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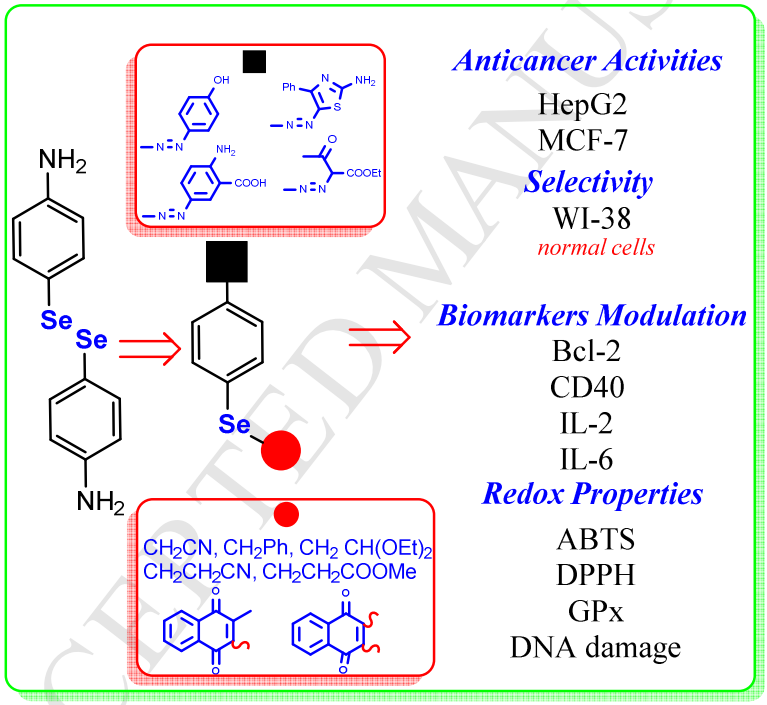

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ABSTRACT

Nineteen organoselenides were synthesized and tested for their intrinsic cytotoxicity in hepatocellular carcinoma (HepG2) and breast adenocarcinoma (MCF-7) cell lines and their corresponding selective cytotoxicity (SI) was estimated using normal lung fibroblast (WI-38) cells. Most of the organic selenides exhibited good anticancer activity, and this was more pronounced in HepG2 cells. Interestingly, the napthoquinone- (**5**), thiazol- (**12**), and the azo-based (**13**) organic selenides demonstrated promising SI (up to 76). Furthermore, the amine **4c**, napthoquinone **5**, and azo-based **13** and **15** organic selenides were able to down-regulate the expression of Bcl-2 and up-regulate the expression levels of IL-2, IL-6 and CD40 in HepG2 cells compared to untreated cells. Moreover, most of the synthesized candidates manifested good free radical-scavenging and GPx-like activities comparable to vitamin C and ebselen. The obtained results suggested that some of the presented organoselenium candidates have promising anti-HepG2 and antioxidant activities.

Keywords: Selenides; diselenides, selenocyanates, hepatocellular carcinoma; breast adenocarcinoma; antioxidant; Michael-type reaction; azo coupling.

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1. Introduction

Oxidative stress (OS) is involved in the aetiology of several diseases, including cancer, rheumatoid arthritis, Alzheimer's disease, and Huntington's disease [1, 2]. The development of agents able to counteract OS progression is becoming crucial in disease prevention and adjuvant therapy. Therefore, various exogenous antioxidants have been extensively investigated to inhibit/reduce oxidative damage and prevent/slow the development of diseases. This approach depends on the 'one-shot' administration of antioxidants (e.g., ascorbic acid, tocopherols, and flavonoids), which react in stoichiometrical ratios with stressors (e.g., reactive oxygen and nitrogen species (ROS and RNS) to form benign compounds [3, 4]. This strategy is, however, limited by the side effects or the cytotoxicity associated with the high concentrations of administered antioxidants [5-7]. In view of the former, interest in synthesizing novel antioxidants that catalytically inhibit cellular oxidation and retard disease progression has recently arisen. Of particular therapeutic significance, organic selenides have been vastly studied owing to their significant chemopreventive and antioxidant activities [8-11]. Furthermore, selenium is crucial for the proper immune system function and viral suppression [12]. An overwhelming number of reports have related selenium-body disorders with an increased risk of many illnesses, including cancer and liver diseases [13, 14]. In the past few years, an enormous effort has been directed towards the synthesis of new organic selenides that could be used as potential cancer chemopreventive agents (Figure 1). For example, the synthetic organic selenides 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) (**I**) and *p*-methoxy-benzyl selenocyanate (BSC) (**II**) have shown excellent cancer chemopreventive activities in several experimental tumour models (e.g., colon, lung, and oral) [15-21]. Furthermore, the water-soluble cyclic selenide trans-3,4-dihydroxyselenolane (DHS) (**III**) exhibited good GPx-like catalytic activity and interesting radioprotective effects [22, 23].

Figure 1

Since 2009, we reported several organic selenides (**IV** and **V**) with promising antioxidant and GPx-like activities [24-27]. The possible mode-of-action(s) by which these agents manifest their antioxidant effects was(were) found to be related to the ROS and glutathione (GSH) levels

modulation in tumour cells. Interestingly, we reported organoselenium candidates with better antioxidant and GPx-like activities than vitamin C and ebselen, respectively [28].

Very recently, we also reported the synthesis of a novel series of *N*-substituted amidic acid organic selenides, and their cytotoxicity, antioxidant and anti-apoptotic activities were tested in oligodendrocytes [29]. Among the tested compounds, (Z)-4-((4-((4-bromobenzyl)selanyl)phenyl)amino)-4-oxobut-2-enoic acid (**VI**) didn't show any cytotoxic effect on the viability of the 158 N oligodendrocytes (IC_{50}>100). On the other hand, it showed remarkable anti-apoptotic properties at low micromolar concentration (1, 5, and 10 µM) by diminishing the formation of the sub-G1 peak of oligodendrocytes and therefore was considered a promising target for chemopreventive applications [29].

Based on our findings, we synthesized new organoselenium compounds to obtain deeper insight into the potential use of these agents as anticancer and antioxidant candidates. The anticancer activity of the compounds was assessed using HepG2 and MCF-7 cell lines and compared with their cytotoxicity to normal WI-38 cells. Furthermore, the antioxidant potential of the compounds was investigated by employing different chemical assays, such as the ABTS, DPPH, bleomycin-dependent DNA damage, and GPx-like assays. Finally, the druggability of the prepared organic selenides was computed using the free SwissADME web tool.

2. Results and Discussions

2.1. Design and synthesis of organic selenides

As a part of our project aimed towards the synthesis of organic selenides-based chemotherapeutic agents [30, 31, 36-38], we herein report the synthesis of a novel class of organic selenide compounds (**4**-**20**) using readily available organoselenium building blocks. The key starting building blocks for the synthesis of the organoselenium agents were the 4-selenocyanatoaniline (**2**) [28], 4-(2-(4-aminophenyl)diselanyl)benzenamin (**3**) [25] and 4-methyl-5-selenocyanatothiazol-2-amine (**10**).

Organoselenium compounds **4**-**6** were synthesized via the nucleophilic substitution of the halogen atom at the corresponding alkyl, benzyl or quinone halides by the *in situ*-generated 4-aminobenzeneselenolate anion, while organic selenides **7a-b** were synthesized via a Michael-type addition (Scheme 1) by employing different activated olefins. On the other hand, N^1,N^3-

bis(4-selenocyanatophenyl)malonamide (**8**) was synthesized in good yields (87 %) by the reaction with malonyl chloride and selenoaniline **2** in dry benzene (Scheme 1).

Scheme 1

Notably, selenaheterocycles constitute an important subclass of organic selenides because of their widespread use in medicinal chemistry [29, 32-36, 39, 40]. In this context, 4-methyl-5-selenocyanatothiazol-2-amine (**10**) was synthesized in 85 % yield by the selenocyanation of 4-methylthiazol-2-amine (**9**) using the known selenocyanating agent triselenium dicyanide (TSD). Similarly, organoselenium compounds 2-((2-amino-4-methylthiazol-5-yl)selanyl)acetonitrile (**11**) and methyl 3-((2-amino-4-methylthiazol-5-yl)selanyl)propanoate (**12**) were synthesized from the *in situ*-formed 2-amino-4-methylthiazole-5-selenolate sodium salt either via Michael-type addition or nucleophilic substitution (Scheme 2).

Scheme 2

We next directed our attention to the development of azo -based organic selenides as many azoic derivatives have been reported to inhibit the synthesis of DNA and RNA, as well as several proteins [41, 42]. The -N=N- motif is assumed to be responsible for the interaction with the active site in proteins [43]. Subsequently, a series of selenocyanate- (**13**, **14**, **15** and **19**) and diselenide-based (**16**, **17**, **18** and **20**) azoic-based organoselenium compounds were prepared by the classical coupling of the 4-selenocyanatoaniline (**2**) and 4-(2-(4-aminophenyl)diselanyl)benzenamin (**3**) with different activated substrates (e.g., ethyl acetoacetate, phenol, anthranilic acid, and 4-methylthiazol-2-amine) (Scheme 3).

Scheme 3

2.2. *In vitro* biological studies:

2.2.1. Organic selenide effects on the viability of HepG2, MCF-7 and WI-38 cells

Not surprisingly, organoselenium compounds have received much attention as potential drug candidates owing to their redox-modulating activity [44-48]. Therefore, they have become a major focus of modern drug development as antioxidants and chemopreventive agents [49, 50]. GPx-like activity was reported mainly for symmetrical diselenides [33, 48, 51-53]. Our group

has explored the antioxidant activity of several organic selenides, such as tetrazole-derived and quinone-based organic selenides. Within this context, we reported several selenium-containing compounds with better antioxidant properties and GPx-like activity than the classic antioxidants ascorbic acid and ebselen, respectively [32, 33, 36, 54].

Based on our previous broad-spectrum cytotoxicity screening of different classes of organoselenium compounds [27, 31-36], we found that the organic selenides cytotoxicity is generally more pronounced in HepG2 and MCF-7 cells. Therefore, the antiproliferative activities of the newly synthesized compounds were preliminarily evaluated in these cancer cells (Table 1).

Table 1

Furthermore, to narrow our focus to the most interesting compounds, the selective cytotoxicity of these compounds was further evaluated using normal WI-38 cells.

In the case of HepG2 cells, most of the organoselenium compounds exhibited good cytotoxicity with IC_{50} values >10 µM and, in some cases, showed better cytotoxicity than the known anticancer medication fluorouracil (5-FU). Selenonapthoquinone **5** and azoic selenocyanates **14** and **15** were found to be the most cytotoxic with IC_{50} values ≤ 1 µM. On the other hand, compared with the effects on HepG2 cells, the cytotoxicity to MCF-7 cells was less pronounced, and most of the compounds exhibited moderate to low cytotoxicity. Selenopropanenitrile **7a** and selenopropanoate **7b** were the most cytotoxic with IC_{50} values ≤ 10 µM, while compounds **5**, **6**, **11**, **12**, and **16** exhibited a cytotoxic range with IC_{50} values ≤ 20 µM.

In chemotherapy, minimal cytotoxicity to healthy cells and high cytotoxicity towards tumour cells is desirable. The SI therefore reflects the differential cytotoxicity of a compound to healthy and tumour cells. The higher the SI value of a compound is, the greater its selectivity. In general, SI values above 10 indicate cytotoxic selectivity and are recommended to ensure the safety of a drug candidate [55]. In this regard, most of the synthesized compounds showed promising SI values in the case of HepG2 cells compared to MCF-7 cells. Among these compounds, organic selenides **5**, **4c**, **7a**, **12**, **13**, **14**, **15**, and **16** were highly selective for HepG2 cells with SI values of 76, 63, 57, 39, 29, 22 and 14, respectively.

Another selectivity pattern was observed in MCF-7 cells, indicating that selenium cytotoxicity is not general. In this regard, compounds **5**, **7a**, **7b**, **11**, **12,** and **16** were the most selective compounds with SI values of 12, 9, 8, 6, 5 and 4. This result is quite interesting and supports more studies employing a wider panel of cell models, including *in vivo* investigations.

2.2.2. Evaluation of Bcl-2, CD40, IL-2 and IL-6 molecular biomarkers in HepG2 cells

We have previously shown that the underlying death mechanism(s) of organoselenium compounds may be due to apoptosis induction [24, 30]. This was confirmed via the estimation of various cellular alterations (e.g., cell morphology, cell cycle delay, and activation of caspase 3/7 and caspase 8) [24, 30]. Very recently, we have also described that organic selenides can modulate the expression levels of some tumor protein biomarkers (e.g., Ki-67, Bcl-2, and caspase-8) leading to apoptosis induction [25, 28].

In order to further understand the expected mode(s) of action of the synthesized compounds, here, we selected the most promising and diverse organic selenides **4c**, **5**, **13**, and **15** and investigated their ability to induce apoptosis in HepG2 cells via modulation the expression of some pro-inflammatory cytokines (IL-2 and IL-6), the CD40 protein (necessary for mediating a broad variety of inflammatory responses and member of the tumor necrosis factor receptor family), and the anti-apoptotic Bcl-2 protein.

Figure 2

Experimental results (Figure 2) showed that compounds **4c**, **5**, **13** and **15** were able to down-regulate the expression of Bcl-2 and up-regulate the expression levels of IL-2, IL-6 and CD40 in HepG2 cells compared to untreated cells. Interestingly, compound **5** increased the expression levels of IL-2 by 1.5-fold compared to the untreated control; whereas, compounds **4c**, **5**, and **13** modulated the IL-6 level at most a 2.2-fold increase in expression when compared to the untreated control cells. Furthermore, compound **15** was able to down-regulate, by 55%, the expression levels of Bcl-2 compared to the untreated cells. Finally, compounds **13** and **15** demonstrated a 1.3-fold increase in the expression level of CD40 compared to the untreated cells.

2.2.3. Assessment of the antioxidant profiles of the organic selenides

Recently, redox modulators have gained great interest due to their chemotherapeutic potential not only as anticancer candidates but also as chemopreventive agents [56, 57]. The behaviour of such compounds depends on their surrounding environment, as they may function as pro-oxidant catalysts in cells with diminished antioxidant capacity or as antioxidants in normal cells [58-60]. Among these agents, organic selenides have been reported to effectively

and selectively attack the disturbed intracellular redox balance in different microorganisms and various tumours rich in ROS [18, 61-63]. Fortuitously, organic selenides are generally good nucleophiles, which has led to the rational design of several synthetic organic selenides as antioxidant agents [64].

2.2.3.1. DPPH and ABTS radical scavenging assays

There are various methods used for the evaluation of the antioxidant properties of organic compounds; however, the ABTS and DPPH assays are considered rapid tools to evaluate the radical-scavenging activities of natural products and foods as well as several organic selenides [65-69].

The antioxidant activity of a compound is assessed by its ability to decolorize the $DPPH^{\cdot}$ and $ABTS^{\cdot}$ radicals, and the corresponding radical-scavenging activity is estimated by the decrease in the absorbance at 517 and 734 nm, respectively. Vitamin C was used as the positive control (Table 2).

Table 2

As depicted in Table 1, compounds **7a**, **7b**, **11**, **12** and **16** were the most active organic selenides in both assays, manifesting good free radical-scavenging activity comparable to that of vitamin C. This result was not surprisingly, as all of these compounds possess only the selenium redox functionality that might be responsible for the antioxidant activity.

There is, of course, also the chance that such agents may possess pro-oxidant activity. Therefore, the bleomycin-induced DNA damage assay was used to evaluate the pro-oxidant activity of the synthesized compounds. This assay has been routinely used as a preliminary method to estimate the potential pro-oxidant nature of drugs and antineoplastic agents [70, 71]. Bleomycin is a glycopeptide antibiotic that complexes with dioxygen and divalent metal ions (mainly Fe^{2+}), generating free radicals [70]. In the modified assay used [72, 73], an increase in the optical density indicates that more bleomycin-Fe^{3+} molecules are converted into bleomycin-Fe^{2+}, which induces DNA damage, supporting the idea of the pro-oxidant activity of these compounds, and vice versa. As shown in Table 2, most of the compounds did not show any remarkable pro-oxidant activity except for compound **8**, which induced DNA degradation significantly more than the other investigated compounds (Table 2).

2.2.3.2. Glutathione peroxidase-like activity assay

Because organic selenides manifest an antioxidant mode-of-action like that of the glutathione peroxidase (GPx) human selenoenzyme, the GPx-like activity of the synthesized candidates was further estimated by employing the NADPH-reductase coupled assay [74-77]. Spectrophotometrically, the GPx activity of the synthesized compounds was estimated by the decrease in absorbance (340 nm) due to the oxidation of NADPH to $NADP^+$, and ebselen was used as the positive control (Figure 3).

Figure 3

As shown in Figure 2, organoselenium compounds **5**, **6**, **11**, **12**, **13**, **16** and **7b** were the most active in this assay, showing comparable activity to that of ebselen. The rest of the compounds showed good to moderate GPx-like activity.

3. Conclusion

A novel library of organoselenium compounds has been prepared with comparable ease using a plenty of readily available substrates, and the resulting organic selenides were obtained in good to excellent yields. The antiproliferative activity of the compounds was evaluated in two cancer cell lines, Hep G2 and MCF-7. The selective cytotoxicity of the synthesized compounds was evaluated using normal WI-38 cells.

In general, most of the organoselenium compounds exhibit good cytotoxicity, and this cytotoxic effect was more pronounced in HepG2 cells than in MCF-7 cells. In the case of HepG2, selenonapthoquinone **5** and azoic selenocyanates **14** and **15** were found to be the most cytotoxic with IC_{50} values $\leq$ 1 µM. In the case of MCF-7 cells, selenopropionitrile **7a** and selenopropanoate **7b** were the most cytotoxic ones with IC_{50} values $\leq$10 µM. Interestingly, a promising selectivity was observed when comparing the cytotoxicity in HepG2 and MCF-7 cells with that in normal WI-38 cells. In this context, most non-heterocyclic organic selenides (**5**, **4c**, **7a**, **12**, **13**, **14**, **15**, and **16)** were highly selective for HepG2 cells with SI values of above 14 and up to useful 76 times. On the other hand, selectivity for MCF-7 cells was generally lower than that for HepG2. Such a differential selectivity pattern is quite interesting and is a strong hint that general selenium toxicity is not the governing factor of the observed cytotoxic effects.

We also showed that organic selenides can induce apoptosis in HepG2 cells by potentially modulation the expression of the IL-2 and IL-6 pro-inflammatory cytokines, the Bcl-2 anti-apoptotic protein, and the CD40 protein which in turn is involved in the inflammatory responses. Within this context, compounds **4c**, **5**, **13** and **15** were able to up-regulate the expression levels of IL-2, IL-6 and CD40 and down-regulate the expression of Bcl-2 and in HepG2 cells compared to control cells. This provides additional hints for the existence of alternative mode-of-action of anti-HepG2; however, the cellular and molecular mechanisms underlying the role of organic selenides in facilitating tumour cell apoptosis remain ambiguous.

Furthermore, the selenium redox centres point towards antioxidant activity. Especially compounds **7a**, **7b**, **11**, **12** and **16** exhibited interesting free radical-scavenging activity comparable to that of vitamin C. These results were in line with those obtained from the bleomycin-dependent DNA damage assay, as none of the synthesized compounds, except for compound **8**, showed any pro-oxidant activity. Of course, any such redox activity, because of the underlying general selenium toxicity above a certain recommended daily intake, is only relevant in the context of a use as adjuvants or co-treatment in a cancer therapy, and of course not as a substitute for vitamin C.

Furthermore, most of the organoselenium compounds exhibited good GPx-like activity, and again compounds also performing best as antioxidants or HepG2 cytotoxins were the most active in this assay (**5**, **6**, **11**, **12**, **13**, **16** and **7b**), showing comparable activity to that of ebselen. Finally, based on the SwissADME web interface, most of these compounds featured good pharmacokinetic parameters.

To this point, the preliminary cell-based studies and the *in vitro* redox assays point towards a selective cytotoxicity and good antioxidant activity of some of these compounds. The distinct selectivity patterns warrant more studies using a wider arsenal of primary and cancer cells. To obtain a clear QSAR, diverse structural sets of organoselenium compounds are required to determine the underlying cytotoxicity and selectivity mechanism(s) of these classes of compounds. Finally, any emerging top compounds need extended to studies including animal models to assess whether a suitable lead can be identified.

Overall, we have clearly shown that synthetic organic selenides can be tailored to achieve increased selectivity in anticancer activity. Based on the above results, we believe that even better candidates than those shown here can be developed. At this stage, it is too premature to

disclose the results of pharmacokinetic studies in animals or the degradation/accumulation of such candidates in specific tissues. Nonetheless, these cases are highly important and will form part of our future studies in appropriate animal models.

4. Experimental

4.1. Material and methods

All chemical reagents for the synthesis of compounds were purchased from Sigma-Aldrich-Fluka or Merck (AMD) and used without further purification unless stated otherwise. Reactions in inert atmosphere were carried out under argon (4.6) using standard Schlenck techniques. Silica gel 60 (Macherey-Nagel, 50-200 μm) was used for column chromatography. Unless noted otherwise, the dimensions of columns used were 2.5 cm (diameter) and 25-30 cm (height of silica gel). TLC plates (silica gel 60 F_{254}, 0.20 mm) were purchased from Merck. NMR spectroscopy*:* ^{1}H NMR spectra were recorded at 400 MHz, ^{13}C NMR spectra at 100 MHz on a Bruker DRX 500 or Avance 500 spectrometer. Chemical shifts are reported in δ (ppm), expressed relative to the solvent signal at 7.26 ppm ($CDCl_3$, ^{1}H NMR) and at 77.16 ppm ($CDCl_3$, ^{13}C NMR), as well as 3.31 ppm (^{1}H NMR, CD_3OD) and 49.00 ppm (^{13}C NMR, CD_3OD). Coupling constants (*J*) are given in Hz. MS analysis: analyses were performed using a TSQ quantum mass spectrometer equipped with an ESI source and a triple quadrupole mass detector (Thermo Finnigan). HRMS: high-resolution mass spectrometry was performed on an Accela UPLC-system (Thermo-Fisher) coupled to a linear trap-FT-Orbitrap combination (LTQ-Orbitrap), operating in positive ionization mode. These spectra indicated ≥ 99% MS-purity of the prepared compounds.

DNA (Calf Thymus type1), bleomycin sulfate, thiobarbituric acid (TBA), 1,1-diphenyl-1-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), and ascorbic acid were obtained from Sigma. All other chemicals were of analytical grade. Compound 1, 3 and 4 were synthesized according to the literature reported method [25, 28, 81].

4.2. Biological assays

4.2.1. Cytotoxicity assay

The HepG2 human liver carcinoma, breast adenocarcinoma (MCF-7) and lung fibroblast (WI-38) cell lines were purchased from American Type Culture Collection (HTB□37; Rockville,

MD, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) calf serum (Hyclone Laboratories, Ogden, UT), 60 mg/mL penicillin G and 100 mg/mL streptomycin sulfate maintained at 37 °C in a humidified atmosphere containing about 15% (v/v) CO_2 in air.

MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma) was used to measure the metabolic activity of cells which can reduce it by dehydrogenases to a violet colored formazan product. Briefly, 120 µL aliquots of a cell suspension (50,000 cells mL^{-1}) in 96-well microplates were incubated at 37 °C and 10% CO_2 and allowed to grow for two days. Then 60 µL of serial dilutions of the test compounds were added. After 48 h of incubation at 37 °C and 10% CO_2, 75 µL MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg mL^{-1}. After 2 h the precipitate of formazan crystals was centrifuged, and the supernatant discarded. The precipitate was washed three times with 100 µL PBS and dissolved in 100 µL DMSO. The resulting color was measured at 590 nm using an ELISA plate reader. All investigations were carried out in two parallel experiments. The IC_{50} values were determined as the concentrations of tested materials, which showed 50% of the absorbance of untreated control cells as estimated from the dose-response curves. 5-Fluorouracil (5-Fu) was used as a positive control.

4.2.2. Detection of Bcl-2, IL-2, IL-6 and CD40 protein expression levels.

Bcl-2, CD40, IL-2 and IL-6 levels were evaluated in HepG2 cells treated with the corresponding IC_{50}s of each compound and incubated for 48 hrs and compared with their levels in control untreated HepG2 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 enzyme-linked

immunosorbent assay (ELISA) according to the manufacturers' instructions (Biomatik, USA). Enzyme-linked immunosorbent assay was used for quantitative detection of CD40, IL-2 andIL-6 (ebioscience).

4.2.3. DPPH free radical scavenging activity

The hydrogen atom or electron donation ability of the corresponding compounds was measured by estimating the bleaching of the purple color of a methanolic solution of DPPH. This spectrophotometric assay uses stable $DPPH^{\cdot}$ as a reagent. The sample was prepared by adding 200 µL of the organic selenides (1 µM in methanol) to 400 µl DPPH in methanol. After 30 min of incubation in the dark, the absorbance was read against a blank at 517 nm. Ascorbic acid (vitamin C) and ebselen were used as a standard antioxidant (positive control). A blank sample was run without DPPH. A negative control sample was run using methanol instead of the sample. The radical scavenging activity was calculated using the following equation: $I\% = (A_{blank}-A_{sample})/(A_{blank})x100$

4.2.4. Bleomycin-dependent DNA damage

The assay was performed according to the reported method with minor modifications [29, 32, 33, 36]. The reaction mixture contained 0.5 mg/ml calf thymus DNA, 0.05 mg/ml bleomycin sulfate, 5 mM magnesium chloride, 50 mM ferric chloride, 2 mM tested compound. L-ascorbic acid (2 mM) was used as a positive control. The mixture was incubated at 37 $^{\circ}$C for 1 hour. The activity of test compounds was evaluated as malondialdehyde equivalents. Thiobarbituric acid reactive substances, which arose from deoxyribose degradation of DNA were assessed. The reaction was terminated by addition of 0.05 ml 0.1 M EDTA. The color was developed by adding 0.5 ml of 1% w/v thiobarbituric acid and 0.5 ml of 25% v/v HCl (25% v/v). The tube was capped with a screw cap and heated at 80 $^{\circ}$C for 30 min. After cooling in ice water, the mixture was then shaken and centrifuged and the extent of DNA damage was measured by an increase in absorbance at wavelength 532 nm.

4.2.5. ABTS assay

The antioxidant activity of the investigated compounds was assessed using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) method. The radical cation derived from ABTS was prepared by the reaction of 60 mM ABTS solution with 0.3 M Manganese dioxide solution in 0.1 M phosphate buffer, pH 7. Then, the mixture was shaken, centrifuged, filtered, and the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was measured at wavelength 734 nm. Then, 50 µl of 1 mg/ml test compound in phosphate buffered methanol was added. The absorbance (A_{test}) was measured. The reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the following equation

$$\text{Inhibition\%} = (A_{control}-A_{testmple})/(A_{control})x100$$

Ascorbic acid (vitamin C) was used as standard anti-oxidant (positive control). A blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of the sample. A negative control sample was run with MeOH/phosphate buffer (1:1) instead of tested compound.

4.2.6. Glutathione peroxidase like activity

GPx kit (Biodiagnostic, Egypt) was used for the determination of GPx according to Paglia *et al.* [40] The reaction mixture contained 1 ml assay buffer (50 mM phosphate buffer containing 0.1 % triton X-100) and 0.1 ml NADPH reagent (24 µmol Glutathione, 12 unit Glutathione reductase and 4.8 µmol NADPH) and 0.01 ml (41 µM) tested compounds and the reaction was started by the addition of H_2O_2 (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 (A_{340nm} / min) was estimated using ebselen (41 µM) as the positive control. The values represented in figure 3 are expressed after background correction for the

reaction with H_2O_2 and GSH. In case of colored compounds, their activities were estimated after subtracting their own absorbances at the used wave length.

4.2.7. Statistical analysis.

Statistical analysis, curve fitting and graphs were performed using IBM SPSS Statistics Version 16 (SPSS Inc, Chicago) and GraphPad Prism V6.02 (GraphPad Software Inc). Data were analyzed by One-Way ANOVA and Tukey post Hoc tests. Data are given as mean ±SD of three independent experiments. P value less than 0.05 was considered statistically significant.

4.3. Synthesis and characterization

Synthesis of 2-methyl-3-bromo-l,4-naphthoquinone [32]

In 25 ml glacial acetic acid, sodium acetate (0.55 mg, 17 mmol) and 2-methyl-1,4-naphthoquinone (6.5 mg, 31.5 mmol) were mixed together. Bromine (1 ml) was then added and the mixture was lifted to settle for four days in the dark. The yellow crystals formed mass formed was removed and the supernatant liquid was poured on to 250 gm ice. The yellow precipitate formed was filtered off and recrystallized from methanol.

Synthesis of 4-selenocyanatoaniline (2)

Selenium dioxide (6 mmol) was added under stirring to a solution of malononitrile (3 mmol) in DMSO (15 ml). The mixture was stirred at room temperature for 15 min in order to obtain triselenium dicyanide. When the exothermic reaction had finished aniline (5 mmol) was added. The mixture was stirred for 20 min. Water (150 ml) was added to the reaction mixture and the resulting precipitate (4-selenocyanatobenzenamine) was filtered off, dried and used without further purifications.

Synthesis of 4-(2-(4-aminophenyl)diselanyl)benzenamine (3)

Under argon, $NaBH_4$ (3 mmol) was added in small portions with caution to a solution of 4-selenocyanatobenzenamine (1 mmol) in absolute ethanol (40 ml). The mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the remaining residue was dissolved in dichloromethane, washed with water (3x50 ml). The organic layer was

separated, dried with anhydrous Na_2SO_4 and removed under vacuum. The residue was purified by chromatography on silica gel (petroleum ether: ethyl acetate 4:2).

Synthesis of 4-methyl-5-selenocyanatothiazol-2-amine (10)

To a well stirred solution of malononitrile (40 mg, 0.6 mmol) in DMF (0.5 mL), SeO_2 (84 mg, 0.75 mmol) was added. The mixture became reddish after 5-10 min and an exothermic reaction with vigorous gas evolution began during the next 10 min. When the gas evolution was ceased the reaction mixture was filtered to remove any solids present, then aminothiazole (114 mg, 1 mmol) was added with stirring. Stirring was continued for additional 1 h at room temperature. The homogenous solution was diluted with ice-cold water, the precipitate formed was filtered off and dried to give **10**.

General procedure I: *Synthesis of organic selenides* **4a-c** and **7a-b** *via reduction of* **3** *and subsequent* nucleophilic *substitution or Michael addition type reactions*

Compound **3** (1 mmol) and appropriate halo/activated olefin derivatives (2.2 mmol) were dissolved in EtOH (15 ml). $NaBH_4$ (113.49 mg, 3 mmol) was added portion wise over 1h. Then the reaction was stirred for additional 5 hrs. The organic layer was dried and evaporated under vacuum. The residue was purified by silica gel chromatography.

General procedure II: *Synthesis of organic selenides* **5** and **6** *via reduction of* **3** *and subsequent* nucleophilic *substitution reaction*

Compound **3** (1 mmol) and appropriate halo quinone derivative (2.2 mmol) were dissolved in EtOAc (20 ml). Water (20 ml) and tricaprylmethylammonium chloride (45 mg, 5% mol) were then added. $NaBH_4$ (189.15 mg, 5 mmol) was added portion wise over 1h. Then the reaction was stirred for additional 3 hrs. The organic layer was dried and evaporated under vacuum. The residue was purified by silica gel chromatography.

General procedure III: *Synthesis of organic selenide* **8** *via reaction with malonyl chloride*

To a solution of **2** (1 mmol) in dry benzene (15 mL), malonyl chloride (0.5 mmol) was added and the mixture was stirred at room temperature for 3h. The formed precipitate was collected and

further washed with benzene and dried under vacuum. The purity of compound **8** was satisfactory for further use. If necessary, further purification using silica gel chromatography was performed.

General procedure IV: *Synthesis of organic selenides* **11** and **12** *via reduction of* **10** *and subsequent* nucleophilic *substitution or Michael addition type reactions*

Compound **10** (1 mmol) and appropriate halo/activated olefin derivatives (2.2 mmol) were dissolved in EtOH (15 ml). $NaBH_4$ (113.49 mg, 2.5 mmol) was added portion wise over 1h. Then the reaction was stirred for additional 5 hrs. The organic layer was dried and evaporated under vacuum. The residue was purified by silica gel chromatography.

General procedure V: *Synthesis of organic selenocyanate azoic dyes* **16**, **17**, **18** and **20**.

4-aminophenylselenocyanate (**2**) (1.97g, 0.01 mol) was dissolved in aqueous acetic acid (8 ml, 1:1), cooled to 0-5°C, then added a cold solution of sodium nitrite (0.7g, 10 mmol, in 3 ml water), while maintaining the temperature at 0-5°C. The formed diazonium salt solution was added dropwise to a cooled and stirred mixture of active methylene/aromatic compounds (0.01 mol) and sodium acetate (2.0g), dissolved in (10 ml of 50% aqueous ethanol). Stirring was continued for 1.5 h. The resulting crystals were collected, washed with water, and purified by silica gel chromatography.

2-((4-Aminophenyl)selanyl)acetonitrile (4a)

2-((4-Aminophenyl)selanyl)acetonitrile was synthesized from 4-(2-(4-aminophenyl)diselanyl)benzenamine **3** (170 mg, 0.5 mmol), $ClCH_2CN$ (69.5 µl, 1.1 mmol), and $NaBH_4$ (57 mg, 1.5 mmol) in ethanol (10 ml). The product formation was followed by TLC petroleum ether: EtOAc= 8:2, R_f = 0.36. Yellow oil; Yield: 113 mg (53%). ^{1}H NMR (400 MHz, DMSO) δ 7.40 – 7.29 (m, 2H, Ar-H), 6.64 – 6.55 (m, 2H, Ar-H), 5.45 (s, 2H, NH_2), 3.67 – 3.62 (s, 2H, $SeCH_2$). ^{13}C NMR (100 MHz, DMSO) δ 149.94, 136.71, 119.10, 114.72, 110.66, 8.65. MS (ESI): *m/z* = found 212.20 [M^++1], 235.40 [M^++Na]; calcd. 211.98 [M^+].

4-(Benzylselanyl)aniline (4b)

4-(Benzylselanyl)aniline was synthesized from 4-(2-(4-aminophenyl)diselanyl)benzenamine **3** (170 mg, 0.5 mmol), $PhCH_2Cl$ (126.5 µl, 1.1 mmol), Starks' catalyst (22.5 mg, 2.5% mol) and $NaBH_4$ (57 mg, 1.5 mmol). The product formation was followed by TLC petroleum ether: EtOAc= 12:2, R_f = 0.36, purified by column silica gel chromatography with petroleum ether: EtOAc = 6:1.5. Colorless oil; Yield: 224 mg (85%).^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 – 7.18 (m, 2H, Ar-H), 7.14 – 7.08 (m, 2H, Ar-H), 6.94 – 6.81

(m, 2H, Ar-H), 6.50 – 6.39 (m, 2H, Ar-H), 3.85 (s, 2H, $SeCH_2$), 3.62 (s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.68, 138.65, 137.03, 131.32, 130.48, 120.33, 116.48, 115.63, 32.51; MS (ESI): *m/z* = found 263.85 [M^++1]; calcd. 263.02 [M^+]; HRMS calcd. for $C_{13}H_{13}NSe$ [M^++1]: 264.02860, found 264.02748 [M^++1].

4-((2,2-Diethoxyethyl)selanyl)aniline (4c)

4-((2,2-Diethoxyethyl)selanyl)aniline was synthesized from 4-(2-(4-aminophenyl)diselanyl)benzenamine **3** (170 mg, 0.5 mmol), 2-bromo-1,1-diethoxyethane (166 µl, 1.1 mmol), Starks' catalyst (22.5 mg, 2.5% mol) and $NaBH_4$ (57 mg, 1.5 mmol). The product formation was followed by TLC petroleum ether: EtOAc= 12:2, R_f= 0.39, purified by column silica gel chromatography with petroleum ether: EtOAc= 6:1.5. Colorless oil; Yield: 257 mg (89%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 – 7.34 (d, *J* = 7.37 HZ, 2H, Ar-H), 6.62 – 6.55 (d, *J* = 6.58, 2H, Ar-H), 4.66 (t, *J* = 5.7 Hz, 1H, CH), 3.67 – 3.46 (m, 4H, $2CH_2$), 3.03 (d, *J* = 5.7 Hz, 2H, $SeCH_2$), 1.19 (t, *J* = 7.1 Hz, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.17, 135.90, 117.11, 115.70, 102.30, 61.70, 32.23, 15.23; MS (ESI): *m/z* = found 312.04 [M^++Na]; calcd. 289.06 [M^+]; HRMS calcd. for $C_{12}H_{19}NO_2Se$ [M^++Na]: 312.0498, found 312.04597 [M^++Na].

2-((4-Aminophenyl)selanyl)-3-methylnaphthalene-1,4-dione (5)

2-((4-Aminophenyl)selanyl)-3-methylnaphthalene-1,4-dione was synthesized from 4-(2-(4-aminophenyl)diselanyl)benzenamine **3** (170 mg, 0.5 mmol), 2-methyl-3-bromo-l,4-naphthoquinone (275 mg, 1.1 mmol), Starks' catalyst (Aliquat 336) (22.5 mg, 2.5% mol) and $NaBH_4$ (57 mg, 1.5 mmol). The product formation was followed by TLC petroleum ether: EtOAc= 8:3, R_f = 0.32, purified by column silica gel chromatography with petroleum ether: EtOAc= 3:1. Brown solid; Yield: 305.50 mg (89%); mp 162-164 ^oC. ^1H NMR (300 MHz, DMSO) δ 7.97 (ddt, *J* = 11.8, 6.9, 3.4 Hz, 2H, Ar-H), 7.87 – 7.77 (m, 2H, Ar-H), 7.28 – 7.19 (m, 2H, Ar-H), 6.55 – 6.44 (m, 2H, Ar-H), 5.39 (s, 2H, NH_2), 1.98 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.84, 181.73, 149.10, 147.31, 147.24, 135.69, 134.08, 133.77, 131.69, 131.65, 126.34, 126.27, 114.65, 111.92, 16.50; MS (ESI): *m/z* = found 344.95 [M^++1], 366.20 [M^++Na]; calcd. 343.01 [M^+]; HRMS calcd. for $C_{17}H_{13}NO_2Se$ [M^++Na]: 366.00037, found 365.99930 [M^++Na].

2,3-bis((4-Aminophenyl)selanyl)naphthalene-1,4-dione (6)

Compound **6** was prepared from compound **3** (344 mg, 1 mmol), 2,3-dichloronaphthalene-1,4-dione (226 mg, 1 mmol), Starks' catalyst (45 mg, 5% mol) and sodium tetrahydridoborate (189.15 mg, 5 mmol). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1.5, R_f = 0.28, purified by column silica gel chromatography with petroleum ether: EtOAc= 3:1. Brown solid; Yield: 610.50 mg (78%).^1H NMR (400 MHz, DMSO) δ 7.96 (m, 3H, Ar-H), 7.84 (m, 3H, Ar-H), 7.29 (m, 3H, Ar-H), 6.52 (m, 3H, Ar-H), 3.36 (s, 4H, $2NH_2$). ^{13}C NMR (100 MHz, DMSO) δ 180.06, 175.00, 149.62, 148.64, 142.37, 136.48, 134.23, 131.90, 131.10, 126.82, 114.47, 111.04. MS (ESI): *m/z* = found 522.40 [M^++Na]; calcd. 499.95 [M^+].

3-((4-Aminophenyl)selanyl)propanenitrile (7a)

Compound **7a** was prepared from compound **3** (340 mg, 1 mmol), 3-chloropropanenitrile (197 mg, 2.2 mmol), and sodium borohydride (113.49 mg, 3 mmol) in EtOH (15 ml). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.32. White powder; Yield: 357 mg (79%). ^1H NMR (400 MHz, DMSO) δ 7.26 – 7.22 (m, 2H, Ar-H), 6.56 – 6.52 (m, 2H, Ar-H), 5.29 (s, 2H, NH_2), 2.88 (t, *J* = 7.49 Hz, 2H, CH_2), 2.75 – 2.70 (t, *J* = 6.84 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ 149.06, 136.09, 134.21, 119.77, 114.74, 110.94, 22.60, 18.17. MS (ESI): *m/z* = found 248.60 [M^++Na]; calcd. 226.09 [M^+]; HRMS calcd. for $C_9H_{10}N_2Se$ [M^++Na]: 226.0009, found 248.9901 [M^++Na].

Methyl 3-((4-aminophenyl)selanyl)propanoate (7b)

Compound **7b** was prepared from compound **3** (340 mg, 1 mmol), methyl 3-chloropropanoate (269 µl, 2.2 mmol), and sodium borohydride (113.49 mg, 3 mmol) in EtOH (15 ml). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.31. Yellow oil; Yield: 404 mg (78%). ^1H NMR (400 MHz, DMSO) δ 7.21 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.53 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.24 (s, 2H, NH_2), 3.58 (s, 3H, CH_3), 2.86 (t, *J* = 7.1 Hz, 2H, CH_2), 2.62 (t, *J* = 7.2 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO) δ 172.11, 148.78, 135.95, 114.69, 111.95, 51.37, 34.57, 22.71. MS (ESI): *m/z* = found 282.10 [M^++Na]; calcd. 259.01 [M^+]; HRMS calcd. for $C_{10}H_{13}NO_2Se$ [M^++Na]: 259.0112, found 260.0184 [M^++1].

N1,N3-bis(4-Selenocyanatophenyl)malonamide (8)

Compound **8** was prepared from compound **2** (197 mg, 1 mmol), and malonyl chloride (49 µl, 0.5 mmol) in benzene (15 ml). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.34. White powder; Yield: 361 mg (78%). ^{1}H NMR (400 MHz, DMSO) δ 10.43 (s, 1H, NH), 7.69 (m, 8H, Ar-H), 3.53 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 165.64, 140.16, 134.86, 120.38, 116.73, 105.35, 46.03. MS (ESI): *m/z* = found 463.20 [M^+]; calcd. 463.92 [M^+]; HRMS calcd. for $C_{17}H_{12}N_4O_2Se_2$ [M^++Na]: 463.9291, found 464.9219 [M^++1].

4-Methyl-5-selenocyanatothiazol-2-amine (10)

Compound **10** was prepared from malononitrile (40 mg, 0.6 mmol) in DMF (0.5 mL), SeO_2 (84 mg, 0.75 mmol), and 4-methylthiazol-2-amine (114 mg, 1 mmol). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 3:1, R_f = 0.32. Reddish brown powder; Yield: 186.0 mg (85%). ^{1}H NMR (400 MHz, DMSO) δ 5.75 (s, 2H, NH_2), 1.99 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 167.81, 147.59, 105.20, 100.35, 17.14. MS (ESI): *m/z* = found 259.20 [M^++1+K]; calcd. 218.93 [M^+].

2-((2-Amino-4-methylthiazol-5-yl)selanyl)acetonitrile (11)

Compound **11** was prepared from compound **10** (218 mg, 1 mmol), chloroacetonitrile (70 µl, 1.1 mmol) and sodium borohydride (95 mg, 2.5 mmol) in EtOH (15 ml). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.35. Red powder; Yield: 100 mg (43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18 (s, 2H, NH_2), 3.23 (s, 2H, CH_2), 2.43 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.67, 158.42, 117.14, 98.25, 16.43, 9.81. MS (ESI): *m/z* = found 256.00 [M^++Na]; calcd. 232.95 [M^+]; HRMS calcd. for $C_6H_7N_3SSe$ [M^+]: 232.9526, found 455.941 [M^++Na].

Methyl 3-((2-amino-4-methylthiazol-5-yl)selanyl)propanoate (12)

Compound **12** was prepared from compound **10** (218 mg, 1 mmol), methyl 3-chloropropanoate (122 µl, 1.1 mmol) and sodium borohydride (95 mg, 2.5 mmol) in EtOH (15 ml). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.35. Red powder; Yield: 243 mg (87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (s, 2H, NH_2), 3.69 (s, 3H, OCH_3), 2.85 (t, *J* = 7.4 Hz, 2H, CH_2), 2.71 (t, *J* = 8.8 Hz, 2H, CH_2), 2.30 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.49, 169.83, 155.31, 99.87, 51.78, 34.79, 24.22, 16.26. MS (ESI): *m/z* = found 281.10 [M^++1]; calcd. 279.978 [M^+]; HRMS calcd. for $C_8H_{12}N_2O_2SSe$ [M^+]: 279.978, found 280.985 [M^++1].

4-((4-Selenocyanatophenyl)diazenyl)phenol (13)

Compound **13** was prepared from compound **2** (1.97g, 0.01 mol), phenol (0.94 ml, 0.01 mmol), and sodium nitrite (0.7g, 10 mmol, in 3 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.39. Yellowish powder; Yield: 2.5 g (83%). ^{1}H NMR (400 MHz, DMSO) δ 7.91 – 7.72 (m, 6H, Ar-H), 7.52 (s, 1H, OH), 7.00 – 6.92 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 161.36, 152.11, 145.15, 133.87, 126.32, 125.19, 123.37, 115.99, 105.09. MS (ESI): *m/z* = found 302.00 [M^+]; calcd. 302.991 [M^+]; HRMS calcd. for $C_{13}H_9N_3OSe$ [M^+]: 302.991, found 303.984 [M^++1].

Ethyl 3-oxo-2-((4-selenocyanatophenyl)diazenyl)butanoate (14)

Compound **14** was prepared from compound **2** (1.97g, 0.01 mol), ethyl acetoacetate (1.28 ml, 0.01 mmol), and sodium nitrite (0.7g, 10 mmol, in 3 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.36. Yellowish powder; Yield: 2.7 g (82%). ^{1}H NMR (400 MHz, DMSO) δ 11.55 (s, 1H, NH tautomer), 7.75 – 7.69 (m, 2H, Ar-H), 7.50 (m, 2H, Ar-H), 4.36 – 4.28 (q, *J* = 7.37 Hz, 2H, CH_2), 3.35 (s, 1H, CH), 2.39 (s, 3H, $COCH_3$), 1.28 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO) δ 193.74, 162.32, 143.61, 135.36, 132.60, 116.62, 116.40, 61.28, 25.30, 13.85. MS (ESI): *m/z* = found 362.30 [M^++Na]; calcd. 339.01 [M^+]; HRMS calcd. for $C_{13}H_{13}N_3O_3Se$ [M^+]: 339.991, found 338.004 [M^+-H].

2-Amino-5-((4-selenocyanatophenyl)diazenyl)benzoic acid (15)

Compound **15** was prepared from compound **2** (1.97g, 0.01 mol), anthranilic acid (1.37 ml, 0.01 mmol), and sodium nitrite (0.7g, 10 mmol, in 3 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.30. Yellowish powder; Yield: 2.86 g (83%). ^{1}H NMR (400 MHz, DMSO) δ 7.96 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.87 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.77 (dd, *J* = 13.2, 6.6

Hz, 2H, Ar-H), 7.67 – 7.61 (m, 2H, Ar-H), 7.18 (t, *J* = 7.5 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 168.94, 143.22, 136.40, 135.11, 134.55, 133.97, 131.18, 122.77, 122.27, 114.92, 105.23. MS (ESI): *m/z* = found 369.1 [M^++Na]; calcd. 345.99 [M^+]; HRMS calcd. for $C_{14}H_{10}N_4O_2Se$ [M^+]: 345.996, found 344.989 [M^+-H].

4,4'-((Diselanediylbis(4,1-phenylene))bis(diazene-2,1-diyl))diphenol (16)

Compound **16** was prepared from compound **3** (3.43 g, 0.01 mol), phenol (1.88 ml, 0.02 mmol), and sodium nitrite (1.4g, 20 mmol, in 6 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.39. Yellowish powder; Yield: 5.08 g (92%). ^{1}H NMR (400 MHz, DMSO) δ 10.49 (s, 2H, 2OH), 7.89 – 7.71 (m, 12H, Ar-H), 6.95 (t, *J* = 6.0 Hz, 4H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 151.50, 145.20, 134.80, 133.34, 132.68, 131.57, 124.98, 122.98, 115.91. MS (ESI): *m/z* = foun 553.2 [M^+]; calcd. 553.97 [M^+]; HRMS calcd. for $C_{24}H_{18}N_4O_2Se_2$ [M^+]: 553.976, found 552.968 [M^+-H].

Diethyl 2,2'-((diselanediylbis(4,1-phenylene))bis(diazene-2,1-diyl))bis(3-oxobutanoate) (17)

Compound **17** was prepared from compound **3** (3.43 g, 0.01 mol), ethyl acetoacetate (2.56 ml, 0.02 mmol), and sodium nitrite (1.4g, 20 mmol, in 6 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.37. Yellowish powder; Yield: 5.6 g (91%). ^{1}H NMR (400 MHz, DMSO) δ 11.58 (s, 2H, NH tautomer), 7.69 – 7.58 (m, 4H, Ar-H), 7.45 – 7.38 (m, 4H, Ar-H), 4.31 (q, *J* 7.09 Hz, 4H, 2CH_2), 3.36 (s, 2H, 2CH), 2.43 (s, 6H, 2$COCH_3$), 1.28 (t, *J* = 9.2, 5.1 Hz, 6H, 2CH_3). ^{13}C NMR (100 MHz, DMSO) δ 193.68, 162.43, 142.70, 133.86, 124.20, 116.00, 61.22, 54.90, 25.40, 13.88. MS (ESI): *m/z* = foun 649.4 [M^++Na]; calcd. 626.01 [M^+]; HRMS calcd. for $C_{24}H_{26}N_4O_6Se_2$ [M^+]: 626.018, found 649.007 [M^++Na].

5,5'-((Diselanediylbis(4,1-phenylene))bis(diazene-2,1-diyl))bis(2-aminobenzoic acid) (18)

Compound **18** was prepared from compound **3** (3.43 g, 0.01 mol), anthranilic acid (2.74 ml, 0.02 mmol), and sodium nitrite (1.4g, 20 mmol, in 6 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.30. Yellowish powder; Yield: 5.94 g (93%). ^{1}H NMR (400 MHz, DMSO) δ 12.71 (s, 2H, COOH), 8.99 (s, *2*H, NH tautomer), 8.29 – 8.24 (m, 3H, Ar-H), 8.22 – 8.17 (m, 3H, Ar-H), 8.03 (d, *J* = 7.1 Hz, 2H, Ar-H), 7.95 (dd, *J* = 9.6, 5.8 Hz, 6H, Ar-H), 4.59 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO) δ 182.13, 166.26, 135.79, 134.80, 133.93, 133.61, 132.28, 127.10, 126.50, 125.25, 122.57. MS (ESI): *m/z* = foun 703.60 [M^++Na+K]; calcd. 639.98 [M^+].

4-Phenyl-5-((4-selenocyanatophenyl)diazenyl)thiazol-2-amine (19)

Compound **19** was prepared from compound **2** (1.97g, 0.01 mol), 4-phenylthiazol-2-amine (1.76 ml, 0.01 mmol), and sodium nitrite (0.7g, 10 mmol, in 3 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.32. Yellowish powder; Yield: 2.86 g (86%). ^{1}H NMR (400 MHz, DMSO) δ 8.59 (s, 1H, NH), 8.22 (dd, *J* = 8.0, 1.3 Hz, 2H, Ar-H), 7.80 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.67 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.51 (dt, *J* = 6.3, 4.4 Hz, 3H, Ar-H). ^{13}C NMR (101 MHz, DMSO) δ 170.13, 157.00, 152.76, 139.60, 134.14, 133.43, 130.10, 129.85, 128.47, 123.65, 122.90, 105.12. MS (ESI): *m/z* = found 384.00 [M^+]; calcd. 384.99 [M^+].

5,5'-((Diselanediylbis(4,1-phenylene))bis(diazene-2,1-diyl))bis(4-phenylthiazol-2-amine) (20)

Compound **20** was prepared from compound **3** (3.43 g, 0.01 mol), 4-phenylthiazol-2-amine (3.52 ml, 0.02 mmol), and sodium nitrite (1.4g, 20 mmol, in 6 ml water). The progress of the product formation was followed by TLC petroleum ether: EtOAc= 4:1, R_f = 0.29. Yellowish powder; Yield: 6.82 g (95%). ^{1}H NMR (400 MHz, DMSO) δ 8.49 (s, H, NH), 8.22 – 8.19 (m, 4H, Ar-H), 7.76 (d, *J* = 8.6 Hz, 4H, Ar-H), 7.60 (d, *J* = 8.6 Hz, 4H, Ar-H), 7.53 – 7.45 (m, 6H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 175.66, 169.83, 156.08, 152.07, 139.77, 133.51, 132.00, 130.01, 129.68, 128.45, 122.50. MS (ESI): *m/z* = found 742.50 [M^++Na]; calcd. 717.973 [M^+].

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References

[1] Johannesson, T., Kristinsson, J., Snaedal, J., Neurodegenerative diseases, antioxidative enzymes and copper. A review of experimental research. *Laeknabladid*, **2003**, *89*, (9), 659-671.

[2] Perry, G., Raina, A. K., Nunomura, A., Wataya, T., Sayre, L. M., Smith, M. A., How important is oxidative damage? Lessons from Alzheimer's disease. *Free Radic Biol Med*, **2000**, *28*, (5), 831-834.

[3] Pisoschi, A.M., Pop, A., The role of antioxidants in the chemistry of oxidative stress. *Eur J Med Chem*, **2015**, *97*, 55-74.

[4] Bruckdorfer, K.R., Antioxidants and CVD. *Proc Nutr Soc*, **2008**, *67*, (2), 214-222.

[5] Wang, J., Yi, J., Cancer cell killing via ROS: to increase or decrease, that is the question. *Cancer Biol Ther*, **2008**, *7*, (12), 1875-1884.

[6] Laurent, A., Nicco, C., Chereau, C., Goulvestre, C., Alexandre, J., Alves, A., Levy, E., Goldwasser, F., Panis, Y., Soubrane, O., Weill, B., Batteux, F., Controlling tumor growth by modulating endogenous production of reactive oxygen species. *Cancer Res.*, **2005**, *65*, (3), 948-956.

[7] Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*, **2007**, *39*, (1), 44-84.

[8] De Souza, D., Mariano, D. O., Nedel, F., Schultze, E., Campos, V. F., Seixas, F., da Silva, R. S., Munchen, T. S., Ilha, V., Dornelles, L., Braga, A. L., Rocha, J. B., Collares, T., Rodrigues, O. E., New organochalcogen multitarget drug: synthesis and antioxidant and antitumoral activities of chalcogenozidovudine derivatives. *J Med Chem*, **2015**, *58*, (8), 3329-3339.

[9] Seo, J.Y., Lee, C. H., Cho, J. H., Choi, J. H., Yoo, K. Y., Kim, D. W., Park, O. K., Li, H., Choi, S. Y., Hwang, I. K., Won, M. H., Neuroprotection of ebselen against ischemia/reperfusion injury involves GABA shunt enzymes. *J Neurol Sci*, **2009**, *285*, (1-2), 88-94.

[10] Posser, T., Franco, J. L., dos Santos, D. A., Rigon, A. P., Farina, M., Dafre, A. L., Teixeira Rocha, J. B., Leal, R. B., Diphenyl diselenide confers neuroprotection against hydrogen peroxide toxicity in hippocampal slices. *Brain Res*, **2008**, *1199*, 138-147.

[11] Salom, J.B., Perez-Asensio, F. J., Burguete, M. C., Marin, N., Pitarch, C., Torregrosa, G., Romero, F. J., Alborch, E., Single-dose ebselen does not afford sustained neuroprotection to rats subjected to severe focal cerebral ischemia. *Eur J Pharmacol*, **2004**, *495*, (1), 55-62.

[12] Kieliszek, M., Błażejak, S.J.M., Current knowledge on the importance of selenium in food for living organisms: a review. *Molecules*, **2016**, *21*, (5), 609.

[13] Fairweather-Tait, S.J., Bao, Y., Broadley, M.R., Collings, R., Ford, D., Hesketh, J.E., Hurst, R., Selenium in human health and disease. *Antioxid Redox Signal*, **2011**, *14*, (7), 1337-1383.

[14] Boosalis, M.G., The role of selenium in chronic disease. *Nutr Clin Pract*, **2008**, *23*, (2), 152-160.
[15] Plano, D., Ramisetti, S., Kim, S.-Y., Pandey, M., Aliaga, C., Karelia, D.N., Berg, A., Sanmartin, C., Lu, J., Amin, S., Selective chemopreventive efficacy of 1,4-phenylenebis(methylene)seleno-aspirin (p-XS-Asp) towards lung cancer. *AACR Annual Meeting*, **2018**; April 14-18.
[16] Sanmartin, C., Plano, D., Sharma, A.K., Palop, J.A., Selenium compounds, apoptosis and other types of cell death: an overview for cancer therapy. *Int J Mol Sci*, **2012**, *13*, (8), 9649-9672.
[17] Plano, D., Baquedano, Y., Moreno-Mateos, D., Font, M., Jiménez-Ruiz, A., Palop, J.A., Sanmartín, C., Selenocyanates and diselenides: A new class of potent antileishmanial agents. *Eur J Med Chem*, **2011**, *46*, (8), 3315-3323.
[18] Plano, D., Sanmartin, C., Moreno, E., Prior, C., Calvo, A., Palop, J.A., Novel potent organoselenium compounds as cytotoxic agents in prostate cancer cells. B*ioorg Med Chem Lett*, **2007**, *17*, (24), 6853-6859.
[19] Rocha, J.B., Oliveira, C.S., Nogara, P.A. Toxicology and anticancer activity of synthetic organoselenium compounds. Organoselenium Compounds in Biology and Medicine, *Royal Society of Chemistry*, **2017**, pp 342-376, ISBN:978-1-78801-029-0.
[20] Fernandes, Aristi P., and Valentina Gandin. Selenium compounds as therapeutic agents in cancer. Biochim Biophys Acta, **2015**, *1850*, (8), 1642-1660.
[21] El-Bayoumy, K., Sinha, R., Richie Jr., Forms of selenium in cancer prevention. Diversity of selenium functions in health and disease, *CRC Press-Taylor Francis*, **2015**, *38*, 137, ISBN 9781482251265.
[22] Verma, P., Kunwar, A., Arai, K., Iwaoka, M., Priyadarsini, K.I.Mechanism of radioprotection by dihydroxy-1-selenolane (DHS): Effect of fatty acid conjugation and role of glutathione peroxidase (GPx). *Biochimie*, **2018**, *144*, 122-133.
[23] Verma, P., Kunwar, A., Priyadarsini, K., Iwaoka, M. Radioprotection studies by small water-soluble, glutathione peroxidise active cyclic selenium compound. Proceedings of the international conference on radiation biology: Frontiers in radiobiology-immunomodulation, countermeasures and therapeutics: *abstract book, souvenir and scientific programme*, **2014**.
[24] Shabaan, S., Ba, L. A., Abbas, M., Burkholz, T., Denkert, A., Gohr, A., Wessjohann, L. A., Sasse, F., Weber, W., Jacob, C., Multicomponent reactions for the synthesis of multifunctional agents with activity against cancer cells. *Chem Commun (Camb)*, **2009**, (31), 4702-4704.
[25] Shaaban, S., Negm, A., Sobh, M. A., Wessjohann, L. A., Expeditious Entry to Functionalized Pseudo-peptidic Organoselenide Redox Modulators via Sequential Ugi/SN Methodology. *Anticancer Agents Med Chem*, **2016**, *16*, (5), 621-632.
[26] Shaaban, S., Gaffer, H. E., Alshahd, M., Elmorsy, S. S., Cytotoxic Symmetrical Thiazolediselenides with Increased Selectivity Against MCF-7 Breast Cancer Cells. *International Journal of Research and Development in Pharmacy & Life Sciences*, **2015**, *4*, (4), 1654-1668.
[27] (a) Shaaban, S., Arafat, M. A., Gaffer, H. E., Hamama, W. S., Synthesis and anti-tumor evaluation of novel organoselenocyanates and symmetrical diselenides dyestuffs. *Der Pharma Chemica*, **2014**, *6*, (3), 186-193. (b) Hamama, W. S., Ismail, M. A, Soliman, M., Shaaban, S., Zoorob, Hanafi H., Behavior of 2□Iminothiazolidin□4□one with Different Reagents. *J Het Chem*, **2011** 48(5): 1169.

[28] Shaaban, S., Negm, A., Ashmawy, A. M., Ahmed, D. M., Wessjohann, L. A., Combinatorial synthesis, in silico, molecular and biochemical studies of tetrazole-derived organic selenides with increased selectivity against hepatocellular carcinoma. *Eur J Med Chem*, **2016**, *122*, 55-71.
[29] Shaaban, S., Vervandier-Fasseur, D., Andreoletti, P., Zarrouk, A., Richard, P., Negm, A., Manolikakes, G., Jacob, C., Cherkaoui-Malki, M. Cytoprotective and antioxidant properties of organic selenides for the myelin-forming cells, oligodendrocytes. *Bioorg Chem*, **2018**, *80*, 43-56.
[30] Mecklenburg, S., Shaaban, S., Ba, L.A., Burkholz, T., Schneider, T., Diesel, B., Kiemer, A.K., Roseler, A., Becker, K., Reichrath, J., Stark, A., Tilgen, W., Abbas, M., Wessjohann, L.A., Sasse, F., Jacob, C., Exploring synthetic avenues for the effective synthesis of selenium- and tellurium-containing multifunctional redox agents. *Org Biomol Chem*, **2009**, *7*, (22), 4753-4762.
[31] Shaaban, S., Negm, A., Ibrahim, E. E., Elrazak, A. A. Chemotherapeutic agents for the treatment of hepatocellular carcinoma: efficacy and mode of action. *Oncology reviews*, **2014**, 8(1)
[32] Cherkaoui-Malki, M., Shaaban, S., Tahri-Joutey, M., Elshobaky, A., Fatima-Ezzahra, S., Vervandier-Fasseur, D., Jacob, C., Nasser, B., Latruffe, N., Andreoletti, P. Cytoprotective and Antioxidants in Peroxisomal Neurodegenerative Diseases. *Proceedings*, **2019**, 11 (1), 33.
[33] Gaffer, H. E., Shaaban, S., Abed, N. A., Abdel-latif, E. Synthesis of some new symmetrical diselenide dyestuffs for dyeing polyester fabrics. *Pigment & Resin Technology*, **2016**, 45(6), 431-438.
[34] Shaaban, S., Gaffer, E.H., Alshahd, M., Elmorsy, S.S., Cytotoxic symmetrical thiazolediselenides with increased selectivity against mcf-7 breast cancer cells. *International Journal of Research and Development in Pharmacy & Life Sciences*, **2015**, *4*, (4), 1654-1668.
[35] Shaaban, S., Gaffer, E.H., Jabar, Y., Elmorsy, S.S., Cytotoxic naphthalene based-symmetrical diselenides with increased selectivity against MCF-7 breast cancer cells. *International Journal of Pharmacy*, **2015**, *5*, (3), 738-746.
[36] Shaaban, S., Negm, A., Sobh, M.A., Wessjohann, L.A., Organoselenocyanates and symmetrical diselenides redox modulators: Design, synthesis and biological evaluation. *European J Med Chem*, **2015**, *97*, 190-201.
[37] Shaaban, S., Arafat, M.A., Hamama, W.S., Vistas in the domain of organo selenocyanates. *Arkivoc*, **2014**, *i*, 470-505.
[38] Abdel-Wahab, B. F., Shaaban, S. Thiazolothiadiazoles and thiazolooxadiazoles: synthesis and biological applications. *Synthesis*, **2014**, 46(13), 1709-1716.
[39] Rusakov, Y.Y., Krivdin, L.B., Potapov, V.A., Penzik, M.V., Amosova, S.V., Conformational analysis and diastereotopic assignments in the series of selenium-containing heterocycles by means of 77Se-1H spin-spin coupling constants: a combined theoretical and experimental study. *Magnetic Resonance in Chemistry : MRC*, **2011**, *49*, (7), 389-398.
[40] (a) Padmavathi, V., Mahesh, K., Nagendra Mohan, A.V., Padmaja, A., Synthesis and bioassay of oxazolyl/thiazolyl selenadiazoles, thiadiazoles and diazaphospholes. *Chemical & Pharmaceutical Bulletin*, **2009**, *57*, (6), 561-566. (b) Hamama, W. S., El-Bana, G. G.,Shaaban, S., Habib, OMO., Zoorob, H. H. Advances in the domain of 4-amino-3-mercapto-1, 2, 4-triazine-5-ones. *RSC advances*, **2016**, 6(29): 24010-24049.
[41] Beharry, A.A., Woolley, G.A., Azobenzene photoswitches for biomolecules. Chem Soc Rev, **2011**, *40*, (8), 4422-4437.
[42] García, C.C., Candurra, N.A., Damonte, E.B. Differential inhibitory action of two azoic compounds against arenaviruses. Int J Antimicrob Agents, **2003**, *21*, (4), 319-324.

[43] Favre-Besse, F.-C., Poirel, O., Bersot, T., Kim-Grellier, E., Daumas, S., El Mestikawy, S., Acher, F.C., Pietrancosta, N. Design, synthesis and biological evaluation of small-azo-dyes as potent Vesicular Glutamate Transporters inhibitors. *Eur J Med Chem*, **2014**, *78*, 236-247.
[44] Shaaban, S., Sasse, F., Burkholz, T., Jacob, C., Sulfur, selenium and tellurium pseudopeptides: Synthesis and biological evaluation. *Bioorg Med Chem,* **2014**, *22*, (14), 3610-3619.
[45] De Oliveira, I.M., Degrandi, T.H., Jorge, P.M., Saffi, J., Rosa, R.M., Guecheva, T.N., Henriques, J.A., Dicholesteroyl diselenide: cytotoxicity, genotoxicity and mutagenicity in the yeast Saccharomyces cerevisiae and in Chinese hamster lung fibroblasts. *Mutation research,* **2014**, *763*, 1-11.
[46] Glaser, V., Martins Rde, P., Vieira, A.J., Oliveira Ede, M., Straliotto, M.R., Mukdsi, J.H., Torres, A.I., de Bem, A.F., Farina, M., Da Rocha, J.B., De Paul, A.L., Latini, A., Diphenyl diselenide administration enhances cortical mitochondrial number and activity by increasing hemeoxygenase type 1 content in a methylmercury-induced neurotoxicity mouse model. *Mol Aspects Med,* **2014**, *390*, (1-2), 1-8.
[47] De Oliveira, J., Moreira, E.L., Mancini, G., Hort, M.A., Latini, A., Ribeiro-do-Valle, R.M., Farina, M., da Rocha, J.B., de Bem, A.F., Diphenyl diselenide prevents cortico-cerebral mitochondrial dysfunction and oxidative stress induced by hypercholesterolemia in LDL receptor knockout mice. *Neurochem Res,* **2013**, *38*, (10), 2028-2036.
[48] Straliotto, M.R., De Oliveira, J., Mancini, G., Bainy, A.C., Latini, A., Deobald, A.M., Rocha, J.B., de Bem, A.F., Disubstituted diaryl diselenides as potential atheroprotective compounds: Involvement of TrxR and GPx-like systems. *Eur J Pharm Sci,* **2013**, *48*, (4-5), 717-725.
[49] Soriano-Garcia, M., Organoselenium compounds as potential therapeutic and chemopreventive agents: a review. *Curr Med Chem,* **2004**, *11*, (12), 1657-1669.
[50] Drake, E. N., Cancer chemoprevention: selenium as a pro-oxidant, not an antioxidant. *Medical hypotheses*, **2006**, *67*, (2), 318-322.
[51] Bruning, C.A., Prigol, M., Luchese, C., Pinton, S., Nogueira, C.W., Diphenyl diselenide ameliorates behavioral and oxidative parameters in an animal model of mania induced by ouabain. *Prog Neuropsychopharmacol Biol Psychiatry,* **2012**, *38*, (2), 168-174.
[52] Krehl, S., Loewinger, M., Florian, S., Kipp, A.P., Banning, A., Wessjohann, L.A., Brauer, M.N., Iori, R., Esworthy, R.S., Chu, F.-F., Brigelius-Flohé, R., Glutathione peroxidase-2 and selenium decreased inflammation and tumors in a mouse model of inflammation-associated carcinogenesis whereas sulforaphane effects differed with selenium supply. *Carcinogenesis,* **2012**, *33*, (3), 620-628.
[53] Nascimento, V., Alberto, E.E., Tondo, D.W., Dambrowski, D., Detty, M.R., Nome, F., Braga, A.L., GPx-Like activity of selenides and selenoxides: experimental evidence for the involvement of hydroxy perhydroxy selenane as the active species. *J Am Chem Soc,* **2012**, *134*, (1), 138-141.
[54] Shaaban, S., Diestel, R., Hinkelmann, B., Muthukumar, Y., Verma, R.P., Sasse, F., Jacob, C., Novel peptidomimetic compounds containing redox active chalcogens and quinones as potential anticancer agents. *Eur J Med Chem,* **2012**, *58*, 192-205.
[55] Badisa, R.B., Darling-Reed, S.F., Joseph, P., Cooperwood, J.S., Latinwo, L.M., Goodman, C.B., Selective cytotoxic activities of two novel synthetic drugs on human breast carcinoma MCF-7 cells. *Anticancer Research,* **2009**, *29*, (8), 2993-2996.

[56] Jamier, V., Ba, L.A., Jacob, C., Selenium□and Tellurium□Containing Multifunctional Redox Agents as Biochemical Redox Modulators with Selective Cytotoxicity. *Chemistry–A European Journal,* **2010**, *16*, (36), 10920-10928.
[57] Sredni, B., Kfar, S., Michael, A., Ramat, G., Use of tellurium containing compounds as nerve protecting agents. *US Patent Publication,* **2009**, *US 7,629,382, B2*.
[58] Fry, F.H.; Jacob, C., Sensor/effector drug design with potential relevance to cancer. *Curr Pharm Des,* **2006**, *12*, (34), 4479-4499.
[59] Fry, F.H., Holme, A.L., Giles, N.M., Giles, G.I., Collins, C., Holt, K., Pariagh, S. Gelbrich, T. Hursthouse, M.B. Gutowski, N.J. Jacob, C., Multifunctional redox catalysts as selective enhancers of oxidative stress. *Org Biomol Chem,* **2005**, *3*, (14), 2579-2587.
[60] Giles, N.M., Giles, G.I., Holley, J.E., Gutowski, N.J., Jacob, C., Targeting oxidative stress-related diseases: organochalcogen catalysts as redox sensitizers. *Biochem Pharmacol,* **2003**, *66*, (10), 2021-2028.
[61] Giles, N.M., Gutowski, N.J., Giles, G.I., Jacob, C., Redox catalysts as sensitisers towards oxidative stress. *FEBS letters,* **2003**, *535*, (1-3), 179-182.
[62] Nogueira, C.W., Zeni, G., Rocha, J.B., Organoselenium and organotellurium compounds: toxicology and pharmacology. *Chem Rev,* **2004**, *104*, (12), 6255-6285.
[63] Soriano-Garcia, M., Organoselenium compounds as potential therapeutic and chemopreventive agents: a review. *Curr Med Chem,* **2004**, *11*, (12), 1657-1669.
[64] Shaaban, S., Diestel, R., Hinkelmann, B., Muthukumar, Y., Verma, R. P., Sasse, F., Jacob, C., Novel peptidomimetic compounds containing redox active chalcogens and quinones as potential anticancer agents. *Eur J Med Chem,* **2012**, *58*, 192-205.
[65] Liang, C.P., Chang, C.H., Liang, C.C., Hung, K.Y., Hsieh, C.W., In Vitro Antioxidant Activities, Free Radical Scavenging Capacity, and Tyrosinase Inhibitory of Flavonoid Compounds and Ferulic Acid from Spiranthes sinensis (Pers.) Ames. *Molecules (Basel, Switzerland),* **2014**, *19*, (4), 4681-4694.
[66] Meriane, D., Genta-Jouve, G., Kaabeche, M., Michel, S., Boutefnouchet, S., Rapid Identification of Antioxidant Compounds of Genista saharae Coss. & Dur. by Combination of DPPH Scavenging Assay and HPTLC-MS. *Molecules (Basel, Switzerland),* **2014**, *19*, (4), 4369-4379.
[67] Uddin, R., Saha, M.R,Subhan, N., Hossain, H., Jahan, I.A., Akter, R., Alam, A., HPLC-Analysis of Polyphenolic Compounds in Gardenia jasminoides and Determination of Antioxidant Activity by Using Free Radical Scavenging Assays. *Adv Pharm Bull,* **2014**, *4*, (3), 273-281.
[68] Meriane, D., Genta-Jouve, G., Kaabeche, M., Michel, S., Boutefnouchet, S., Rapid identification of antioxidant compounds of Genista saharae Coss. & Dur. by combination of DPPH scavenging assay and HPTLC-MS. *Molecules,* **2014**, *19*, (4), 4369-4379.
[69] Tian, X., Schaich, K.M., Effects of molecular structure on kinetics and dynamics of the trolox equivalent antioxidant capacity assay with ABTS(+*). *J Agric Food Chem,* **2013**, *61*, (23), 5511-5519.
[70] Mira, A., Gimenez, E. M., Bolzan, A. D., Bianchi, M. S., Lopez-Larraza, D. M., Effect of thiol compounds on bleomycin-induced DNA and chromosome damage in human cells. *Arch Environ Occup Health,* **2013**, *68*, (2), 107-116.
[71] Wang, Q., Cui, K., Espin-Garcia, O., Cheng, D., Qiu, X., Chen, Z., Moore, M., Bristow, R. G., Xu, W., Der, S., Liu, G., Resistance to bleomycin in cancer cell lines is characterized by prolonged doubling time, reduced DNA damage and evasion of G2/M arrest and apoptosis. *PLoS One,* **2013**, *8*, (12), e82363.

[72] Aruoma, O.I., Murcia, A., Butler, J., Halliwell, B., Evaluation of the antioxidant and pro-oxidant actions of gallic acid and its derivatives. *J Agric Food Chem*, **1993**, *41*, 1880-1885.
[73] Evans, P.J., Halliwell, B., Measurement of iron and copper in biological systems: bleomycin and copper-phenanthroline assays. *Methods Enzymol*, **1994**, *233*, 82-92.
[74] Nascimento, V., Alberto, E. E., Tondo, D. W., Dambrowski, D., Detty, M. R., Nome, F., Braga, A. L., GPx-Like activity of selenides and selenoxides: experimental evidence for the involvement of hydroxy perhydroxy selenane as the active species. *J Am Chem Soc*, **2012**, *134*, (1), 138-141.
[75] Hodage, A.S., Phadnis, P. P., Wadawale, A., Priyadarsini, K. I., Jain, V. K., Synthesis, characterization and structures of 2-(3,5-dimethylpyrazol-1-yl)ethylseleno derivatives and their probable glutathione peroxidase (GPx) like activity. *Org Biomol Chem*, **2011**, *9*, (8), 2992-2998.
[76] Sarma, B.K., Manna, D., Minoura, M., Mugesh, G., Synthesis, structure, spirocyclization mechanism, and glutathione peroxidase-like antioxidant activity of stable spirodiazaselenurane and spirodiazatellurane. *J Am Chem Soc*, **2010**, *132*, (15), 5364-5374.
[77] Kumakura, F., Mishra, B., Priyadarsini, K. I., Iwaoka, M., A Water-Soluble Cyclic Selenide with Enhanced Glutathione Peroxidase-Like Catalytic Activities. *Eur J Org Chem*, **2010**, *2010*, (3), 440-445.
[78] Daina, A., Michielin, O., Zoete, V.J.S.r., SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*, **2017**, *7*, 42717.
[79] Lohidakshan, K., Rajan, M., Ganesh, A., Paul, M., Jerin, J., Pass and Swiss ADME collaborated in silico docking approach to the synthesis of certain pyrazoline spacer compounds for dihydrofolate reductase inhibition and antimalarial activity. *Bangladesh J Pharmacol*, **2018**, *13*, (1), 23-29.
[80] (a) Abdel-Wahab, B.F., Shaaban, S. and El-Hiti, G.A.,. Synthesis of sulfur-containing heterocycles via ring enlargement. *Mol Divers*, **2018**, 22(2), pp.517-542.(b) Hamama, W. S., Ismail, M. A., Shaaban, S., Zoorob. H. H. Synthesis and biological evaluation of some new Thiazolo [3, 2-a][1, 3, 5] triazine derivatives. *Med Chem Res*, **2012**, 21, 9, 2615-2623.
[81] Gouda, M.A., Sherif, Y.E. S., Elsherbini, M.S., Synthesis, Anti-Inflammatory, and Analgesic Evaluation of Some 2-Amino-5-Selenothiazoles. *Phosphorus Sulfur*, **2014**, *189*, (11), 1633-1643.

Figures

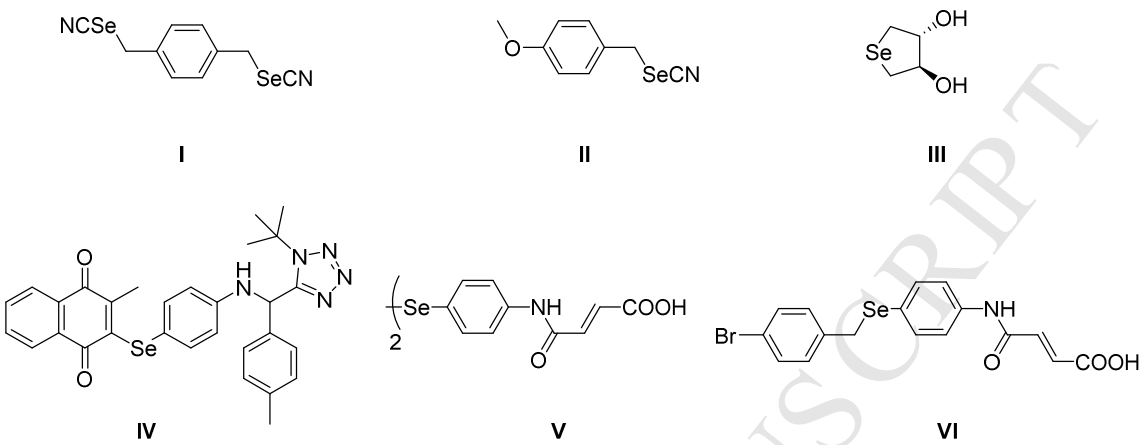


Figure 1. Chemical structures of selected chemopreventive and antioxidant organoselenium compounds.

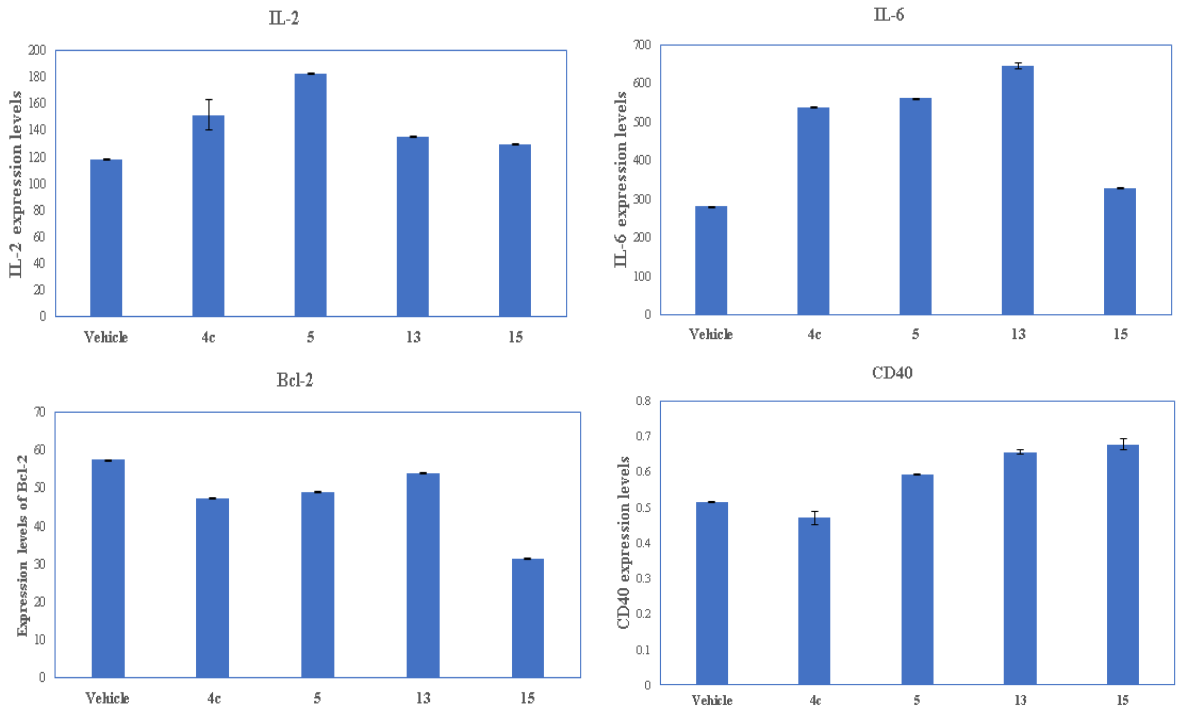


Figure 2. Expression levels of IL-2, IL-6, Bcl-2, and CD40 in HepG2 cells after 48 hrs incubation with compounds **4c**, **5**, **13** and **15** at their respective IC_{50}s compared to vehicles (untreated cells). Shown are the mean ±SD of 3 independent experiments.

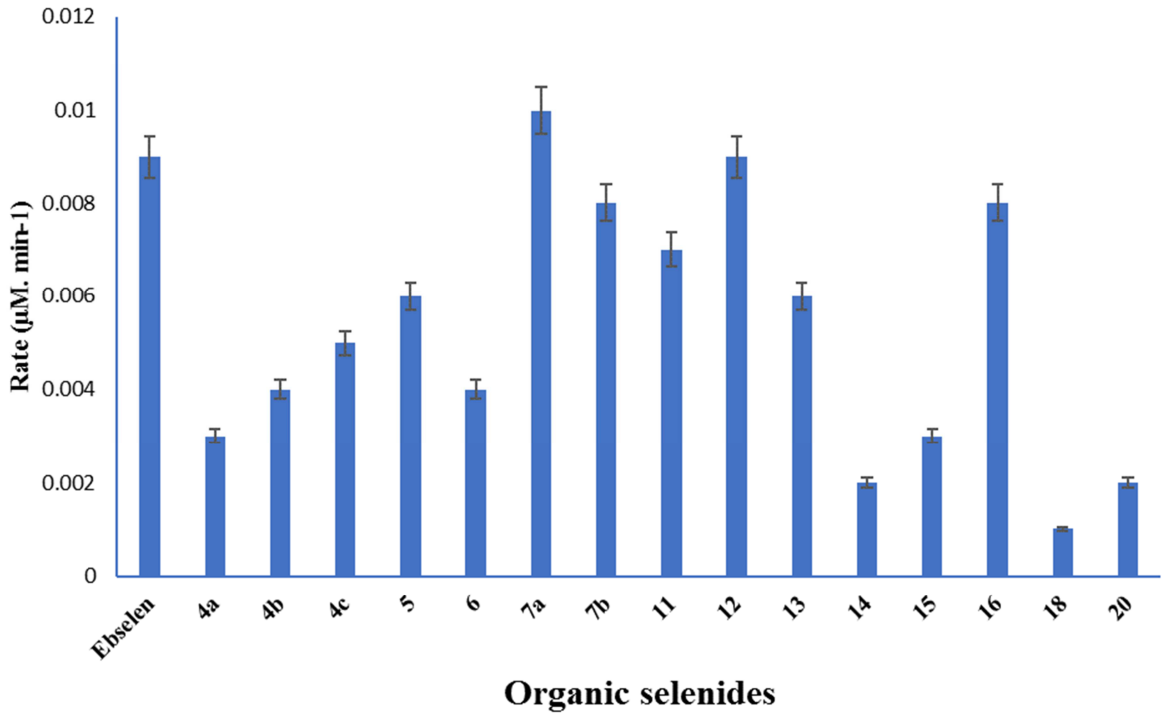
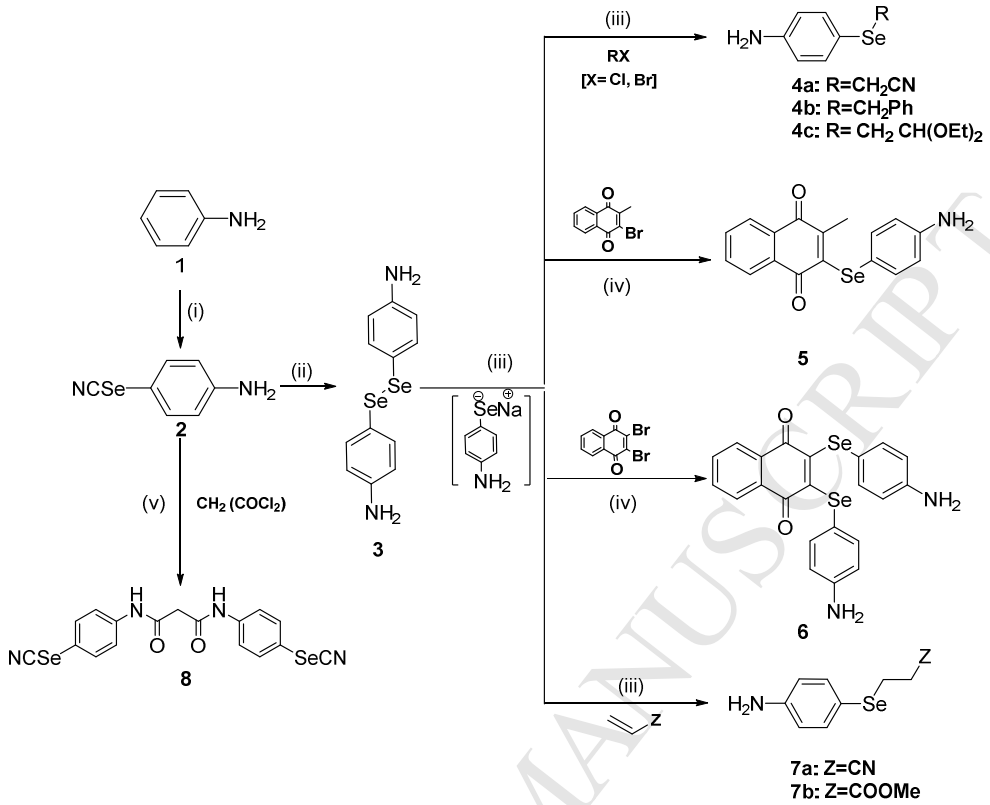
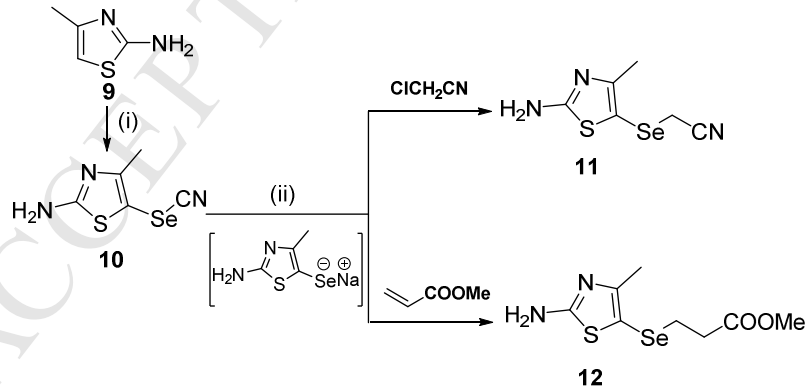


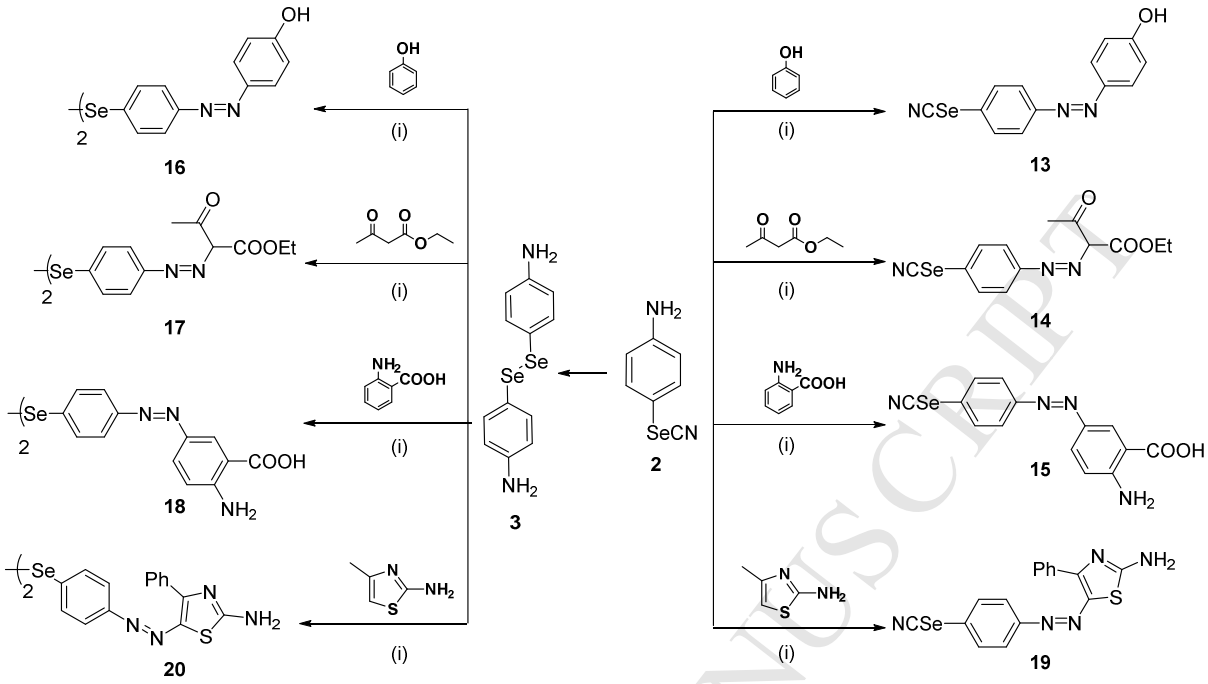
Figure 3. Results of the glutathione peroxidase-like activity assay of the investigated compounds in µM. min^{-1}. The reaction was monitored to completion, and the reaction rate was linear throughout the entire time course.



Scheme 1. Synthesis of organoselenium agents **4**-**8**. Reagents and conditions: (i) Aniline (1 mmol), malononitrile (0.6 mmol), selenium dioxide (1.2 mmol), DMSO (0.5 ml); (ii) Amine **2** (1 mmol), $NaBH_4$ (3 mmol), 2 h; (iii) Amine **3** (1 mmol), $NaBH_4$ (3 mmol), EtOH (15 ml), 6 h, rt; (iv) Amine **3** (1 mmol), AcOEt:H_2O (1:1), tricaprylmethylammonium chloride (5 mol %), $NaBH_4$ (5 mmol), 4 h, rt; (v) Amine **2** (1 mmol), malonyl chloride (0.5 mmol), benzene (15 ml).



Scheme 2. Synthesis of organic selenides **10**-**12**. Reagents and conditions: i) 4-Methylthiazol-2-amine (1 mmol), malononitrile (0.6 mmol), selenium dioxide (0.75 mmol), DMF (0.5 ml); ii) 4-Methyl-5-selenocyanatothiazol-2-amine **10** (1 mmol), $NaBH_4$ (2.5 mmol), EtOH (15 ml), 6 h, rt.



Scheme 3. Synthesis of selenocyanate- and diselenide-based azo compounds. Reagents and conditions: Amine (1 mmol), AcOH (8 mL), $NaNO_2$ (10 mmol), 0 °C.

Table 1 Effect of organoselenium compounds **4-20** on the viability of HepG2, MCF-7 and WI-38 cells and their corresponding selectivity indices.

Compounds	**HepG2**		**MCF-7**		**WI-38**
	IC_{50} (µM)[a]	SI[c]	IC_{50}(µM)[a]	SI[c]	IC_{50}(µM)[a]
5-Fu[b]	8.8±0.68	1	5.4±0.2	1	4.1±0.6
4a	9.2±1.13	4	42.8±3.6	1	34.1±2.4
4b	6.8±0.49	8	92.9±4.7	1	51.5±3.1
4c	3.1±0.37	14	27.1±2.7	2	42.6±2.9
5	0.9±0.07	76	14.7±1.6	5	68.1±3.8
6	3±0.26	13	23.0±2.1	2	39.7±2.6
7a	4.2±0.37	22	7.6±0.6	12	91.9±4.8
7b	5.8±0.63	14	8.4±0.9	9	79.2±4.2
8	n.d[d]	-	84.5±4.5	0	10.3±1.0
11	8.6±0.74	9	18.9±1.9	4	75.5±3.8
12	1.3±0.05	63	10.5±1.3	8	82.4±4.4
13	1.1±0.08	57	32.4±2.8	2	62.5±3.6
14	0.8±0.06	39	56.3±3.8	1	31.0±2.2
15	1±0.08	56	38.7±3.2	2	56.4±3.5
16	2.5±0.18	29	12.8±1.6	6	72.6±3.9
17	3.2±0.23	4	71.3±4.2	0	13.3±1.3
18	2.8±0.17	6	61.2±3.9	0	17.6±1.6
19	4.9±0.53	3	65.5±4.1	0	15.8±1.6
20	2.6±0.13	9	51.2±3.9	1	23.2±1.9

The metabolic activity of the cells was estimated after 48 h of incubation with different concentrations of the investigated compounds by means of an MTT assay; [a] The IC_{50} value was determined from the dose-response curves as the mean of two parallel experiments; [b] Fluorouracil (5-Fu) was used as a positive control; [c] Selectivity index (SI) is the ratio of the IC_{50} value for normal cells (WI-38) to the IC_{50} values for HepG2 and MCF-7 cells; [d] No growth inhibition was recorded.

Table 2. Evaluation of the redox-modulating activity of the compounds using the DPPH, ABTS and bleomycin-dependent DNA damage assays.

Compound No.	DPPH Assay		ABTS assay		Bleomycin-dependent DNA damage assay
	Inhibition %	Fold	Inhibition %	Fold	Absorbance[a]
4a	14.2 ± 1.3	0.2	11.3 ± 1.9	0.1	0.088 ± 0.0075
4b	19.1 ± 2.6	0.2	9.9 ± 1.1	0.1	0.129 ± 0.0106
4c	17.4 ± 2.3	0.2	20.2 ± 2.5	0.2	0.107 ± 0.0083
5	30.1 ± 1.9	0.3	40.7 ± 3.6	0.4	0.054 ± 0.0043
6	16.3 ± 2.2	0.2	31.3 ± 2.9	0.3	0.072 ± 0.0063
7a	88.8 ± 5.3	1.0	75.2 ± 6.2	0.8	n.d
7b	80.9 ± 8.1	0.9	63.5 ± 4.3	0.7	0.087 ± 0.0074
8	4.7 ± 0.9	0.1	10.4 ± 1.1	0.1	0.163 ± 0.0123
11	63.6 ± 4.8	0.7	75.1 ± 4.6	0.8	0.097 ± 0.0082
12	84.1 ± 4.1	0.9	95.1 ± 5.3	1.0	n.d
13	24.2 ± 3.9	0.3	17.5 ± 1.6	0.2	0.091 ± 0.0077
14	13.1 ± 1.1	0.1	32.5 ± 2.7	0.4	0.08 ± 0.0064
15	20.3 ± 2.1	0.2	32.1 ± 2.3	0.4	0.096 ± 0.0081
16	62.3 ± 4.3	0.7	51.7 ± 3.9	0.6	0.072 ± 0.0062
17	9.5 ± 0.85	0.1	4.8 ± 0.56	0.1	0.105 ± 0.0096
18	10.4 ± 0.52	0.1	13.1 ± 1.1	0.1	0.109 ± 0.0082
19	9.8 ± 0.65	0.1	15.2 ± 0.92	0.2	0.072 ± 0.0051
20	12.3 ± 1.9	0.1	3.7 ± 0.51	0.0	0.114 ± 0.0095
Vitamin C	89.6 ± 3.5	1	91.4 ± 5.9	1	0.288 ± 0.026

[a] The absorbance (Au) was read at 517 nm after 30 minutes. Vitamin C was used as positive control in the DPPH and the ABTS assays and as a reducing agent in the bleomycin-dependent DNA damage assay. Values are expressed as the mean ± SD. Compounds were dissolved in methanol to obtain a final concentration of 1 mM, 200 µL of each sample was added to 400 µl of 0.1 mM DPPH in methanol. For the ABTS assay, the absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was measured at 734 nm after the addition of 50 µl of 1 mg/ml solutions of the tested compound in phosphate-buffered methanol. [a] Absorbance was not recorded.

Synthesis and biochemical studies of novel organic selenides with increased selectivity for hepatocellular carcinoma and breast adenocarcinoma

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Highlights

- Nineteen organoselenides were synthesized.
- Cytotoxicity was evaluated in HepG2 and MCF-7 cell lines.
- Selective cytotoxicity was estimated using normal WI-38 cells.
- Compounds modulated the expression of Bcl-2, IL-2, IL-6 and CD40 in HepG2 cells.
- Redox profiles were estimated employing ABTS, DPPH, and GPx-like activity assays.
- Compound exhibited good anti-HepG2 and antioxidant activities.

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