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In vitro radical scavenging and cytotoxic activities of novel hybrid selenocarbamates

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ABSTRACT

Novel selenocyanate and diselenide derivatives containing a carbamate moiety were synthesised and evaluated in vitro to determine their cytotoxic and radical scavenging properties. Cytotoxic activity was tested against a panel of human cell lines including CCRF-CEM (lymphoblastic leukaemia), HT-29 (colon carcinoma), HTB-54 (lung carcinoma), PC-3 (prostate carcinoma), MCF-7 (breast adenocarcinoma), 184B5 (non-malignant, mammary gland derived) and BEAS-2B (non-malignant, derived from bronchial epithelium). Most of the compounds displayed high antiproliferative activity with GI_{50} values below 10 μM in MCF-7, CCRF-CEM and PC-3 cells. Radical scavenging properties of the new selenocompounds were confirmed testing their ability to scavenge DPPH and ABTS radicals. Based on the activity of selenium-based glutathione peroxidases (GPxs), compounds **1a**, **2e** and **2h** were further screened for their capacity to reduce hydrogen peroxide under thiol presence. Results suggest that compound **1a** mimics GPxs activity. Cytotoxic parameters, radical scavenging activity and ADME profile point to **1a** as promising drug candidate.

1. Introduction

Due to the lack of selectivity and increased resistance to anticancer drugs, there is an urgent need to develop new and safer drugs for effective treatment of cancer.[1,2] In this field selenium derivatives have caught great attention due to their wide variety of biological activities.[3–6] Although it is noticeable that the chemical form of selenium is a determinant for its biological activity,[7,8] the mechanisms that drive the selenium-anticancer action are not fully understood. Nevertheless, since oxidative stress is a hallmark of a variety of chronic human diseases commonly attributed to radical scavenging and enzymatic decomposition of oxygen metabolites, the antioxidant properties of organoselenium compounds have been extensively investigated.[9–11] Interestingly, recent data from our laboratory have demonstrated that some organoselenium derivatives presented both antiproliferative and antioxidant activities.[12]

Among the different synthetic selenium compounds that have been developed, the chemical forms selenocyanate and diselenide have received significant attention.[13–19] Selenocyanate function has proven effectiveness in the prevention and treatment of a variety of cancers, both in vitro and in vivo. Among their actions inhibition of m-TOR signalling,[13] tubulin polymerization[14] and nitric oxide levels,[15] and modulation of antioxidative enzymes such as glutathione peroxidase, glutathione-S-transferase, thioredoxin reductase, superoxide reductase and catalase[14–17] have all been described. Diselenide compounds could play a beneficial role in oxidative diseases acting as antioxidants that can protect against cancer lesions. In fact, a series of substituted diaryl diselenides behaved as cytotoxic and apoptotic inducers in HT-29 cells,[18] while other diselenide derivatives have shown ability to inhibit histone deacetylase and PI3K/Akt signalling in WM35 and UACC 903 melanoma cells.[19] In addition, diphenyl diselenide is able to protect both MCF-7 breast cancer cells from oxidative DNA damage induced by tamoxifen[20] and J774 macrophage-like cells from oxidized LDL-mediated effects.[21] Moreover, some diselenides have been designed as potential therapeutic agents mimicking the peroxidase activity of selenium based glutathione peroxidases.[22]

Taking into account our previous experience in selenocyanate and diselenide chemistry[23–25] and the fact that we have recently reported some carbamates as potential antiproliferative drugs,[26] we decided to further extend our research by synthesising thirty new hybrid compounds in which selenocyanate or diselenide and carbamate moieties were combined. In these compounds the carbamate function, a group commonly found in antitumour active

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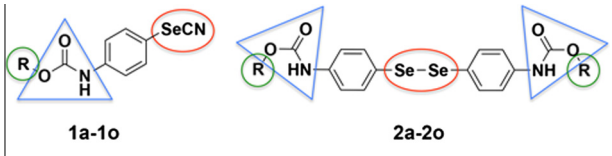
compounds[27–32] acts as a link between the central and ending scaffolds, thus enabling their chemical accessibility and increasing polarity in this area as compared to our previously described structures.[23,33–35] Besides, this group might facilitate the hydrolysis of the compounds towards anionic species that could act as prodrugs (Fig. 1).

To explore the influence on the activity of the molecular modifications of the ending cores we decided to compare the effect of different aryl and short-chain alkyl substituents commonly used in medicinal chemistry. During the last years it has been established that short-chain and medium-chain length fatty acids, as well as long-chain polyunsaturated fatty acids can be used as chemotherapeutic agents for cancer treatment. For instance, while monounsaturated fatty acids from sixteen to twenty two carbon atoms have shown to inhibit proliferation in breast cancer cells,[36] lauric acid derivatives induced apoptosis in colon cancer,[37] and the carbamate scaffold linked to tert-butyl[38] or butyl chains resulted active against lung and colon cancer.[39] Additionally, it has been described that some fatty acids can act synergistically with classic chemotherapeutic agents.[40] Keeping this in mind, here we analyse how short chain fatty acids (4–7 atoms in length) linked to the carbamate scaffold affect activity.

To study the impact on the activity of different aromatic rings, and considering the synthetic accessibility, we also synthesised a diverse pool of phenyl derivatives either with one electron-donating or one electron-withdrawing substituent that carried or not a methylene group as a separating moiety, increasing both bond flexibility and adaptability of bulky derivatives. In some cases the aromatic ring was either a polycyclic system such as Fmoc, that has been used previously as an antitumour agent conjugated to peptides,[41] or a heteroaromatic unit such as the benzo[*b*]thiophene-1,1-dioxide.[42]



Compounds	R
1a/2a	Ethyl
1b/2b	Propyl
1c/2c	Butyl
1d/2d	Hexyl
1e/2e	Isobutyl
1f/2f	Allyl
1g/2g	Phenyl
1h/2h	4-Methoxyphenyl
1i/2i	4-Methylphenyl
1j/2j	4-Chlorophenyl
1k/2k	4-Fluorophenyl
1l/2l	Benzyl
1m/2m	4-Nitrobenzyl
1n/2n	(fluoren-9-yl)methyl (Fmoc)
1o/2o	1,1-dioxobenzo[*b*]thiophen-2-yl

Figure 1. Structure of new synthesised hybrids.

Inspired by aforementioned above, we herein report the design, synthesis, and in vitro cytotoxic and cytostatic activities, as well as the selectivity, against a panel of human cell lines for 30 new selenocarbamate derivatives. The capability of the title compounds to interact with the stable radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), their efficiency to scavenge 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{+\cdot}$) and their ability to mimic glutathione peroxidase activity was also examined.

2. Results and discussion

2.1. Chemistry

General procedure for selenocyanate (compounds **1a–o**) and diselenide (compounds **2a–o**) derivatives synthesis is illustrated in Scheme 1. The first step for **1a–o** preparation was the generation of 4-aminophenylselenocyanate according to a previous reported procedure.[31] Treatment of 4-aminophenylselenocyanate with the appropriated chloroformates, synthesised according to standard procedures[43,44] or purchased commercially, for 24 h at room temperature in dry chloroform and 1:1 molar ratio, gave **1a–o** in a 4–29% yield. Formation of carbamates was confirmed following the wavenumber position in infrared spectroscopy peaks: carbonyl group showed up around 1780 cm^{-1} in the starting chloroformates whereas the carbonyl in the carbamates arose around 1706–1735 cm^{-1}. In addition, the triple bond of SeCN group was detected between 2141 and 2159 cm^{-1}. Crude reaction products were purified by recrystallization and/or washed with organic solvents.

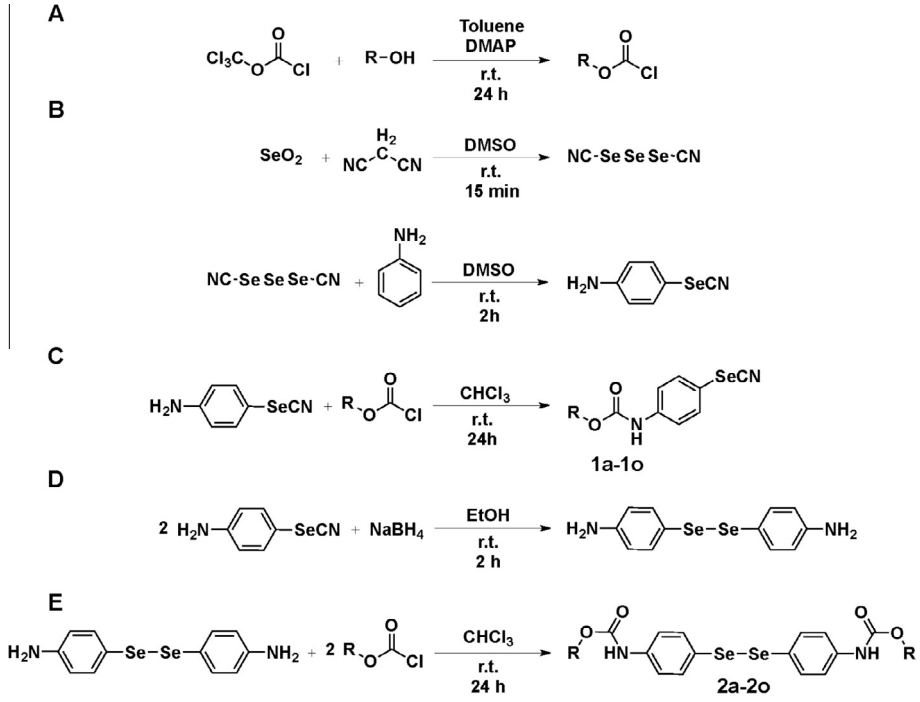
The synthetic route for **2a–o** was based on the reaction between 4,4-diaminodiphenyldiselenide with the corresponding chloroformates in a 1:2 molar ratio, in dry chloroform at room temperature for 24 h. The starting intermediate 4,4-diaminodiphenyldiselenide was prepared according to the method described by Banks et al.[45] **2a–o** diselenide derivatives, whose carbamate carbonyl group was detected at 1684–1726 cm^{-1}, were recovered at variable yields (2–63%) after washing with ethyl ether or hexane.

Chemical structures for every synthesised compound were confirmed by ^{1}H NMR, ^{13}C NMR, MS spectral and elemental analysis. NMR spectral data of the compounds showed all proton and carbon signals at their expected chemical shift values. No sophisticated instrumentation, conditions or reagents were required for the transformations that took place under mild conditions.

2.2. Biological evaluation

2.2.1. Cytotoxicity

Synthesised compounds were screened for their cytotoxic and antiproliferative activities against a panel of human tumour cell lines as a representative selection of solid, liquid and hormone-dependent tumours, including lung carcinoma (HTB-54), colon carcinoma (HT-29), lymphocytic leukaemia (CCRF-CEM), breast adenocarcinoma (MCF-7) and prostate adenocarcinoma (PC-3). As a guide with regard to selectivity all of the compounds were further examined for toxicity in two cell lines derived from non-malignant cells: 184B5, a cell line established from normal breast tissue,[46] and BEAS-2B, a cell line derived from non-malignant bronchial epithelium cells.[47] Cytotoxicity assays were based on the reactivity of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] as previously described.[48] The cytotoxic effect of each substance was tested at five different concentrations between 0.01 and 100 μM. GI_{50}, that is, the concentration that reduces by 50% the growth of treated cells with respect to untreated controls, TGI, the concentration that completely inhibits cell growth, and LC_{50}, the concentration that kills 50% of the cells



Scheme 1. Synthesis of selenocyanates **1a**–**o** and diselenides **2a**–**o**. (A) Preparation of chloroformates. (B) Preparation of 4-aminophenylselenocyanate. (C) Synthesis of compounds **1a**–**o**. (D) Preparation of 4,4-diaminodiphenyldiselenide. (E) Synthesis of compounds **2a**–**o**.

for each compound are shown in Tables 1 and 2. As shown in Table 1, all the compounds were highly active under our experimental conditions displaying toxicities against HT-29 and MCF-7 cells stronger than those of the traditional anticancer agent etoposide. Moreover, as attested by the high number of compounds showing GI_{50} values <10 μM, MCF-7, CCRF-CEM and PC-3 cell lines were the most sensitive towards the newly synthesised compounds. On the other hand, HTB-54 cells were the least sensitive cells. Thus, only two compounds (**1o** and **2o**) exhibited GI_{50} values smaller than 10 μM in HTB-54 cells, while 18 compounds (**1a**, **1b**, **1c**, **1d**, **1g**, **1i**, **1j**, **1k**, **1l**, **1n**, **1o**, **2a**, **2g**, **2h**, **2i**, **2k**, **2l** and **2o**) displayed GI_{50} values <10 μM in MCF-7 cells, 17 compounds (**1a**, **1b**, **1c**, **1e**, **1f**, **1m**, **1n**, **1o**, **2b**, **2d**, **2e**, **2f**, **2h**, **2k**, **2l**, **2m** and **2o**) did it in CCRF-CEM cells and 19 compounds (**1a**, **1g**, **1h**, **1i**, **1j**, **1l**, **1m**, **1n**, **1o**, **2d**, **2g**, **2h**, **2i**, **2j**, **2k**, **2l**, **2m**, **2n** and **2o**) did it in PC-3 cells. Besides, most of the compounds exhibited similar activities in both tumour and non-tumour cell lines (Tables 1 and 2).

Results of the in vitro antitumoural activity did not allow us to determine a structure-activity relationship since:

(1) No great differences in the cytotoxic activity could be detected among selenocyanate and diselenide derivatives in spite of their structural differences (i.e. double amount of selenium in weight percentage and symmetry, a relevant factor for biological active entities widely reviewed,[49] for diselenide derivatives). Other structural differences were the variability of molecular weights and polarity of the selenium-bond for both series. To evaluate the polarizability of Se–CN and Se–Se bonds, the atomic charge distribution over the selenium atom was theoretically calculated.[50] Results, reported as Supplementary material, showed that Se–Se bond in diselenide compounds **2a**–**o** presented no polarizability, as expected. On the contrary, the polarizability of Se–CN bond, measured as charge difference, in selenocyanate derivatives **1a**–**o** ranged from 0.553 to 2.000. The list of compounds exhibiting GI_{50} values <10 μM includes eight selenocyanates and nine diselenides in CCRF-CEM cells.

(2) Modification of the ending cores resulted in a slight or no change in the antitumour activity of the compounds. Thus, neither ramification of the alkyl chain (butyl vs isobutyl fragment), nor the attachment of an unsaturated allyl unit instead of a saturated propyl one on the aliphatic derivatives altered activity in any of the tested cell lines.

(3) Similarly, both electron-withdrawing and electron-releasing groups on aromatic derivatives yielded similar cytotoxicity values *f* (e.g. **1h** and **1i** vs **1j** and **1k**, **2h** and **2i** vs **2j** and **2k**). However, it is interesting to note that the presence of a methylene group as spacer between the carbamate and the phenyl ring plays an important role in the antitumour activity of diselenide function (e.g. when compared to **2g**, the benzyl derivative **2l** increased the cytostatic activity by 59 folds in PC-3 cells, by 7 folds in HT-29 cells and by 3.5 folds in CCRF-CEM cells), though it has little or no influence on the activity of selenocyanate entities (**1g** vs **1l**).

(4) Finally, incorporation of bulky moieties with high bond flexibility such as polycyclic groups (**1n**) or 1,1-dioxobenzo[*b*]thiophen-2-ylmethyl ring (**1o** and **2o**) improved activity and led to LC_{50} values smaller than 10 μM for **1o** in CCRF-CEM cells and for **2o** in every tested cell line.

To compare activity among compounds, the average sensitivity of a cell line towards the tested compounds (MG-MID) was calculated using their GI_{50} values. Obtained results are shown in Figures 2 and 3 for tumour and non-malignant cell lines respectively. Selectivity of a compound towards a cell line of the panel is characterized by a high negative deviation (D) of its GI_{50} from the MG-MID value. On the other hand, positive deviations point to less powerful compounds. As shown in Figure 2, **1a**, **2h**, 2l and 2o displayed negative values in every tested tumour cell line. Additionally, Figure 3 shows positive values for **1a** in BEAS-2B cells. Upon these results, **1a** emerges as a promising cytostatic and/or cytotoxic candidate.

Table 1
Average GI_{50}, TGI and LC_{50} values (μM) for selenocyanates **1a–o** and diselenides **2a–o** against tumour cell lines

Refs.	R	MCF-7[a]			HT-29[b]			CCRF-CEM[c]			PC-3[d]			HTB-54[e]		
		GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1a	Ethyl	8.9	36.7	67.8	9.3	38.0	66.6	9.8	39.1	68.8	9.9	39.2	68.4	24.9	49.3	72.7
1b	Propyl	8.0	34.4	67.5	28.4	51.9	75.4	9.3	38.4	68.8	21.1	47.4	73.8	28.9	51.9	74.9
1c	Butyl	6.6	20.2	59.7	8.1	31.3	63.7	6.5	27.9	63.5	12.4	40.8	69.3	17.2	43.4	69.5
1d	Hexyl	6.6	30.5	63.9	10.7	40.3	69.9	19.7	46.2	72.7	17.3	44.6	72.0	23.6	48.5	73.0
1e	Isobutyl	18.1	45.5	73.0	8.1	31.3	63.7	9.3	38.3	68.7	21.6	47.3	73.0	27.1	51.2	75.4
1f	Allyl	15.0	43.1	71.2	24.5	48.6	72.8	9.2	38.1	68.6	17.6	44.7	71.8	27.0	50.1	73.3
1g	Phenyl	4.0	35.9	67.8	11.7	41.7	71.7	22.9	48.0	73.2	9.1	37.5	68.0	26.2	50.2	74.4
1h	4-Methoxyph.	22.4	48.2	74.1	13.6	41.5	69.6	13.0	41.5	70.0	7.5	44.6	71.6	23.7	48.5	73.3
1i	4-Metylph.	9.9	39.9	69.8	8.8	38.8	68.7	17.4	44.7	72.0	7.6	32.3	65.1	17.3	44.2	71.1
1j	4-Chloroph.	6.0	26.3	63.6	11.8	40.3	68.7	18.3	45.1	71.8	6.4	18.7	57.9	13.8	41.4	69.0
1k	4-Fluoroph.	8.3	33.3	65.7	8.8	37.6	66.5	12.6	41.9	71.1	11.8	40.4	69.0	25.0	48.4	71.9
1l	Benzyl	8.7	38.8	68.9	13.3	41.2	69.1	23.2	48.4	73.7	3.7	27.8	62.5	27.6	50.8	74.0
1m	4-Nitrobenzyl	11.8	40.7	69.5	22.4	47.1	71.9	9.3	38.3	68.8	9.0	36.5	67.6	22.9	47.6	72.4
1n	Fmoc	7.6	34.6	67.0	15.5	42.8	70.2	7.5	34.2	66.7	2.0	34.3	70.0	23.8	48.4	73.0
1o	Benzothiophen.	8.3	34.9	66.1	26.1	50.2	74.3	3.3	6.1	8.9	6.3	21.3	59.8	6.8	26.9	62.7
2a	Ethyl	9.9	39.0	68.3	26.0	51.1	76.1	24.6	49.5	74.3	28.8	52.2	75.7	31.2	56.6	82.0
2b	Propyl	31.7	55.8	80.0	18.3	47.2	76.1	4.3	8.8	49.1	18.4	45.0	71.5	48.5	68.4	88.3
2c	Butyl	17.3	46.3	75.2	27.6	55.0	82.4	16.7	44.5	72.3	28.6	52.5	76.4	35.2	57.3	79.4
2d	Hexyl	15.8	52.9	89.9	11.0	42.6	74.2	6.2	34.4	67.3	0.6	25.7	66.7	31.0	54.3	77.2
2e	Isobutyl	19.0	49.7	80.5	19.9	48.6	77.4	9.8	39.8	70.2	24.2	48.9	73.7	29.0	52.9	76.9
2f	Allyl	20.8	47.2	73.6	25.9	52.7	79.5	4.6	8.7	46.9	25.0	49.9	74.7	29.8	56.6	83.3
2g	Phenyl	8.0	36.7	69.0	13.0	41.6	70.2	22.4	48.1	73.7	5.3	27.2	62.9	22.7	56.2	89.8
2h	4-Methoxyph.	9.7	42.8	76.8	13.0	46.1	79.2	5.3	25.8	63.1	4.4	28.2	64.6	18.0	46.3	74.5
2i	4-Metylph.	6.9	37.7	74.0	7.9	39.4	70.9	21.4	48.5	75.6	4.4	28.2	64.8	24.9	57.0	89.1
2j	4-Chloroph.	12.4	46.0	79.6	9.9	43.4	76.9	10.8	43.4	76.0	1.0	28.1	82.6	28.6	60.8	93.0
2k	4-Fluoroph.	8.0	33.7	64.3	18.5	51.9	85.3	8.7	37.6	68.8	4.9	31.3	64.5	26.7	51.8	77.0
2l	Benzyl	5.2	38.6	74.5	1.8	35.4	69.0	6.4	34.6	67.9	0.1	33.5	70.4	23.8	50.9	78.0
2m	4-Nitrobenzyl	20.6	52.8	85.0	16.7	46.3	75.9	8.6	36.5	68.1	6.4	27.1	69.2	31.8	55.6	79.4
2n	Fmoc	22.6	52.9	83.3	17.8	49.0	80.3	14.9	43.2	71.4	9.2	38.8	70.3	36.8	60.5	84.3
2o	Benzothiophen.	3.2	5.7	8.2	3.0	5.4	7.8	2.3	4.9	7.5	3.8	5.8	7.9	4.4	6.3	8.2
Etoposide[f]		19.9	>100	>100	31.6	>100	>100	1.6	50.5	89.9	0.6	4.0	79.4	n.d.[g]	n.d.	n.d.
Cisplatin[f]		3.2	>100	>100	7.9	>100	>100	1.7	>100	>100	5.0	50.1	>100	9.6	32.7	50.0

[a] Breast adenocarcinoma.
[b] Colon carcinoma.
[c] Lymphoblastic leukaemia.
[d] Prostate carcinoma.
[e] Lung carcinoma.
[f] NCI data (http://dtp.nci.nih.gov).
[g] No data.

2.2.2. Radical scavenging activity

Since selenium anticancer activity has been related with its ability to interact with the redox status of the cell[20,51] and it has been reported that some selenium-containing organic molecules are stronger antioxidants than "classical" ones,[52] we decided to test the newly synthesised compounds to determine their radical scavenging activity. With this purpose we measured the ability of the compounds to scavenge both DPPH· and $ABTS^{+\cdot}$. Compounds displaying interesting radical scavenging profiles were also screened for its ability to mimic GPx by reducing peroxides.

2.2.2.1. DPPH radical-scavenging activity. The ability of the selenocyanates and diselenides to scavenge the DPPH· was determined following decolorization of the DPPH· at 517 nm.[53] Determinations were performed at 6 different concentrations of the compounds ranging from 0.016 to 0.5 mg/mL and were recorded at different time lengths. Due to technical problems, as lack of solubility of the compounds at the working conditions and formation of coloured complexes with funny absorbance profiles, only 23 compounds were evaluated (Fig. 4A).

As shown in Figure 4A, every tested compound was able to scavenge the DPPH·, with values above 25% at 0.5 mg/mL for 11 of them (**1a**, **1e**, **1n**, **2a**, **2b**, **2c**, **2e**, **2f**, **2g**, **2h** and **2i**). As depicted by **2b**, **2e**, **2g**, **2h** and **2i**, whose DPPH· scavenging values were higher than 50%, diselenides displayed higher activities than their selenocyanate analogues. Homolytic Se–Se bond cleavage frequently generates free radicals that can react directly with DPPH· explaining the higher scavenge activity observed for diselenide derivatives. Besides, a decrease on the scavenging activity was detected in both series upon elongation of the aliphatic chain (e.g. activity fell down from around 37% for **1a** and **2a** to less than 16% for **1c** and **2d**). On the other hand, saturation and ramification of the side chain led to an increase on activity (e.g. activity rose up from 30% of **2f** to 52.4% of **2b**, from 6.6% of **1c** to 27.1% of **1e** and from 36.1% of **2c** to 66.5% of **2e**). As for the aromatic derivatives, the presence of a methylene between the carbamate and the phenyl group nearly abolished the DPPH· scavenging activity (e.g. activity fell down from 66.9% of **2g** to 7.4% of **2l** and from 19.3% of **1g** to 3.7% of **1l**) indicating that the spacer is not well tolerated. Finally, DPPH· scavenging activity was time- and dose-dependent as illustrated for **2e** in Figure 4B and C.

2.2.2.2. ABTS radical-scavenging assay. To confirm the radical scavenging activity of the compounds we next determined their capacity to reduce $ABTS^{+\cdot}$. Reduction of $ABTS^{+\cdot}$ was determined by measuring absorbance at 741 nm after 6 min incubation with 0.1 mg/mL of each compound. Trolox, a water soluble analogue of Vitamin E, was used as a positive control of the technique. Obtained results, expressed as the percentage of scavenged $ABTS^{+\cdot}$ are shown in Figure 5.

As shown, this assay confirmed the radical scavenging properties of the new hybrid selenocarbamates. For instance, **1a**, **1j**, **1k**, **1g**, **2e** and **2h** scavenged more than 25% of the $ABTS^{+\cdot}$. As a general rule, the same trend observed in DPPH assay was obtained again.

Table 2
Average GI_{50}, TGI and LC_{50} values (μM) for selenocyanates **1a–o** and diselenides **2a–o** against 184B5 and BEAS-2B cells

Refs.	184B5[a]			BEAS-2B[b]		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1a	5.5	18.6	59.0	21.7	46.9	72.2
1b	3.7	9.1	50.9	19.2	46.3	73.5
1c	5.3	14.5	56.8	5.0	12.2	54.5
1d	4.6	20.0	60.1	2.8	35.9	68.0
1e	5.8	21.6	60.9	26.9	49.5	72.1
1f	5.0	9.8	53.8	21.4	47.4	73.5
1g	0.4	22.7	61.2	8.9	38.2	69.3
1h	4.5	19.2	59.6	25.2	48.4	71.6
1i	4.4	8.7	46.9	5.6	15.3	58.3
1j	5.6	19.9	59.8	9.3	38.0	68.7
1k	6.9	29.5	64.4	19.8	45.6	71.4
1l	5.0	21.9	60.7	0.1	34.4	66.6
1m	6.0	18.0	58.5	7.7	32.8	65.8
1n	5.5	25.8	62.5	7.9	38.4	68.2
1o	5.3	19.2	59.7	7.7	29.0	64.3
2a	5.7	16.7	58.7	16.1	44.0	71.9
2b	7.2	24.5	62.0	14.4	43.3	72.2
2c	7.2	26.0	63.1	24.2	49.6	75.0
2d	4.6	9.8	53.4	0.01	6.9	51.8
2e	5.5	10.5	56.6	13.1	42.7	72.4
2f	6.4	21.5	60.4	14.3	42.9	71.4
2g	4.5	9.0	48.9	1.4	8.6	50.7
2h	3.4	8.1	43.5	0.6	19.9	62.3
2i	3.5	7.6	37.2	0.7	19.2	61.8
2j	2.5	8.9	52.6	0.1	26.6	64.7
2k	3.7	8.6	47.8	0.7	23.5	61.8
2l	0.1	5.8	36.2	0.01	17.3	58.5
2m	6.0	13.8	56.5	6.3	22.5	61.6
2n	6.5	19.7	59.4	7.1	27.9	64.1
2o	2.6	5.0	7.5	3.5	5.7	7.9

[a] Mammary gland cell culture derived from non-malignant cells.
[b] Bronchial epithelium cell culture derived from non-malignant cells.

Thus, elongation and unsaturation of the alkyl chain led to a noticeable decrease in radical scavenging activity. Thus, percentage of scavenged $ABTS^{+\cdot}$ fell from 26.3% and 21.3% of **1a** and **2a** to 12.6% and 7.3% of **1c** and **2c**, and also from 22.5% of **2b** to 10.9% of **2f**. However, ramification of the side chain only affected activity of diselenide derivatives (29.6% of **2e** vs 7.3% of **2c**). Analysis of the influence of the aromatic moiety also revealed that the presence of the methylene group decreased the percentage of $ABTS^{+\cdot}$ scavenged. Finally, the Trolox equivalent antioxidant capacity (TEAC) was calculated using a calibration curve and is shown in Table 3.

2.2.2.3. Determination of GPx-like activity. Glutathion peroxidases (GPxs) are a family of selenoproteins, key on the maintenance of the redox status of the cells. They remove hydrogen peroxide as well as lipid, phospholipid and cholesterol hydroperoxides.[54] To evaluate whether **1a**, **2e** and **2h** are able to reduce hydrogen peroxides modelling GPx activity we used the method developed by Iwaoka and Tomoda.[55] In this method, tiophenol (PhSH) is used for the catalytic reduction of H_2O_2 according to the equation:

$$H_2O_2 + 2\text{phSH} \xrightarrow{\text{GPx or } \mathbf{1a},\mathbf{2e},\mathbf{2h}} 2H_2O + \text{phSSph}$$

Then, the shift in UV absorption at 305 nm due to formation of diphenyl disulphide (PhSSPh) is used to estimate GPx-like activity. While **2e** and **2h** were not able to mimic GPx activity (no shown), Figure 6 shows that **1a** increased reduction of H_2O_2 as compared to the negative control (vehicle). This result suggests that compound **1a** follows a mechanism similar to the catalytic GPxs enzymes.

2.3. Drug-likeness properties

Taking in consideration the results obtained from the cytotoxic and radical scavenging evaluation, compounds **1a**, **2e**, **2h**, **2l** and **2o** were selected to analyse their drug-likeness properties. Since Lipinski parameters describe important molecular properties for drug pharmacokinetics in the human body, especially their oral absorption, Lipinski descriptors for **1a**, **2e**, **2h**, **2l** and **2o** bioavailability were calculated using the freely accessible program MIPC[56] (Molinspiration Property Calculator). As a rule, when using these descriptors an oral active drug must not violate more than one of the following criteria: <5 hydrogen donors (nOHNH), <10 hydrogen acceptors (nON), MW <500, log*P* calcd <5. As shown in Table 4, 1a possess drug like molecule (DLM) features.

3. Conclusions

In summary, thirty new hybrid selenocyanate and diselenide carbamates were synthesised and tested for their cytotoxic potential against a panel of human cancer and non tumoural cell lines. Ten compounds exhibited promising broad spectrum antiproliferative activity with GI_{50} below 10 μM in at least three tumour cell lines. Active compounds displayed modest selectivity for human normal cells. Compounds **1a**, **2h**, **2l** and **2o** showed the lowest GI_{50} values (MG-MID) along with potential radical scavenging activity for DPPH and ABTS assays. In addition, compound **1a**

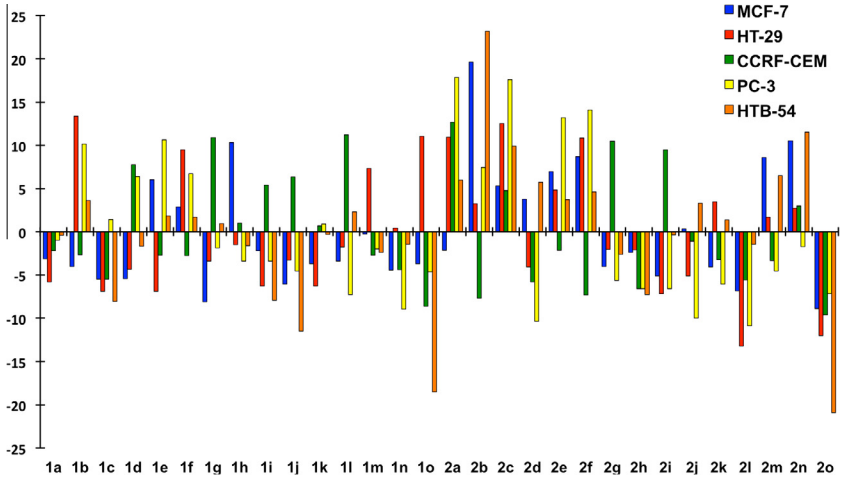


Figure 2. MG-MID for compounds **1a–2o** in tumour cell lines. Compounds are depicted at the horizontal axis. Vertical bars represent the deviation respect GI_{50} MG-MID value. A positive value indicates that GI_{50} is bigger than the mean, whereas negative values point to GI_{50} values smaller than the mean.

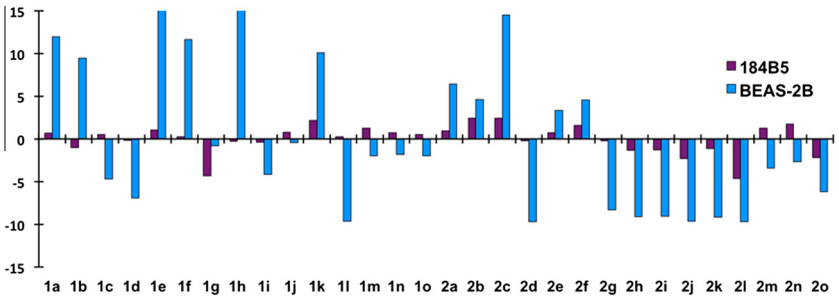


Figure 3. MG-MID for compounds **1a**–**2o** in non-malignant cell lines.

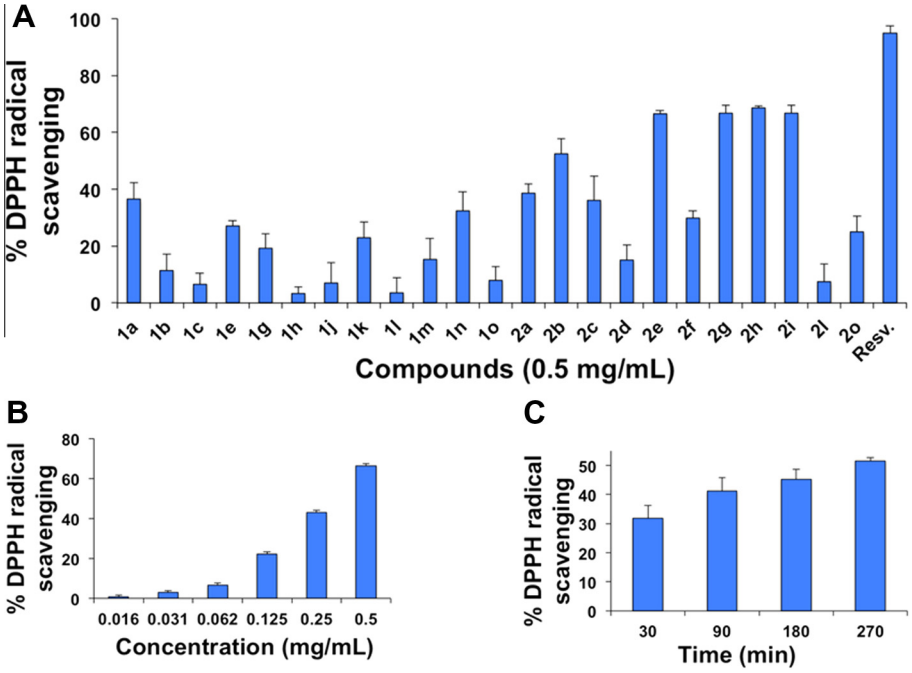


Figure 4. Analysis of DPPH radical scavenging activity of the novel compounds. (A) Percentage of DPPH· scavenging after 180 min of incubation with 0.5 mg/mL of the tested compound. The concentration values, expressed as μM, for each compound are collected in the Supplementary material. (B) Concentration dependence analysis of the scavenging activity of **2e** (0.016–0.5 mg/mL). (C) Time course analysis of reduction of DPPH· by **2e** (0.25 mg/mL). Results are presented as the mean ± SD of four replicate assays. Resveratrol (Resv.) was used as a positive control.

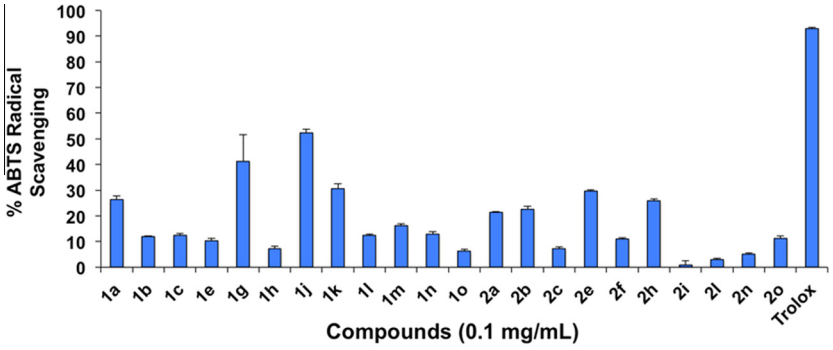


Figure 5. ABTS radical scavenging activity of the new selenocyanates and diselenides. Results are presented as the mean ± SD of four replicate assays. The concentration values, expressed as μM, for each compound are collected in the Supplementary material.

exhibited GPx-like activity. Despite the structural differences previously mentioned, such as bond polarizability, or sterical hindrance, both functional groups (selenocyanate and diselenide) have shown to be active in the different biological activities tested. Since analysis of the bioavailability and toxicity of the compounds revealed **1a** as a non-toxic compound with drug-likeness properties, results of this study may have found a leader for development of new therapeutic agents to fight cancer.

Table 3
TEAC (Trolox equivalent antioxidant capacity) of selenocyanates **1a–o** and diselenides **2a–o**

Compound	TEAC (μg/μL)	Compound	TEAC (μg/μL)
1a	17.43	**2a**	13.58
1b	6.27	**2b**	14.51
1c	6.77	**2c**	2.67
1d	n.d.[a]	**2d**	n.d.
1e	5.09	**2e**	20.01
1f	n.d.	**2f**	5.50
1g	29.00	**2g**	n.d.
1h	2.58	**2h**	n.d.
1i	n.d.	**2i**	n.d.
1j	37.60	**2j**	n.d.
1k	20.76	**2k**	n.d.
1l	6.74	**2l**	n.d.
1m	9.66	**2m**	n.d.
1n	7.02	**2n**	0.93
1o	1.92	**2o**	5.78

[a] No data.

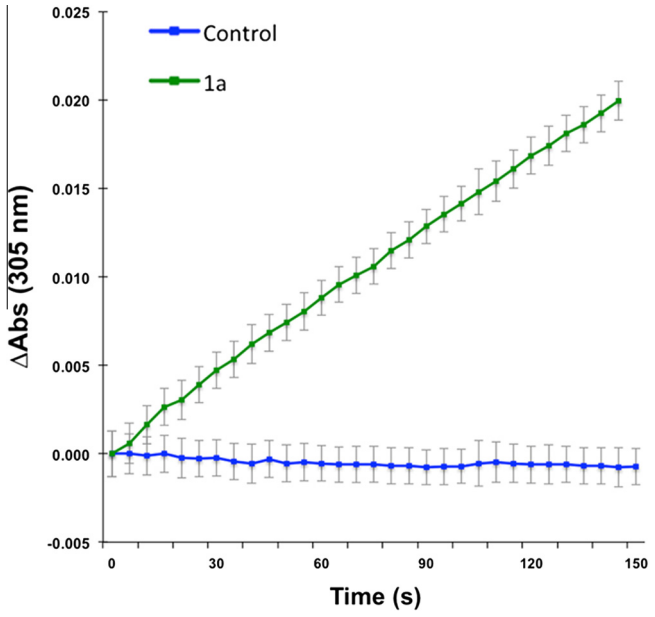


Figure 6. Determination of GPx-like activity. GPx-like activity of **1a** was tested at 0.033 mg/mL. Data were collected every 5 s. Results are presented as the mean ± SD of three replicate assays.

Table 4
Theoretical structural properties of lead compounds

Compd	Log*P*	MW	TPSA	n-OH acceptors	n-OHNH donors	Volume
1a	2.73	269	62.12	4	1	198.13
2e	7.07	542	76.66	6	2	416.68
2h	7.23	642	95.13	8	2	477.08
2l	7.57	610	76.66	6	2	459.60
2o	6.87	786	144.95	10	2	555.61

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and have not been corrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 Ultrashield™ spectrometer (Rheinstetten, Germany) using TMS as the internal standard. The IR spectra were obtained on a Thermo Nicolet FT-IR Nexus spectrophotometer with KBr pellets. Elemental microanalyses were carried out on vacuum-dried samples using a LECO CHN-900 Elemental Analyzer. Alugram®SIL G/UV_{254} (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG. Postfach 101352, D-52313 Düren, Germany) was used for Thin Layer Chromatography. Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Madrid, Spain), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, Belgium) and Lancaster (Bischheim–Strasbourg, France).

4.1.1. General procedure for compounds 1a–o

Appropriate chloroformates (5 mmol) dissolved in dry chloroform (25 mL) were added dropwise, while stirring, to 4-aminophenylselenocyanate (5 mmol) dissolved in dry chloroform (25 mL). The mixture was kept with stirring at room temperature for another 24 h and during stirring a solid precipitate formed. The resulting solids were collected by filtration and purified to afford the target compounds. In order to assign the signals in NMR spectroscopy the substituents have been named according to Figure 7.

4.1.1.1. Ethyl *N*-(4-cyanoselanylphenyl)carbamate (1a). From ethyl chloroformate. The compound was purified washed with ethyl ether (20 mL). Yellow solid; mp: 107–108 °C. Yield: 27%. IR (KBr) cm^{-1}: 3308 (N–H), 2967 (C–H_{ali}), 2153 (CN), 1718 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.25 (t, 3H, CH_3, $J_{CH3-CH2}$ = 7.2 Hz); 4.14 (q, 2H, CH_2, $J_{CH2-CH3}$ = 7.2 Hz); 7.54 (d, 2H, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.64 (d, 2H, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 9.89 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 18 (1C, CH_3); 64.1 (1C, CH_2); 109.0 (1C, SeCN); 118.8 (1C, C_4); 122.9 (2C, C_2, C_6); 138.7 (2C, C_3, C_5); 144.4 (1C, C_1); 157.1 (1C, C=O). MS (*m*/*z*% abundance): 270 ($M^{+\cdot}$, 2), 156 (69), 118 (100). Elemental Analysis for $C_{10}H_{10}N_2O_2Se$, Calcd/Found (%): C: 44.62/44.97; H: 3.74/3.54; N: 10.41/10.38.

4.1.1.2. Propyl *N*-(4-cyanoselanylphenyl)carbamate (1b). From propyl chloroformate. The compound was purified washed with ethyl ether (20 mL). Yellow solid; mp: 100 °C. Yield: 18%. IR (KBr) cm^{-1}: 3313 (N–H), 2975 (C–H), 2146 (CN), 1707 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, 3H, CH_3, $J_{CH3-CH2}$ = 7.6 Hz); 1.65 (m, 2H, βCH_2); 4.05 (t, 2H, αCH_2, $J_{\alpha CH2-\beta CH2}$ = 7.6 Hz); 7.54 (d, 2H, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.64 (d, 2H, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 9.90 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.1 (1C, CH_3); 23.3 (1C, βCH_2); 66.7 (1C, αCH_2); 106.2 (1C, SeCN); 116.3 (1C, C_4); 119.9 (2C, C_2, C_6); 135.9 (2C, C_3, C_5); 142.2 (1C, C_1); 154.1 (1C, C=O). MS (*m*/*z*% abundance): 284 ($M^{+\cdot}$, 40), 118 (100), 57 (35). Elemental Analysis for $C_{11}H_{12}N_2O_2Se$, Calcd/Found (%): C: 46.65/46.47; H: 4.27/4.76; N: 9.89/9.63.

4.1.1.3. Butyl *N*-(4-cyanoselanylphenyl)carbamate (1c). From butyl chloroformate. The compound was purified washed with ethyl ether (20 mL). Yellow solid; mp: 83–85 °C. Yield: 22%. IR (KBr) cm^{-1}: 3327 (N–H), 2961 (C–H), 2144 (CN), 1706 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.92 (t, 3H, CH_3, $J_{CH3-CH2}$ = 7.4 Hz); 1.38 (dq, 2H, γCH_2, $J_{\gamma CH2-CH3} = J_{\gamma CH2-\beta CH2}$ = 7.4 Hz); 1.60 (dt, 2H, βCH_2, $J_{\beta CH2-\alpha CH2} = J_{\beta CH2-\gamma CH2}$ = 7 Hz); 4.10 (t, 2H, αCH_2, $J_{\alpha CH2-\beta CH2}$ = 6.7 Hz); 7.54 (d, 2H, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.64 (d, 2H, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 9.91 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.5 (1C, CH_3); 19.4 (1C, γCH_2); 31.4 (1C, βCH_2); 65.0 (1C, αCH_2); 106.3 (1C, SeCN); 116.1 (1C, C_4); 120.2 (2C, C_2, C_6); 135.9 (2C, C_3, C_5); 141.6 (1C, C_1); 154.4 (1C, C=O). MS (*m*/*z*% abundance): 298 ($M^{+\cdot}$,

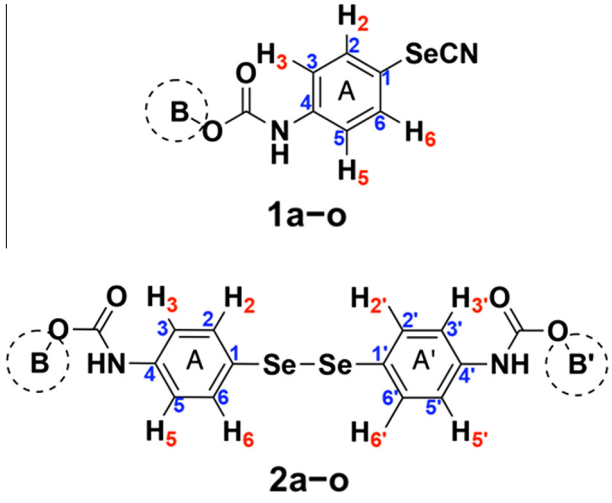


Figure 7. Assignment of chemical shifts in NMR spectroscopy.

43), 118 (72), 57 (100), 41 (42). Elemental Analysis for $C_{12}H_{14}N_2O_2Se$, Calcd/Found (%): C: 48.49/48.26; H: 4.75/4.72; N: 9.43/9.06.

4.1.1.4. Hexyl *N*-(4-cyanoselanylphenyl)carbamate (1d). From hexyl chloroformate. The compound was purified washed with ethyl ether (20 mL) and hexane (20 mL). Yellow solid; mp: 62–63 °C. Yield: 19%. IR (KBr) cm^{-1}: 3336 (N–H), 2961 (C–H), 2150 (CN), 1735 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.96 (t, 3H, CH_3, $J_{CH3-CH2}$ = 6 Hz); 1.43 (m, 6H, γ+δ+εCH_2); 1.70 (m, 2H, βCH_2); 4.18 (t, 2H, αCH_2, $J_{CH2-CH2}$ = 6 Hz); 7.63 (d, 2H, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8 Hz); 7.73 (d, 2H, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8 Hz); 9.99 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.8 (1C, CH_3); 22.9 (1C, εCH_2); 25.9 (1C, δCH_2); 29.3 (1C, γCH_2); 31.8 (1C, βCH_2); 65.3 (1C, αCH_2); 106.3 (1C, SeCN); 116.1 (1C, C_4); 120.2 (2C, C_2, C_6); 135.9 (2C, C_3, C_5); 141.7 (1C, C_1); 154.4 (1C, C=O). MS (*m*/*z*% abundance): 326 ($M^{+\cdot}$, 90), 118 (100), 43 (86). Elemental Analysis for $C_{14}H_{18}N_2O_2Se$, Calcd/Found (%): C: 51.69/51.27; H: 5.54/5.44; N: 8.62/8.65.

4.1.1.5. Isobutyl *N*-(4-cyanoselanylphenyl)carbamate (1e). From isobutyl chloroformate. The compound was purified washed with ethyl ether (20 mL). Yellow solid; mp: 88–90 °C. Yield: 14%. IR (KBr) cm^{-1}: 3318 (N–H), 2959 (C–H), 2141 (CN), 1706 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.95 (d, 6H, $2CH_3$, J_{CH3-CH} = 6.6 Hz); 1.92 (m, 1H, CH); 3.89 (d, 2H, CH_2, J_{CH2-CH} = 6.5 Hz); 7.55 (d, 2H, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.5 Hz); 7.64 (d, 2H, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.5 Hz); 9.91 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 19.7 (2C, $2CH_3$); 28.4 (1C, CH); 71.2 (1C, CH_2); 106.2 (1C, SeCN); 116.1 (1C, C_4); 120.2 (2C, C_2, C_6); 135.9 (2C, C_3, C_5); 141.6 (1C, C_1); 154.4 (1C, C=O). MS (*m*/*z*% abundance): 298 ($M^{+\cdot}$, 77), 118 (100), 57 (39). Elemental Analysis for $C_{12}H_{14}N_2O_2Se$, Calcd/Found (%): C: 48.49/48.44; H: 4.75/4.66; N: 9.43/9.11.

4.1.1.6. Allyl *N*-(4-cyanoselanylphenyl)carbamate (1f). From allyl chloroformate. The compound was purified washed with ethyl ether (20 mL). Yellow solid; mp: 91–92 °C. Yield: 28%. IR (KBr) cm^{-1}: 3239 (N–H), 2981 (C–H), 2148 (CN), 1707 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.62 (d, 2H, CH_2, $J_{CH2=CH}$ = 6 Hz); 5.26 (d, 1H, CH_{cis}, $J_{CHcis=CH}$ = 6 Hz); 5.39 (d, 1H, CH_{trans}, $J_{CHtrans=CH}$ = 6.1 Hz); 5.98 (m, 1H, CH); 7.54 (d, 2H, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.7 Hz); 7.65 (d, 2H, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.7 Hz); 10.00 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 65.8 (1C, CH_2); 106.2 (1C, SeCN); 116.3 (1C, C_4); 118.6 (1C, = CH_2); 120.3 (2C, C_2, C_6); 133.9 (1C, CH); 135.9 (2C, C_3, C_5); 141.5 (1C, C_1); 153.9 (1C, C=O). MS (*m*/*z*% abundance): 283 ($M^{+\cdot}$, 20), 118 (100), 56 (23). Elemental Analysis for $C_{11}H_{10}N_2O_2Se$, Calcd/Found (%): C: 46.99/46.65; H: 3.57/3.59; N: 9.96/9.35.

4.1.1.7. Phenyl *N*-(4-cyanoselanylphenyl)carbamate (1g). From phenyl chloroformate, prepared with phenol and trichloromethylchloroformate in toluene as solvent and with DMAP as catalyst according to the literature.[44] Recrystallized from methanol and washed with hexane (20 mL). White powder; mp: 120 °C. Yield: 16%. IR (KBr) cm^{-1}: 3299 (N–H), 2156 (CN), 1733 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.25–7.29 (m, 3H, B, H_3+H_4+H_5); 7.43 (d, 2H, B, H_2+H_6, $J_{2-3} = J_{6-5}$ = 7.5 Hz); 7.59 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.7 Hz); 7.70 (d, 2H, A, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.7 Hz); 10.51 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 106.3 (1C, SeCN); 117.1 (1C, A, C_1); 120.6 (2C, A, C_3, C_5); 122.8 (2C, B, C_2, C_6); 126.5 (1C, B, C_4); 130.3 (2C, B, C_3, C_5); 135.9 (2C, A, C_2, C_6); 140.9 (1C, A, C_4); 151.2 (1C, B, C_1); 152.5 (1C, C=O). MS (*m*/*z*% abundance): 319 ($M^{+\cdot}$, 2), 144 (45), 94 (100). Elemental Analysis for $C_{14}H_{10}N_2O_2Se$, Calcd/Found (%): C: 53.01/52.76; H: 3.18/3.05; N: 8.83/8.36.

4.1.1.8. 4-Methoxyphenyl *N*-(4-cyanoselanylphenyl)carbamate (1h). From 4-methoxyphenyl chloroformate, prepared with 4-methoxyphenol and trichloromethylchloroformate in toluene as solvent and with DMAP as catalyst according to the literature.[44] Recrystallized from methanol. White solid; mp: 163–164 °C. Yield: 12%. IR (KBr) cm^{-1}: 3299 (N–H), 2157 (CN), 1715 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.77 (s, 3H, OCH_3); 6.97 (d, 2H, B, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.16 (d, 2H, B, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 7.58 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 7.69 (d, 2H, A, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 10.44 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.3 (1C, OCH_3); 106.3 (1C, SeCN); 115.2 (2C, B, C_3, C_5); 116.9 (1C, A, C_1); 120.5 (2C, A, C_3, C_5); 123.7 (2C, B, C_2, C_6); 135.9 (2C, A, C_2, C_6); 141.1 (1C, A, C_4); 144.6 (1C, B, C_1); 152.9 (1C, C=O); 157.6 (1C, B, C_4). MS (*m*/*z*% abundance): 144 (65), 124 (100), 109 (82), 81 (41), 31 (55). Elemental Analysis for $C_{15}H_{12}N_2O_3Se$, Calcd/Found (%): C: 51.87/51.82; H: 3.48/3.61; N: 8.07/8.07.

4.1.1.9. 4-Methylphenyl *N*-(4-cyanoselanylphenyl)carbamate hydrochloride (1i). From 4-methylphenyl chloroformate. Recrystallized from methanol. Green solid; mp: 163–164 °C. Yield: 2%. IR (KBr) cm^{-1}: 3304 (N–H), 2156 (CN), 1712 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, CH_3); 7.11 (d, 2H, B, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 7.23 (d, 2H, B, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 7.58 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.69 (d, 2H, A, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 10.46 (s, 1H, NH). ^{13}C NMR (100 MHz,DMSO-d_6) δ: 106.3 (1C, SeCN); 116.9 (1C, A, C_1); 121.2 (2C, A, C_3, C_5); 121.9 (2C, B, C_2, C_6); 130.1 (2C, B, C_3, C_5); 135.2 (1C, B, C_4); 135.9 (2C, A, C_2, C_6); 140.9 (1C, A, C_4); 147.8 (1C, B, C_1); 151.9 (1C, C=O). MS (*m*/*z*% abundance): 332 ($M^{+\cdot}$, 2), 224 (18), 144 (36), 108 (100). Elemental Analysis for $C_{15}H_{12}N_2O_2Se{\cdot}HCl$, Calcd/Found (%): C: 48.98/48.40; H: 3.54/3.08; N: 7.62/7.71.

4.1.1.10. 4-Chlorophenyl *N*-(4-cyanoselanylphenyl)carbamate (1j). From 4-chlorophenyl chloroformate. Recrystallized from methanol and washed with hexane (20 mL). White solid; mp: 135–136 °C. Yield: 4%. IR (KBr) cm^{-1}: 3299 (N–H), 2151 (CN), 1734 (C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.16 (d, 2H, B, H_3+H_5, $J_{3-2} = J_{5-6}$ = 7.8 Hz); 7.27 (d, 2H, B, H_2+H_6, $J_{2-3} = J_{6-5}$ = 7.8 Hz); 7.59 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.68 (d, 2H, A, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 10.54 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 106.3 (1C, SeCN); 117.3 (1C, A, C_1); 120.6 (2C, A, C_3, C_5); 124.7 (2C, B, C_2, C_6); 130.2 (2C, B, C_3, C_5); 130.6 (1C, B, C_4); 135.9 (2C, A, C_2, C_6); 140.8 (1C, A, C_4); 150.0 (1C, B,

C_1); 152.2 (1C, C=O). MS (*m*/*z*% abundance): 224 (38), 196 (22) 144 (76), 128 (100). Elemental Analysis for $C_{14}H_9ClN_2O_2Se$, Calcd/Found (%): C: 47.82/47.44; H: 2.58/2.52; N: 7.97/8.04.

4.1.1.11. 4-Fluorophenyl *N*-(4-cyanoselanylphenyl)carbamate (1k). From 4-fluorophenyl chloroformate. Recrystallized from methanol. White powder; mp: 168–169 °C. Yield: 14%. IR (KBr) cm^{-1}: 3299 (N–H), 2157 (CN), 1715 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.27–7.29 (m, 4H, B, H_2+H_3+H_5+H_6); 7.58 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.5 Hz); 7.70 (d, 2H, A, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.5 Hz); 10.52 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 106.2 (1C, SeCN); 116.8 (2C, B, C_3, C_5, J^2_{C-F} = 24 Hz); 116.9 (1C, A, C_1); 120.6 (2C, A, C_3, C_5); 124.6 (2C, B, C_2, C_6); 135.9 (2C, A, C_2, C_6); 140.9 (1C, A, C_4); 147.3 (1C, B, C_1); 152.5 (1C, C=O); 159.2 (1C, B, C_4, J^1_{C-F} = 241 Hz). MS (*m*/*z*% abundance): 336 ($M^{+\cdot}$, 2), 224 (28), 144 (52), 112 (100). Elemental Analysis for $C_{14}H_9FN_2O_2Se$, Calcd/Found (%): C: 50.15/50.04; H: 2.69/2.72; N: 8.36/8.32.

4.1.1.12. Benzyl *N*-(4-cyanoselanylphenyl)carbamate (1l). From benzyl chloroformate. Recrystallized from methanol. White powder; mp: 98–99 °C. Yield: 29%. IR (KBr) cm^{-1}: 3342 (N–H), 2948 (C–H), 2155 (CN), 1709 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 5.17 (s, 2H, CH_2); 7.40–7.43 (m, 5H, B, H_2+H_3+H_4+H_5+H_6); 7.55 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.5 Hz); 7.65 (d, 2H, A, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.5 Hz); 10.06 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 66.9 (1C, CH_2); 106.3 (1C, SeCN); 116.4 (1C, A, C_1); 120.2 (2C, A, C_3, C_5); 129.3 (5C, B, C_2, C_3, C_4, C_5, C_6); 135.9 (2C, A, C_2, C_6); 137.2 (1C, B, C_1); 141.5 (1C, A, C_4); 154.1 (1C, C=O). MS (*m*/*z*% abundance): 333 ($M^{+\cdot}$, 42), 224 (37), 196 (40), 91 (100). Elemental Analysis for $C_{15}H_{12}N_2O_2Se$, Calcd/Found (%): C: 54.38/53.93; H: 3.63/3.59; N: 8.46/8.33.

4.1.1.13. 4-Nitrobenzyl *N*-(4-cyanoselanylphenyl)carbamate (1m). From 4-nitrobenzyl chloroformate. Recrystallized from methanol. Green powder; mp: 167–168 °C. Yield: 18%. IR (KBr) cm^{-1}: 3326 (N–H), 2149 (CN), 1725 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 5.32 (s, 2H, CH_2); 7.55 (d, 2H, B, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.66 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.70 (d, 2H, A, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 8.27 (d, 2H, B, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 10.19 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 65.6 (1C, CH_2); 106.2 (1C, SeCN); 116.6 (1C, A, C_1); 120.3 (2C, A, C_3, C_5); 124.5 (2C, B, C_3, C_5); 129.4 (2C, B, C_2, C_6); 135.9 (2C, A, C_2, C_6); 141.3 (1C, A, C_4); 145.2 (1C, B, C_1); 147.9 (1C, B, C_4); 153.8 (1C, C=O). MS (*m*/*z*% abundance): 377 ($M^{+\cdot}$, 46), 224 (37), 196 (25), 136 (100). Elemental Analysis for $C_{15}H_{11}N_3O_4Se$, Calcd/Found (%): C: 47.89/47.75; H: 2.95/2.89; N: 11.17/11.28.

4.1.1.14. (Fluoren-9-yl)methyl *N*-(4-cyanoselanylphenyl)-carbamate (1n). From fluorenylmethyloxycarbonyl chloride. Recrystallized from methanol. White powder; mp: 190 °C. Yield: 15%. IR (KBr) cm^{-1}: 3347 (N–H), 2159 (CN), 1728 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.32 (t, 1H, B, H_9, $J_{9\text{-}CH2}$ = 6 Hz); 4.53 (d, 2H, CH_2, J_{CH2-9} = 6.5 Hz); 7.36 (t, 2H, B, H_2+H_7, $J_{1-2} = J_{2-3} = J_{6-7} = J_{7-8}$ = 7.5 Hz); 7.44 (t, 2H, B, H_3+H_6, $J_{2-3} = J_{3-4} = J_{5-6} = J_{6-7}$ = 7.2 Hz); 7.51–7.52 (m, A, 2H, H_2+H_6); 7.62 (d, 2H, A, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 7.76 (d, 2H, B, H_1+H_8, $J_{1-2} = J_{8-7}$ = 7.4 Hz); 7.92 (d, 2H, B, H_4+H_5, $J_{3-4} = J_{5-6}$ = 7.4 Hz); 9.95 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 47.5 (1C, B, C_9); 66.6 (1C, CH_2); 106.3 (1C, SeCN); 116.4 (1C, A, C_1); 120.4 (2C, B, C_1, C_8); 121.1 (2C, A, C_3, C_5); 125.9 (2C, B, C_2, C_7); 128.0 (2C, B, C_4, C_5); 128.6 (2C, B, C_3, C_6); 135.9 (2C, A, C_2, C_6); 141.7 (1C, A, C_4); 144.6 (4C, B, C_{4a}, C_{4a1}, C_{8a}, C_{9a}); 154.2 (1C, C=O). MS (*m*/*z*% abundance): 418 ($M^{+\cdot}$, 1), 196 (10), 178 (100), 165 (36). Elemental Analysis for $C_{22}H_{16}N_2O_2Se$, Calcd/Found (%): C: 63.01/62.41; H: 3.82/3.99; N: 6.68/6.70.

4.1.1.15. 1,1-Dioxobenzo[b]thiophen-2-ylmethyl *N*-(4-cyanoselanylphenyl)carbamate (1o). From 1,1-dioxobenzo[*b*]thiophen-2-ylmethyl chloroformate. Recrystallized from methanol. White powder; mp: 197 °C. Yield: 19%. IR (KBr) cm^{-1}: 3334 (N–H), 2159 (CN), 1726 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 5.19 (s, 2H, CH_2); 7.56 (d, 2H, A, H_2+H_6, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.63–7.66 (m, 5H, A, H_2+H_6, B, H_3+H_5+H_6); 7.71 (d, 1H, B, H_4, J_{4-5} = 7.3 Hz); 7.90 (d, 1H, B, H_7, J_{7-6} = 7.3 Hz); 10.2 (s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 57.1 (1C, CH_2); 106.2 (1C, SeCN); 116.7 (1C, A, C_1); 120.5 (1C, B, C_7); 122.2 (2C, A, C_3, C_5); 127.1 (1C, B, C_4); 130.7 (1C, B, C_6); 131.9 (2C, A, C_2, C_6); 135.2 (2C, B, C_A, C_3); 135.9 (1C, B, C_5); 137.3 (1C, B, C_2); 139.3 (1C, A, C_4); 141.1 (1C, B, C_B); 153.5 (1C, C=O). MS (*m*/*z*% abundance): 420 ($M^{+\cdot}$, 4), 224 (41), 196 (44), 144 (84), 131 (100), 118 (58). Elemental Analysis for $C_{17}H_{12}N_2O_4SSe$, Calcd/Found (%): C: 48.69/49.01; H: 2.86/2.77; N: 6.68/6.69.

4.1.2. General procedure for compounds 2a–o

Appropriate chloroformates (6 mmol) dissolved in dry chloroform (25 mL) were added dropwise while stirring to 4,4-diaminodiphenyldiselenide (3 mmol) dissolved in dry chloroform (25 mL). The mixture was kept with stirring at room temperature for another 24 h and during stirring a solid precipitate formed. The resulting solid was collected by filtration and solvent was evaporated under vacuum. The residue was treated with ethyl ether (3 × 25 mL) to obtain the desired compounds. Compounds **2j** and **2m** were additionally washed with hexane (20 mL) for purification.

4.1.2.1. Diethyl (diselanediyldibenzene-4,1-diyl)biscarbamate (2a). From ethyl chloroformate. Yellow solid; mp: 125–126 °C. Yield: 32%. IR (KBr) cm^{-1}: 3370 (N–H), 1707 (C=O), 818 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, 6H, $2CH_3$, $J_{CH3-CH2}$ = 7.1 Hz); 4.13 (q, 4H, $2CH_2$, $J_{CH2-CH3}$ = 7.1 Hz); 7.43 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.5 Hz); 7.51 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.5 Hz); 9.79 (s, 2H, 2NH). ^{13}C NMR (100 MHz,$CDCl_3$) δ: 15.3 (2C, $2CH_3$); 61.2 (2C, $2CH_2$); 119.7 (4C, A+A′, C_3, C_5); 123.6 (2C, A+A′, C_1); 134.3 (4C, A+A′, C_2, C_6); 140.6 (2C, A+A′, C_4); 154.3 (2C, 2C=O). MS (*m*/*z*% abundance): 488 ($M^{+\cdot}$, 40), 442 (20), 244 (100), 198 (23), 172 (60). Elemental Analysis for $C_{18}H_{20}N_2O_4Se_2$, Calcd/Found (%): C: 44.46/44.26; H: 4.13/3.82; N: 5.76/5.83.

4.1.2.2. Dipropyl (diselanediyldibenzene-4,1-diyl)biscarbamate (2b). From propyl chloroformate. Yellow solid; mp: 119 °C. Yield: 45%. IR (KBr) cm^{-1}: 3406 (N–H), 1684 (C=O), 807 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.92 (t, 6H, $2CH_3$, $J_{CH3-CH2}$ = 6.8 Hz); 1.60–1.62 (m, 4H, $2\beta CH_2$); 4.03 (t, 4H, $2\alpha CH_2$, $J_{\alpha CH2-\beta CH2}$ = 6.4 Hz); 7.43–7.50 (m, 8H, A+A′, H_2+H_3+H_5+H_6); 9.80 (s, 2H, 2NH). ^{13}C NMR (100 MHz,$CDCl_3$) δ: 11.1 (2C, $2CH_3$); 22.7 (2C, $2\beta CH_2$); 66.7 (2C, 2 αCH_2); 119.7 (4C, A+A′, C_3, C_5); 123.6 (2C, A+A′, C_1); 134.3 (4C, A+A′, C_2, C_6); 140.6 (2C, A+A′, C_4); 154.4 (2C, 2C=O). MS (*m*/*z*% abundance): 516 ($M^{+\cdot}$, 29), 456 (21), 258 (96), 172 (100), 43 (56). Elemental Analysis for $C_{20}H_{24}N_2O_4Se_2$, Calcd/Found (%): C: 46.70/47.00; H: 4.70/4.67; N: 5.45/5.40.

4.1.2.3. Dibutyl (diselanediyldibenzene-4,1-diyl)biscarbamate hydrochloride (2c). From butyl chloroformate. Yellow solid; mp: 89 °C. Yield: 46%. IR (KBr) cm^{-1}: 3327 (N–H), 2958 (C–H), 1702 (C=O), 818 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, 6H, $2CH_3$, $J_{CH3-CH2}$ = 7.3 Hz); 1.38 (m, 4H, $2\gamma CH_2$); 1.60 (m, 4H, $2\beta CH_2$); 4.08 (t, 4H, $2\alpha CH_2$, $J_{\alpha CH2-\beta CH2}$ = 7.4 Hz); 7.43 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 7.8 Hz); 7.50 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 7.8 Hz); 9.79 (s, 2H, 2NH). ^{13}C NMR (100 MHz,$CDCl_3$) δ: 14.4 (2C, $2CH_3$); 19.5 (2C, $2\gamma CH_2$); 31.4 (2C, $2\beta CH_2$); 64.9 (2C, 2 αCH_2); 119.7 (4C, A+A′, C_3, C_5); 123.6 (2C, A+A′, C_1); 134.3 (4C, A+A′, C_2, C_6); 140.6 (2C, A+A′, C_4); 154.4 (2C, 2C=O). MS (*m*/*z*% abundance):

544 ($M^{+\cdot}$, 24), 470 (27), 272 (100), 198 (39), 172 (82), 57 (49). Elemental Analysis for $C_{22}H_{28}N_2O_4Se_2$·HCl, Calcd/Found (%): C: 45.60/45.81; H: 5.00/4.68; N: 4.84/4.77.

4.1.2.4. Dihexyl (diselanediyldibenzene-4,1-diyl)biscarbamate (2d). From hexyl chloroformate. Yellow solid; mp: 84 °C. Yield: 63%. IR (KBr) cm^{-1}: 3359 (N–H), 2954 (C–H), 1704 (C=O), 814 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.86–0.87 (m, 6H, $2CH_3$); 1.25–1.28 (m, 8H, 2δ+$2\varepsilon CH_2$); 1.58–1.60 (m, 4H, $2\gamma CH_2$) 3.35–3.37 (m, 4H, $2\beta CH_2$); 4.05–4.07 (m, 4H, $2\beta CH_2$ + $2\alpha CH_2$); 7.43 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.7 Hz); 7.50 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.7 Hz); 9.79 (s, 2H, 2NH). ^{13}C NMR (100 MHz,$CDCl_3$) δ: 14.8 (2C, $2CH_3$); 22.9 (2C, $2\varepsilon CH_2$); 25.9 (2C, $2\delta CH_2$); 31.8 (2C, $2\gamma CH_2$); 33.6 (2C, $2\beta CH_2$); 61.6 (2C, 2 αCH_2); 119.7 (4C, A+A′, C_3, C_5); 123.5 (2C, A+A′, C_1); 134.3 (4C, A+A′, C_2, C_6); 140.6 (2C, A+A′, C_4); 154.4 (2C, 2C=O). MS (*m*/*z*% abundance): 600 ($M^{+\cdot}$, 16), 498 (36), 300 (78), 198 (66), 172 (78), 43 (100). Elemental Analysis for $C_{26}H_{36}N_2O_4Se_2$, Calcd/Found (%): C: 52.18/52.10; H: 6.06/5.85; N: 4.68/4.98.

4.1.2.5. Diisobutyl (diselanediyldibenzene-4,1-diyl)biscarbamate (2e). From isobutyl chloroformate. Yellow solid; mp: 110 °C. Yield: 32%. IR (KBr) cm^{-1}: 3360 (N–H), 2959 (C–H), 1705 (C=O), 816 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.93 (d, 12H, $4CH_3$, $J_{CH3-CH2}$ = 6.7 Hz); 1.90–1.93 (m, 2H, 2CH); 3.87 (d, 4H, $2CH_2$, J_{CH2-CH} = 6.8 Hz); 7.43 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.7 Hz); 7.51 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.7 Hz); 9.80 (s, 2H, 2NH). ^{13}C NMR (100 MHz,$CDCl_3$) δ: 19.8 (4C, $4CH_3$); 28.4 (2C, 2CH); 71.1 (2C, $2CH_2$); 119.7 (4C, A+A′, C_3, C_5); 123.6 (2C, A+A′, C_1); 134.3 (4C, A+A′, C_2, C_6); 140.6 (2C, A+A′, C_4); 154.4 (2C, 2C=O). MS (*m*/*z*% abundance): 544 ($M^{+\cdot}$, 21), 470 (28), 272 (62), 198 (41), 172 (87), 57 (100). Elemental Analysis for $C_{22}H_{28}N_2O_4Se_2$, Calcd/Found (%): C: 48.72/48.44; H: 5.20/4.94; N: 5.16/5.10.

4.1.2.6. Diallyl (diselanediyldibenzene-4,1-diyl)biscarbamate (2f). From allyl chloroformate. Yellow solid; mp: 108 °C. Yield: 34%. IR (KBr) cm^{-1}: 3348 (N–H), 2924 (C–H), 1704 (C=O), 814 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.61 (d, 4H, $2CH_2$, J_{CH2-CH} = 5.4 Hz); 5.30–5.33 (m, 4H, $2CH_2$=); 5.94–5.97 (m, 2H, 2CH); 7.43 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 7.52 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.8 Hz); 9.90 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 65.7 (2C, $2CH_2$); 118.6 (2C, $2CH_2$=); 119.8 (4C, A+A′, C_3, C_5); 123.7 (2C, A+A′, C_1); 134.1 (4C, A+A′, C_2, C_6); 134.3 (2C, 2CH); 140.4 (2C, A+A′, C_4); 153.9 (2C, 2C=O). MS (*m*/*z*% abundance): 512 ($M^{+\cdot}$, 26), 256 (48), 213 (41), 172 (22), 156 (98), 92 (36), 41 (100). Elemental Analysis for $C_{20}H_{20}N_2O_4Se_2$, Calcd/Found (%): C: 47.07/46.57; H: 3.95/3.72; N: 5.49/5.50.

4.1.2.7. Diphenyl (diselanediyldibenzene-4,1-diyl)biscarbamate (2g). From phenyl chloroformate, prepared with phenol and trichloromethylchloroformate in toluene as solvent and with DMAP as catalyst according to the literature.[44] Yellow solid; mp: 167 °C. Yield: 7%. IR (KBr) cm^{-1}: 3317 (N–H), 1718 (C=O), 820 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.23–7.25 (m, 6H, B+B′, H_2+H_4+H_6); 7.49–7.55 (m, 8H, A+A′, H_2+H_6, B+B′, H_3+H_5); 7.57 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.8 Hz); 10.4 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 120.1 (2C, A+A′, C_1); 122.8 (8C, A+A′, C_3, C_5, B+B′, C_2, C_6); 126.4 (2C, B+B′, C_4); 130.3 (4C, B+B′, C_3, C_5); 134.3 (4C, A+A′, C_2, C_6); 139.9 (2C, A+A′, C_4); 151.3 (2C, 2C=O); 152.5 (2C, B+B′, C_1). MS (*m*/*z*% abundance): 396 (60), 198 (100), 94 (83). Elemental Analysis for $C_{26}H_{20}N_2O_4Se_2$, Calcd/Found (%): C: 53.62/53.30; H: 3.46/3.22; N: 4.81/4.75.

4.1.2.8. Bis(4-methoxyphenyl) (diselanediyldibenzene-4,1-diyl)biscarbamate (2h). From 4-methoxyphenyl chloroformate, prepared with 4-methoxyphenol and trichloromethylchloroformate in toluene as solvent and with DMAP as catalyst according to the literature.[44] Yellow solid; mp: 169–170 °C. Yield: 30%. IR (KBr) cm^{-1}: 3344 (N–H), 2927 (C–H), 1717 (C=O), 817 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.76 (s, 6H, $2OCH_3$); 6.96 (d, 4H, B+B′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 7.3 Hz); 7.14 (d, 4H, B+B′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 7.3 Hz); 7.47 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 7.2 Hz); 7.56 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 7.2 Hz); 10.34 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 56.3 (2C, $2CH_3$); 115.2 (4C, B+B′, C_3, C_5); 119.9 (4C, A+A′, C_3, C_5); 123.7 (4C, B+B′, C_2, C_6); 124.2 (2C, A+A′, C_1); 134.3 (4C, A+A′, C_2, C_6); 140.1 (2C, A+A′, C_4); 144.6 (2C, B+B′, C_1); 152.8 (2C, 2C=O); 157.6 (2C, B+B′, C_4). MS (*m*/*z*% abundance): 396 (53), 198 (98), 124 (99), 109 (100). Elemental Analysis for $C_{28}H_{24}N_2O_6Se_2$, Calcd/Found (%): C: 52.34/51.96; H: 3.74/3.95; N: 4.36/4.18.

4.1.2.9. Bis(4-methylphenyl) (diselanediyldibenzene-4,1-diyl)biscarbamate hemihydrochloride (2i). From 4-methylphenyl chloroformate. Yellow solid; mp: 174–175 °C. Yield: 12%. IR (KBr) cm^{-1}: 3391 (N–H), 1726 (C=O), 819 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.31 (s, 6H, $2CH_3$); 7.10 (d, 4H, B+B′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 7.22 (d, 4H, B+B′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 7.47 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 7.57 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 10.35 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 129.9 (4C, A+A′, C_3, C_5); 122.2 (4C, B+B′, C_2, C_6); 124.3 (2C, A+A′, C_1); 129.9 (4C, B+B′, C_3, C_5); 134.1 (4C, A+A′, C_2, C_6); 135.2 (2C, B+B′, C_4); 140.4 (2C, A+A′, C_4); 147.9 (2C, B+B′, C_1); 154.2 (2C, 2C=O). MS (*m*/*z*% abundance): 396 (56), 198 (100), 107 (82). Elemental Analysis for $C_{28}H_{24}N_2O_4Se_2$ (½) HCl, Calcd/Found (%): C: 53.50/53.68; H: 4.14/4.45; N: 4.45/4.64.

4.1.2.10. Bis(4-chlorophenyl) (diselanediyldibenzene-4,1-diyl)biscarbamate hydrochloride (2j). From 4-chlorophenyl chloroformate. Yellow solid; mp: 165–166 °C. Yield: 2%. IR (KBr) cm^{-1}: 3368 (N–H), 1724 (C=O), 819 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.28 (d, 4H, B+B′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 7.47–7.49 (m, 8H, A+A′, H_3+H_5, B+B′, H_2+H_6); 7.58 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 10.45 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 120.2 (4C, A+A′, C_3, C_5); 124.2 (2C, A+A′, C_1); 125.3 (4C, B+B′, C_2, C_6); 130.3 (4C, B+B′, C_3, C_5); 130.9 (2C, B+B′, C_4); 134.4 (4C, A+A′, C_2, C_6); 140.3 (2C, A+A′, C_4); 150.1 (2C, B+B′, C_1); 154.3 (2C, 2C=O). MS (*m*/*z*% abundance): 396 (55), 198 (100), 396 (55), 128 (80). Elemental Analysis for $C_{26}H_{18}Cl_2N_2O_4Se_2$·HCl, Calcd/Found (%): C: 45.38/45.22; H: 2.79/2.61; N: 4.30/4.07.

4.1.2.11. Bis(4-fluorophenyl) (diselanediyldibenzene-4,1-diyl)biscarbamate (2k). From 4-fluorophenyl chloroformate. Yellow solid; mp: 174–175 °C. Yield: 2%. IR (KBr) cm^{-1}: 3333 (N–H), 1723 (C=O), 820 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.25–7.27 (m, 8H, B+B′, H_2+H_3+H_5+H_6); 7.47 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6}$ = 8.4 Hz); 7.56 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 10.42 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 117.2 (4C, B+B′, C_3, C_5, J^2_{C-F} = 24 Hz); 120.2 (4C, A+A′, C_3, C_5); 124.3 (2C, A+A′, C_1); 134.2 (4C, A+A′, C_2, C_6); 136.1 (4C, B+B′, C_2, C_6); 140.4 (2C, A+A′, C_4); 152.1 (2C, B+B′, C_1); 154.3 (2C, 2C=O); 162.1 (2C, B+B′, C_4, J^1_{C-F} = 241 Hz). MS (*m*/*z*% abundance): 396 (55), 198 (100), 112 (86). Elemental Analysis for $C_{26}H_{18}F_2N_2O_4Se_2$, Calcd/Found (%): C: 50.50/49.75; H: 2.93/2.71; N: 4.53/4.50.

4.1.2.12. Dibenzyl (diselanediyldibenzene-4,1-diyl)biscarbamate (2l). From benzyl chloroformate. Yellow solid; mp: 158 °C. Yield: 24%. IR (KBr) cm^{-1}: 3359 (N–H), 1708 (C=O), 814 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 5.16 (s, 4H, $2CH_2$); 7.34–7.40 (m, 12H, A+A′, H_3+H_5, B+B′, H_2+H_3+H_5+H_6); 7.56 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5}$ = 8.4 Hz); 9.95 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 66.8 (2C, $2CH_2$); 119.7 (4C, A+A′, C_3, C_5); 123.8 (2C, A+A′, C_1); 128.9 (10C, B+B′, C_2, C_3, C_4, C_5, C_6); 134.4

(4C, A+A′, C_2, C_6); 137.3 (2C, B+B′, C_1); 140.4 (2C, A+A′, C_4); 154.1 (2C, 2C=O). MS (*m*/*z*% abundance): 396 (28), 198 (62), 91 (100). Elemental Analysis for $C_{28}H_{24}N_2O_4Se_2$, Calcd/Found (%): C: 55.09/55.10; H: 3.96/3.85; N: 4.59/4.57.

4.1.2.13. Bis(4-nitrobenzyl) (diselanediyldibenzene-4,1-diyl)biscarbamate (2m). From 4-nitrobenzyl chloroformate. Yellow solid; mp: 198 °C. Yield: 5%. IR (KBr) cm^{-1}: 3375 (N–H), 1718 (C=O), 813 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 5.35 (s, 4H, $2CH_2$); 7.47 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6} = 7.8$ Hz); 7.56 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5} = 7.8$ Hz); 7.67 (d, 4H, B+B′, H_3+H_5, $J_{3-2} = J_{5-6} = 8.8$ Hz); 8.25 (d, 4H, B+B′, H_2+H_6, $J_{2-3} = J_{6-5} = 8.8$ Hz); 10.01 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 65.9 (1C, CH_2); 120.2 (4C, A+A′, C_3, C_5); 124.1 (2C, A+A′, C_1); 125.3 (4C, B+B′, C_3, C_5); 129.1 (4C, B+B′, C_2, C_6); 134.2 (4C, A+A′, C_2, C_6); 140.1 (2C, A+A′, C_4); 145.2 (2C, B+B′, C_1); 148.3 (2C, B+B′, C_4); 154.1 (2C, 2C=O). MS (*m*/*z*% abundance): 396 (32), 198 (60), 136 (100). Elemental Analysis for $C_{28}H_{22}N_4O_8Se_2$, Calcd/Found (%): C: 48.01/47.75; H: 3.17/2.98; N: 8.00/7.63.

4.1.2.14. Bis[(fluoren-9-yl)methyl] (diselanediyldibenzene-4,1-diyl)biscarbamate (2n). From fluorenylmethyloxycarbonyl chloride. Yellow solid; mp: 175 °C. Yield: 50%. IR (KBr) cm^{-1}: 3370 (N–H), 1708 (C=O), 818 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.31 (t, 2H, B+B′, H_9, $J_{9\text{-}CH2} = 6.5$ Hz); 4.51 (d, 4H, $2CH_2$, $J_{CH2\text{-}9} = 6.5$ Hz); 7.35 (t, 4H, B+B′, H_2+H_7, $J_{1-2} = J_{2-3} = J_{6-7} = J_{7-8} = 7.4$ Hz); 7.40–7.42 (m, 8H, A+A′, H_2+H_6, B+B′, H_3+H_6); 7.49 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6} = 7.4$ Hz); 7.75 (d, 4H, B+B′, H_1+H_8, $J_{1-2} = J_{8-7} = 7.4$ Hz); 7.91 (d, 4H, B+B′, H_4+H_5, $J_{3-4} = J_{5-6} = 7.4$ Hz); 9.88 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 47.5 (2C, B+B′, C_9); 66.6 (2C, $2CH_2$); 119.9 (4C, B+B′, C_1, C_8); 121.1 (4C, A+A′, C_3, C_5); 123.8 (2C, A+A′, C_1); 125.9 (4C, B+B′, C_2, C_7); 127.9 (4C, B+B′, C_4, C_5); 128.6 (4C, B+B′, C_3, C_6); 134.2 (4C, A+A′, C_2, C_6); 140.3 (2C, A+A′, C_4); 141.7 (4C, B+B′, C_C, C_D); 144.6 (4C, B+B′, C_A, C_B); 154.2 (2C, 2C=O). MS (*m*/*z*% abundance): 213 (37), 156 (100), 92 (42). Elemental Analysis for $C_{42}H_{32}N_2O_4Se_2$, Calcd/Found (%): C: 64.12/63.58; H: 4.07/4.48; N: 3.56/3.68.

4.1.2.15. Bis[(1,1-dioxobenzo[b]thiophen-2-yl)methyl] (diselanediyldibenzene-4,1-diyl)biscarbamate (2o). From 1,1-dioxobenzo[*b*]thiophen-2-ylmethyl chloroformate. Yellow solid; mp: 182 °C. Yield: 47%. IR (KBr) cm^{-1}: 3342 (N–H), 1706 (C=O), 821 (Se–Se). ^1H NMR (400 MHz, $CDCl_3$) δ: 5.17 (s, 4H, $2CH_2$); 7.46 (d, 4H, A+A′, H_3+H_5, $J_{3-2} = J_{5-6} = 8.8$ Hz); 7.53 (d, 4H, A+A′, H_2+H_6, $J_{2-3} = J_{6-5} = 8.8$ Hz); 7.64–7.67 (m, 8H, B+B′, H_3+H_4+H_5+H_6); 7.90 (d, 2H, B+B′, H_7, $J_{2-3} = J_{6-5} = 7.6$ Hz); 10.10 (s, 2H, 2NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 56.9 (2C, $2CH_2$); 119.9 (4C, A+A′, C_3, C_5); 122.2 (2C, B+B′, C_7); 124.1 (2C, A+A′, C_1); 126.4 (2C, B+B′, C_4); 130.7 (2C, B+B′, C_6); 134.3 (4C, A+A′, C_2, C_6); 135.2 (4C, B+B′, C_A, C_3); 136.1 (2C, B+B′, C_5); 137.3 (2C, B+B′, C_2); 138.5 (2C, B+B′, C_B); 140.1 (2C, A+A′, C_4); 153.5 (2C, 2C=O). MS (*m*/*z*% abundance): 396 (18), 200 (45), 179 (59), 131 (100). Elemental Analysis for $C_{32}H_{24}N_2O_8S_2Se_2$, Calcd/Found (%): C: 48.86/48.84; H: 3.08/3.16; N: 3.56/3.08.

4.2. Biological evaluation

4.2.1. Cytotoxic and antiproliferative activities

Human tumour cell lines were provided by the European Collection of Cell Cultures (ECACC) or the American Type Culture Collection (ATCC). Five tumour cell lines (CCRF-CEM, HT-29, HTB-54, PC-3 and MCF-7) and two non-malignant cell lines were used (184B5 and BEAS-2B). CCRF-CEM, HT-29, PC-3 and HTB-54 cells were grown in RPMI 1640 medium (Invitrogen) supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin and 10 mM HEPES buffer (pH = 7.4). MCF-7 cells were grown in EMEM medium (Clonetics) supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin.184B5 cells were grown in Hams F-12/DMEM (50:50) supplemented as described by Li et al.[57] BEAS-2B were grown in Hams F-12/DMEM (50:50) supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin.

The cytotoxic effect of each substance was tested by the MTT method as previously described.[48] Cytotoxicity was determined at five different concentrations ranging from 0.01 μM to 100 μM. The plates with cells from the different lines, to which medium containing the substance under test was added, were incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2. Results are expressed as GI_{50}, the concentration that reduces by 50% the growth of treated cells with respect to untreated controls, TGI, the concentration that completely inhibits cell growth, and LC_{50}, the concentration that kills 50% of the cells. Data were obtained from at least 3 independent experiments performed in quadruplicate.

4.2.2. Radical scavenging activity evaluation

4.2.2.1. DPPH radical-scavenging assay. DPPH· assay was performed as described by Svinyarov.[53] Briefly, each compound was initially dissolved in absolute ethanol at a concentration of 1 mg/mL and serial dilutions were prepared. 150 μL of the ethanolic solutions were added to 0.2 μM DPPH· in ethanol (150 μL). The resulting mixtures were incubated at 37 °C in the dark. Decolourization of the purple radical to the yellow reduced form was followed at 517 nm. Determinations were recorded at several time intervals. Resveratrol was used as a positive control. All the measurements were carried out in quadruplicate. Results are expressed as the percentage of the radical scavenged, calculated using the following formula:

$$\%\ \text{DPPh radical scavenging} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} refers to the absorbance of the negative control (vehicle) and A_{sample} refers to the absorbance of the tested compound.

4.2.2.2. ABTS radical-scavenging assay. ABTS radical scavenging was assayed with a colorimetric test.[58] Briefly, ABTS was solved in deionized water at 1 mg/mL and oxidised to $ABTS^{+\cdot}$ with potassium persulfate (2.45 mM final concentration). This reaction mixture was kept overnight at room temperature in the dark. After radical generation, $ABTS^{+\cdot}$ was diluted with 50% ethanol until $A_{741} = 0.700 \pm 0.02$. Tested compounds were solved in absolute ethanol at a concentration of 1 mg/mL and 20 μL of this solution were added to 180 μL of the diluted $ABTS^{+\cdot}$. After 6 min of incubation the decrease in A_{741} was recorded using 50% ethanol as blank. Trolox was used as a positive control. All determinations were carried out in quadruplicate. The ability to scavenge $ABTS^{+\cdot}$ was calculated using the following formula:

$$\%\ \text{ABTS radical scavenging} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} refers to the absorbance of the negative control, vehicle plus $ABTS^{+\cdot}$, and A_{sample} refers to the absorbance of the tested compounds.

4.2.2.3. Determination of GPx-like activity. The GPx catalytic activity of selected selenoderivatives was evaluated using the method previously described by Iwaoka and Tomoda.[55] Briefly, compounds were solved in ethanol at 0.1 mg/mL. Then, 200 μL of this solution were added to a solution containing 200 μL of 2 mM PhSH in ethanol and 200 μL of 5 mM H_2O_2 in ethanol. The H_2O_2 reduction was monitored by measuring UV absorption of PhSSPh at 305 nm for 150 s. Data were collected every 5 s. A solution of

the appropriate compound in ethanol was used as a blank. Negative control, a solution containing the test compound, vehicle and PhSH (in the absence of H_2O_2), was also measured. All determinations were carried out in triplicate.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.02.048.

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