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► To cite this version:

Xianran He, Yousong Nie, Min Zhong, Shaolei Li, Xiaolong Li, et al.. New organoselenides (NSAIDs-Se derivatives) as potential anticancer agents: Synthesis, biological evaluation and in silico calculations. European Journal of Medicinal Chemistry, 2021, 218, pp.113384. 10.1016/j.ejmech.2021.113384 . hal-03188603

HAL Id: hal-03188603

<https://hal.sorbonne-universite.fr/hal-03188603>

Submitted on 2 Apr 2021

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

Xianran He^a, Yousong Nie^b, Min Zhong^a, Shaolei Li^c, Xiaolong Li^c, Yi Guo^d,
Zhenming Liu^d, Yangguang Gao^a, Fei Ding^a, Dan Wen^a, Yongmin Zhang^{a,e,*}

^a *Institute for Interdisciplinary Research, Jiangnan University, Wuhan Economic and
Technological Development Zone, Wuhan 430056, China*

^b *School of Environmental Ecology and Biological Engineering, Wuhan Institute of
Technology, LiuFang Campus, Guanggu 1st road, Wuhan 430205, China*

^c *Shenzhen Fushan Biological Technology Co., Ltd, Kexing Science Park A1 1005,
Nanshan Zone, Shenzhen 518057, China*

^d *State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical
Sciences, Peking University, Beijing 100191, China*

^e *Institut Parisien de Chimie Moléculaire, CNRS UMR 8232, Sorbonne Université, 4
Place Jussieu, 75005 Paris, France*

*Corresponding author: Yongmin Zhang

E-mail: yongmin.zhang@upmc.fr

Abstract:

Herein we reported the synthesis of twenty new organoselenium compounds (**2a-2j** and **3a-3j**) based on the hybridization of nonsteroidal antiinflammatory drugs (NSAIDs) skeleton and organoselenium motif (-SeCN and -SeCF₃), the anticancer activity was evaluated against four types of cancer cell lines, Caco-2 (human colon adenocarcinoma cells), BGC-823 (human gastric cancer cells), MCF-7 (human breast adenocarcinoma cells), PC-3 (human prostatic cancer cells). Interestingly, the introduction of the -SeCN or -SeCF₃ moiety in corresponding parent NSAIDs results in the significant effect on cancer cell lines. Moreover, the most active compound **3a** showed IC₅₀ values lower than 5 μM against the four cancer cell lines, particularly to BGC-823 and MCF-7 with IC₅₀ values of 2.5 and 2.7 μM, respectively. Furthermore, three compounds **3a**, **3g** and **3i** were selected to investigate their ability to induce apoptosis in BGC-823 cells via modulating the expression of anti-apoptotic Bcl-2 protein, pro-inflammatory cytokines (IL-2) and proapoptotic caspase-8 protein. The redox properties of the NSAIDs-Se derivatives prepared herein were conducted by 2, 2-didiphenyl-1-picrylhydrazyl (DPPH), bleomycin dependent DNA damage and glutathione peroxidase (GPx)-like assays. Finally, molecular docking study revealed that an interaction with the active site of **thioredoxin reductase 1** (TrxR1) and predicted the anticancer activity of the synthesized candidates. Overall, these results could serve a promising launch point for further design of NSAIDs-Se derivatives as potential anticancer agents.

Keywords: selenium; selenocyanates; trifluoromethyl selenides; anticancer; *in silico* 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-, -SeCF₃) 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

on design and synthesis of new NSAIDs-Se derivatives as potential anticancer agents [28, 29], twenty new NSAIDs-SeCN and NSAIDs-SeCF₃ derivatives were designed by the incorporation of an appropriate Se moiety into various NSAIDs with a general model consist of three essential fragments in their molecular: i) NSAIDs fragment; ii) electron donating group (X = NH, O, Se); iii) functional group bearing the Se atom (**Fig 2**). Their anticancer activities against the human cancer cell lines Caco-2, BGC-823, MCF-7 and PC-3 *in vitro* using the MTT assay. Three compounds **3a**, **3g** and **3i** were selected to test the protein expression levels of Bcl-2, IL-8 and caspase-8 biomarkers in BGC-823 cells. Furthermore, the antioxidant potential of the compounds was investigated by employing DPPH, bleomycin-dependent DNA damage and GPx-like assays. Finally, TrxR1 (Thioredoxin Reductase) was selected as docking protein in order to predict the target and anticancer activity of the prepared NSAIDs-Se hybrid compounds.

Fig. 1. Organic selenium compounds previously reported with anticancer activity

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

2. Results and Discussion

2.1 Chemistry

The synthetic route for target compounds (**2a-2j** and **3a-3j**) were prepared as outlined in Scheme 1 according to the procedure described in the literature with some modifications [30]. Compound **1** was obtained by the nucleophilic substitution of -Br atom in 3-bromo-1-propanol by -SeCN, using KSeCN as nucleophilic donor, in acetonitrile as solvent and under a nitrogen atmosphere. The selenocyanate derivatives **2a-2j** were readily obtained by reacting 3-selenocyanatopropan-1-ol with commercially available NSAIDs in the presence of DCC and DMAP as condensation agent. The trifluoromethyl selenide derivatives were obtained by conducting corresponding selenocyanate derivative with trimethyl(trifluoromethyl)silane (TMSCF₃) in the presence of tetrabutylammonium fluoride (TBAF) as catalyst to afford **3a-3j** in good yields (yield $\geq 80\%$) (Scheme 1) [31].

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

Scheme 1. i) KSeCN, CH₃CN, 80 °C, 24 h, 90 %; ii) DCC, DMAP, DCM, 25 °C, 16 h 70% - 90%; iii) TBAF, TMSCF₃, 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 IC₅₀ 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 IC₅₀ values obtained for the

1 NSAIDs-Se derivatives **2j**, **3b** and **3f**, showed that introduction of the -SeCN or
2 -SeCF₃ moiety in corresponding parent NSAIDs scaffold result in the significant
3 effect on cancer cell line.

4 An overview analysis of the IC₅₀ values obtained and summarized in Table 1
5 showed that most of the NSAIDs-SeCF₃ 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-SeCF₃ derivatives **3a**, **3g** and **3i**. These
9 three compounds show IC₅₀ values below 10 µM in all of tested cancer cell lines.
10 Compound **3a** emerges the most potent agent with IC₅₀ 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-SeCF₃
14 derivatives except **3f** and **3h** displayed IC₅₀ 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.

Table 1

Cytotoxic activity expressed by IC₅₀ of NSAIDs-Se hybrid compounds (**2a-2j** and **3a-3j**) on different cancer cell lines.

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

^a IC₅₀ values (±SD) of % cell viability determined by the MTT assay of three repetitions

^b Patent NSAIDs

^c Standard benchmark compound.

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

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

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

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

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

2.4. Antioxidant assay

Reactive oxygen species (ROS) is a broad term that encompasses both oxygen free radicals, which have unpaired electrons, such as superoxide, hydroxyl and peroxy as well as oxidizing agents that are not free radicals such as hydrogen 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].

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-SeCF₃ 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-SeCF₃ derivatives is better than the
25 corresponding NSAIDs-SeCN derivatives on this assay except for the compare of **2d**
26 and **3d**.

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

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

Table 2

Redox modulation activity of NSAID-Se hybrid compounds.

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

3i	66.3±2.6	0.7	128.4±1.38
3j	32.4±1.8	0.4	88.7±1.32

2.4.3. Glutathione peroxidase-like activity assay.

Glutathione peroxidase (GPx) is a selenoenzyme that protects cells by catalyzing the reduction of peroxides with the stoichiometric reductant glutathione (GSH) [50, 51]. The potential antioxidant activity of all of the NSAIDs-Se derivatives were estimated using NADPH-reductase coupled assay [52]. 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

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

3D structure complexes cocrystallized of human TrxR1 with inhibitors, flexible docking was considered to be a practical method according to the literature [56]. With good antioxidant activity, compounds **3a**, **3g** and **3i** were docked into the TrxR1 protein (PDB id: 1H6V) using Flexible Docking Protocol as reported in the literature [56]. All three compounds showed acceptable docking results (**Table 3-5** are reported in the supporting information). It is thought that the distance between the selenium atom and Cys497/Cys498 is closely related to the accessibility of cysteine thiol attacking the selenide. Therefore, for each structure, the selection of the best pose of the docking results is related to the value of binding energy, while the distance would also be focused. Among the three compounds, Pose 3 of **3a** showed a better docking results with the relatively good value of -CDOCKER energy (30.184 kcal/mol). Meanwhile, the distance between the selenium atom and Cys498 was only 4.388 Å (**Table 3**, Pose 3). This good result may be related to the key hydrogen bond interaction between the Fluorine on benzene group and His472 (2.11 Å). In addition, **3a** also formed two hydrogen bonds, which are the hydrogen bond between -SeCF₃ group and His472 (2.97 Å) and the hydrogen bond between the oxygens of ester groups and Cys475 (2.62 Å) (**Figure 5**). For compound **3g**, the interactions shown in pose 3 are not only the hydrogen bonds, but also a π - π stacking between the benzene ring and Phe406 (**Figure 6**). However, the distance between the selenium atom and Cys497/Cys498 is far than **3a** (**Table 4**). For **3i**, although there are multiple hydrogen bonds near the carbonyl group, the long distance between the selenium atom and Cys497/Cys498 may be related to the long linear structure of the whole compound (**Figure 7**, **Table 5**). This structure makes it difficult for molecule to penetrate into the pocket as a whole, thus affecting the interaction between molecule and protein.

Fig. 5. The pose 3 of **3a**. Three interactions are shown: hydrogen bonding between the Fluorine on benzene group and His472 (2.11 Å); hydrogen bonding between the Fluorine of -SeCF₃ group and His472 (2.97 Å) and hydrogen bonding between the oxygens of ester groups and Cys475 (2.62 Å).

Fig. 6. The pose 3 of **3g**. Three interactions are shown: hydrogen bonding between the Fluorine of -CF₃ group and LEU409 (2.57 Å); hydrogen bonding between the oxygens of ester groups and Trp407 (2.78 Å) and π - π stacking between the benzene ring and Phe406.

Fig. 7. The pose 4 of **3i**. Three interactions are shown: hydrogen bonding between the Fluorine of -SeCF₃ group and Ser404 (2.80 Å); hydrogen bonding between the two hydrogens on carbonyl group α position and Ile492 (2.54 Å, 2.64 Å) or Gln494 (2.90 Å); hydrogen bonding between the oxygens of ester groups and Phe406 (2.46 Å).

3. Conclusions

In summary, twenty new organoselenium compounds were synthesized and characterized. Four human cell lines (Caco-2, BGC-823, MCF-7 and PC-3) were selected to test anticancer activity of the compounds. Compound **3a** showed most potent anticancer activity with IC₅₀ values below 5 μ m against four cancer cell lines. Moreover, three compounds were selected to test their ability to induce apoptosis in BGC-823 cells via modulation the expression of anti-apoptotic Bcl-2 protein, pro-inflammatory cytokines (IL-2) and proapoptotic caspase-8 protein. Compounds **3a**, **3g** and **3i** were able to downregulate the expression of Bcl-2 and upregulate the expression of IL-2 and Caspase-8 in BGC-823 cells. Furthermore, most of the organoselenium compounds exhibited moderate to good CPx-like activity compared to ebselen. Finally, in flexible docking study performed into TrxR1 enzyme, compound **3a** showed a promising binding energies and binding mode that the distance between the selenium atom and Cys497/Cys498. At this point, compound **3a** may act as TrxR inhibitors.

4. Experimental section

4.1. General methods

All chemical reagents for the synthesis of the compounds were purchased from Macklin (Shanghai, China) or TCI (Shanghai, China) and used without further purification unless stated otherwise. Thin-layer chromatography (TLC) was performed on aluminium pre-coated sheets (E. Merck Silica gel 60 F254). Melting points were recorded on an Electrothermal apparatus and are uncorrected. NMR spectra were recorded in CDCl₃ on a Bruker Avance 400 MHz (for ¹H), 100 MHz (for ¹³C) and 376 MHz (for ¹⁹F) spectrometer with 5 mm PABBO probe. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Chemical shifts (δ) are reported in parts per million (ppm) downfield from TMS and the coupling constants (*J*) are expressed in Hertz (Hz). High-resolution MS were 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 24 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°C for 30 minutes. Then 2-selenocyanatoethanamine hydrobromide (1.2 eq) or 2-selenocyanatopropanamine hydrobromide (1.2 eq.) was added into the mixture. The mixture was stirred at 25°C for 16 hrs. TLC showed the reaction was complete. The

mixture was diluted with H₂O (20 mL), the aqueous layer was extracted with DCM (15 mL×2), the combined organic layer was washed with brine (20 mL×2), dried over Na₂SO₄, 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.

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

Yield: 78 %. White solid. Mp: 103-105°C. ¹H NMR (400 MHz, CDCl₃): δ 1.54 (d, 3H, *J* = 8.00 Hz, -CH₃), 2.20-2.23 (m, 2H, -CH₂), 2.94-2.99 (m, 2H, -CH₂), 3.76 (q, 1H, *J* = 8.00 Hz, -CH), 4.23-4.24 (m, 2H, -CH₂), 7.09-7.15 (m, 2H, ArH), 7.37-7.43 (m, 4H, ArH), 7.46-7.54 (m, 2H, ArH). ¹³C NMR (100 MHz, CDCl₃): δ 18.2, 25.7, 29.8, 45.0, 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), 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* = 7.0Hz), 159.5 (d, *J* = 247.0 Hz), 173.8. HRMS calcd. For C₁₉H₁₈FNO₂Se [M+Na]⁺: 414.0385, found 414.0365 [M+Na]⁺.

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

White solid. Mp: 97-99°C. ¹H NMR (400 MHz, CDCl₃): δ 0.89 (d, 6H, *J* = 8.00Hz, 2-CH₃), 1.49 (d, 3H, *J* = 8.00Hz, -CH₃), 1.84 (q, 1H, *J* = 8.00Hz, -CH), 2.14-2.17 (m, 2H, -CH₂), 2.45 (d, 2H, *J* = 8.00Hz, -CH₂), 2.78-2.88 (m, 2H, -CH₂), 3.69 (q, 1H, *J* = 8.00 Hz, -CH), 4.12-4.27 (m, 2H, -CH₂), 7.10 (d, 2H, ArH), 7.18 (d, 2H, ArH). ¹³C NMR (100 MHz, CDCl₃): δ 18.1, 22.4, 25.7, 29.7, 30.2, 45.0, 45.1, 62.5, 127.1, 129.5, 137.6, 140.9, 174.6. HRMS calcd. For C₁₇H₂₃NO₂Se [M+Na]⁺: 376.0792, found 376.0770 [M+Na]⁺.

4.2.2.3.3-selenocyanatopropyl2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetate (2c). Yield: 78 %. White solid. Mp: 110-112°C. ¹H NMR (400 MHz, CDCl₃): δ 2.19-2.25 (m, 2H, -CH₂), 2.40 (s, 3H, -CH₃), 2.92-3.00 (m, 2H, -CH₂), 3.69 (s, 2H, -CH₂), 3.84 (s, 3H, -CH₃), 4.24-4.26 (m, 2H, -CH₂), 6.66 (d, 1H, *J* = 4.00 Hz, 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}C NMR (100 MHz, 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. ^1H NMR (400 MHz, CDCl_3): δ 2.17 (s,
8 3H, - CH_3), 2.33 (s, 3H, - CH_3), 2.40-2.43 (m, 2H, - CH_2), 3.21-3.24 (m, 2H, - CH_2),
9 4.46-4.49 (m, 2H, - 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, -NH). ^{13}C NMR (100 MHz,
12 CDCl_3): δ 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. ^1H NMR (400 MHz, CDCl_3): δ 0.84
19 (t, 3H, $J = 8.00\text{Hz}$, - CH_3), 1.37 (t, 3H, $J = 8.00\text{Hz}$, - CH_3), 1.63 (s, 2H, - CH_2),
20 1.94-2.22 (m, 4H, 2- CH_2), 2.71-3.04 (m, 8H, 4 $\times$ - CH_2), 3.93-4.06 (m, 2H, - CH_2),
21 4.18-4.30 (m, 2H, - CH_2), 7.01-7.09 (m, 2H, ArH), 7.36 (d, 1H, $J = 8.00\text{Hz}$, ArH),
22 8.78 (s, 1H, -NH). ^{13}C NMR (100 MHz, CDCl_3): δ 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. ^1H NMR (400 MHz, CDCl_3): δ 1.58 (d,
29 3H, $J = 8.00\text{Hz}$, - CH_3), 2.11-2.18 (m, 2H, - CH_2), 2.76-2.90 (m, 2H, - CH_2), 3.83-3.88
30 (m, 2H, - CH_2), 3.92(s, 3H, - OCH_3), 4.15-4.26 (m, 1H, -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).

¹³C NMR (100 MHz, CDCl₃): δ 18.2, 25.7, 29.7, 45.2, 55.4, 62.7, 101.3, 105.6, 119.3, 126.0, 126.1, 127.3, 128.9, 129.2, 133.7, 135.4, 157.8, 174.5. HRMS calcd. For C₁₈H₁₉NO₃Se[M+H]⁺: 378.0608, found 378.0596 [M+H]⁺.

4.2.2.7. 3-selenocyanatopropyl 2-((3-(trifluoromethyl)phenyl)amino)benzoate

(**2g**). Yield: 77%. White solid. Mp: 121-123°C. ¹H NMR (400 MHz, CDCl₃): δ 2.38-2.45 (m, 2H, -CH₂), 3.19-3.23 (m, 2H, -CH₂), 4.46-4.49 (m, 2H, -CH₂), 6.82 (t, 1H, *J* = 8.00 Hz, ArH), 7.28-7.49 (m, 6H, ArH), 7.96 (d, 1H, *J* = 1.0 Hz, ArH), 9.54 (s, 1H, -NH). ¹³C NMR (100 MHz, CDCl₃): δ 26.0, 30.1, 62.9, 101.1, 112.3, 114.3, 118.2 (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, 131.6, 131.9 (q, *J* = 32.0 Hz), 134.7, 141.4, 147.1, 168.1. HRMS calcd. For C₁₈H₁₅F₃N₂O₂Se[M+H]⁺: 429.0329, found 429.0318 [M+H]⁺.

4.2.2.8. 3-selenocyanatopropyl (Z)-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1H-inden-3-yl)acetate

(**2h**). Yield: 82%. White solid. Mp: 91-93°C. ¹H NMR (400 MHz, CDCl₃): δ 2.21-2.26 (m, 2H, -CH₂), 2.22 (s, 3H, -CH₃), 2.82 (s, 3H, -CH₃), 3.00 (t, 2H, *J* = 8.00 Hz, -CH₂), 4.26 (t, 2H, *J* = 8.00 Hz, -CH₂), 6.56-6.61 (m, 1H, ArH), 6.87 (d, 1H, *J* = 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* = 8.00 Hz, ArH). ¹³C NMR (100 MHz, CDCl₃): δ 10.7, 25.7, 29.7, 31.8, 43.9, 63.2, 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), 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 (d, *J* = 9.0 Hz), 163.3 (d, *J* = 245.0 Hz), 170.1. HRMS calcd. For C₂₄H₂₂FO₃SSe[M+H]⁺: 504.0548, found 504.0528 [M+H]⁺.

4.2.2.9. 3-selenocyanatopropyl 2-(3-benzoylphenyl)propanoate

(**2i**). Yield: 85%. White solid. Mp: 96-98°C. ¹H NMR (400 MHz, CDCl₃): δ 1.55 (d, 3H, *J* = 8.00 Hz, -CH₃), 2.18-2.21 (m, 2H, -CH₂), 2.94-2.98 (m, 2H, -CH₂), 3.82 (q, 1H, *J* = 8.00 Hz, -CH), 4.21-4.25 (m, 2H, -CH₂), 7.43-7.54 (m, 4H, ArH), 7.61 (t, 1H, *J* = 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). ¹³C NMR (100 MHz, CDCl₃): δ 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 $[M+H]^+$.

4 *4.2.2.10. 3-selenocyanatopropyl 2-acetoxybenzoate (2j)*. Yield: 90%. White solid. Mp:

5 117-118°C. 1H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H, -CH₃), 2.33-2.40 (m, 2H,

6 -CH₂), 3.11-3.17 (m, 2H, -CH₂), 4.44-4.46 (m, 2H, -CH₂), 7.12 (d, 1H, J = 8.00Hz,

7 ArH), 7.33 (t, 1H, J = 8.00Hz, ArH), 7.58 (t, 1H, J = 8.00Hz, ArH), 7.99 (d, 1H, J =

8 8.00Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 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 $[M+Na]^+$.

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.

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, $CDCl_3$): δ 1.54

20 (d, 3H, J = 8.00 Hz, -CH₃), 2.09-2.12 (m, 2H, -CH₂), 2.90-2.93 (m, 2H, -CH₂), 3.75 (q,

21 1H, J = 8.00 Hz, -CH), 4.20-4.22 (m, 2H, -CH₂), 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, $CDCl_3$): δ 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, -SeCF₃), 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 = 7.0Hz),

26 159.7 (d, J = 247.0 Hz), 173.8. ^{19}F NMR ($CDCl_3$, 376 MHz): δ -34.3 (s, -SeCF₃),

27 -117.5 (s, F). HRMS calcd. For $C_{19}H_{18}F_4O_2Se$ $[M+H]^+$: 435.0486, found 435.0462

28 $[M+H]^+$.

4.2.3.2. 3-((trifluoromethyl)selanyl)propyl 2-(4-isobutylphenyl)propanoate (**3b**).

Yield: 82%. Yellow solid. Mp: 102-104°C. ¹H NMR (400 MHz, CDCl₃): δ 0.89 (d, 6H, *J* = 8.00 Hz, 2-CH₃), 1.49 (d, 3H, *J* = 8.00Hz, -CH₃), 1.84 (q, 1H, *J* = 8.00Hz, -CH), 2.04-2.07 (m, 2H, -CH₂), 2.44 (d, 2H, *J* = 8.00Hz, -CH₂), 2.78-2.83 (m, 2H, -CH₂), 3.68 (q, 1H, *J* = 8.00Hz, -CH), 4.12-4.21 (m, 2H, -CH₂), 7.09 (d, 2H, ArH), 7.18 (d, 2H, ArH). ¹³C NMR (100 MHz, CDCl₃): δ 18.2, 21.8, 22.4, 29.4, 30.2, 45.0, 45.1, 63.1, 122.5 (q, *J* = 328.0 Hz, -SeCF₃), 127.1, 129.4, 137.6, 140.7, 174.6. ¹⁹F NMR (CDCl₃, 376 MHz): δ -34.3 (s, -SeCF₃). HRMS calcd. For C₁₇H₂₃F₃O₂Se [M+H]⁺: 397.0893, found 397.0883 [M+H]⁺.

4.2.3.3. 3-((trifluoromethyl)selanyl)propyl

2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetate (**3c**). Yield: 80%.

White solid. Mp: 131-133°C. ¹H NMR (400 MHz, CDCl₃): δ 2.09-2.15 (m, 2H, -CH₂), 2.40 (s, 3H, -CH₃), 2.91-2.95 (m, 2H, -CH₂), 3.68 (s, 2H, -CH₂), 3.84 (s, 3H, -CH₃), 4.20-4.23 (m, 2H, -CH₂), 6.67 (d, 1H, *J* = 4.00 Hz, ArH), 6.85 (d, 1H, *J* = 8.00Hz, ArH), 6.94 (s, 1H, ArH), 7.48 (d, 2H, *J* = 8.00 Hz, ArH), 7.66 (d, 2H, *J* = 8.00Hz, ArH). ¹³C NMR (100 MHz, CDCl₃): δ 13.3, 21.9, 29.4, 30.3, 55.7, 63.7, 101.3, 111.6, 112.3, 115.0, 122.5 (q, *J* = 329 Hz, -SeCF₃), 129.2, 130.5, 130.8, 131.2, 133.9, 136.0, 139.3, 156.1, 168.3, 170.7. ¹⁹F NMR (CDCl₃, 376 MHz): δ -34.3 (s, -SeCF₃). HRMS calcd. For C₂₃H₂₁ClF₃NO₄Se [M+H]⁺: 548.0354, found 508.0305 [M+H]⁺.

4.2.3.4. 3-((trifluoromethyl)selanyl)propyl 2-((2,3-dimethylphenyl)amino)benzoate

(**3d**). Yield: 82%. White solid. Mp: 116-118°C. ¹H NMR (400 MHz, CDCl₃): δ 2.18

(s, 3H, -CH₃), 2.28-2.31 (m, 2H, -CH₂), 2.33 (s, 3H, -CH₃), 3.13-3.17 (m, 2H, -CH₂), 4.41-4.44 (m, 2H, -CH₂), 6.67 (t, 1H, *J* = 8.00 Hz, ArH), 6.75 (d, 1H, *J* = 8.00 Hz, ArH), 7.02 (d, 1H, *J* = 8.00 Hz, ArH), 7.10-7.15 (m, 2H, ArH), 7.23-7.27 (m, 1H, ArH), 7.93 (d, 1H, *J* = 8.00 Hz, ArH), 9.23 (s, 1H, -NH). ¹³C NMR (100 MHz, CDCl₃): δ 14.0, 20.7, 22.2, 29.7, 63.1, 110.4, 113.8, 116.1, 122.6 (q, *J* = 328.0 Hz, -SeCF₃), 123.2, 126.0, 126.9, 131.3, 132.5, 134.4, 138.3, 138.6, 149.7, 168.5. ¹⁹F

1 NMR (CDCl₃, 376 MHz): δ -34.2 (s, -SeCF₃). HRMS calcd. For C₁₉H₂₀F₃NO₂Se
2 [M+H]⁺: 432.0611, found 432.0675 [M+H]⁺.

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. ¹H NMR (400 MHz, CDCl₃): δ 0.84 (t, 3H, *J* = 8.00 Hz,
7 -CH₃), 1.37 (t, 3H, *J* = 8.00 Hz, -CH₃), 1.99-2.16 (m, 4H, 2 × -CH₂), 2.75-3.04 (m, 8H,
8 4-CH₂), 3.93-4.06 (m, 2H, -CH₂), 4.18-4.30 (m, 2H, -CH₂), 7.01-7.07 (m, 2H, ArH),
9 7.36 (d, 1H, *J* = 8.00 Hz, ArH), 8.94 (s, 1H, -NH). ¹³C NMR (100 MHz, CDCl₃): δ
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, -SeCF₃), 126.2, 126.6, 134.5, 135.7, 172.6. ¹⁹F NMR
12 (CDCl₃, 376 MHz): δ -34.2 (s, -SeCF₃). HRMS calcd. For C₂₁H₂₆F₃NO₃Se [M+H]⁺:
13 478.1108, found 478.1089 [M+H]⁺.

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. ¹H NMR (400 MHz, CDCl₃): δ 1.58 (d,
17 3H, *J* = 8.00 Hz, -CH₃), 2.03-2.07 (m, 2H, -CH₂), 2.82-2.86 (m, 2H, -CH₂), 3.87 (q,
18 1H, *J* = 8.00 Hz, -CH), 3.91 (s, 3H, -OCH₃), 4.16-4.19 (m, 2H, -CH₂), 7.12 (t, 1H, *J* =
19 8.00 Hz, ArH), 7.38 (d, 1H, *J* = 8.00 Hz, ArH), 7.65 (s, 1H, ArH), 7.70 (d, 2H, *J* =
20 8.00 Hz, ArH). ¹³C NMR (100 MHz, CDCl₃): δ 18.3, 21.8, 29.4, 45.4, 55.3, 63.3,
21 105.6, 119.1, 122.5 (d, *J* = 328.0 Hz, -SeCF₃), 125.9, 126.1, 127.2, 128.9, 129.3,
22 133.7, 135.5, 157.7, 174.6. ¹⁹F NMR (CDCl₃, 376 MHz): δ -34.3 (s, -SeCF₃). HRMS
23 calcd. For C₁₈H₁₉F₃O₃Se[M+Na]⁺: 443.0350, found 443.0337 [M+Na]⁺.

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. ¹H NMR (400 MHz, CDCl₃): δ 2.27-2.33 (m, 2H, -CH₂), 3.12-3.16 (m, 2H,
28 -CH₂), 4.42-4.45 (m, 2H, -CH₂), 6.83 (t, 1H, *J* = 8.00 Hz, ArH), 7.27-7.49 (m, 6H,
29 ArH), 7.97 (d, 1H, *J* = 1.0 Hz, ArH), 9.58 (s, 1H, -NH). ¹³C NMR (100 MHz, CDCl₃):
30 δ 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, -SeCF₃), 124.0 (q, $J = 270$ Hz, -CF₃), 124.7, 129.9, 131.6,
2 131.8 (q, $J = 32.0$ Hz), 134.5, 141.5, 147.0, 168.2. ¹⁹F NMR (CDCl₃, 376 MHz): δ
3 -34.2 (s, -SeCF₃), -62.8(s, -CF₃). HRMS calcd. For C₁₈H₁₅F₆NO₂Se [M+H]⁺:
4 472.0250, found 472.0233 [M+H]⁺.

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. ¹H NMR (400 MHz, CDCl₃): δ
9 2.10-2.13 (m, 2H, -CH₂), 2.21 (s, 3H, -CH₃), 2.82 (s, 3H, -CH₃), 2.94 (t, 2H, $J = 8.00$
10 Hz, -CH₂), 3.58 (s, 2H, -CH₂), 4.22 (t, 2H, $J = 8.00$ Hz, -CH₂), 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). ¹³C NMR (100 MHz, CDCl₃): δ 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 -SeCF₃), 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. ¹⁹F NMR (CDCl₃, 376 MHz): δ -34.3 (s, -SeCF₃), -112.8(s, -F).
17 HRMS calcd. For C₂₄H₂₂F₄O₃SSe [M+Na]⁺: 569.0289, found 569.0263 [M+Na]⁺.

18
19 4.2.3.9. *3-((trifluoromethyl)selanyl)propyl 2-(3-benzoylphenyl)propanoate (3i)*. Yield:
20 85%. White solid. Mp: 87-89°C. ¹H NMR (400 MHz, CDCl₃): δ 1.55 (d, 3H, $J =$
21 8.00Hz, -CH₃), 2.05-2.12(m, 2H, -CH₂), 2.87-2.89 (m, 2H, -CH₂), 3.82 (q, 1H, $J =$
22 8.00Hz, -CH), 4.19-4.21 (m, 2H, -CH₂), 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). ¹³C NMR (100 MHz, CDCl₃): δ 18.3, 21.8, 29.4, 45.4, 63.5, 122.5 (q, $J = 329.0$
25 Hz, -SeCF₃), 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. ¹⁹F NMR (CDCl₃, 376 MHz): δ -34.2 (s, -SeCF₃). HRMS calcd. For
27 C₂₀H₁₉F₃O₃Se [M+H]⁺: 445.0530, found 445.0491 [M+H]⁺.

28
29 4.2.3.10. *3-((trifluoromethyl)selanyl)propyl 2-acetoxybenzoate (3j)*. Yield: 80%.
30 White solid. Mp: 104-106°C. ¹H NMR (400 MHz, CDCl₃): δ 2.23-2.28 (m, 2H, -CH₂),

2.35 (s, 3H, -CH₃), 3.07-3.10 (m, 2H, -CH₂), 4.38-4.41 (m, 2H, -CH₂), 7.11 (d, 1H, *J* = 8.00 Hz, ArH), 7.30-7.57 (m, 1H, ArH), 7.58-7.60 (m, 1H, ArH), 7.99 (d, 1H, *J* = 8.00 Hz, ArH). ¹³C NMR (100 MHz, CDCl₃): δ 21.0, 22.0, 29.6, 63.7, 122.6 (q, *J* = 328 Hz, -SeCF₃), 123.0, 123.9, 131.6, 134.1, 150.8, 164.3, 169.7. ¹⁹F NMR (CDCl₃, 376 MHz): δ -34.2 (s, -SeCF₃). HRMS calcd. For C₁₃H₁₃F₃O₄Se [M+H]⁺: 392.9829, found 392.9827 [M+H]⁺.

4.3. Cell lines and culture conditions

Four human cancer cell lines Caco-2, BGC-823, MCF-7 and PC-3 cells were maintained in RPMI 1640 medium with 10% fetal bovine serum (FBS) and 100 units/mL of penicillin and streptomycin (Thermo Fisher Scientific, Shanghai, China) at 37 °C and 5% CO₂ in a humidified atmosphere. Cells were passaged at confluent densities, using a solution containing 0.05% trypsin and 0.5 mM EDTA. Human cancer cell lines Caco-2, BGC-823, MCF-7 and PC-3 used in this work were obtained from the American Type Culture Collection (ATCC, Manassas, VA).

All the tested NSAIDs-Se derivatives were evaluated in vitro for their antitumor activity against four cancer cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay according to the method as described before [57-58]. 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% CO₂ to allow cell attachment, the cells in the wells were respectively treated with target compounds at various concentrations for 24 h, 48 h and 72 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-8 protein expression levels

Bcl-2, IL-2 and capase-8 levels were evaluated in BGC-823 cells treated with the corresponding IC₅₀s of each compound and incubated for 48 h and compared with their levels in control untreated BGC-823 cell line. The cells were harvested by applying trypsin and lysed by freezing with liquid nitrogen and then thawing with gentle mixing and the total proteins were isolated. Protein levels of the anti-apoptotic marker Bcl-2 were then measured using enzymelinked immunosorbent assay (ELISA) according to the manufacturers' instructions (Merck, USA). Enzyme-linked immunosorbent assay was used for quantitative detection of IL-2 and caspase-8 (Platinum ELISA). The reaction product was detected at 450 nm using enzyme-linked immunosorbent assay (Platinum ELISA; Merck) according to the instructions of the manufacturer.

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 [59]. 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), MgCl₂ (5 mM), FeCl₃ (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 [60].

4.7. Glutathione peroxidase-like activity

GPx kit (Biodiagnostic, Egypt) was used for the determination of GPx according to Paglia et al [61]. The reaction mixture contained 1ml assay buffer (50mM phosphate buffer containing 0.1% Triton X-100) and 0.1ml NADPH reagent (24 mmol Glutathione, 12 unit Glutathione reductase and 4.8 mmol NADPH) and 0.01ml (41 mM) tested compounds and the reaction was started by the addition of H₂O₂ (0.8 mM). The contents were mixed well and the absorbances were recorded at 340 nm over a period of 3 min against deionized water. The change of absorbance per minute (A₃₄₀ nm/min) was estimated using ebselen (41 mM) as positive control. The values represented in Fig 3 are expressed after background correction for the reaction with H₂O₂ and GSH. In case of colored compounds, their activities were estimated after subtracting their own absorbances at the used wave length.

4.8. Molecular Modeling

4.8.1 Protein and Ligand Preparation

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

4.8.2 Ligand Docking

The docking task was completed on Discovery Studio Client 3.1. and the binding site of TrxR1 was defined as a docking sphere with dimensions X: 27.757, Y: 6.510, 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 π - π 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 17 **Acknowledgments**

18 This investigation was made possible through the financial support of National
19 Natural Science Foundation of China (Grant No: 21302065) and Shenzhen Fushan
20 Biological Technology Co., Ltd. China.

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