2	0.7	92.7±1.62
3i	72.5±2.8	0.8	130.4±1.46

2.4.3. Glutathione peroxidase-like activity assay.

Glutathione peroxidase (GPx) is an important selenoenzyme found in humans that is responsible for the reduction of toxic peroxides at the expense of glutathione (GSH), an endogenous thiol [51, 52]. The potential antioxidant activity of all of the NSAIDs-Se derivatives were estimated using NADPH-reductase coupled assay [53, 54]. The GPx activity of the synthesized compounds was estimated by the decrease in absorbance (340 nm) due to the oxidation of NADPH to $NADP^+$. Ebselen was used as the positive control.

As shown in **Fig. 4**, compounds **2h**, **2i**, **3b**, **3e**, **3h** and **3i** displayed a GPx-like activity better than other derivatives. Compound **3h** was the most active derivatives in this assay, up to 3 fold to the GPx mimetic ebselen.

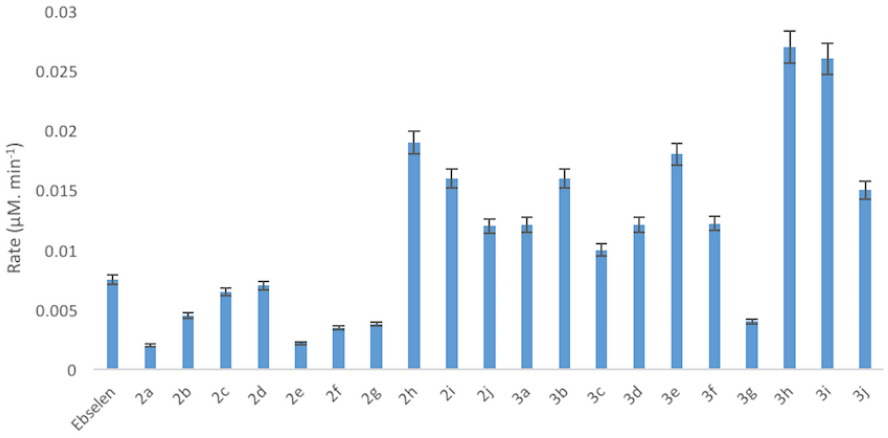


Fig. 4. GPx-like activity assay of NSAID-Se hybrid compounds in µM. Min^{-1}.

2.5. Docking Studies

Compound drugability against another selenium-contained enzyme, Thioredoxin Reductase 1(TrxR1), was investigated *in silico* based on the previously revealed binding mode of ethaselen featuring two selenenyl covalent bonds respectively with Cys497 and Sec498 [55]. Here, we adopted a noncovalent docking method given that binding modes of covalent ligands are mostly determined by noncovalent interactions.

Compound **2h**, **3h**, **3i** with promising antioxidant activity were docked into the rat Sec498Cys mutant TrxR1 protein (PDB id: 1H6V) using Flexible Docking

Protocol as reported in the literature [56]. Distance between the selenium atom and either Cys497 or Cys498 with a 0.5 nm cut-off was used to assess the accessibility of the cysteine thiol attacking the selenide, according to the proximity rule of disulfide bonding [57]. For each tested compound, multiple binding poses were generated, the best of which was elected with balanced consideration of binding energy and spatial proximity to Cys497/Cys498.

Overall analysis of all potential poses, as shown in **Table 4**, gave an evaluation of the binding affinity and covalent reaction possibility, presenting compound **3h** as the most probable TrxR1 inhibitor with the highest average –CDocker energy and greater likeliness of selenium-cysteine interaction. Compared with **3h**, apart from bearing the same scaffold, compound **2h** possesses a cyano group that, through hydrogen bonding, directly orients the reactive selenium atom, in most cases, away from Cys497/Cys498, which accounts for the increased average Se-S_{Cys498} distance. Docking performance of compound **3i** is rather unsatisfying probably due to a less compatible NSAID core with the binding cavity. Nonetheless, **3i** is somehow the most competent one to interfere with Cys497.

For each top pose in **Fig. 5**, access of the selenium atom to the reactive Cys497/Cys498 is facilitated with the assistance of hydrogen bonds between the carbonyl oxygen and neighboring residues (e.g. Ser404), as well as π-π stacking of the benzene ring and aromatic residues (e.g. Trp 407).

Table 4. Analysis of the flexible docking poses clustered by featured compound

	Average -CDocker energy /kcal·mol^{-1} (Mean±S.D.)	Average Se-S_{Cys498} Distance[a] (d_{498}) /Å (Mean±S.D.)	Average Se-S_{Cys497} Distance[a] (d_{497})/Å (Mean±S.D.)	Number (percentage[a,b]) of potentially reactive complexes[c]: Total/Cys498-reacting/ Cys497-reacting[d]	Average -CDocker energy of potentially reactive complexes /kcal·mol^{-1} (Mean±S.D.)
3h	35.68±4.20	8.39±4.00	11.62±3.08	10(19.6) / 10(19.6) / 0	37.06±2.41
2h	33.75±4.25	8.99±2.69	11.4±2.06	5(12.7) / 4(10.5) / 1(2.2)	34.34±2.27
3i	26.26±3.70	8.85±4.20	10.65±3.49	8(19.4) / 5(12.8) / 3(6.6)	25.50±2.68

a. energy weighted; b. in terms of all poses of the corresponding compound; c,d. Complexes with d_{498} or d_{497} no more than 0.5Å, referred to as Cys498-reacting and Cys497-reacting complexes, respectively.

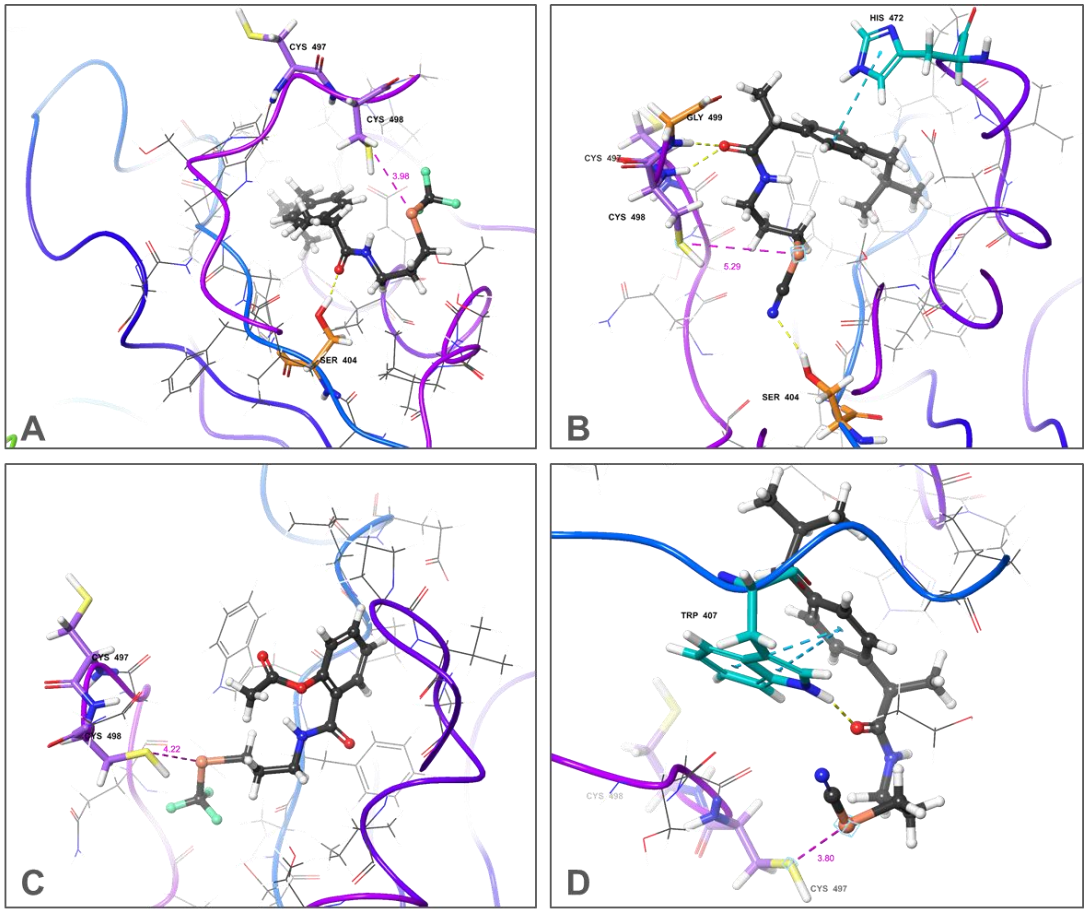


Fig. 5. Top pose of each compound (ball-and-stick in black) and cysteine497/498 (thick tube in purple). A. Top pose with respect to both **3h** and Cys498. The carbonyl oxygen of **3h** is hydrogen-bonded with Ser404. (–CDOCKER ENERGY = 39.24 kcal/mol, distance Se-S_{Cys498} = 3.98 Å.) B. Top pose of **2h**, showing that the carbonyl oxygen of **2h** interacts with Cys498 and Gly499 through hydrogen bonds with their amino groups. Π-π stacking between the benzene ring of **2h** and His472 also contributes to the binding, while the cyano group oriented towards Ser404 acts as a downside. (–CDOCKER ENERGY = 37.14 kcal/mol, distance Se-S_{Cys498} = 5.29 Å.) C. Top pose of **3i**, with no significant interaction detected. (–CDOCKER ENERGY = 30.95 kcal/mol, distance Se-S_{Cys498} = 4.22 Å.) D. Top pose regarding Cys497, featured by compound **2h** that shares hydrogen bond and π-π interactions with Trp407. (–CDOCKER ENERGY = 30.20 kcal/mol, distance Se-S_{Cys497} = 3.80 Å.)

3. Conclusions

In summary, the present study reports the synthesis of new organoselenium derivatives including NSAIDs scaffolds and Se functionalities (-SeCN and -$SeCF_3$), Compound **3h** exhibited the most potent activity in MTT assay with remarkable anticancer activity against MCF-7 (2.8 μM at 72 h) and SW480 (3.3 μM at 48 h). Compounds **2h, 2i, 3h** and **3i** were selected to verify if organic selenides can induce

apoptosis in MCF-7 cells by modulating the expression of the Bcl-2, IL-2 and caspase-3 molecular biomarkers, the selected compounds were able to downregulate the expression of Bcl-2 and upregulate the expression of IL-2 and Caspase-3 in MCF-7 cells compared with untreated cells. Furthermore, some of the synthesized NSAIDs-Se hybrid compounds (e.g., **2d**, **2h**, **2i**, **3b**, **3d**, **3e**, **3g**, **3h, 3i**) exhibited antioxidant activity in antioxidant evaluation including DPPH, bleomycin-dependent DNA damage and Gpx-like assays.

Overall, considering the potency of these NSAIDs-Se derivatives on cancer cell viability, antioxidant activity and docking study, it appears that introduction of selenocyanate (-SeCN) or trifluoromethyl selenides (-$SeCF_3$) moiety to some NSAIDs could serve as a promising launch point for the further design of this type of NSAIDs-Se anticancer agents.

4. Materials and methods

4.1 Materials

All chemical reagents for the synthesis of the compounds were perchased from Macklin (Shanghai, China) or TCI (Shanghai, China) and used without further purification unless stated otherwise. TLCs were performed on aluminium pre-coated sheets (E. Merck Silica gel 60 F254). Melting points (uncorrected) were recorded on an Electrothermal apparatus. ^{1}H (400 MHz), ^{13}C (100 MHz) NMR and ^{19}F (376 MHz) spectra were recorded at 25℃ on a Bruker Avance 400 MHz spectrometer with 5 mm PABBO probe. Chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants(*J*) are expressed in Hertz (Hz). Mass analysis was recorded on an ESI source mass detector (Thermo LCQ FLEET). HRMS spectrometry was performed on a SCIEX，TripleTOF 5600+, operating in ionization mode.

4.2. Experimental procedures

*4.2.1. Procedure for the synthesis of compound **1***

To a solution of 3-bromopropan-1-amine hydrobromide (3g, 13.7 mmol) in anhydrous acetonitrile (40 mL) was added KSeCN (1.97 g, 13.7mmol). The mixture

was stirred at 80°C for 18 hours. Then the mixture was cooled to 25°C and filtered. The filter cake was washed with acetonitrile (5mL×2) and dried under vacuum to obtain the brown solid (3.1g yield = 91%). The isolated solid was used without purification for further reactions.

4.2.2. General procedure for the synthesis of compounds ***2a-2j***

To a solution of patent NSAIDs (1.0 eq) in DCM (5 mL) and DMF (5 mL) was added EDCI (1.2 eq.), HOBT (1.2 eq.) and TEA (3.0 eq.). The mixture was stirred at 25℃ for 30 minutes under nitrogen atmosphere. Then 3-selenocyanatopropanamine hydrobromide (1.2 eq.) was added into the mixture. The mixture was stirred at 25℃ for 16 hrs under inert atmophere. TLC showed the reaction was complete. The mixture was diluted with H_2O (20 mL), the aqueous layer was extracted with DCM (15 mL×2), the combined organic layer was washed with brine (20 mL×3), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with dichloromethane /methanol solution to obtain the desire compound [58].

4.2.2.1.(Z)-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)-N-(3-selenocyanatopropyl)acetamide **(2a)**[58]. Yield: 60%. White solid. Mp: 117-118°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.06 (t, 2H, J = 4.00 Hz, CH_2), 2.23 (s, 3H, -CH_3), 2.82 (s, 3H, -CH_3), 2.97 (t, 2H, J = 4.00 Hz, CH_2), 3.38-3.42 (m, 2H, CH_2), 3.52 (s, 2H, -CH_2), 6.02 (brs, 1H, NH), 6.58-6.62 (m, 1H, ArH), 6.84 (d, 1H, J = 8.00Hz, ArH), 7.18-7.21 (m, 2H, ArH), 7.68 (d, 2H, J = 8.00Hz, Ar-H), 7.75(d, 2H, J = 8.00Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.6, 27.1, 31.0, 33.7, 38.4, 43.9, 102.1 (-CN), 105.8 (d, $J_{c\text{-}f}$ = 24.0 Hz), 111.3 (d, $J_{c\text{-}f}$ = 22.0 Hz), 124.0 (d, $J_{c\text{-}f}$ = 12.0 Hz), 129.0, 129.6 (d, $J_{c\text{-}f}$ = 3.0 Hz), 130.3, 132.2 (d, $J_{c\text{-}f}$ = 2.0 Hz), 138.9, 139.4, 141.4, 145.6, 146.2 (d, $J_{c\text{-}f}$ = 9.0 Hz), 162.1, 164.6, 170.0. MS(ESI): m/z = found 524.9 ([M+Na]$^+$); calcd. 524.5 [M+Na]$^+$; HRMS calcd. For $C_{24}H_{23}FN_2O_2SSe$[M+H]$^+$: 503.0699, found 503.0658 [M+H]$^+$.

4.2.2.2. 2-(2-Fluoro-biphenyl-4-yl)-N-(2-selenocyanato-ethyl)-propinonamide **(2b)**[58]. Yield: 62%. White solid. Mp: 88-90°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53 (d, 3H, *J* = 4.00 Hz, $-CH_3$), 1.86-1.91 (m, 2H, $-CH_2$), 2.81 (t, 2H, *J* = 8.00 Hz, $-CH_2$), 3.26-3.30 (m, 2H, $-CH_2$), 3.59 (q, 1H, *J* = 4.00 Hz, -CH), 6.04 (s, 1H, -NH), 7.11-7.15 (m, 2H, ArH), 7.34-7.45 (m, 4H, ArH), 7.51-7.53 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5, 27.2, 31.1, 38.4, 46.6, 102.3 (-CN), 115.2 (d, J_{C-F} = 23.0 Hz), 123.5 (d, J_{C-F}= 3.0 Hz), 127.8, 128.1 (d, J_{C-F} = 14.0 Hz), 128.5, 128.9 (d, J_{C-F} = 3.0 Hz), 131.2 (d, J_{C-F}= 3.0 Hz), 135.2, 142.4 (d, J_{C-F}= 7.0 Hz), 160.3 (d, J_{C-F} = 248.0 Hz), 174.4. MS(ESI): m/z = found 413.0 ($[M+Na]^+$); calcd. 412.3 $[M+Na]^+$; HRMS calcd. For $C_{19}H_{19}FN_2OSe[M+H]^+$: 391.0717, found 391.0718 $[M+H]^+$.

4.2.2.3. 2-(2,3-Dimethyl-phenylamino)-N-(2-selenocyanato-propyl)-benzamide (**2c**)[58]. Yield: 55%. White solid. Mp: 95-96°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.18 (s, 3H, $-CH_3$), 2.14-2.23 (m, 2H, $-CH_2$), 2.31 (s, 3H, $-CH_3$), 3.09 (t, 2H, *J* = 8.00 Hz, $-CH_2$), 3.60 (q, 2H, *J* = 8.00 Hz, $-CH_2$), 6.54-6.56 (m, 1H, Ar-H), 6.68 (t, 1H, *J* = 8.00 Hz, ArH), 6.91 (d, 1H, *J* = 8.00 Hz, ArH), 6.95 (d, 1H, *J* = 8.00 Hz, Ar-H), 7.06 (t, 1H, *J* = 8.00 Hz, ArH), 7.13 (d, 1H, *J* = 8.00 Hz, ArH), 7.19-7.24 (m, 1H, ArH), 7.41 (d, 1H, *J* = 8.00 Hz, ArH), 9.10 (s, 1H, -NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 20.8, 27.6, 31.3, 38.5, 102.7(-CN), 115.2, 116.4, 117.1, 121.0, 125.9, 126.0, 127.6, 131.0, 132.7, 138.3, 139.5, 147.3, 170.5. MS(ESI): m/z = found 410.1 ($[M+Na]^+$); calcd. 409.3$[M+Na]^+$; HRMS calcd. For $C_{19}H_{21}N_3OSe$ $[M+H]^+$: 388.0920, found 388.0917 $[M+H]^+$.

4.2.2.4.
*2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-N-2(2-selenocyanato-propyl)-acetamide (***2d**)[58]. Yield: 65%. White solid. Mp: 101-102°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.03 (t, 2H, *J* = 8.00 Hz, $-CH_2$), 2.39 (s, 3H, $-CH_3$), 2.96 (t, 2H, *J* = 8.00 Hz, $-CH_2$), 3.35-3.39 (m, 2H, $-CH_2$), 3.64 (s, 2H, $-CH_2$), 3.82 (s, 3H, $-OCH_3$), 5.93 (brs, 1H, -NH), 6.68-6.71 (m, 1H, ArH), 6.83-6.86 (m, 2H, ArH), 7.47 (d, 2H, *J* = 8.00 Hz, ArH), 7.63 (d, 2H, *J* = 8.00Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.3,

27.2, 30.9, 32.2, 38.3, 55.8, 100.8, 102.1(-CN), 112.2, 112.5, 115.2, 129.2, 130.2, 131.0, 131.2, 133.5, 136.5, 139.6, 156.3, 168.4, 170.8. MS(ESI): m/z = found 526.0 ($[M+Na]^+$); calcd. 525.9 $[M+Na]^+$; HRMS calcd. For $C_{23}H_{22}ClN_3O_3Se$ $[M+H]^+$: 504.0585, found 504.0575 $[M+H]^+$.

4.2.2.5. (s)-2-(3-Benzoyl-phenyl)-N-(2-selenocyanato-propyl)-propionamide ***(2e)****.* Yield: 65%. White solid. Mp: 87-89°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.53 (d, 3H, *J* = 8.00 Hz, $-CH_3$), 2.02-2.06 (m, 2H, $-CH_2$), 2.96 (t, 2H, *J* = 8.00 Hz, $-CH_2$), 3.35-3.38 (m, 2H, $-CH_2$), 3.63 (q, 1H, *J* = 8.00 Hz, -CH), 5.96 (brs, 1H, -NH), 7.44-7.51 (m, 3H, ArH), 7.56-7.61 (m, 3H, ArH), 7.74-7.79 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.6, 27.2, 31.1, 38.4, 46.9, 102.3(-CN), 128.4, 128.9, 129.0, 129.3, 130.1, 131.4, 132.7, 137.3, 138.1, 141.7, 174.5, 196.6. MS(ESI): m/z = found 423.0 ($[M+Na]^+$); calcd.422.3 $[M+Na]^+$; HRMS calcd. For $C_{20}H_{20}N_2O_2Se$ $[M+H]^+$: 401.0760, found 401.0765 $[M+H]^+$.

*4.2.2.6.(S)-2-(6-methoxynaphthalen-2-yl)-N-(3-selenocyanatopropyl)propanamide****(2f)*** [58].Yield: 75%. White solid. Mp: 96-98 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58 (d, *J* = 8.00 Hz, 3H, $-CH_3$), 1.96-1.99 (m, 2H, $-CH_2$), 2.86-2.92 (m, 2H, $-CH_2$), 3.30-3.33 (m, 2H, $-CH_2$), 3.65-3.68 (q, *J* = 8.00 Hz, 1H, -CH), 3.91 (s, 3H, $-OCH_3$), 5.69 (brs, 1H, -NH), 7.12-7.18 (m, 2H, ArH), 7.35 (d, 1H, *J* = 8.00 Hz), 7.64 (s, 1H, ArH), 7.69-7.14 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.6, 27.3, 31.1, 38.3, 47.0, 55.4, 102.4(-CN), 105.7, 119.4, 126.0, 126.2, 127.7, 129.0, 129.2, 133.8, 136.2, 157.9, 175.3. MS(ESI): m/z = found 399.0 ($[M+Na]^+$); calcd.399.1 $[M+Na]^+$; HRMS calcd. For $C_{18}H_{20}N_2O_2Se[M+H]^+$: 377.0760, found 377.0759 $[M+H]^+$.

4.2.2.7.

2-(1,8-Diethyl-1,3,4,9-tetrahydro-pyranol[3,4-b]indol-1-yl)-N-(3-selenocyanato-prop yl)-acetamide ***(2g)*** [58]. Yield: 65%. White solid. Mp: 104-106 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.92 (t, 3H, *J* = 8.00 Hz, $-CH_3$), 1.31 (t, 3H, *J* = 8.00 Hz, $-CH_3$), 1.87-1.97 (m, 2H, $-CH_2$), 2.08-2.17 (m, 2H, $-CH_2$), 2.40-2.44 (m, 1H, -CH), 2.79-2.88

(m, 5H, CH_2, CH_2, CH), 2.88-2.32 (m, 1H, -CH), 3.09-3.16 (m, 1H, -CH), 3.52-3.60 (m, 1H, -CH), 4.05-4.15 (m, 2H, -CH_2), 6.88 (brs, 1H, -NH), 7.00-7.02 (m, 1H, ArH), 7.05-7.09 (m, 1H, ArH), 7.33 (d, 1H, *J* = 8.00 Hz, ArH), 9.35 (brs, 1H, -NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.7, 14.2, 22.4, 24.1, 26.4, 31.1, 31.7, 37.9, 44.0, 60.4, 76.0, 102.4(-CN), 107.4, 115.8, 120.0, 120.9, 126.1, 127.0, 134.7, 135.8, 172.2. MS(ESI): m/z = found 456.2 ($[M+Na]^+$); calcd.456.1 $[M+Na]^+$; HRMS calcd. For $C_{21}H_{27}N_3O_2Se[M+H]^+$: 434.1338, found 434.1302 $[M+H]^+$.

*4.2.2.8. 2-(4-isobutylphenyl)-N-(3-selenocyanatopropyl)propanamide (**2h**)*[58]. Yield: 68%. White solid. Mp: 109-111°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ0.89 (d, 6H, *J* = 8.00 Hz, 2×-CH_3), 1.48 (d, 3H, *J* = 8.00 Hz, -CH_3), 1.81-1.87 (m, 1H, -CH), 1.98-2.03 (m, 2H, -CH_2), 2.44 (d, 1H, *J* = 4.00 Hz, -CH_2), 2.91 (td, 2H, *J* = 8.00 and 1.00 Hz, -CH_2), 3.29-3.34 (m, 2H, -CH_2), 3.49-3.54 (m, 1H, -CH), 5.72 (brs, 1H, -NH), 7.11 (d, 2H, *J* = 8.00 Hz, ArH), 7.16 (d, 2H, *J* = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3, 22.4, 27.3, 30.2, 31.1, 38.2, 45.0, 46.7, 102.4(-CN), 127.2, 129.8, 138.3, 141.0, 175.0. MS(ESI): m/z = found 353.0 ($[M+H]^+$); calcd.353.1 $[M+H]^+$; HRMS calcd. For $C_{17}H_{24}N_2OSe[M+H]^+$: 353.1124, found 353.1129 $[M+H]^+$.

*4.2.2.9.2-((3-selenocyanatopropyl)carbamoyl)phenyl acetate (**2i**).* Yield: 72%. White solid. Mp: 72-74°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13-2.19 (m, 2H, -CH_2), 2.32 (s, 3H, -CH_3), 3.10 (d, 2H, *J* = 8.00 Hz, -CH_2), 3.54-3.59 (m, 1H, -CH_2), 6.50 (brs, 1H, -NH), 7.10 (d, 1H, *J* = 8.00 Hz, ArH), 7.27-7.29 (m, 1H, ArH), 7.31-7.50 (m, 1H, ArH), 7.65 (d, 1H, *J* = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.2, 27.2, 31.2, 38.5, 102.4 (-CN), 123.2, 126.3, 128.1, 129.2, 132.1, 148.1, 166.8, 169.3. MS(ESI): m/z = found 326.9 ($[M+H]^+$); calcd. 327.0 $[M+H]^+$; HRMS calcd. For $C_{13}H_{14}N_2O_3Se$ $[M+H]^+$: 327.0240, found 327.0238 $[M+H]^+$.

*4.2.2.10.N-(3-selenocyanatopropyl)-2-((3-(trifluoromethyl)phenyl)amino)benzamide (**2j**).* Yield: 75%. White solid. Mp: 132-134 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13-2.19 (m, 2H, -CH_2), 2.32 (s, 3H, -CH_3), 3.10 (d, 2H, *J* = 8.00 Hz, -CH_2),

3.54-3.59 (m, 1H, -CH_2), 6.50 (brs, 1H, -NH), 7.10 (d, 1H, *J* = 8.00 Hz, ArH), 7.27-7.29 (m, 1H, ArH), 7.31-7.50 (m, 1H, ArH), 7.65 (d, 1H, *J* = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.2, 31.1, 38.6, 102.1(-CN), 116.0, 116.4 (q, J_{C-F} = 4.0 Hz), 118.60 (m, J_{C-F} = 4.0 Hz), 119.2, 122.7, 123.1(q, J_{C-F} = 271 Hz, -CF_3), 127.5, 129.9, 131.6, 131.7 (q, J_{C-F} = 32 Hz), 132.7, 142.2, 144.5, 170.0. MS(ESI): m/z = found 450.0 ($[M+Na]^+$); calcd. 450.0 $[M+Na]^+$; HRMS calcd. For $C_{18}H_{16}F_3N_3OSe$ $[M+H]^+$: 428.0481, found 428.0483 $[M+H]^+$.

*4.2.3. General procedure for the synthesis of compounds **3a-3j***

To a solution of compound **2(a-j)** (300mg, 1.0eq.) in THF (10ml) was added TBAF (1 eq.) and $TMSCF_3$ (10 eq.). The mixture was stirred at 25℃ for 6 hours. TLC showed the reaction was completed. The mixture was concentrated under reduced pressure. The desire compound was purified by column chromatography on silica gel.

4.2.3.1.(Z)-5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-N-(3-(trifluoromethylselanyl)propyl)-1H-indene-3-carboxamide (**3a**). Yield: 72%. White solid. Mp: 134-136 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.94-1.96 (m, 2H, -CH_2), 2.22 (s, 3H, -CH_3), 2.82 (s, 3H, -CH_3), 2.89 (t, 2H, *J* = 8.00 Hz, -CH_2), 3.33-3.36 (m, 2H, -CH_2), 3.53(s, 2H, -CH_2), 5.76 (brs, 1H, -NH), 6.58-6.63 (m, 1H, ArH), 6.83-6.86 (m, 1H, ArH), 7.18-7.20 (m, 1H, ArH), 7.21(s, 1H, CH), 7.68 (d, 2H, *J* = 8.00 Hz, ArH), 7.74 (d, 2H, *J* = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.6, 22.8, 30.2, 33.8, 39.1, 43.9, 105.9 (d, J_{C-F} = 24.0 Hz), 111.3 (d, J_{C-F} = 22.0 Hz), 122.5 (q, J_{C-F} = 313.0 Hz, -$SeCF_3$), 123.9, 128.9, 129.5 (d, J_{C-F} = 3.0 Hz), 130.2, 132.3 (d, J_{C-F} = 2.0 Hz), 138.8, 139.4, 141.4, 145.8, 146.2 (d, J_{C-F} = 8.0 Hz), 163.4 (d, J_{C-F} = 246.0 Hz), 169.4. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.3 (s, -$SeCF_3$), -112.1 (s, F). MS(ESI): m/z = found 546.1 ($[M+H]^+$); calcd. 546.1$[M+H]^+$; HRMS calcd. For $C_{24}H_{23}F_4NO_2SSe$ $[M+H]^+$: 546.0621, found 546.0568 $[M+H]^+$.

4.2.3.2. 2-(2-fluorobiphenyl-4-yl)-N-(3-(trifluoromethylselanyl)propyl)propanamide (**3b**). Yield: 75%. White solid. Mp: 113-115 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.55

(d, 3H, J = 8.00 Hz, -CH_3), 1.95-1.99 (m, 2H, -CH_2), 2.89-2.92 (m, 2H, -CH_2), 3.33-3.37 (m, 2H, -CH_2), 3.57 (q, 1H, J = 8.00 Hz, -CH), 5.51 (brs, 1H, -NH), 7.09-7.15 (m, 2H, ArH), 7.36-7.46 (m, 4H, ArH), 7.52-7.55 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5, 22.8, 30.4, 39.1, 46.7, 115.2, 115.2 (d, J_{C-F} = 24 Hz), 122.6 (q, J_{C-F} = 329 Hz, -$SeCF_3$), 127.8, 128.3 (d, J_{C-F} = 13 Hz), 128.5, 128.9 (d, J_{C-F} = 3.0 Hz), 131.2 (d, J_{C-F} = 4.0 Hz), 135.3, 142.5 (d, J_{C-F} = 7.0 Hz), 159.9 (d, J_{C-F} = 250.0 Hz), 173.8. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.3 (s, -$SeCF_3$), -116.9 (s, F). MS(ESI): m/z = found 434.1 ($[M+H]^+$); calcd. 434.1$[M+H]^+$; HRMS calcd. For $C_{19}H_{19}F_4NOSe$ $[M+H]^+$: 434.0638, found 434.0625 $[M+H]^+$.

*4.2.3.3. 2-(2,3-dimethylphenylamino)-N-(3-(trifluoromethylselanyl)propyl)benzamide (**3c**).* Yield: 75 %. White solid. Mp: 113-115 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ2.12-2.15 (m, 2H, -CH_2), 2.19 (s, 3H, -CH_3), 2.32 (s, 3H, -CH_3), 3.04-3.07 (m, 2H, -CH_2), 3.55-3.60 (m, 2H, -CH_2), 6.26 (brs, 1H, -NH), 6.67-6.71 (m, 1H, ArH), 6.90-6.96 (m, 2H, ArH), 7.05-7.08 (m, 1H, ArH), 7.14-7.16 (m, 1H, ArH), 7.19-7.24 (m, 1H, ArH), 7.37-7.40 (m, 1H, ArH), 9.12 (brs, 1H, -NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.9, 20.7, 23.0, 30.6, 39.1, 115.0, 116.6, 116.8, 121.0, 124.9 (q, J_{C-F} = 301.0 Hz, -$SeCF_3$), 125.7, 125.8, 127.3, 130.9, 132.5, 138.1, 139.4, 147.2, 170.0. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.2 (s, -$SeCF_3$). MS(ESI): m/z = found 431.1 ($[M+H]^+$); calcd. 431.1$[M+H]^+$; HRMS calcd. For $C_{19}H_{21}F_3N_2OSe$ $[M+H]^+$: 431.0781, found 431.0831 $[M+H]^+$.

*4.2.3.4. 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N-(3-(trifluoromethylselanyl)propyl)acetamide (**3d**).* Yield: 70 %. White solid. Mp: 162-164 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ1.91-1.94 (m, 2H, -CH_2), 2.39 (s, 3H, -CH_3), 2.85-2.88 (m, 2H, -CH_2), 3.30-3.35 (m, 2H, -CH_2), 3.65 (s, 2H, -CH_2), 3.82 (s, 3H, -CH_3), 5.76 (brs, 1H, -NH), 6.69-6.72 (m, 1H, ArH), 6.84 (s, 1H, ArH), 6.86-6.87 (m, 1H, ArH), 7.50 (d, 2H, J = 8.00 Hz, ArH), 7.64 (d, 2H, J = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.2, 22.7, 30.3, 32.2, 39.0, 55.8, 100.7, 112.4, 112.6, 115.2, 122.5 (q, J_{C-F} = 329.0 Hz,

-$SeCF_3$), 129.3, 130.2, 130.9, 131.2, 133.5, 136.4, 139.7, 156.4, 168.4, 170.3. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.3 (s, -$SeCF_3$). MS(ESI): m/z = found 547.0 ($[M+H]^+$); calcd. 547.0$[M+H]^+$; HRMS calcd. For $C_{23}H_{22}ClF_3N_2O_3Se$ $[M+H]^+$: 547.0506, found 547.0470 $[M+H]^+$.

*4.2.3.5. 2-(3-Benzoyl-phenyl)-N-(3-trifluoromethylselanyl-propyl)-propionamide (**3e**).* Yield: 65 %. White solid. Mp: 101-103 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (d, 3H, *J* = 8.00 Hz, -CH_3), 1.93-1.96 (m, 2H, -CH_2), 2.86-2.90 (m, 2H, -CH_2), 3.31-3.34 (m, 2H, -CH_2), 3.62 (q, 1H, *J* = 8.00 Hz, -CH), 5.59 (brs, 1H, -NH), 7.44-7.51 (m, 3H, ArH), 7.56-7.63 (m, 2H, ArH), 7.67 (d, 1H, *J* = 8.00 Hz, ArH), 7.74 (s, 1H, ArH), 7.79 (d, 2H, *J* = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.6, 22.8, 30.4, 39.0, 47.0, 122.6 (q, J_{C-F} = 328.0 Hz, -$SeCF_3$), 128.4, 128.8, 129.3, 130.0, 131.5, 132.7, 137.3, 138.1, 141.9, 174.1, 196.7. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.3 (s, -$SeCF_3$). MS(ESI): m/z = found 444.1 ($[M+H]^+$); calcd. 444.1$[M+H]^+$; HRMS calcd. For $C_{20}H_{20}F_3NO_2Se$ $[M+H]^+$: 444.0681, found 444.0679 $[M+H]^+$.

*4.2.3.6. (S)-2-(6-methoxynaphthalen-2-yl)-N-(3-(trifluoromethylselanyl)propyl)propanamide (**3f**).* Yield: 78 %. White solid. Mp: 127-129 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 1.60 (d, 3H, *J* = 8.00 Hz, -CH_3), 1.86-1.93 (m, 2H, -CH_2), 2.83-2.86 (m, 2H, -CH_2), 3.27-3.32 (m, 2H, -CH_2), 3.69 (q, 1H, *J* = 8.00 Hz, -CH), 3.92 (s, 3H, -OCH_3), 5.46 (brs, 1H, -NH), 7.13-7.18 (m, 2H, ArH), 7.35 (dd, 1H, J_1 = 4.00Hz, J_2 = 8.00 Hz, ArH), 7.65 (s, 1H, ArH), 7.72 (t, 2H, *J* = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3, 22.8, 30.4, 39.0, 47.1, 55.4, 105.7, 119.3, 122.6 (q, J_{C-F} = 328.0 Hz, -$SeCF_3$), 126.1, 126.2, 127.7, 129.0, 129.2, 133.8, 136.3, 157.9, 174.8. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.4 (s, -$SeCF_3$). MS(ESI): m/z = found 420.1 ($[M+H]^+$); calcd. 420.1$[M+H]^+$; HRMS calcd. For $C_{18}H_{20}F_3NO_2Se$ $[M+H]^+$: 420.0681, found 420.0686 $[M+H]^+$.

4.2.3.7.

*2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)-N-(3-(trifluoromethylselanyl)propyl)acetamide (**3g**).* Yield: 65 %. White solid. Mp: 187-189 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, *J* = 8.00 Hz, -CH_3), 1.31 (t, 3H, *J* = 8.00 Hz, -CH_3), 1.78-1.91 (m, 2H, -CH_2), 1.92-2.17 (m, 2H, -CH_2), 2.60-2.71 (m, 2H, -CH_2), 2.81-2.96 (m, 6H, 3×-CH_2), 3.22-3.46 (m, 2H, -CH_2), 4.03-4.10 (m, 2H, -CH_2), 6.62 (brs, 1H, -NH), 7.00 (d, 1H, *J* = 8.00 Hz, ArH), 7.06 (t, 1H, *J* = 8.00 Hz, ArH), 7.33 (d, 1H, *J* = 4.00 Hz, ArH), 9.35 (brs, 1H, -NH).^{13}C NMR (100 MHz, $CDCl_3$): δ 7.7, 13.9, 22.4, 22.7, 24.1, 30.7, 30.9, 38.5, 44.4, 60.5, 75.8, 107.6, 115.7, 119.7, 120.5, 122.6 (q, $J_{C\text{-}F}$ = 328.0 Hz, -$SeCF_3$), 126.2, 126.9, 134.8, 135.8, 171.5. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.3 (s, -$SeCF_3$). MS(ESI): m/z = found 477.1 ($[M+H]^+$); calcd. 477.1 $[M+H]^+$; HRMS calcd. For $C_{21}H_{27}F_3N_2O_2Se$ $[M+H]^+$: 477.1260, found 477.1220 $[M+H]^+$.

*4.2.3.8. 2-(4-isobutylphenyl)-N-(3-(trifluoromethylselanyl)propyl)propanamide (**3h**).* Yield: 60 %. White solid. Mp: 108-110 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (d, 6H, *J* = 8.00 Hz, 2×-CH_3), 1.51 (d, 3H, *J* = 8.00 Hz, -CH_3), 1.80-1.92 (m, 3H, -CH_2, -CH), 2.46 (d, 2H, *J* = 8.00 Hz, -CH_2), 2.83-2.86 (m, 2H, -CH_2), 3.25-3.32 (m, 2H, -CH_2), 3.52 (q, 1H, *J* = 8.00 Hz, -CH), 5.39 (brs, 1H, -NH), 7.12 (d, 2H, *J* = 8.00 Hz, ArH), 7.17 (d, 2H, *J* = 8.00 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3, 22.4, 22.8, 30.2, 30.4, 38.9, 45.0, 46.8, 122.6 (q, $J_{C\text{-}F}$ = 328.0 Hz, -$SeCF_3$), 127.3, 129.8, 138.4, 141.0, 174.9. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.4 (s, -$SeCF_3$). MS(ESI): m/z = found 396.1 ($[M+H]^+$); calcd. 396.1 $[M+H]^+$; HRMS calcd. For $C_{17}H_{24}F_3NOSe$ $[M+H]^+$: 396.1045, found 396.1034 $[M+H]^+$.

*4.2.3.9. 2-(3-(trifluoromethylselanyl)propylcarbamoyl)phenyl acetate (**3i**).* Yield: 78 %. White solid. Mp: 94-96 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.04-2.12 (m, 2H, -CH_2), 3.02-3.06 (m, 2H, -CH_2), 2.32 (s, 3H, -CH_3), 3.51-3.56 (m, 2H, -CH_2), 6.32 (brs, 1H, -NH), 7.10 (dd, 1H, J_1 = 4.00 Hz, J_2 = 8.00 Hz, ArH), 7.28-7.32 (m, 1H, ArH), 7.45-7.49 (m, 1H, ArH), 7.68 (dd, 1H, J_1 = 4.00 Hz, J_2 = 8.00 Hz, ArH). ^{13}C

NMR (100 MHz, $CDCl_3$): δ 21.1, 22.8, 30.6, 39.2, 122.7 (q, J_{C-F} = 329.0 Hz, -$SeCF_3$), 123.2, 126.3, 128.4, 129.3, 131.9, 148.0, 166.3, 169.2. ^{19}F NMR ($CDCl_3$, 376 MHz): δ = -34.2 (s, -$SeCF_3$). MS(ESI): m/z = found 370.0 ($[M+H]^+$); calcd. 370.0 $[M+H]^+$; HRMS calcd. For $C_{13}H_{14}F_3NO_3Se$ $[M+H]^+$: 370.0161, found 370.0158 $[M+H]^+$.

4.2.3.10.

2-(3-(trifluoromethyl)phenylamino)-N-(3-(trifluoromethylselanyl)propyl)benzamide ***(3j)***. Yield: 63 %. White solid. Mp: 164-166 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 2.10-2.17 (m, 2H, -CH_2), 3.03-3.07 (m, 2H, -CH_2), 3.54-3.59 (m, 2H, -CH_2), 6.29 (brs, 1H, -NH), 6.84-6.88 (m, 1H, ArH), 7.21-7.23 (m, 1H, ArH), 7.31-7.39 (m, 4H, ArH), 7.42-7.44 (m, 2H, ArH), 9.45 (brs, 1H, -NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.9, 30.4, 39.2, 116.0, 116.2 (q, J_{C-F} = 3.0 Hz), 118.5 (q, J_{C-F} = 4.0 Hz), 119.0, 119.2, 122.6 (q, J_{C-F} = 329.0 Hz, -$SeCF_3$), 123.0, 124.0 (q, J_{C-F} = 271.0 Hz, -CF_3), 127.5, 129.9, 131.7 (q, J_{C-F} = 32.0 Hz), 132.5, 142.2, 144.3, 169.7. ^{19}F NMR ($CDCl_3$, 376 MHz): δ -34.2 (s, -$SeCF_3$), -62.8 (s, $PhCF_3$). MS(ESI): m/z = found 471.0 ($[M+H]^+$); calcd. 471.0 $[M+H]^+$; HRMS calcd. For $C_{18}H_{16}F_6N_2OSe$ $[M+H]^+$: 471.0402, found 471.0405 $[M+H]^+$.

4.3. Cell lines and growth conditions

Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1×104 cells/well. After 24 h incubation at 37 °C under a humidified 5% CO_2 to allow cell attachment, the cells in the wells were respectively treated with target compounds at various concentrations for 48 h. The concentration of DMSO was always kept below 1.25%, which was found to be non-toxic to the cells. Three hours prior to experiment termination, MTT solution (20 μL of 5.0 mg/mL solution) was added to each well and incubated at 37°C. At the termination time point, the medium/MTT mixtures were removed, and the formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in 100 μl of DMSO per well. The optical densities were measured at 570 nm using a 96-well multiscanner (Dynex Technologies, MRX Revelation; Chantilly, VA, USA).

4.4 Detection of Bcl-2, IL-2 and caspase-3 molecular biomarkers in MCF-7 cells

Bcl-2, IL-2 and caspase-3 cells were evaluated in MCF-7 cells treated with the corresponding target compounds and incubated for 48 h and compared with their levels in control untreated MCF-7 cell line [59]. The cells were harvested by trypsinization and lysed by lysate buffer (Beyotime Biotech, Najing, China). Protein levels of Bcl-2, IL-2 and caspase-3 were measured using enzyme-linked immunosorbent assay (ELISA) by multifunctional enzyme marker (Molecular Devices i3, USA) at a wavelength of 570 nm.

4.5. DPPH free radical scavenging activity

DPPH free radical scavenging activity of corresponding compounds was measured according to the method as previous reported with little optimization[60]. Briefly, 20 mL of test samples at different concentrations was mixed with 180 mL of or DPPH solution for 30 min in the dark. Then, the change in absorbance at 517 nm for DPPH was measured on a microplate reader. Ascorbic acid (vitamin C) and ebselen were used as a positive control, DMSO was used as a negative control.

4.6. Bleomycin-dependent DNA damage

The reaction mixture contained DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 mM), and tested compound in a conc. of 0.1 mg/mL. L-ascorbic acid was used as positive control. The mixture was incubated at 37°C for 1h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding 0.5 mL TBA (1% w/v) and 0.5 mL HCl (25% v/v), followed by heating at 80°C for 30 minutes. After cooling in ice water, the extent of DNA damage was measured by increase in absorbance at 532 nm [61].

4.7 Molecular Modeling

4.7.1 Protein and Ligand Preparation

The mammalian TrxR1 protein (PDB ID: 1H6V) used for docking was obtained from Protein Data Bank. The original structure was prepared using Protein Preparation Wizard in Maestro 11.5 (Schrödinger Release 2018-1: Maestro, Schrödinger, LLC, New York, NY, 2018.), with all but one subunit (E) discarded, bond orders assigned, hydrogens added, ionization and tautomerization state adjusted, hydrogen bond assignment optimized, waters removed, and structure minimized.

The LigPrep utility in Maestro 11.5 was used to perform ligand preparation applying OPLS3 force field. Generation of tautomers and possible ionization states was mediated by Epik utility. All stereoisomers were considered to be generated, followed by minimization of the resulting 3D comformations. There was no filtration process during preparation.

4.7.2 Ligand Docking

The docking task was carried out in Discovery Studio 2018 (Dassault Systèmes BIOVIA, Discovery Studio 2018, San Diego: Dassault Systèmes, 2018). The prepared TrxR1 protein was typed in CHARMm force field and the docking site was defined as a sphere with center coordinates X: 27.757, Y: 6.510, Z: 33.698 and a radius of 15 Å. Using Flexible Docking protocol, the residue sidechains within the site sphere were allowed to move. 10 protein conformations were created with a maximum alteration of 8 residues. FAST method adopted, up to 25 conformations per ligand were generated with an energy threshold of 20 kcal. With all other parameters as default, ligands were preliminarily docked into each protein structure. After removal of similar poses by clustering, the remaining complexes were refined and minimized, leading to a total of 133 final poses.

4.7.3 Result Analysis

The resulting 133 poses were clustered by ligand (53 for 3h, 40 each for 2h and 3h) and visualized in Maestro 11.5. For each of the poses, the distance between the compound’s selenium atom and the sulfur atom of either Cys497 or Cys498 was calculated as a measurement of covalent bonding probability. Any complex with less

than 5Å of the distance above was counted potentially reactive. For each ligand, average –CDocker energy and average selenium-sulfur distance were calculated, the latter was –CDocker energy weighted.

Statistical analysis

MTT data were given as mean ± SD of three independent experiments, graphs and curve fitting were using origin Version 8.0 (OriginLab Corporation, Northampton, USA). P value less than 0.05 was considered statistically significant.

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