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## New organoselenides (NSAIDs-Se derivatives) as potential anticancer agents: Synthesis, biological evaluation and in silico calculations

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1 **New organoselenides (NSAIDs-Se derivatives) as potential anticancer agents:**  
2 **Synthesis, biological evaluation and *in silico* calculations**

3  
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1 **Abstract:**

2       Herein we reported the synthesis of twenty new organoselenium compounds  
3 (**2a-2j** and **3a-3j**) based on the hybridization of nonsteroidal antiinflammatory drugs  
4 (NSAIDs) skeleton and organoselenium motif (-SeCN and -SeCF<sub>3</sub>), the anticancer  
5 activity was evaluated against four types of cancer cell lines, Caco-2 (human colon  
6 adenocarcinoma cells), BGC-823 (human gastric cancer cells), MCF-7 (human breast  
7 adenocarcinoma cells), PC-3 (human prostatic cancer cells). Interestingly, the  
8 introduction of the -SeCN or -SeCF<sub>3</sub> moiety in corresponding parent NSAIDs results  
9 in the significant effect on cancer cell lines. Moreover, the most active compound **3a**  
10 showed IC<sub>50</sub> values lower than 5 μM against the four cancer cell lines, particularly to  
11 BGC-823 and MCF-7 with IC<sub>50</sub> values of 2.5 and 2.7 μM, respectively. Furthermore,  
12 three compounds **3a**, **3g** and **3i** were selected to investigate their ability to induce  
13 apoptosis in BGC-823 cells via modulating the expression of anti-apoptotic Bcl-2  
14 protein, pro-inflammatory cytokines (IL-2) and proapoptotic caspase-8 protein. The  
15 redox properties of the NSAIDs-Se derivatives prepared herein were conducted by 2,  
16 2-didiphenyl-1-picrylhydrazyl (DPPH), bleomycin dependent DNA damage and  
17 glutathione peroxidase (GPx)-like assays. Finally, molecular docking study revealed  
18 that an interaction with the active site of **thioredoxin reductase 1** (TrxR1) and  
19 predicted the anticancer activity of the synthesized candidates. Overall, these results  
20 could serve a promising launch point for further design of NSAIDs-Se derivatives as  
21 potential anticancer agents.

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26 **Keywords:** selenium; selenocyanates; trifluoromethyl selenides; anticancer; *in silico*  
27 calculations

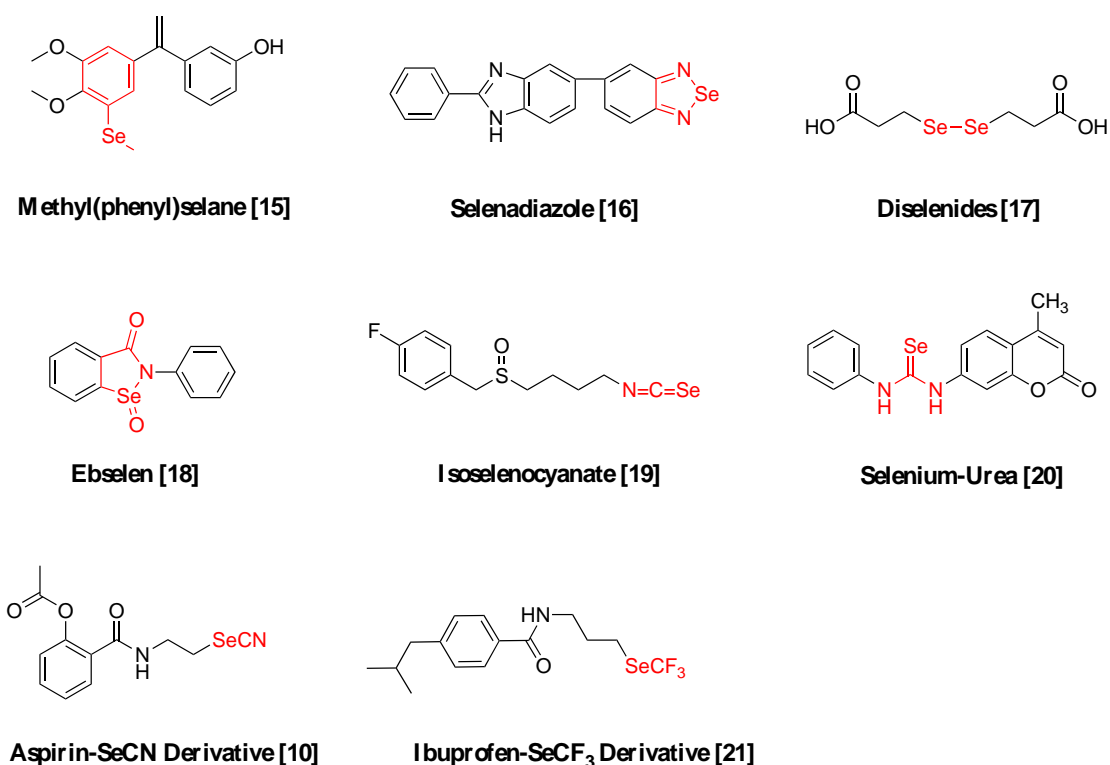
## 1 **Introduction**

2 Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of often chemically  
3 unrelated compounds commonly used to treat symptoms of inflammatory diseases  
4 such as osteoarthritis and rheumatoid arthritis, and are among the most widely used  
5 drugs worldwide [1, 2]. In the field of cancer research, a large body of evidence from  
6 epidemiological and preclinical studies have shown that NSAIDs have used for  
7 chemo-preventive agents, especially in colorectal cancer (CRC) and prostate cancer  
8 [3-6]. Several modifications, based on NSAIDs scaffolds, have demonstrated stronger  
9 cytotoxicity and chemo-preventive than corresponding NSAID alone [7, 8]. NSAIDs  
10 framework modification has become a structure-based medicinal chemistry strategy to  
11 design novel anticancer agents in the past decades [9-12].

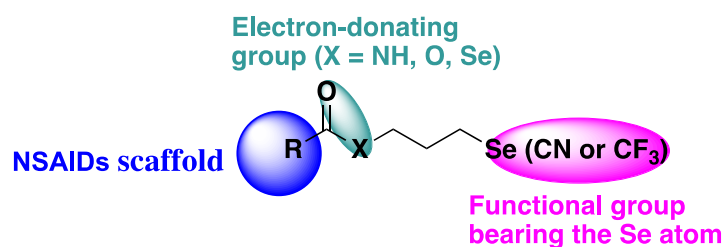
12 Selenium (Se) is an essential trace element that is of importance to human health  
13 and disease [13]. There are three main categories Se-containing compounds  
14 (inorganic, organic and selenoproteins) with potential pharmacological properties, the  
15 most developed and studied are the org-Se derivatives [14]. Different organic  
16 selenium compounds with diverse functional groups, including selenocyanates,  
17 selenoureas, heterocycles with endocyclic selenium, selenides and diselenides, have  
18 been reported to exhibit anticancer activity (**Fig 1**) [10, 15-21]. Although the  
19 mechanisms that underlie the potential anticancer activity of seleno compounds are  
20 very diverse (including protein modification, cell growth arrest, anti-angiogenic  
21 effects, etc) [22], the most frequent one is the reduction of oxidative stress through the  
22 elimination of free radicals [23-25].

23 In the previous study, the modification of NSAID framework with Se  
24 functionalities is the novel celecoxib-Se derivatives, which exhibited  
25 anti-inflammatory and anti-cancer activity [11, 12]. Very recently, we have reported  
26 the synthesis of a series of novel NSAIDs-Selenium derivatives and screened their  
27 anticancer activity by *vitro* study, the modification of NSAIDs scaffolds with Se  
28 functionalities (-SeCN, -Se-Se-, -SeCF<sub>3</sub>) demonstrated potent inhibition of human  
29 tumor cell [21, 26-27]. Along with the reports that support the modification of  
30 NSAIDs scaffolds with Se functionalities and in continuation of our research program

1 on design and synthesis of new NSAIDs-Se derivatives as potential anticancer agents  
 2 [28, 29], twenty new NSAIDs-SeCN and NSAIDs-SeCF<sub>3</sub> derivatives were designed  
 3 by the incorporation of an appropriate Se moiety into various NSAIDs with a general  
 4 model consist of three essential fragments in their molecular: i) NSAIDs fragment; ii)  
 5 electron donating group (X = NH, O, Se); iii) functional group bearing the Se atom  
 6 (**Fig 2**). Their anticancer activities against the human cancer cell lines Caco-2,  
 7 BGC-823, MCF-7 and PC-3 in *vitro* using the MTT assay. Three compounds **3a**, **3g**  
 8 and **3i** were selected to test the protein expression levels of Bcl-2, IL-8 and caspase-8  
 9 biomarkers in BGC-823 cells. Furthermore, the antioxidant potential of the  
 10 compounds was investigated by employing DPPH, bleomycin-dependent DNA  
 11 damage and GPx-like assays. Finally, TrxR1 (Thioredoxin Reductase) was selected as  
 12 docking protein in order to predict the target and anticancer activity of the prepared  
 13 NSAIDs-Se hybrid compounds.



14 **Fig. 1.** Organic selenium compounds previously reported with anticancer activity  
 15  
 16



1

2

3 **Fig. 2.** General pattern of NSAIDs-Se derivatives with anticancer activity

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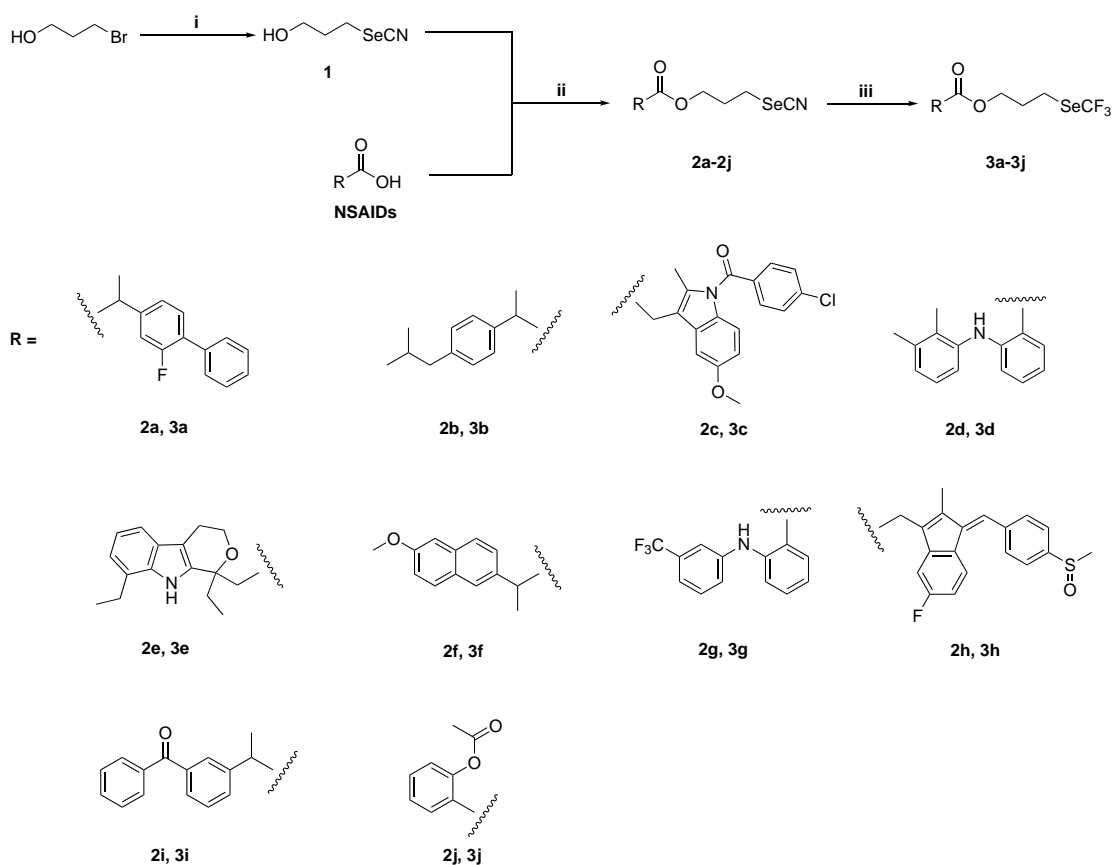
## 5 **2. Results and Discussion**

### 6 **2.1 Chemistry**

7 The synthetic route for target compounds (**2a-2j** and **3a-3j**) were prepared  
 8 as outlined in Scheme 1 according to the procedure described in the literature  
 9 with some modifications [30]. Compound 1 was obtained by the nucleophilic  
 10 substitution of -Br atom in 3-bromo-1-propanol by -SeCN, using KSeCN as  
 11 nucleophilic donor, in acetonitrile as solvent and under a nitrogen atmosphere.  
 12 The selenocyanate derivatives **2a-2j** were readily obtained by reacting  
 13 3-selenocyanatopropan-1-ol with commercially available NSAIDs in the present  
 14 of DCC and DMAP as condensation agent. The trifluoromethyl selenide derivatives  
 15 were obtained by conducting corresponding selenocyanate derivative with  
 16 trimethyl(trifluoromethyl)silane (TMSCF<sub>3</sub>) in the present of tetrabutylammonium  
 17 fluoride (TBAF) as catalyst to afford **3a-3j** in good yields (yield ≥ 80 %) (Scheme 1)  
 18 [31].

19 The purity of all final compounds was 95% or higher and their chemical  
 20 structures were characterized using <sup>1</sup>H NMR, <sup>13</sup>C NMR, <sup>19</sup>F NMR and HRMS (ESI).

21



**Scheme 1.** i) KSeCN, CH<sub>3</sub>CN, 80 °C, 24 h, 90 %; ii) DCC, DMAP, DCM, 25 °C, 16 h 70% - 90%; iii) TBAF, TMSCF<sub>3</sub>, THF, 25 °C, 2 h, 80 % - 85%.

## 2.2. Cell viability assay

The synthesized compounds (**3a**, **3g** and **3i**) and selected patent NSAIDs (Aspirin, Ibuprofen and Naproxen) were evaluated for their anticancer activity towards human tumor cell lines: Caco-2 (human epithelial colorectal adenocarcinoma cell line), BGC-823 (human gastric cancer cell line), MCF-7 (human breast adenocarcinoma cell line) and PC-3 (human prostate cancer cell line). In vitro evaluation of anticancer activity was determined by the MTT assay. 5-Fluorouracil was used as positive control because it is commonly used in adjuvant and palliative cancer chemotherapy.

Overall, the IC<sub>50</sub> values obtained and summarized in Table 1 shows that all of the tested organoselenium compounds exhibit growth inhibition in all cancer cell lines, while the selected patent NSAIDs (Aspirin, Ibuprofen and Naproxen) are inactive against all cells even in the maximum dose of 50 μM. The IC<sub>50</sub> values obtained for the

1 NSAIDs-Se derivatives **2j**, **3b** and **3f**, showed that introduction of the -SeCN or  
2 -SeCF<sub>3</sub> moiety in corresponding parent NSAIDs scaffold result in the significant  
3 effect on cancer cell line.

4 An overview analysis of the IC<sub>50</sub> values obtained and summarized in Table 1  
5 showed that most of the NSAIDs-SeCF<sub>3</sub> derivatives presented better effectiveness  
6 than NSAIDs-SeCN derivatives and previous reported NSAIDs-diselenides  
7 derivatives against all four cancer cell lines [27]. Furthermore, the most active  
8 compounds of these two series are NSAIDs-SeCF<sub>3</sub> derivatives **3a**, **3g** and **3i**. These  
9 three compounds show IC<sub>50</sub> values below 10 μM in all of tested cancer cell lines.  
10 Compound **3a** emerges the most potent agent with IC<sub>50</sub> values below 5 μM in all  
11 cancer cell lines and with remarkable anticancer activity against BGC-823 (2.5 μM)  
12 and MCF-7 (2.7 μM).

13 Interestingly, among the tested compounds, most of the NSAIDs-SeCF<sub>3</sub>  
14 derivatives except **3f** and **3h** displayed IC<sub>50</sub> values below 10 μM against MCF-7 cells.  
15 The anticancer activity of NSAIDs with trifluoromethyl selenides moiety is better  
16 than corresponding NSAIDs with selenocyanates moiety, considering the lipophilicity  
17 and electron withdrawing effect.



1 **Table 1**

2 Cytotoxic activity expressed by IC<sub>50</sub> of NSAIDs-Se hybrid compounds (**2a-2j** and  
3 **3a-3j**) on different cancer cell lines.

Compound	IC <sub>50</sub> ( $\mu$ M) <sup>a</sup>			
	Caco2	BGC-823	MCF-7	PC-3
Aspirin <sup>b</sup>	>50	>50	>50	>50
Ibuprofen <sup>b</sup>	>50	>50	>50	>50
Naproxen <sup>b</sup>	>50	>50	>50	>50
<b>2a</b>	27.5 $\pm$ 3.1	29.4 $\pm$ 3.3	22.4 $\pm$ 2.1	19.7 $\pm$ 1.8
<b>2b</b>	14.5 $\pm$ 1.3	24.5 $\pm$ 2.3	19.5 $\pm$ 1.7	22.5 $\pm$ 3.4
<b>2c</b>	32.4 $\pm$ 3.5	35.5 $\pm$ 3.4	29.3 $\pm$ 1.9	21.8 $\pm$ 1.6
<b>2d</b>	17.2 $\pm$ 1.4	22.1 $\pm$ 1.9	17.4 $\pm$ 2.1	33.2 $\pm$ 3.3
<b>2e</b>	11.5 $\pm$ 1.1	21.4 $\pm$ 2.3	14.4 $\pm$ 1.3	31.4 $\pm$ 3.0
<b>2f</b>	21.5 $\pm$ 2.4	17.3 $\pm$ 2.3	32.8 $\pm$ 3.1	22 $\pm$ 1.7
<b>2g</b>	8.4 $\pm$ 0.8	13.7 $\pm$ 1.2	14.2 $\pm$ 1.1	7.5 $\pm$ 1.3
<b>2h</b>	28.6 $\pm$ 2.5	17.5 $\pm$ 1.8	31.3 $\pm$ 3.2	22.3 $\pm$ 2.1
<b>2i</b>	19.7 $\pm$ 2.0	12.6 $\pm$ 1.4	8.3 $\pm$ 0.7	12.6 $\pm$ 1.5
<b>2j</b>	14.5 $\pm$ 1.8	17.3 $\pm$ 2.3	8.9 $\pm$ 0.8	11.2 $\pm$ 2.3
<b>3a</b>	4.5 $\pm$ 0.6	2.5 $\pm$ 0.4	2.7 $\pm$ 0.2	3.3 $\pm$ 0.3
<b>3b</b>	9.5 $\pm$ 0.6	14.3 $\pm$ 1.5	9.9 $\pm$ 0.7	10.4 $\pm$ 2.0
<b>3c</b>	10.5 $\pm$ 1.1	7.3 $\pm$ 0.5	9.3 $\pm$ 0.7	7.8 $\pm$ 0.7
<b>3d</b>	13.3 $\pm$ 1.6	19.6 $\pm$ 2.1	8.5 $\pm$ 1.3	24.5 $\pm$ 2.3
<b>3e</b>	10.4 $\pm$ 1.3	18.5 $\pm$ 1.7	8.7 $\pm$ 0.7	19.7 $\pm$ 1.9
<b>3f</b>	16.3 $\pm$ 1.4	10.8 $\pm$ 0.8	12.4 $\pm$ 0.4	18.4 $\pm$ 1.7
<b>3g</b>	3.5 $\pm$ 1.8	2.7 $\pm$ 1.8	4.2 $\pm$ 1.8	5.8 $\pm$ 1.8
<b>3h</b>	16.4 $\pm$ 2.2	14.4 $\pm$ 1.6	19.6 $\pm$ 2.4	11.6 $\pm$ 0.7
<b>3i</b>	9.5 $\pm$ 1.1	4.8 $\pm$ 0.3	6.5 $\pm$ 1.8	8.8 $\pm$ 1.3
<b>3j</b>	11.3 $\pm$ 1.5	8.2 $\pm$ 0.7	7.7 $\pm$ 0.6	10.4 $\pm$ 0.9
<b>5-Fu<sup>c</sup></b>	7.8 $\pm$ 3.1	15.4 $\pm$ 1.8	12.3 $\pm$ 2.2	9.5 $\pm$ 1.1

4 <sup>a</sup> IC<sub>50</sub> values ( $\pm$ SD) of % cell viability determined by the MTT assay of three  
5 repetitions

6 <sup>b</sup> Patent NSAIDs

7 <sup>c</sup> Standard benchmark compound.

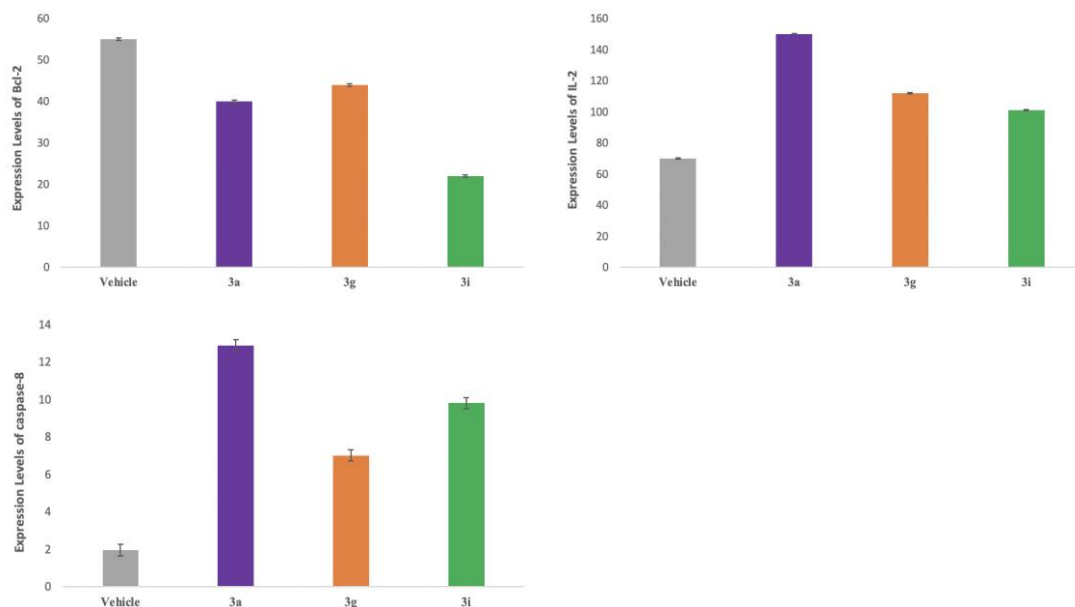
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9 2.3. Detection of Bcl-2, IL-2 and caspase-8 protein expression levels in BGC-823  
10 cells.

11 In order to further understand the possibly addressed signaling pathways and  
12 obtain hints on the mode(s) of action of the synthesized compounds, we selected the  
13 most promising derivatives **3a**, **3g** and **3i** and investigated their ability to induce

1 apoptosis in BGC-823 cells via modulation the expression of anti-apoptotic Bcl-2  
2 protein, pro-inflammatory cytokines (IL-2) and proapoptotic caspase-8 protein.

3 As shown in **Fig 3**, all the three compounds were able to downregulate the  
4 expression of Bcl-2 and upregulate the expression of IL-2 and Caspase-3 in BGC-823  
5 cells compared with untreated cells. Interestingly, compound **1g** downregulate over  
6 50% the expression levels of Bcl-2 compared to untreated cells. Further more,  
7 compounds **1g** and **1h** modulate the Caspase-8 level at most 1.5 fold increase in  
8 expression when compared to the untreated control cells. From these results, it's likely  
9 that organic selenocyanates may induced apoptosis to inhibit tumor cells growth, and  
10 in line with the first selenocyanate (1,4-phenylenebis(methylene)selenocyanate)  
11 which proved to be effective against prostate and oral carcinoma cells [32, 33].



12  
13  
14 **Fig. 3.** Protein expression levels of Bcl-2, IL-2 and caspase-8 in BGC-823 cells after  
15 48 h incubation with compounds **3a**, **3g** and **3i** at their respective  $IC_{50}$ s compared to  
16 untreated cells.

#### 17 18 2.4. Antioxidant assay

19 Reactive oxygen species (ROS) is a broad term that encompasses both oxygen  
20 free radicals, which have unpaired electrons, such as superoxide, hydroxyl and  
21 peroxy as well as oxidizing agents that are not free radicals such as hydrogen  
22 peroxide, hypochlorous acid and ozone [34]. ROS play essential roles in altering

1 protein structure, thereby changing its function and participate in many pathological  
2 processes [35, 36]. Various human diseases, including different types of cancer, are  
3 associated with a disturbed intracellular redox balance and oxidative stress (OS) [37,  
4 38].

5 Owing to the fact that a number of synthetic organoselenium compounds have  
6 been synthesized for their use as redox-modulators in the last few years [39-41], the  
7 antioxidant activity of compounds (**3a**, **3g**, **3i**) are further estimated employing  
8 different biochemical assays such as DPPH, bleomycin-dependent DNA damage and  
9 Gpx-like assays [42, 43].

10

#### 11 2.4.1. Radical scavenging capacity (DPPH) assay.

12 There are various methods which have been developed to provide fast prediction  
13 of antioxidant of natural compounds [44], however, the DPPH chemical assay is  
14 considered to be the rapid tools to evaluate the radical-scavenging activities of  
15 nutritional products and organic selenides [45]. The antioxidant activity of a  
16 compound is assessed by its ability to decolorize DPPH radical (purple color in  
17 methanol) to DPPHH (colorless) and the corresponding radical-scavenging activity is  
18 estimated by the decrease in the absorbance at 517 nm [46]. Vitamin C was used as a  
19 positive control (**Table 2**). Antioxidant activity was calculated as follows:

20  $\% \text{ Antioxidant activity} = [(\text{control absorbance} - \text{sample absorbance}) / \text{control}$   
21  $\text{absorbance}] \times 100\%$

22 As depicted in **Table 2**, NSAIDs-SeCF<sub>3</sub> derivatives **3h** and **3i** were the most  
23 active compounds in this assay, demonstrating a good free-radical scavenging activity  
24 compared to Vitamin C. The family of NSAIDs-SeCF<sub>3</sub> derivatives is better than the  
25 corresponding NSAIDs-SeCN derivatives on this assay except for the compare of **2d**  
26 and **3d**.

27

#### 28 2.4.2. Bleomycin DNA damage assay.

29 Bleomycin (BLM) is a complex of related glycopeptide from *Streptomyces*  
30 *verticillus*, it inhibits DNA metabolism and is used as an antineoplastic, especially for

1 solid tumors [47]. The bleomycin-iron DNA damage assay has been routinely used as  
 2 a preliminary method to test potential of drugs and organic selenium compound [48,  
 3 49]. As shown in **Table 2**, compounds **3a**, **3g** and **3i** induced DNA degradation  
 4 significantly more than other tested compounds.

5

6 **Table 2**

7 Redox modulation activity of NSAID-Se hybrid compounds.

Compd. No.	DPPH assay		Bleomycin-dependent DNA damage assay
	Inhibition %	Fold	Absorbance
<b>Vitamin C</b>	96.4±1.3	1	297±2.83
<b>2a</b>	17.2±1.4	0.2	86.5±0.54
<b>2b</b>	31.2±2.8	0.3	60.3±0.43
<b>2c</b>	44.3±36	0.4	72.4±0.33
<b>2d</b>	29.6±2.7	0.3	95.6±1.82
<b>2e</b>	30.4±1.4	0.3	69.4±0.42
<b>2f</b>	24.6±1.3	0.2	81.6±0.48
<b>2g</b>	51.5±1.2	0.5	76.1±0.39
<b>2h</b>	45.7±4.3	0.5	91.3±1.63
<b>2i</b>	57.1±4.3	0.6	67.6±1.83
<b>2j</b>	27.3±3.1	0.3	78.3±1.17
<b>3a</b>	73.5±4.1	0.8	119.4±1.78
<b>3b</b>	48.5±2.8	0.5	95.7±2.27
<b>3c</b>	36.6±2.2	0.4	62.6±1.18
<b>3d</b>	23.3±1.2	0.3	77.6±1.40
<b>3e</b>	41.4±2.2	0.4	86.4±1.21
<b>3f</b>	37.0±1.0	0.4	91.4±1.13
<b>3g</b>	68.6±2.6	0.7	114.8±2.32
<b>3h</b>	44.9±2.3	0.5	73.7±1.12

<b>3i</b>	66.3±2.6	0.7	128.4±1.38
<b>3j</b>	32.4±1.8	0.4	88.7±1.32

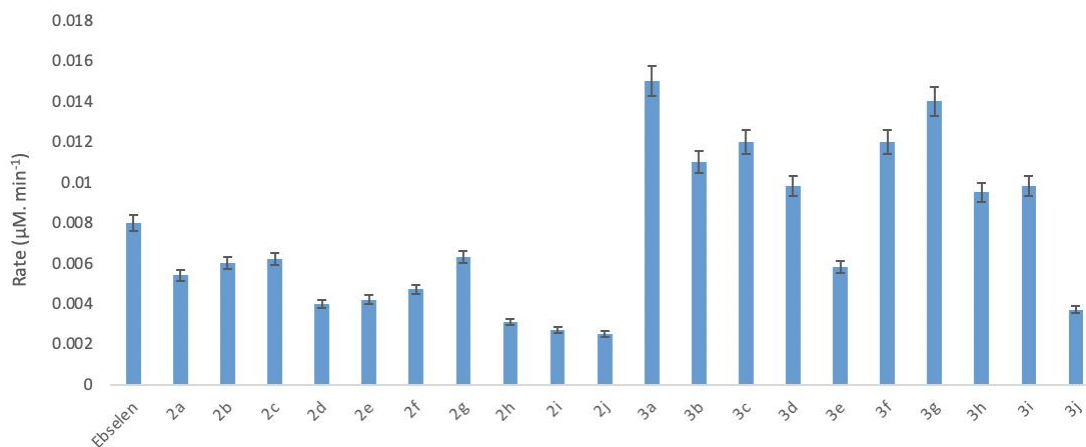
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### 2 2.4.3. Glutathione peroxidase-like activity assay.

3 Glutathione peroxidase (GPx) is a selenoenzyme that protects cells by catalyzing  
4 the reduction of peroxides with the stoichiometric reductant glutathione (GSH) [50,  
5 51]. The potential antioxidant activity of all of the NSAIDs-Se derivatives were  
6 estimated using NADPH-reductase coupled assay [52]. The GPx activity of the  
7 synthesized compounds was estimated by the decrease in absorbance (340 nm) due to  
8 the oxidation of NADPH to NADP<sup>+</sup>. Ebselen was used as the positive control.

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

12



13

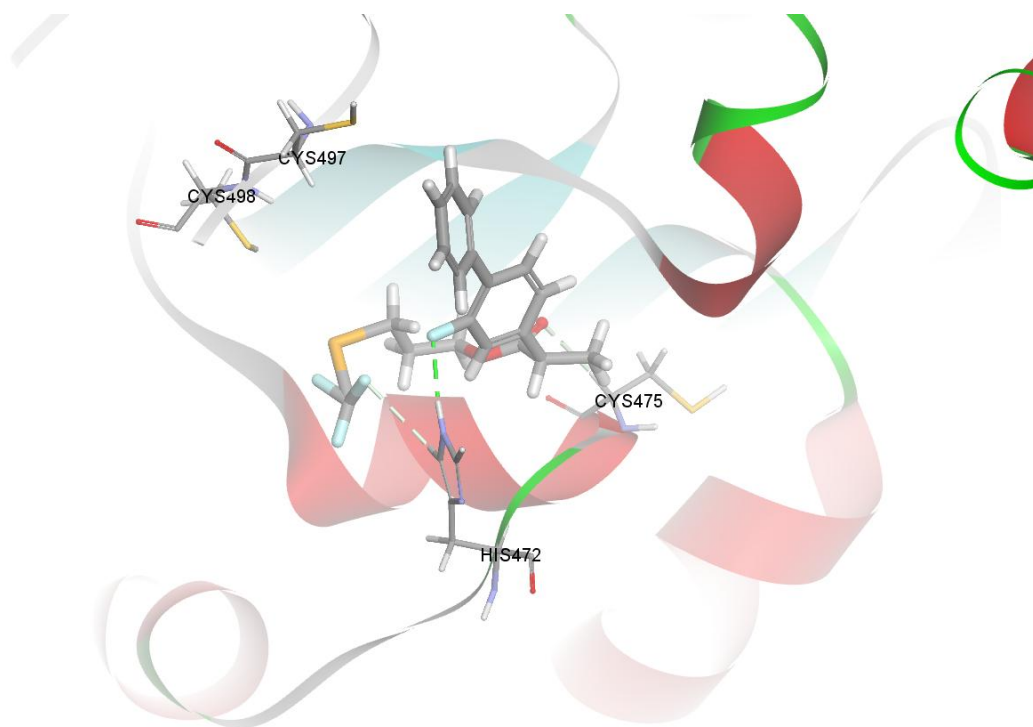
14 **Fig. 4.** GPx-like activity assay of NSAID-Se hybrid compounds in µM. Min<sup>-1</sup>.

15

### 16 2.5. Docking Studies

17 The interaction mode between our organoselenium compounds and Mammalian  
18 TrxR1 protein, which is closely related to the anticancer activity of compounds, need  
19 to be further explained by docking studies. TrxR1 consists of four monimers which  
20 have the FAD and NAD binding domains at the N-terminal and the dimerization  
21 interface domain at the flexible C-terminal side [53-55]. In the insufficiency of human

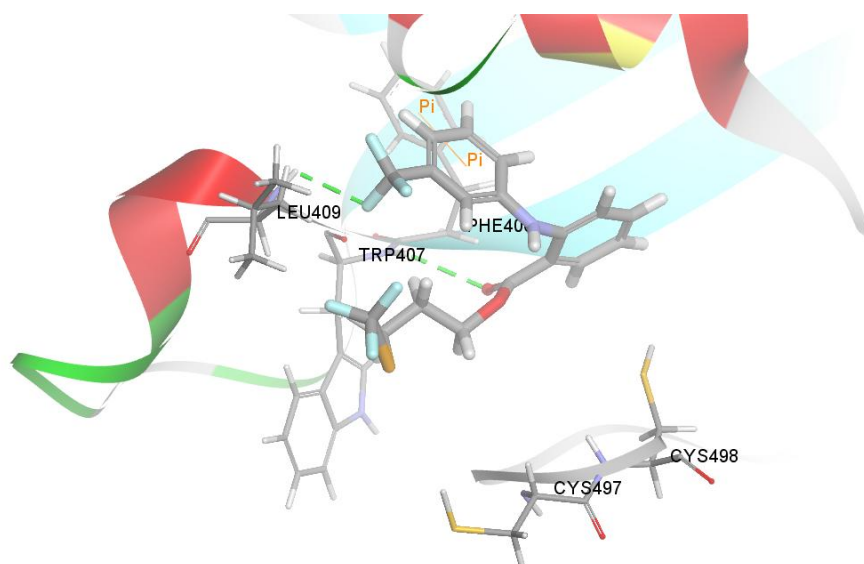
1 3D structure complexes cocrystallized of human TrxR1 with inhibitors, flexible  
2 docking was considered to be a practical method according to the literature [56]. With  
3 good antioxidant activity, compounds **3a**, **3g** and **3i** were docked into the TrxR1  
4 protein (PDB id: 1H6V) using Flexible Docking Protocol as reported in the literature  
5 [56]. All three compounds showed acceptable docking results (**Table 3-5 are reported**  
6 **in the supporting information**). It is thought that the distance between the selenium  
7 atom and Cys497/Cys498 is closely related to the accessibility of cysteine thiol  
8 attacking the selenide. Therefore, for each structure, the selection of the best pose of  
9 the docking results is related to the value of binding energy, while the distance would  
10 also be focused. Among the three compounds, Pose 3 of **3a** showed a better docking  
11 results with the relatively good value of -CDOCKER energy (30.184 kcal/mol).  
12 Meanwhile, the distance between the selenium atom and Cys498 was only 4.388 Å  
13 (**Table 3**, Pose 3). This good result may be related to the key hydrogen bond  
14 interaction between the Fluorine on benzene group and His472 (2.11 Å). In addition,  
15 **3a** also formed two hydrogen bonds, which are the hydrogen bond between -SeCF<sub>3</sub>  
16 group and His472 (2.97 Å) and the hydrogen bond between the oxygens of ester  
17 groups and Cys475 (2.62 Å) (**Figure 5**). For compound **3g**, the interactions shown in  
18 pose 3 are not only the hydrogen bonds, but also a  $\pi$ - $\pi$  stacking between the benzene  
19 ring and Phe406 (**Figure 6**). However, the distance between the selenium atom and  
20 Cys497/Cys498 is far than **3a** (**Table 4**). For **3i**, although there are multiple hydrogen  
21 bonds near the carbonyl group, the long distance between the selenium atom and  
22 Cys497/Cys498 may be related to the long linear structure of the whole compound  
23 (**Figure 7**, **Table 5**). This structure makes it difficult for molecule to penetrate into the  
24 pocket as a whole, thus affecting the interaction between molecule and protein.



1

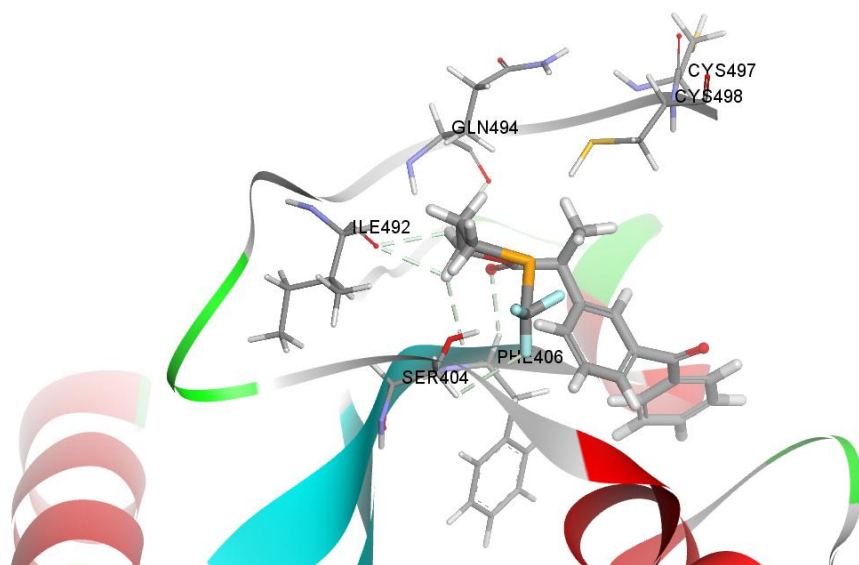
2 **Fig. 5.** The pose 3 of **3a**. Three interactions are shown: hydrogen bonding between the  
 3 Fluorine on benzene group and His472 (2.11 Å); hydrogen bonding between the  
 4 Fluorine of -SeCF<sub>3</sub> group and His472 (2.97 Å) and hydrogen bonding between the  
 5 oxygens of ester groups and Cys475 (2.62 Å).

6



7

8 **Fig. 6.** The pose 3 of **3g**. Three interactions are shown: hydrogen bonding between the  
 9 Fluorine of -CF<sub>3</sub> group and LEU409 (2.57 Å); hydrogen bonding between the  
 10 oxygens of ester groups and Trp407 (2.78 Å) and  $\pi$ - $\pi$  stacking between the benzene  
 11 ring and Phe406.



1

2 **Fig. 7.** The pose 4 of **3i**. Three interactions are shown: hydrogen bonding between the  
 3 Fluorine of -SeCF<sub>3</sub> group and Ser404 (2.80 Å); hydrogen bonding between the two  
 4 hydrogens on carbonyl group  $\alpha$  position and Ile492 (2.54 Å, 2.64 Å) or Gln494 (2.90  
 5 Å); hydrogen bonding between the oxygens of ester groups and Phe406 (2.46 Å).

6

### 7 **3. Conclusions**

8 In summary, twenty new organoselenium compounds were synthesized and  
 9 characterized. Four human cell lines (Caco-2, BGC-823, MCF-7 and PC-3) were  
 10 selected to test anticancer activity of the compounds. Compound **3a** showed most  
 11 potent anticancer activity with IC<sub>50</sub> values below 5 $\mu$ m against four cancer cell lines.  
 12 Moreover, three compounds were selected to test their ability to induce apoptosis in  
 13 BGC-823 cells via modulation the expression of anti-apoptotic Bcl-2 protein,  
 14 pro-inflammatory cytokines (IL-2) and proapoptotic caspase-8 protein. Compounds  
 15 **3a**, **3g** and **3i** were able to downregulate the expression of Bcl-2 and upregulate the  
 16 expression of IL-2 and Caspase-8 in BGC-823 cells. Furthermore, most of the  
 17 organoselenium compounds exhibited moderate to good CPx-like activity compared  
 18 to ebselen. Finally, in flexible docking study performed into TrxR1 enzyme,  
 19 compound **3a** showed a promising binding energies and binding mode that the  
 20 distance between the selenium atom and Cys497/Cys498. At this point, compound **3a**  
 21 may act as TrxR inhibitors.

22



## 1 **4. Experimental section**

### 2 4.1. General methods

3 All chemical reagents for the synthesis of the compounds were purchased from  
4 Macklin (Shanghai, China) or TCI (Shanghai, China) and used without further  
5 purification unless stated otherwise. Thin-layer chromatography (TLC) was  
6 performed on aluminium pre-coated sheets (E. Merck Silica gel 60 F254). Melting  
7 points were recorded on an Electrothermal apparatus and are uncorrected. NMR  
8 spectra were recorded in CDCl<sub>3</sub> on a Bruker Avance 400 MHz (for <sup>1</sup>H), 100 MHz (for  
9 <sup>13</sup>C) and 376 MHz (for <sup>19</sup>F) spectrometer with 5 mm PABBO probe. The following  
10 abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t =  
11 triplet, q = quartet, and m = multiplet. Chemical shifts (δ) are reported in parts per  
12 million (ppm) downfield from TMS and the coupling constants (*J*) are expressed in  
13 Hertz (Hz). High-resolution MS were performed on a SCIEX, TripleTOF 5600+,  
14 operating in ionization mode.

### 15 16 4.2. Experimental procedures

#### 17 *4.2.1. Procedure for the synthesis of compound 1*

18 To a solution of 3-bromopropan-1-amine hydrobromide (3g, 13.7 mmol) in  
19 anhydrous acetonitrile (40 mL) was added KSeCN (1.97 g, 13.7mmol). The mixture  
20 was stirred at 80°C for 24 hours. Then the mixture was cooled to 25°C and filtered.  
21 The filter cake was washed with acetonitrile (5mL×2) and dried under vacuum to  
22 obtain the brown solid 3.1g (Yield = 91%). The isolated solid was used without  
23 purification for further reactions.

#### 24 25 *4.2.2. General procedure for the synthesis of compounds (2a-2j)*

26 To a solution of patent NSAIDs (1.0 eq) in DCM (5 mL) and DMF (5 mL) was  
27 added EDCI (1.2 eq.), HOBT (1.2 eq.) and TEA (3.0 eq.). The mixture was stirred at  
28 25°C for 30 minutes. Then 2-selenocyanatoethanamine hydrobromide (1.2 eq) or  
29 2-selenocyanatopropanamine hydrobromide (1.2 eq.) was added into the mixture. The  
30 mixture was stirred at 25°C for 16 hrs. TLC showed the reaction was complete. The

1 mixture was diluted with H<sub>2</sub>O (20 mL), the aqueous layer was extracted with DCM  
2 (15 mL×2), the combined organic layer was washed with brine (20 mL×2), dried over  
3 Na<sub>2</sub>SO<sub>4</sub>, filtered and the filtrate was concentrated under reduced pressure. The residue  
4 was purified by column chromatography on silica gel, eluting with dichloromethane  
5 /methanol solution to obtain the desire compound.

6

7 *4.2.2.1.3-selenocyanatopropyl 2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanoate (2a).*

8 Yield: 78 %. White solid. Mp: 103-105°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 1.54 (d, 3H,  
9 *J* = 8.00 Hz, -CH<sub>3</sub>), 2.20-2.23 (m, 2H, -CH<sub>2</sub>), 2.94-2.99 (m, 2H, -CH<sub>2</sub>), 3.76 (q, 1H, *J*  
10 = 8.00 Hz, -CH), 4.23-4.24 (m, 2H, -CH<sub>2</sub>), 7.09-7.15 (m, 2H, ArH), 7.37-7.43 (m, 4H,  
11 ArH), 7.46-7.54 (m, 2H, ArH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 18.2, 25.7, 29.8, 45.0,  
12 63.0, 101.1, 115.2 (d, *J* = 23.0 Hz), 123.5 (d, *J* = 3.0 Hz), 127.8, 127.9 (d, *J* = 14.0 Hz),  
13 129.0, 128.9 (d, *J* = 2.0 Hz), 130.9 (d, *J* = 4.0 Hz), 135.3 (d, *J* = 2.0 Hz), 141.4 (d, *J* =  
14 7.0Hz), 159.5 (d, *J* = 247.0 Hz), 173.8. HRMS calcd. For C<sub>19</sub>H<sub>18</sub>FNO<sub>2</sub>Se [M+Na]<sup>+</sup>:  
15 414.0385, found 414.0365 [M+Na]<sup>+</sup>.

16

17 *4.2.2.2. 3-selenocyanatopropyl 2-(4-isobutylphenyl)propanoate (2b).* Yield: 82 %.

18 White solid. Mp: 97-99°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.89 (d, 6H, *J* = 8.00Hz,  
19 2-CH<sub>3</sub>), 1.49 (d, 3H, *J* = 8.00Hz, -CH<sub>3</sub>), 1.84 (q, 1H, *J* = 8.00Hz, -CH), 2.14-2.17 (m,  
20 2H, -CH<sub>2</sub>), 2.45 (d, 2H, *J* = 8.00Hz, -CH<sub>2</sub>), 2.78-2.88 (m, 2H, -CH<sub>2</sub>), 3.69 (q, 1H, *J* =  
21 8.00 Hz, -CH), 4.12-4.27 (m, 2H, -CH<sub>2</sub>), 7.10 (d, 2H, ArH), 7.18 (d, 2H, ArH). <sup>13</sup>C  
22 NMR (100 MHz, CDCl<sub>3</sub>): δ 18.1, 22.4, 25.7, 29.7, 30.2, 45.0, 45.1, 62.5, 127.1, 129.5,  
23 137.6, 140.9, 174.6. HRMS calcd. For C<sub>17</sub>H<sub>23</sub>NO<sub>2</sub>Se [M+Na]<sup>+</sup>: 376.0792, found  
24 376.0770 [M+Na]<sup>+</sup>.

25

26 *4.2.2.3.3-selenocyanatopropyl2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3*

27 *-yl)acetate (2c).* Yield: 78 %. White solid. Mp: 110-112°C. <sup>1</sup>H NMR (400 MHz,  
28 CDCl<sub>3</sub>): δ 2.19-2.25 (m, 2H, -CH<sub>2</sub>), 2.40 (s, 3H, -CH<sub>3</sub>), 2.92-3.00 (m, 2H, -CH<sub>2</sub>), 3.69  
29 (s, 2H, -CH<sub>2</sub>), 3.84 (s, 3H, -CH<sub>3</sub>), 4.24-4.26 (m, 2H, -CH<sub>2</sub>), 6.66 (d, 1H, *J* = 4.00 Hz,  
30 ArH), 6.86(d, 1H, *J* = 8.00 Hz, ArH), 6.93(s, 1H, ArH), 7.48 (d, 2H, *J* = 8.00 Hz,

1 ArH), 7.66 (d, 2H,  $J = 8.00\text{Hz}$ , ArH).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 13.4, 25.8, 29.7,  
2 30.4, 55.8, 63.1, 101.2, 101.4, 111.5, 112.2, 115.1, 129.2, 130.5, 130.8, 131.2, 133.7,  
3 136.1, 139.4, 156.0, 168.3, 170.7. HRMS calcd. For  $\text{C}_{23}\text{H}_{21}\text{ClN}_2\text{O}_4\text{Se}[\text{M}+\text{H}]^+$ :  
4 505.0433, found 505.0400  $[\text{M}+\text{H}]^+$ .

5

6 *4.2.2.4. 3-selenocyanatopropyl 2-((2,3-dimethylphenyl)amino)benzoate*

7 **(2d)**. Yield: 80 %. White solid. Mp: 90-92°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.17 (s,  
8 3H,  $-\text{CH}_3$ ), 2.33 (s, 3H,  $-\text{CH}_3$ ), 2.40-2.43 (m, 2H,  $-\text{CH}_2$ ), 3.21-3.24 (m, 2H,  $-\text{CH}_2$ ),  
9 4.46-4.49 (m, 2H,  $-\text{CH}_2$ ), 6.66 (t, 1H,  $J = 8.00\text{ Hz}$ , ArH), 6.74 (d, 1H,  $J = 8.00\text{Hz}$ ,  
10 ArH), 7.03 (d, 1H,  $J = 8.00\text{ Hz}$ , ArH), 7.11-7.15 (m, 2H, ArH), 7.26-7.27 (m, 1H,  
11 ArH), 7.91 (d, 1H,  $J = 8.00\text{ Hz}$ , ArH), 9.20 (s, 1H,  $-\text{NH}$ ).  $^{13}\text{C}$  NMR (100 MHz,  
12  $\text{CDCl}_3$ ):  $\delta$  14.0, 20.6, 26.1, 30.1, 62.5, 101.2, 110.1, 113.8, 116.1, 123.2, 126.0, 127.0,  
13 131.3, 132.6, 134.5, 138.3, 138.5, 149.8, 168.4. HRMS calcd. For  
14  $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_2\text{Se}[\text{M}+\text{H}]^+$ : 389.0768, found 389.0761  $[\text{M}+\text{H}]^+$ .

15

16 *4.2.2.5. 3-selenocyanatopropyl*

17 *2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate*

18 **(2e)**. Yield: 85%. White solid. Mp: 130-132°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.84  
19 (t, 3H,  $J = 8.00\text{Hz}$ ,  $-\text{CH}_3$ ), 1.37 (t, 3H,  $J = 8.00\text{Hz}$ ,  $-\text{CH}_3$ ), 1.63 (s, 2H,  $-\text{CH}_2$ ),  
20 1.94-2.22 (m, 4H, 2- $\text{CH}_2$ ), 2.71-3.04 (m, 8H, 4 $\times$ - $\text{CH}_2$ ), 3.93-4.06 (m, 2H,  $-\text{CH}_2$ ),  
21 4.18-4.30 (m, 2H,  $-\text{CH}_2$ ), 7.01-7.09 (m, 2H, ArH), 7.36 (d, 1H,  $J = 8.00\text{Hz}$ , ArH),  
22 8.78 (s, 1H,  $-\text{NH}$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.7, 13.8, 22.3, 24.2, 25.6, 29.7,  
23 31.0, 43.1, 60.7, 63.0, 74.7, 101.2, 108.7, 116.0, 119.8, 120.6, 126.2, 126.6, 134.5,  
24 135.5, 172.3. HRMS calcd. For  $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_3\text{Se}[\text{M}+\text{H}]^+$ : 435.1187, found 435.1165  
25  $[\text{M}+\text{H}]^+$ .

26

27 *4.2.2.6. 3-selenocyanatopropyl 2-(6-methoxynaphthalen-2-yl)propanoate*

28 **(2f)**. Yield: 78%. White solid. Mp: 88-90°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.58 (d,  
29 3H,  $J = 8.00\text{Hz}$ ,  $-\text{CH}_3$ ), 2.11-2.18 (m, 2H,  $-\text{CH}_2$ ), 2.76-2.90 (m, 2H,  $-\text{CH}_2$ ), 3.83-3.88  
30 (m, 2H,  $-\text{CH}_2$ ), 3.92(s, 3H,  $-\text{OCH}_3$ ), 4.15-4.26 (m, 1H,  $-\text{CH}$ ), 7.15 (t, 1H,  $J = 8.00\text{Hz}$ ,  
31 ArH), 7.37 (d, 1H,  $J = 8.00\text{Hz}$ , ArH), 7.65 (s, 1H, ArH), 7.71(d, 2H,  $J = 8.00\text{Hz}$ , ArH).

1  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.2, 25.7, 29.7, 45.2, 55.4, 62.7, 101.3, 105.6, 119.3,  
2 126.0, 126.1, 127.3, 128.9, 129.2, 133.7, 135.4, 157.8, 174.5. HRMS calcd. For  
3  $\text{C}_{18}\text{H}_{19}\text{NO}_3\text{Se}[\text{M}+\text{H}]^+$ : 378.0608, found 378.0596  $[\text{M}+\text{H}]^+$ .

4  
5

6 4.2.2.7. *3-selenocyanatopropyl 2-((3-(trifluoromethyl)phenyl)amino)benzoate*  
7 (**2g**). Yield: 77%. White solid. Mp: 121-123°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$   
8 2.38-2.45 (m, 2H,  $-\text{CH}_2$ ), 3.19-3.23 (m, 2H,  $-\text{CH}_2$ ), 4.46-4.49 (m, 2H,  $-\text{CH}_2$ ), 6.82 (t,  
9 1H,  $J = 8.00\text{Hz}$ , ArH), 7.28-7.49 (m, 6H, ArH), 7.96 (d, 1H,  $J = 1.0\text{Hz}$ , ArH), 9.54 (s,  
10 1H,  $-\text{NH}$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  26.0, 30.1, 62.9, 101.1, 112.3, 114.3, 118.2  
11 (q,  $J = 4.0$  Hz), 118.3, 119.8 (q,  $J = 4.0$  Hz), 123.9 (q,  $J = 271.0$  Hz), 124.9, 130.0,  
12 131.6, 131.9 (q,  $J = 32.0$  Hz), 134.7, 141.4, 147.1, 168.1. HRMS calcd. For  
13  $\text{C}_{18}\text{H}_{15}\text{F}_3\text{N}_2\text{O}_2\text{Se}[\text{M}+\text{H}]^+$ : 429.0329, found 429.0318  $[\text{M}+\text{H}]^+$ .

14  
15

16 4.2.2.8. *3-selenocyanatopropyl*  
17 *(Z)-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate*  
18 (**2h**). Yield: 82%. White solid. Mp: 91-93°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$   
19 2.21-2.26 (m, 2H,  $-\text{CH}_2$ ), 2.22 (s, 3H,  $-\text{CH}_3$ ), 2.82 (s, 3H,  $-\text{CH}_3$ ), 3.00 (t, 2H,  $J = 8.00$   
20 Hz,  $-\text{CH}_2$ ), 4.26 (t, 2H,  $J = 8.00$  Hz,  $-\text{CH}_2$ ), 6.56-6.61(m, 1H, ArH), 6.87 (d, 1H,  $J =$   
21 8.00 Hz, ArH), 7.15-7.18 (m, 2H, ArH), 7.67(d, 2H,  $J = 8.00$  Hz, ArH), 7.72 (d, 2H,  $J$   
22 = 8.00 Hz, ArH).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  10.7, 25.7, 29.7, 31.8, 43.9, 63.2,  
23 101.2, 105.9 (d,  $J = 24$  Hz), 110.9 (d,  $J = 23$  Hz), 123.8, 123.9, 128.6 (d,  $J = 2.0$  Hz),  
24 129.5 (d,  $J = 3.0$  Hz), 130.3, 131.4 (d,  $J = 3.0$  Hz), 138.4, 139.5, 141.5, 145.5, 146.5  
25 (d,  $J = 9.0$  Hz), 163.3 (d,  $J = 245.0$  Hz), 170.1. HRMS calcd. For  
26  $\text{C}_{24}\text{H}_{22}\text{FNO}_3\text{SSe}[\text{M}+\text{H}]^+$ : 504.0548, found 504.0528  $[\text{M}+\text{H}]^+$ .

27  
28

29 4.2.2.9. *3-selenocyanatopropyl 2-(3-benzoylphenyl)propanoate*  
30 (**2i**). Yield: 85%. White solid. Mp: 96-98°C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  1.55 (d,  
31 3H,  $J = 8.00\text{Hz}$ ,  $-\text{CH}_3$ ), 2.18-2.21 (m, 2H,  $-\text{CH}_2$ ), 2.94-2.98 (m, 2H,  $-\text{CH}_2$ ), 3.82 (q,  
32 1H,  $J = 8.00\text{Hz}$ ,  $-\text{CH}$ ), 4.21-4.25 (m, 2H,  $-\text{CH}_2$ ), 7.43-7.54 (m, 4H, ArH), 7.61(t, 1H,  $J$   
33 = 8.00 Hz, ArH), 7.67 (d, 1H,  $J = 8.00$  Hz, ArH), 7.76(s, 1H, ArH), 7.80 (d, 2H,  $J =$   
8.00 Hz, ArH).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  18.3, 25.7, 29.7, 45.4, 63.0, 101.2,

1 128.4, 128.6, 129.0, 129.2, 130.1, 131.4, 132.7, 137.4, 138.1, 140.7, 173.8, 196.4.  
2 HRMS calcd. For  $C_{20}H_{19}NO_3Se[M+H]^+$ : 402.0608, found 402.0588  $[M+H]^+$ .

3

4 *4.2.2.10. 3-selenocyanatopropyl 2-acetoxybenzoate (2j)*. Yield: 90%. White solid. Mp:  
5 117-118°C.  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  2.38 (s, 3H,  $-CH_3$ ), 2.33-2.40 (m, 2H,  
6  $-CH_2$ ), 3.11-3.17 (m, 2H,  $-CH_2$ ), 4.44-4.46 (m, 2H,  $-CH_2$ ), 7.12 (d, 1H,  $J = 8.00$ Hz,  
7 ArH), 7.33 (t, 1H,  $J = 8.00$ Hz, ArH), 7.58 (t, 1H,  $J = 8.00$ Hz, ArH), 7.99 (d, 1H,  $J =$   
8 8.00Hz, ArH).  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  21.1, 25.8, 30.0, 63.1, 101.3, 122.8,  
9 123.9, 126.1, 131.5, 134.3, 150.7, 164.3, 169.8. HRMS calcd. For  
10  $C_{24}H_{23}FN_2O_2SSe[M+Na]^+$ : 349.9908, found 349.9896  $[M+Na]^+$ .

11

#### 12 *4.2.3. General procedure for the synthesis of compounds 3a-3j*

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

17

18 *4.2.3.1.3-((trifluoromethyl)selanyl)propyl 2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanoate*  
19 (**3a**). Yield: 80 %. White solid. Mp: 113-115°C.  $^1H$  NMR (400 MHz,  $CDCl_3$ ):  $\delta$  1.54  
20 (d, 3H,  $J = 8.00$  Hz,  $-CH_3$ ), 2.09-2.12 (m, 2H,  $-CH_2$ ), 2.90-2.93 (m, 2H,  $-CH_2$ ), 3.75 (q,  
21 1H,  $J = 8.00$  Hz,  $-CH$ ), 4.20-4.22 (m, 2H,  $-CH_2$ ), 7.13 (t, 2H,  $J = 8.00$  Hz, ArH),  
22 7.37-7.39 (m, 2H, ArH), 7.44 (t, 2H,  $J = 8.00$  Hz, ArH), 7.53(d, 2H,  $J = 8.00$  Hz,  
23 ArH).  $^{13}C$  NMR (100 MHz,  $CDCl_3$ ):  $\delta$  18.2, 21.8, 29.4, 45.0, 63.6, 115.2 (d,  $J = 24.0$   
24 Hz), 122.5 (q,  $J = 329.0$  Hz,  $-SeCF_3$ ), 123.4 (d,  $J = 3.0$  Hz), 127.7, 128.0 (d,  $J = 13.0$   
25 Hz), 128.5, 128.9 (d,  $J = 5.0$  Hz), 130.9 (d,  $J = 4.0$  Hz), 135.4, 141.5 (d,  $J = 7.0$ Hz),  
26 159.7 (d,  $J = 247.0$  Hz), 173.8.  $^{19}F$  NMR ( $CDCl_3$ , 376 MHz):  $\delta$  -34.3 (s,  $-SeCF_3$ ),  
27 -117.5 (s, F). HRMS calcd. For  $C_{19}H_{18}F_4O_2Se [M+H]^+$ : 435.0486, found 435.0462  
28  $[M+H]^+$ .

29

1 4.2.3.2. 3-((trifluoromethyl)selanyl)propyl 2-(4-isobutylphenyl)propanoate (**3b**).  
2 Yield: 82%. Yellow solid. Mp: 102-104°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.89 (d,  
3 6H, *J* = 8.00 Hz, 2-CH<sub>3</sub>), 1.49 (d, 3H, *J* = 8.00Hz, -CH<sub>3</sub>), 1.84 (q, 1H, *J* = 8.00Hz,  
4 -CH), 2.04-2.07 (m, 2H, -CH<sub>2</sub>), 2.44 (d, 2H, *J* = 8.00Hz, -CH<sub>2</sub>), 2.78-2.83 (m, 2H,  
5 -CH<sub>2</sub>), 3.68 (q, 1H, *J* = 8.00Hz, -CH), 4.12-4.21 (m, 2H, -CH<sub>2</sub>), 7.09 (d, 2H, ArH),  
6 7.18 (d, 2H, ArH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 18.2, 21.8, 22.4, 29.4, 30.2, 45.0,  
7 45.1, 63.1, 122.5 (q, *J* = 328.0 Hz, -SeCF<sub>3</sub>), 127.1, 129.4, 137.6, 140.7, 174.6. <sup>19</sup>F  
8 NMR (CDCl<sub>3</sub>, 376 MHz): δ -34.3 (s, -SeCF<sub>3</sub>). HRMS calcd. For C<sub>17</sub>H<sub>23</sub>F<sub>3</sub>O<sub>2</sub>Se  
9 [M+H]<sup>+</sup>: 397.0893, found 397.0883 [M+H]<sup>+</sup>.

10

11 4.2.3.3. 3-((trifluoromethyl)selanyl)propyl  
12 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetate (**3c**). Yield: 80%.  
13 White solid. Mp: 131-133°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 2.09-2.15 (m, 2H, -CH<sub>2</sub>),  
14 2.40 (s, 3H, -CH<sub>3</sub>), 2.91-2.95 (m, 2H, -CH<sub>2</sub>), 3.68 (s, 2H, -CH<sub>2</sub>), 3.84 (s, 3H, -CH<sub>3</sub>),  
15 4.20-4.23 (m, 2H, -CH<sub>2</sub>), 6.67 (d, 1H, *J* = 4.00 Hz, ArH), 6.85 (d, 1H, *J* = 8.00Hz,  
16 ArH), 6.94 (s, 1H, ArH), 7.48 (d, 2H, *J* = 8.00 Hz, ArH), 7.66 (d, 2H, *J* = 8.00Hz,  
17 ArH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 13.3, 21.9, 29.4, 30.3, 55.7, 63.7, 101.3, 111.6,  
18 112.3, 115.0, 122.5 (q, *J* = 329 Hz, -SeCF<sub>3</sub>), 129.2, 130.5, 130.8, 131.2, 133.9, 136.0,  
19 139.3, 156.1, 168.3, 170.7. <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz): δ -34.3 (s, -SeCF<sub>3</sub>). HRMS  
20 calcd. For C<sub>23</sub>H<sub>21</sub>ClF<sub>3</sub>NO<sub>4</sub>Se [M+H]<sup>+</sup>: 548.0354, found 508.0305 [M+H]<sup>+</sup>.

21

22 4.2.3.4. 3-((trifluoromethyl)selanyl)propyl 2-((2,3-dimethylphenyl)amino)benzoate  
23 (**3d**). Yield: 82%. White solid. Mp: 116-118°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 2.18  
24 (s, 3H, -CH<sub>3</sub>), 2.28-2.31 (m, 2H, -CH<sub>2</sub>), 2.33 (s, 3H, -CH<sub>3</sub>), 3.13-3.17 (m, 2H, -CH<sub>2</sub>),  
25 4.41-4.44 (m, 2H, -CH<sub>2</sub>), 6.67 (t, 1H, *J* = 8.00 Hz, ArH), 6.75 (d, 1H, *J* = 8.00 Hz,  
26 ArH), 7.02 (d, 1H, *J* = 8.00 Hz, ArH), 7.10-7.15 (m, 2H, ArH), 7.23-7.27 (m, 1H,  
27 ArH), 7.93 (d, 1H, *J* = 8.00 Hz, ArH), 9.23 (s, 1H, -NH). <sup>13</sup>C NMR (100 MHz,  
28 CDCl<sub>3</sub>): δ 14.0, 20.7, 22.2, 29.7, 63.1, 110.4, 113.8, 116.1, 122.6 (q, *J* = 328.0 Hz,  
29 -SeCF<sub>3</sub>), 123.2, 126.0, 126.9, 131.3, 132.5, 134.4, 138.3, 138.6, 149.7, 168.5. <sup>19</sup>F

1 NMR (CDCl<sub>3</sub>, 376 MHz):  $\delta$  -34.2 (s, -SeCF<sub>3</sub>). HRMS calcd. For C<sub>19</sub>H<sub>20</sub>F<sub>3</sub>NO<sub>2</sub>Se  
2 [M+H]<sup>+</sup>: 432.0611, found 432.0675 [M+H]<sup>+</sup>.

3

4 4.2.3.5. *3-((trifluoromethyl)selanyl)propyl*

5 *2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate (3e)*. Yield: 82%.

6 White solid. Mp: 127-129°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  0.84 (t, 3H, *J* = 8.00 Hz,  
7 -CH<sub>3</sub>), 1.37 (t, 3H, *J* = 8.00 Hz, -CH<sub>3</sub>), 1.99-2.16 (m, 4H, 2×-CH<sub>2</sub>), 2.75-3.04 (m, 8H,  
8 4-CH<sub>2</sub>), 3.93-4.06 (m, 2H, -CH<sub>2</sub>), 4.18-4.30 (m, 2H, -CH<sub>2</sub>), 7.01-7.07 (m, 2H, ArH),  
9 7.36 (d, 1H, *J* = 8.00 Hz, ArH), 8.94 (s, 1H, -NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$   
10 7.6, 13.8, 21.9, 22.4, 24.2, 29.3, 30.8, 43.0, 60.7, 63.6, 74.6, 108.6, 116.0, 119.7,  
11 120.5, 122.5 (q, *J* = 329 Hz, -SeCF<sub>3</sub>), 126.2, 126.6, 134.5, 135.7, 172.6. <sup>19</sup>F NMR  
12 (CDCl<sub>3</sub>, 376 MHz):  $\delta$  -34.2 (s, -SeCF<sub>3</sub>). HRMS calcd. For C<sub>21</sub>H<sub>26</sub>F<sub>3</sub>NO<sub>3</sub>Se [M+H]<sup>+</sup>:  
13 478.1108, found 478.1089 [M+H]<sup>+</sup>.

14

15 4.2.3.6. *3-((trifluoromethyl)selanyl)propyl 2-(6-methoxynaphthalen-2-yl)propanoate*

16 (**3f**). Yield: 85%. White solid. Mp: 125-127°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  1.58 (d,  
17 3H, *J* = 8.00Hz, -CH<sub>3</sub>), 2.03-2.07 (m, 2H, -CH<sub>2</sub>), 2.82-2.86 (m, 2H, -CH<sub>2</sub>), 3.87 (q,  
18 1H, *J* = 8.00 Hz, -CH), 3.91(s, 3H, -OCH<sub>3</sub>), 4.16-4.19 (m, 2H, -CH<sub>2</sub>), 7.12 (t, 1H, *J* =  
19 8.00Hz, ArH), 7.38 (d, 1H, *J* = 8.00Hz, ArH), 7.65 (s, 1H, ArH), 7.70 (d, 2H, *J* =  
20 8.00Hz, ArH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  18.3, 21.8, 29.4, 45.4, 55.3, 63.3,  
21 105.6, 119.1, 122.5 (d, *J* = 328.0 Hz, -SeCF<sub>3</sub>), 125.9, 126.1, 127.2, 128.9, 129.3,  
22 133.7, 135.5, 157.7, 174.6. <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz):  $\delta$  -34.3 (s, -SeCF<sub>3</sub>). HRMS  
23 calcd. For C<sub>18</sub>H<sub>19</sub>F<sub>3</sub>O<sub>3</sub>Se[M+Na]<sup>+</sup>: 443.0350, found 443.0337 [M+Na]<sup>+</sup>.

24

25 4.2.3.7. *3-((trifluoromethyl)selanyl)propyl*

26 *2-((3-(trifluoromethyl)phenyl)amino)benzoate (3g)*. Yield: 80%. White solid. Mp:

27 99-101°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.27-2.33 (m, 2H, -CH<sub>2</sub>), 3.12-3.16 (m, 2H,  
28 -CH<sub>2</sub>), 4.42-4.45 (m, 2H, -CH<sub>2</sub>), 6.83 (t, 1H, *J* = 8.00Hz, ArH), 7.27-7.49 (m, 6H,  
29 ArH), 7.97 (d, 1H, *J* = 1.0Hz, ArH), 9.58 (s, 1H, -NH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  
30  $\delta$  22.1, 29.6, 63.5, 112.5, 114.3, 118.2 (q, *J* = 4.0 Hz), 118.3, 119.7 (q, *J* = 4.0 Hz),

1 122.6 (q,  $J = 328.0$  Hz, -SeCF<sub>3</sub>), 124.0 (q,  $J = 270$  Hz, -CF<sub>3</sub>), 124.7, 129.9, 131.6,  
2 131.8 (q,  $J = 32.0$  Hz), 134.5, 141.5, 147.0, 168.2. <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz):  $\delta$   
3 -34.2 (s, -SeCF<sub>3</sub>), -62.8(s, -CF<sub>3</sub>). HRMS calcd. For C<sub>18</sub>H<sub>15</sub>F<sub>6</sub>NO<sub>2</sub>Se [M+H]<sup>+</sup>:  
4 472.0250, found 472.0233 [M+H]<sup>+</sup>.

5  
6 4.2.3.8. *3-((trifluoromethyl)selanyl)propyl*  
7 *(Z)-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate*

8 **(3h)**. Yield: 80%. White solid. Mp: 116-118°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$   
9 2.10-2.13 (m, 2H, -CH<sub>2</sub>), 2.21 (s, 3H, -CH<sub>3</sub>), 2.82 (s, 3H, -CH<sub>3</sub>), 2.94 (t, 2H,  $J = 8.00$   
10 Hz, -CH<sub>2</sub>), 3.58 (s, 2H, -CH<sub>2</sub>), 4.22 (t, 2H,  $J = 8.00$  Hz, -CH<sub>2</sub>), 6.55-6.60(m, 1H, ArH),  
11 6.87 (d, 1H,  $J = 8.00$  Hz, ArH), 7.14-7.18 (m, 2H, ArH), 7.67 (d, 2H,  $J = 8.00$  Hz,  
12 ArH), 7.72 (d, 2H,  $J = 8.00$  Hz, ArH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  10.5, 21.8,  
13 29.3, 31.8, 43.9, 63.7, 105.9 (d,  $J = 23$  Hz), 110.4 (d,  $J = 23$  Hz), 122.5 (q,  $J = 328$  Hz,  
14 -SeCF<sub>3</sub>), 123.7 (d,  $J = 9.0$  Hz), 123.8, 128.4 (d,  $J = 2.0$  Hz), 129.5 (d,  $J = 3.0$ Hz),  
15 130.3, 131.5 (d,  $J = 2.0$ Hz), 138.3, 139.6, 141.6, 145.5, 146.5 (d,  $J = 9.0$  Hz), 163.3 (d,  
16  $J = 245$  Hz), 170.0. <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz):  $\delta$  -34.3 (s, -SeCF<sub>3</sub>), -112.8(s, -F).  
17 HRMS calcd. For C<sub>24</sub>H<sub>22</sub>F<sub>4</sub>O<sub>3</sub>SSe [M+Na]<sup>+</sup>: 569.0289, found 569.0263 [M+Na]<sup>+</sup>.

18  
19 4.2.3.9. *3-((trifluoromethyl)selanyl)propyl 2-(3-benzoylphenyl)propanoate (3i)*. Yield:  
20 85%. White solid. Mp: 87-89°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  1.55 (d, 3H,  $J =$   
21 8.00Hz, -CH<sub>3</sub>), 2.05-2.12(m, 2H, -CH<sub>2</sub>), 2.87-2.89 (m, 2H, -CH<sub>2</sub>), 3.82 (q, 1H,  $J =$   
22 8.00Hz, -CH), 4.19-4.21 (m, 2H, -CH<sub>2</sub>), 7.43-7.59 (m, 4H, ArH), 7.60 (t, 1H,  $J = 8.00$   
23 Hz, ArH), 7.67 (d, 1H,  $J = 8.00$  Hz, ArH), 7.76 (s, 1H, ArH), 7.79 (d, 2H,  $J = 8.00$  Hz,  
24 ArH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  18.3, 21.8, 29.4, 45.4, 63.5, 122.5 (q,  $J = 329.0$   
25 Hz, -SeCF<sub>3</sub>), 128.4, 128.6, 129.1, 129.2, 130.1, 131.4, 132.6, 137.4, 138.0, 140.7,  
26 174.0, 195.5. <sup>19</sup>F NMR (CDCl<sub>3</sub>, 376 MHz):  $\delta$  -34.2 (s, -SeCF<sub>3</sub>). HRMS calcd. For  
27 C<sub>20</sub>H<sub>19</sub>F<sub>3</sub>O<sub>3</sub>Se [M+H]<sup>+</sup>: 445.0530, found 445.0491 [M+H]<sup>+</sup>.

28  
29 4.2.3.10. *3-((trifluoromethyl)selanyl)propyl 2-acetoxybenzoate (3j)*. Yield: 80%.  
30 White solid. Mp: 104-106°C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  2.23-2.28 (m, 2H, -CH<sub>2</sub>),



1 2.35 (s, 3H, -CH<sub>3</sub>), 3.07-3.10 (m, 2H, -CH<sub>2</sub>), 4.38-4.41 (m, 2H, -CH<sub>2</sub>), 7.11 (d, 1H, *J*  
2 = 8.00 Hz, ArH), 7.30-7.57 (m, 1H, ArH), 7.58-7.60 (m, 1H, ArH), 7.99 (d, 1H, *J* =  
3 8.00 Hz, ArH). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 21.0, 22.0, 29.6, 63.7, 122.6 (q, *J* =  
4 328 Hz, -SeCF<sub>3</sub>), 123.0, 123.9, 131.6, 134.1, 150.8, 164.3, 169.7. <sup>19</sup>F NMR (CDCl<sub>3</sub>,  
5 376 MHz): δ -34.2 (s, -SeCF<sub>3</sub>). HRMS calcd. For C<sub>13</sub>H<sub>13</sub>F<sub>3</sub>O<sub>4</sub>Se [M+H]<sup>+</sup>: 392.9829,  
6 found 392.9827 [M+H]<sup>+</sup>.

7

### 8 4.3. Cell lines and culture conditions

9 Four human cancer cell lines Caco-2, BGC-823, MCF-7 and PC-3 cells were  
10 maintained in RPMI 1640 medium with 10% fetal bovine serum (FBS) and 100  
11 units/mL of penicillin and streptomycin (Thermo Fisher Scientific, shanghai, China)  
12 at 37 °C and 5% CO<sub>2</sub> in a humidified atmosphere. Cells were passaged at  
13 confluent densities, using a solution containing 0.05% trypsin and 0.5 mM EDTA.  
14 Human cancer cell lines Caco-2, BGC-823, MCF-7 and PC-3 used in this work were  
15 obtained from the American Type Culture Collection (ATCC, Manassas, VA).

16 All the tested NSAIDs-Se derivatives were evaluated in vitro for their antitumor  
17 activity against four cancer cell lines by  
18 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay  
19 according to the method as described before [57-58]. Exponentially growing cells  
20 were harvested and plated in 96-well plates at a concentration of 1×10<sup>4</sup> cells / well.  
21 After 24 h incubation at 37 °C under a humidified 5% CO<sub>2</sub> to allow cell attachment,  
22 the cells in the wells were respectively treated with target compounds at various  
23 concentrations for 24 h, 48 h and 72 h. The concentration of DMSO was always kept  
24 below 1.25%, which was found to be non-toxic to the cells. Three hours prior to  
25 experiment termination, MTT solution (20 μL of 5.0 mg/mL solution) was added to  
26 each well and incubated at 37°C. At the termination time point, the medium/MTT  
27 mixtures were removed, and the formazan crystals formed by the mitochondrial  
28 dehydrogenase activity of vital cells were dissolved in 100 μL of DMSO per well.  
29 The optical densities were measured at 570 nm using a 96-well multiscanner (Dynex  
30 Technologies, MRX Revelation; Chantilly, VA, USA).

#### 1 4.4. Detection of Bcl-2, IL-2 and caspase-8 protein expression levels

2 Bcl-2, IL-2 and capase-8 levels were evaluated in BGC-823 cells treated with the  
3 corresponding IC<sub>50</sub>s of each compound and incubated for 48 h and compared with  
4 their levels in control untreated BGC-823 cell line. The cells were harvested by  
5 applying trypsin and lysed by freezing with liquid nitrogen and then thawing with  
6 gentle mixing and the total proteins were isolated. Protein levels of the anti-apoptotic  
7 marker Bcl-2 were then measured using enzymelinked immunosorbent assay (ELISA)  
8 according to the manufacturers' instructions (Merck, USA). Enzyme-linked  
9 immunosorbent assay was used for quantitative detection of IL-2 and caspase-8  
10 (Platinum ELISA). The reaction product was detected at 450 nm using enzyme-linked  
11 immunosorbent assay (Platinum ELISA; Merck) according to the instructions of the  
12 manufacturer.

#### 14 4.5. DPPH free radical scavenging activity

15 DPPH free radical scavenging activity of corresponding compounds was  
16 measured according to the method as previous reported with little optimization [59].  
17 Briefly, 20 mL of test samples at different concentrations was mixed with 180 mL of  
18 or DPPH solution for 30 min in the dark. Then, the change in absorbance at 517 nm  
19 for DPPH was measured on a microplate reader. Ascorbic acid (vitamin C) and  
20 ebselen were used as a positive control, DMSO was used as a negative control.

#### 22 4.6. Bleomycin-dependent DNA damage

23 The reaction mixture contained DNA (0.5 mg/mL), bleomycin sulfate  
24 (0.05 mg/mL), MgCl<sub>2</sub> (5 mM), FeCl<sub>3</sub> (50 mM), and tested compound in a conc. of  
25 0.1 mg/mL. L-ascorbic acid was used as positive control. The mixture was incubated  
26 at 37°C for 1h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M).  
27 The color was developed by adding 0.5 mL TBA (1% w/v) and 0.5 mL HCl (25% v/v),  
28 followed by heating at 80°C for 30 minutes. After cooling in ice water, the extent of  
29 DNA damage was measured by increase in absorbance at 532 nm [60].

#### 1 4.7. Glutathione peroxidase-like activity

2 GPx kit (Biodiagnostic, Egypt) was used for the determination of GPx according  
3 to Paglia et al [61]. The reaction mixture contained 1ml assay buffer (50mM  
4 phosphate buffer containing 0.1% Triton X-100) and 0.1ml NADPH reagent (24  
5 mmol Glutathione, 12 unit Glutathione reductase and 4.8 mmol NADPH) and 0.01ml  
6 (41 mM) tested compounds and the reaction was started by the addition of H<sub>2</sub>O<sub>2</sub> (0.8  
7 mM). The contents were mixed well and the absorbances were recorded at 340 nm  
8 over a period of 3 min against deionized water. The change of absorbance per minute  
9 (A<sub>340 nm/min</sub>) was estimated using ebselen (41 mM) as positive control. The values  
10 represented in Fig 3 are expressed after background correction for the reaction with  
11 H<sub>2</sub>O<sub>2</sub> and GSH. In case of colored compounds, their activities were estimated after  
12 subtracting their own absorbances at the used wave length.

#### 14 4.8. Molecular Modeling

##### 15 4.8.1 Protein and Ligand Preparation

16 Prepared by Protein Preparation Wizard in Maestro 11.5 (Schrödinger, LLC,  
17 New York, NY, 2019.), the Mammalian TrxR1 protein (PDB ID: 1H6V) was obtained  
18 from Protein Data Bank. The other subunits were deleted and only one monomer F  
19 was retained. Next, subunits F was assigned in sequence, hydrogen was added,  
20 ionization and tautomerism were adjusted, hydrogen bond distribution was optimized,  
21 water was removed, and structure was minimized. The LigPrep utility in Maestro 11.5  
22 was used to perform ligand preparation applying OPLS2005 force field. Generation of  
23 tautomers and possible ionization states was mediated by Epik utility, followed by  
24 minimization of the resulting 3D conformations.

##### 26 4.8.2 Ligand Docking

27 The docking task was completed on Discovery Studio Client 3.1. and the binding  
28 site of TrxR1 was defined as a docking sphere with dimensions X: 27.757, Y: 6.510,  
29 Z: 33.698 and a radius of 15 Å. Before using Flexible Docking Protocol, TrxR1

1 protein was typed in CHARMM field force. 10 protein conformations were generated  
2 with a maximum alteration of 8 residues.

3 Under the conformation method FAST, every ligand were generated 25  
4 conformations with the value of 20 kcal in the energy threshold. With all other  
5 parameters as default, three ligands were docked into protein structure in the Flexible  
6 Docking Protocol. For each poses, the distance between the compound's selenium  
7 atom and the sulfur atom of either Cys497 or Cys498 was calculated by the distance  
8 monitor in the Discovery Studio. For each ligand, average -CDocker energy and  
9 average selenium-sulfur distance were calculated. The hydrogen bond interaction and  
10  $\pi$ - $\pi$  stacking between the compounds and protein were analyzed.

11

### 12 **Statistical analysis**

13 Data were given as mean  $\pm$  SD of three independent experiments, graphs and  
14 curve fitting were using origin Version 8.0 (OriginLab Corporation, Northampton,  
15 USA). P value less than 0.05 was considered statistically significant.

16

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21

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2 **References**

- 3 [1] S. Bacchi, P. Palumbo, A. Sponta, M. F. Coppolino, Clinical pharmacology of.  
4 non-steroidal anti-Inflammatory drugs: a review, *Antiinflamm Antiallergy Agents*  
5 *Med Chem.* 11(1) (2012) 52-64.
- 6 [2] R. A. Moore, S. Derry, P. J. Wiffen, S. Straube. Effects of food on  
7 pharmacokinetics of immediate release oral formulations of aspirin, dipyrene,  
8 paracetamol and NSAIDs - a systematic review, *Br. J. Clin. Pharmacol.* 80 (3)  
9 (2015) 381-388.
- 10 [3] K. Miura, W. Fujibuchi, K. Ishida, T. Naitoh, H. Ogawa, T. Ando, N. Yazaki, K.  
11 Watanabe, S. Haneda, C. Shibata, I. Sasaki, Inhibitor of apoptosis protein family as  
12 diagnostic markers and therapeutic targets of colorectal cancer, *Surg Today.* (41)  
13 2011, 175-182.
- 14 [4] A.P. Fernandes, V. Gandin, Selenium compounds as therapeutic agents in cancer,  
15 *Biochimica. et. Biophysica. Acta.* 1850 (2015) 1642-1660.
- 16 [5] M. R. Smith, J. Manola, D.S. Kaufman, W.K. Oh, G.J. Bubley, P.W. Kantoff,  
17 Celecoxib versus placebo for men with prostate cancer and a rising serum  
18 prostate-specific antigen after radical prostatectomy and/or radiation therapy, *J.*  
19 *Clin. Oncol.* 24 (2006) 2723–2728.
- 20 [6] R.S. Pruthi, J.E. Derksen, D. Moore, C.C. Carson, G. Grigson, C. Watkins, E.  
21 Wallen, Phase II trial of celecoxib in prostate specific antigen recurrent prostate  
22 cancer after definitive radiation therapy or radical prostatectomy, *Clin. Cancer. Res.*  
23 12 (2006) 2172–2177.
- 24 [7] D. Basudhar, G. Bharadwaj, R. Y. Cheng, S. Jain, S. Shi, J. L. Heinecke, R. J.  
25 Holland, L. A. Ridnour, V. M. Caceres, R. C. Spadari-Bratfisch, N. Paolocci, C. A.  
26 Velazquez-Martinez, D. A. Wink, K. M. Miranda, Synthesis and chemical and  
27 biological comparison of nitroxyl- and nitric oxide-releasing  
28 diazeniumdiolate-based aspirin derivatives, *J. Med. Chem.* 56 (2013) 7804–7820.

- [8] J. L. Williams, N. Nath, J. Chen, T. R. Hundley, J. Gao, L. Kopelovich, K. Kashfi, B. Rigas, Growth inhibition of human colon cancer cells by nitric oxide (NO)-donating aspirin is associated with cyclooxygenase-2 induction and beta-catenin/T-cell factor signaling, nuclear factor-kappaB, and NO synthase inhibition: implications for chemoprevention, *Cancer Res.* 63 (2003) 7613-7618.
- [9] Y.A. Ammar, M.A. Salem, E.A. Fayed, M.H. Helal, M.S.A. El-Gaby, H. K. Thabet, Naproxen derivatives: Synthesis, reactions, and biological applications, *Synth. Commun.* 47(15) (2017) 1341-1367.
- [10] D. Plano, D. N. Karelia, M. K. Pandey, J. E. Spallholz, S. Amin, A. K. Sharma, Design, synthesis, and biological evaluation of novel selenium (Se-NSAID) molecules as anticancer agents, *J. Med. Chem.* 59 (2016) 1946-1959.
- [11] D. Desai, N. Kaushal, U. H. Gandhi, R. J. Arner, C. D'Souza, G. Chen, H. Vunta, K. El-Bayoumy, S. Amin, K. S. Prabhu, Synthesis and evaluation of the anti-inflammatory properties of selenium-derivatives of celecoxib, *Chem. Biol. Interact.* 188 (2010) 446-456.
- [12] D. Desai, I. Sinha, K. Null, W. Wolter, M. A. Suckow, T. King, S. Amin, R. Sinha, Synthesis and antitumor properties of selenocoxib-1 against rat prostate adenocarcinoma cells, *Int. J. Cancer.* 127 (2010) 230-238.
- [13] R. Alhasan, A. Kharm, P. Leroy, C. Jacob, C. Gaucher, Selenium Donors at the Junction of Inflammatory Diseases, *Curr. Pharm. Des.* 25 (15) (2019) 1707-1716.
- [14] H. J. Reich, R. J. Hondal, Why nature chose selenium. *ACS Chem. Biol.* 11 (2016) 821-841.
- [15] Y. Pang, B. An, L. Lou, J. Zhang, J. Yan, L. Huang, X. Li, S. Yin, Design, synthesis, and biological evaluation of novel selenium-containing *isocombretastatins* and phen-statins as antitumor agents, *J. Med. Chem.* 60 (17) (2017) 7300-7314.
- [16] Y. Yang, S. Deng, Q. Zeng, W. Hu, T. Chen, Highly stable selenadiazole derivatives. induce bladder cancer cell apoptosis and inhibit cell migration and

- 1 invasion through the activation of ROS-mediated signaling pathways, Dalton  
2 Trans. 45 (46) (2016) 18465-18475.
- 3 [17] A. Kunwar, B. Mishra, A. Barik, L.B. Kumbhare, R. Pandey, V.K. Jain, K.I.  
4 Priyadarsini, 3,3'-Diselenodipropionic acid, an efficient peroxy radical scavenger  
5 and GPx mimic, protects erythrocytes (RBCs) from AAPH-induced hemolysis,  
6 Chem. Res. Toxicol. 20 (2007) 1482–1487.
- 7 [18] Y. Wang, J. Wallach, S. Duane, Y. Wang, J. Wu, J. Wang, A. Adejare, H. Ma,  
8 Developing selective histone deacetylases (HDACs) inhibitors through ebselen  
9 and analogs, Drug. Des. Dev. Ther. 11 (2017) 1369–1382.
- 10 [19] T. Cierpień, J. Łuczak, M. Kwiatkowska, P. Kielbasiński, L. Mielczarek, K.  
11 Wiktorska, Z. Chilmonczyk, M. Milczarek, K. Karwowska, Organofluorine  
12 isoselenocyanate analogues of sulforaphane: synthesis and anticancer activity,  
13 ChemMedChem. 11 (21) (2016) 2398-2409.
- 14 [20] I. Lagunes, P. Begines, A. Silva, A.R. Galán, A. Puerta, M.X. Fernandes, I. Maya,  
15 J.G. Fernández-Bolaños, Ó. López, J.M. Padrón, Selenocoumarins as new  
16 multitarget antiproliferative agents: Synthesis, biological evaluation and in silico  
17 calculations, Eur. J. Med. Chem. 179 (2019) 493-501.
- 18 [21] X. He, M. Zhong, S. Li, X. Li, Y. Li, Z. Li, Y. Gao, F. Ding, D. Wen, Y. Lei, Y.  
19 Zhang, Synthesis and biological evaluation of organoselenium (NSAIDs-SeCN  
20 and SeCF<sub>3</sub>) derivatives as potential anticancer agents, Eur. J. Med. Chem. 208  
21 (2020) 112864.
- 22 [22] R. J. Jariwalla, B. Gangapurkar, D. Nakamura, Differential sensitivity of various  
23 human tumour-derived cell types to apoptosis by organic derivatives of selenium,  
24 Br. J. Nutr. 101 (2009) 182-189.
- 25 [23] J. E. Spallholz, Free radical generation by selenium compounds and their  
26 prooxidant toxicity, Biomed. Environ. Sci. 10 (1997) 260-270.
- 27 [24] C. Storkey, M. J. Davies, J. M. White, C. H. Schiesser, Synthesis and antioxidant  
28 capacity of 5-selenopyranose derivatives, Chem. Commun. 47 (2011) 9693-9695.

- 1[25] K. N. Sands, T. G. Back, Key steps and intermediates in the catalytic mechanism  
2 for the reduction of peroxides by the antioxidant ebselen, *Tetrahedron*. 74 (38),  
3 (2018) 4959-4967.
- 4[26] L. Liu, S. Li, X. Li, M. Zhong, Y. Lu, J. Yang, Y. Zhang, X. He, Synthesis of  
5 NSAIDs-Se derivatives as potent anticancer agents, *Med. Chem. Res.* 27  
6 (2018) 2071-2078.
- 7[27] Y. Nie, M. Zhong, S. Li, X. Li, Y. Zhang, Y. Zhang, X. He, Synthesis and  
8 potential anticancer activity of some novel selenocyanates and diselenides, *Chem.*  
9 *Biodivers.* 17(5) (2020) e1900603.
- 10[28] A.S. Hodage, P.P. Phadnis, A. Wadawale, K.I. Priyadarsini, V.K. Jain, Synthesis,  
11 characterization and structures of 2-(3,5-dimethylpyrazol-1-yl) ethylseleno  
12 derivatives and their probable glutathione peroxidase (GPx) like activity, *Org.*  
13 *Biomol. Chem.* 9 (2011) 2992-2998.
- 14 [29] V. Nascimento, E. E. Alberto, D. W. Tondo, D. Dambrowski, M. R. Detty, F.  
15 Nome, A. L. Braga, GPx-Like activity of selenides and selenoxides: experimental  
16 evidence for the involvement of hydroxy perhydroxy selenane as the active species,  
17 *J. Am. Chem. Soc.* 134 (1) (2012) 138-141.
- 18 [30] S. Ficht, L. Röglin, M. Ziehe, D. Breyer, O. Seitz, Direct carbodiimide-mediated  
19 conjugation of carboxylates using pyridinium p-toluenesulfonate and tertiary  
20 amines as additives, *Synlett.* 14 (2004) 2525-2528.
- 21 [31] P. Nikolaienko, M Rueping, Trifluoromethylselenolation of Aryldiazonium  
22 Salts: A Mild and Convenient Copper-Catalyzed Procedure for the Introduction  
23 of the SeCF<sub>3</sub> Group, *Chem. Eur. J.* 22 (2016) 2620 – 2623.
- 24 [32] J. T. Pinto, R. Sinha, K. Papp, N. D. Facompre, D. Desai, K. El-Bayoumy,  
25 Differential effects of naturally occurring and synthetic organoselenium  
26 compounds on biomarkers in androgen responsive and androgen independent  
27 human prostate carcinoma cells, *Int. J. Cancer.* 120 (2007) 1410-1417.
- 28 [33] A. Ghose, J. Fleming, K. El-Bayoumy, P. R. Harrison, Enhanced sensitivity of  
29 human oral carcinomas to induction of apoptosis by selenium compounds:



- 1 involvement of mitogen-activated protein kinase and Fas pathways, *Cancer. Res.*  
2 61 (2001) 7479-7487.
- 3 [34] T. C. W. Chan, J. L. Wilkinson Berka, D. Deliyanti, D. Hunter, A. Fung, G. Liew,  
4 A. White, The role of reactive oxygen species in the pathogenesis and treatment  
5 of retinal diseases, *Exp. Eye. Res.* 201(2020) 108255.
- 6 [35] G. M. Gordillo, C. K. Sen, Revisiting the essential role of oxygen in wound  
7 healing, *Am. J. Surg.* 186 (2003) 259-263.
- 8 [36] K. R. Martin, J. C. Barrett, Reactive oxygen species as double-edged swords in  
9 cellular processes: low-dose cell signaling versus high-dose toxicity, *Hum. Exp.*  
10 *Toxicol.* 21 (2002) 71-75.
- 11 [37] J. E. Klaunig, Oxidative stress and cancer, *Curr. Pharm. Des.* 24 (40) (2018)  
12 4771-4778.
- 13 [38] D. Pathania, M. Sechi, M. Palomba, V. Sanna, F. Berrettini, A. Sias, L. Taheri, N.  
14 Neamati, Design and discovery of novel quinazolinedione-based redox  
15 modulators as therapies for pancreatic cancer, *Biochim. Biophys. Acta Gen. Subj.*  
16 1840 (1) (2014) 332-343.
- 17 [39] I. Rohn, N. Kroepfl , M. Aschner , J. Bornhorst , D. Kuehnelt , T. Schwerdtle,  
18 Selenoneine ameliorates peroxide-induced oxidative stress in *C. elegans*, *J. Trace.*  
19 *Elem. Med. Bio.* 55 (2019) 78-81.
- 20 [40] M. T. Melo, I. M. de Oliveira, I. Grivicich, T. N. Guecheva, J. Saffi, J. A.  
21 Henriques, R. M. Rosa, Diphenyl diselenide protects cultured MCF-7 cells  
22 against tamoxifen-induced oxidative DNA damage, *Biomed. Pharmacother.* 67  
23 (2013) 329–335.
- 24 [41] S. Shaaban, A.M. Ashmawy, A. Negm, L.A. Wessjohann, Synthesis and  
25 biochemical studies of novel organic selenides with increased selectivity for  
26 hepatocellular carcinoma and breast adenocarcinoma, *Eur. J. Med. Chem.* 179  
27 (2019) 515-526.
- 28 [42] D. Meriane, G. Genta-Jouve, M. Kaabeche, S. Michel, S. Boutefnouchet, Rapid  
29 identification of antioxidant compounds of *Genista saharae* coss. & dur. By

- 1 combination of DPPH scavenging assay and HPTLC-MS, *Molecules*. 19 (4)  
2 (2014) 4369-4379.
- 3 [43] R. Uddin, M.R. Saha, N. Subhan, H. Hossain, I.A. Jahan, R. Akter, A. Alam,  
4 HPLC-analysis of polyphenolic compounds in gardenia jasminoides and  
5 determination of antioxidant activity by using free radical scavenging assays,  
6 *Adv. Pharmaceut. Bull.* 4 (3) (2014) 273-281
- 7 [44] A. A. Bunaciu, A. F. Danet, Ş. Fleschin, H. Y. Aboul-Enein, Recent applications  
8 for in vitro antioxidant activity assay, *Crit. Rev. Anal. Chem.* 46 (5) (2016)  
9 389-399.
- 10 [45] X. Tian, K.M. Schaich, Effects of molecular structure on kinetics and dynamics  
11 of the trolox equivalent antioxidant capacity assay with ABTS(t\*), *J. Agric. Food*  
12 *Chem.* 61 (23) (2013) 5511-5519.
- 13 [46] M. Ibrahim, W. Hassan, J. Anwar, A.M. Deobald, J.P. Kamdem, D.O. Souza, J.B.  
14 Rocha, 1-(2-(2-(2-(1-Aminoethyl)phenyl)diselanyl)phenyl)ethanamine: an amino  
15 organoselenium compound with interesting antioxidant profile, *Toxicol. In. Vitro.*  
16 28 (4) (2014) 524-530.
- 17 [47] J. L. Rose, K. C. Reeves, R. I. Likhovtorik, D. G. Hoyt, Base excision repair  
18 proteins are required for integrin-mediated suppression of bleomycin-induced  
19 DNA breakage in murine lung endothelial cells, *J. Pharmacol. Exp. Ther.* 321 (1)  
20 (2007) 318-326.
- 21 [48] A. Mira, E. M. Gimenez, A. D. Bolzan, M.S. Bianchi, D. M. Lopez-Larraz, A.  
22 Effect of thiol compounds on bleomycin-induced DNA and chromosome damage  
23 in human cells, *Arch. Environ. Occup. Health.* 68 (2) (2013) 107-116.
- 24 [49] B. Laffon, V. Valdiglesias, E. Pásaro, J. Méndez. The Organic selenium  
25 compound selenomethionine modulates bleomycin-induced DNA damage and  
26 repair in human leukocytes, *Biol. Trace. Elem. Res.* 133 (1) (2010) 12–19.
- 27 [50] V. Nascimento, E. E. Alberto, D. W. Tondo, D. Dambrowski, M. R. Detty, F.  
28 Nome, A. L. Braga, GPx-like activity of selenides and selenoxides: experimental  
29 evidence for the involvement of hydroxy perhydroxy selenane as the active  
30 species, *J. Am. Chem. Soc.* 134 (1) (2012) 138-141.

- 1 [51] C.W. Nogueira, J.B.T. Rocha, Toxicology and pharmacology of selenium:  
2 emphasis on synthetic organoselenium compounds. *Arch. Toxicol.* 85 (11) (2011)  
3 1313-1359.
- 4 [52] A.S. Hodage, P.P. Phadnis, A. Wadawale, K.I. Priyadarsini, V.K. Jain, Synthesis,  
5 characterization and structures of 2-(3,5-dimethylpyrazol-1-yl)ethylseleno  
6 derivatives and their probable glutathione peroxidase (GPx) like activity, *Org.*  
7 *Biomol. Chem.* 9 (8) (2011) 2992-2998.
- 8 [53] S. Gromer, L.A. Wessjohann, J. Eubel, W. Brandt, Mutational studies confirm.  
9 the catalytic triad in the human selenoenzyme thioredoxin reductase predicted by  
10 molecular modeling, *Chem biochem* 7 (2006) 1649-1652.
- 11 [54] W. Brandt, L.A. Wessjohann, The functional role of selenocysteine (Sec) in the  
12 catalysis mechanism of large thioredoxin reductases: proposition of a swapping  
13 catalytic triad including a Sec-His-Glu state, *Chembiochem* 6 (2005) 386-394.
- 14 [55] T. Sandalova, L. Zhong, Y. Lindqvist, A. Holmgren, G. Schneider,  
15 Threedimensional structure of a mammalian thioredoxin reductase: implications  
16 for mechanism and evolution of a selenocysteine-dependent enzyme, *Proc. Natl.*  
17 *Acad. Sci. U. S. A.* 98 (2001) 9533-9538.
- 18 [56] S. Shaaban, Amr Negm, A.M. Ashmawy, D.M. Ahmed, L.A. Wessjohann,  
19 Combinatorial synthesis, in silico, molecular and biochemical studies of  
20 tetrazole-derived organic selenides with increased selectivity against  
21 hepatocellular carcinoma, *Eur. J. Med. Chem.* 122 (2016) 55-71.
- 22 [57] S. A. Arafa, A. H. Abdelazeem, H. H. Arab, H. A. Omar, OSU-CG5, a novel  
23 energy restriction mimetic agent, targets human colorectal cancer cells in vitro,  
24 *Acta Pharmacol. Sin.* 35 (2014) 394-400.
- 25 [58] H. A. Omar, S. A. Arafa, I. A. Maghrabi, J. R. Weng, Sensitization of  
26 hepatocellular carcinoma cells to Apo2l/TRAIL by a novel Akt/NF- $\kappa$ B  
27 signalling Inhibitor, *Basic Clin. Pharmacol. Toxicol.* 114 (2014) 464-471.
- 28 [59] A.R. Verma, M. Vijayakumar, C.V. Rao, C.S. Mathela, In vitro and in  
29 vivo antioxidant properties and DNA damage protective activity of green fruit  
30 of *Ficus glomerata*, *Food. Chem. Toxicol.* 48 (2) (2010) 704-709.

- 1 [60] A.B.A. El-Gazzar, M.M. Youssef, A.M.S. Youssef, A.A. Abu-Hashem, F.A.  
2 Badria, Design and synthesis of azolopyrimidoquinolines, pyrimidoquinazolines  
3 as anti-oxidant, anti-inflammatory and analgesic activities, *Eur. J. Med. Chem.* 44  
4 (2009) 609–624.
- 5 [61] N. M. Giles, G. I. Giles, J. E. Holley, N. J. Gutowski, C. Jacob, Targeting oxidative  
6 stress-related diseases: organochalcogen catalysts as redox sensitizers, *Biochem.*  
7 *Pharmacol.* 66 (2014), 2021-2028.  
8