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Synthesis and antiproliferative activity of novel selenoester derivatives

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ABSTRACT

A series of 31 new selenoesters were synthesized and their cytotoxic activity was evaluated against a prostate cancer cell line (PC-3). The most active compounds were also tested against three tumoural cell lines (MCF-7, A-549 and HT-29) and one non-tumour prostate cell line (RWPE-1). Thirteen compounds showed significant activity towards all tumour cells investigated, and some of them were even more potent than etoposide and cisplatin, which were used as reference drugs. Because of their pronounced potency and/or selectivity, four analogues (**5**, **21**, **28** and **30**), were selected in order to assess their redox properties related to a possible redox modulating activity. The glutathione peroxidase (GPx) assay showed slight activity for compound **30** and the 2,2-diphenyl-1-picrylhydrazyl-(DPPH) assay showed a weak activity for compounds **5** and **28**. The present results revealed that analogues **5**, **21**, **28** and **30** might serve as a useful starting point for the design of improved anti-tumour agents.

1. Introduction

Cancer represents one of the most serious clinical problems in the world and has become the second major cause of death in developed countries. Over 1.5 million cases of cancer occur in the United States annually and cancer-related deaths are estimated to reach 12 million worldwide in 2015 [1]. Unlimited replication, self-sufficiency in growth signals, insensitivity to antigrowth signals, sustained angiogenesis, metastasis, and evasion of apoptosis are the hallmarks of cancer cells [2]. Most of the anticancer agents currently used in clinical practice exhibit undesirable side effects such as reduced bioavailability, toxicity and drug-resistance [3]. Therefore, cancer treatment has been a major endeavour of research and development in academia and pharmaceutical industry for the last decades. For this reason, the discovery and development of novel molecules that possess ideally both, a chemoprotective effect and an anticancer chemotherapeutic effect, are pertinent.

Amidst the wide range of compounds tested as potential anticancer agents, derivatives comprising functionalities based on a selenium moiety have attracted reasonable attention [4–9]. The role of selenium as part of potential redox-modulating, chemotherapeutic and chemopreventive agents has been supported by epidemiological, preclinical and clinical studies, having shown many authors associations among them [10–13]. Several mechanisms have been suggested to explain the anticancer effects of selenium derivatives. The most accepted ones are reduction of DNA damage, oxidative stress, inflammation, enhancement of the immune response, incorporation into selenoproteins, tumour suppressor gene silencing by alteration of their DNA methylation status, cell cycle arrest, inhibition of angiogenesis and induction of apoptosis [14,15]; depending on the chemical form of selenium involved, the type of cancer and its dosage [16,17]. Related to chemopreventive activity, different mechanisms of action have been reported. Among them, the action of certain selenoderivatives as interleukin-18 expression suppressors [18] and angiogenesis inhibitors [19] can be highlighted; being the control of the level of oxidative stress the point of convergence of both activities [20–27]. This control can be exerted by modulating the expression of selenoproteins [20,23,25], regulating the intracellular redox state [21,22,24] or scavenging the reactive oxygen species [26].

Furthermore, it is well known that the selenium metabolism is critical for the anticancer activity of selenocompounds. Amidst the active metabolites, α-keto acids as well as methylselenol have been

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hypothesized as responsible of the anticancer and chemopreventive effects [28–30]. Recently, glutaredoxin proteins, which exert a critical role in the maintenance of the cell redox homeostasis, have been proposed to be implicated in a novel pathway for selenium metabolism [31].

In the past few years, we have been involved in the development, design as well as the synthesis of structurally modified selenium derivatives, some of which have exhibited significant anticancer activity as cytotoxic and antiproliferative agents acting through the induction of apoptosis, as antioxidants or even as kinase modulators [32–40].

Among these various selenium compounds, our research group described in 2009 the synthesis and antiproliferative activity of particular selanylacetic acid derivatives [33]. Prompted by the abovementioned results and by the promising activity profile associated with several selanylacetic derivatives, and as an extension of our previous studies, the aim of this study is to design and synthesis of a range of novel selenoester-substituted derivatives using the structural derivatization approach. It is expected that the potential of these compounds as anticancer and chemopreventive agents should be higher than the one of the original compounds.

The rationale behind this strategy is to retain the selenoester group as scaffold, for considering that its possible hydrolysis represents a crucial "redox activation" step which could be modulated by the inclusion of different functional groups in the moiety bound to the selenium atom of the selenoester. The hydrolysis subsequently generates ionic species of selenium (such as selenols) which can readily participate in redox processes. These charged selenocompounds may possess inherent biological activity, and it would be beneficial if they could enhance the cytotoxic impact on cancer cells.

The strategy to optimize the activity of these new selenoester derivatives (Fig. 1) involved the modification of the lead molecules previously reported [33], taking into account the synthetic accessibility.

Therefore, all the compounds share as common feature a selenoester group whose carbonylic carbon is bounded to an aromatic or heteroaromatic ring with or without substituents through a linker of variable length (0–2 carbons), whereas the selenium atom is linked to different functionalities through an alkyl bridge of the same length as the aforementioned linker; the functionalities being considered include amides, ketones, methyl groups and methyl, *tert*-butyl and phenyl esters. These functional groups have been selected in order to obtain compounds with different physicochemical characteristics related to hydrophobic or hydrophilic properties and hydrogen bond donors or acceptors, with the purpose of maintaining a gradient in their polarity and to modify their ability to establish electrostatic interactions. The placement of different substituents in the aryl ring leads to variations of the electronic, hydrophobic and steric properties of the molecule, thus exerting a noteworthy influence over the magnitude of the hydrolysis process as well as over the compounds' expected biological activity.

The selection of the substituents is mainly based on the results obtained for the previously reported selanylacetic acids [33].

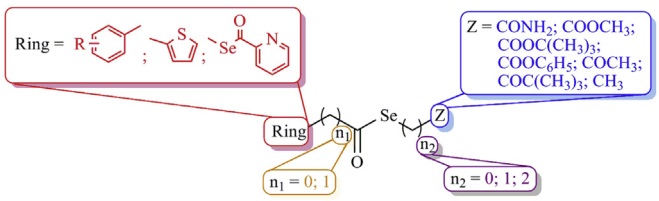


Fig. 1. General scheme of structural modulation.

Furthermore, it seems reasonable to bear in mind the biological activity of related compounds which contain heterocyclic rings instead of carbocycles, hence certain representative pyridyl and thienyl derivatives have been also synthesized. Finally, we have investigated whether or not the length of the aliphatic side chain is a key feature for *in vitro* activity.

Herein, we report the synthesis of thirty-one new compounds and their *in vitro* cytotoxic activity against prostate human tumour cell line (PC-3). The most active compounds of the preliminary screening have been evaluated further against the immortalized non-tumour cell line RWPE-1 in order to assess the selectivity of the compounds for cancer cells (compared to non-cancer cells). In addition, some of these active compounds have been tested against other tumour cell lines (*i.e.* breast, lung and colon) with the aim of ascertain whether there are differences of susceptibility amidst the cancer cell lines derived from different tissues. Furthermore, the involvement of the compounds in redox-modulating processes is determined through the evaluation of their electrochemical properties, their glutathione peroxidase (GPx)-like activity as well as the assessment of their capability to interact with a stable free radical as 2,2-diphenyl-1-picrylhydrazyl (DPPH).

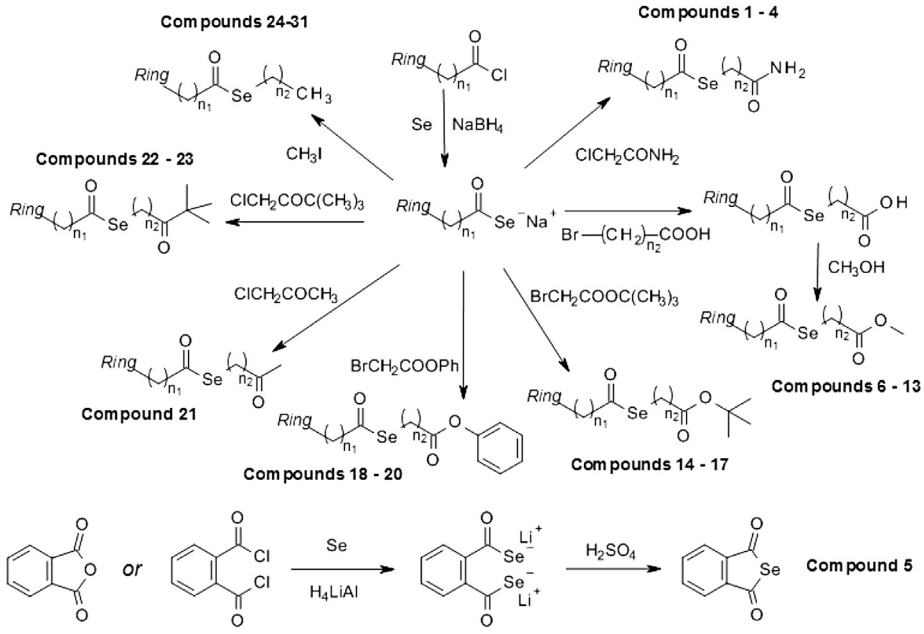
2. Results and discussion

2.1. Synthetic chemistry

The key step in the synthetic strategy for the majority of the compounds synthesized (**1**–**4** and **14**–**31**) was the reaction between sodium hydrogen selenide, produced by the reaction of powdered grey selenium with sodium borohydride in an adequate medium, and the aroyl or heteroaroyl chloride, followed by the treatment of the intermediate sodium aroylselenide generated with the corresponding α-halo derivative according to Scheme 1. This synthesis was carried out following our own protocol [33], which was based on a published procedure [41]. Derivative **5** was isolated with a 94% yield, whereby the selenating agent was generated by the reaction of lithium aluminium hydride with selenium in anhydrous THF, the subsequent reaction with 2-benzofuran-1,3-dione or phthaloyl chloride and the final dehydration with sulphuric acid. Compound **5** could also be obtained following the sodium borohydride based-procedure, although in this case the yield is lower (18%). In general, the yields are moderate with the exception of compounds **25**–**29**, for which yields up to 70% could be achieved. The derivatives **6**–**13** were synthesized from the corresponding selanylacetic acids [33] by Fischer esterification with yields ranging from 18 to 63%.

All the newly synthesized compounds were pure and chemically stable on air, according to the spectroscopic (IR, ^{1}H and ^{13}C NMR, MS) and the elemental analysis carried out to confirm the structures of the different derivatives. The patterns observed in the IR spectra were similar for all derivatives, including the carbonyl stretch of the selenoester as the most remarkable band observed in the IR spectra. On the other hand, no general features were found in ^{1}H and ^{13}C NMR spectra due to the high structural variations amidst the different compounds. Multidimensional COSY, HMQC and HMBC NMR experiments were performed for selected compounds to confirm their respective structures.

In the mass spectra of methylselenoesters (derivatives **24**–**31**), the molecular ion peak is observed, although with a low abundance; whereas the most intense peak in the mass spectra of the majority of the selenocompounds is either the carbonyl-containing cation generated after the selenoester bond breakdown or the fragment resulting when carbon dioxide is liberated from the aforesaid carbonyl ion.



Comp.	Ring	n_1	n_2	Comp.	Ring	n_1	n_2
1	Phenyl	0	1	17	Phenyl	1	1
2	4–Chlorophenyl	0	1	18	Phenyl	0	1
3	3,5–Dimethoxyphenyl	0	1	19	4–Chlorophenyl	0	1
4	Phenyl	1	1	20	3,5–Dimethoxyphenyl	0	1
6	Phenyl	0	1	21	4–Chlorophenyl	0	1
7	4–Chlorophenyl	0	1	22	4–Chlorophenyl	0	1
8	2–Chlorophenyl	0	1	23	3,5–Dimethoxyphenyl	0	1
9	3,5–Dimethoxyphenyl	0	1	24	Phenyl	0	0
10	3,4,5–Trimethoxyphenyl	0	1	25	4–Chlorophenyl	0	0
11	2–Thienyl	0	1	26	4–Cyanophenyl	0	0
12	Phenyl	1	1	27	3,5–Dimethoxyphenyl	0	0
13	Phenyl	1	2	28	4–Methylselenoacylphenyl	0	0
14	Phenyl	0	1	29	3–Methylselenoacylphenyl	0	0
15	4–Chlorophenyl	0	1	30	2–Methylselenoacylpyridyl	0	0
16	3,5-Dimethoxyphenyl	0	1	31	5–Methylselenoacylthienyl	0	0

Scheme 1. Synthesis of compounds **1**–**31**.

2.2. Biological evaluation

2.2.1. Cytotoxicity

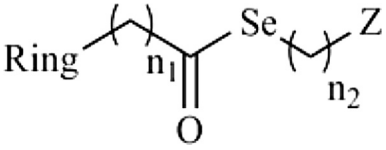
Many of the studies addressing a prospective anticancer activity of selenium compounds have been performed in prostate cancer cell lines [42–46]. Thus, the 31 derivatives synthesized have been tested *in vitro* against cultured prostate cancer cells (PC-3). To evaluate the antiproliferative potential of the aforementioned compounds, the values of cytostatic and cytotoxic parameters, such as GI_{50}, TGI and LC_{50}, are determined after an incubation of the cells with the compounds for 72 h. The LC_{50} indicates the cytotoxic potency of a derivative, whereas the TGI and the GI_{50} measure the antiproliferative properties as they represent the concentrations at which the cell growth of treated cells is suppressed completely or reduced by 50%, respectively, in comparison to the untreated control.

Cytotoxicity assays were based on the reactivity of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] as

described by Denizot and Lang [47], measured over a range of concentrations of each substance between 0.01 and 100 μM. To place the data in perspective, cisplatin and etoposide were selected as a positive control. The analyses were all performed with a minimum of three independent experiments. As shown in Table 1, 20 of the 31 derivatives assayed (*i.e.* compounds **3**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **18**, **19**, **21**, **22**, **23**, **25**, **28**, **29**, **30** and **31**) exhibited potent antiproliferative activity against PC-3 cells, with GI_{50} values lower than 10 μM. 13 of them (*i.e.* compounds **6**, **8**, **9**, **11**, **12**, **13**, **21**, **22**, **23**, **28**, **29**, **30** and **31**) were also active in improving the GI_{50} of cisplatin (5 μM). Furthermore, the three most cytostatic compounds (**29**, **30** and **31**) show GI_{50} values in nanomolar range, *i.e.* between 8.4 and 57 nM. Amazingly, it appears that these compounds are 11–70-fold more active than etoposide. Interestingly, the derivatives with the selenium-moiety showing higher polarity used to have a lower activity. In other words, the replacement of the original acid group of selanylacetic acids by an ester or ketone group might improve the cytostatic efficiency against this cancer cell line. In fact, this hypothesis is confirmed through the comparison of the activity of derivatives **1** versus **6** and **18**; and **2** versus **7**, **19**, **21** and **22**. In contrast, steric hindrance can explain that the R′-oxicarbonylselenoesters whose R′ is a bulky substituent, such as a *tert*-butyl group (compounds **6**, **7**, **9** and **12**), exhibit a lower activity compared to similar compounds in which R′ is a methyl group. According to a previous work of our group the number of methylene groups of the n_1 chain that maximizes the activity were 0 and 1, as further extensions of the chain inactivates the compounds [33]. For this reason only these values of n_1 were considered, finding that, in compounds which contain an amide or a *tert*-butyl ester group, the molecule with a methylene group in n_1 has a slightly higher activity than the comparable arylselenoester; whereas the effect is the opposite one in the derivatives with a methyl ester group in the Se-bounded alkyl chain. Regarding the length of this second alkyl chain (n_2), it is observed that its extension (from 1 to 2 methylene groups) entails a higher increase of the toxicity in non-tumoural prostate cells than the observed increase of the activity in PC-3 cell line.

Finally, several methylselenoesters (compounds **24**–**31**) have been synthesized and evaluated since methylselenol has been hypothesized to be a selenium metabolite with its very own

Table 1
Average GI_{50}, TGI and LC_{50} values (μM) for compounds.



Comp	Ring	n_1	n_2	Z	PC-3			RWPE-1			SI[e]
					GI_{50}[a]	TGI[b]	LC_{50}[c]	GI_{50}	TGI	LC_{50}	
1	Phenyl	0	1	$CONH_2$	46.25	97.55	>100	ND[d]	ND	ND	ND
2	4-Chlorophenyl	0	1	$CONH_2$	40.96	>100	>100	ND	ND	ND	ND
3	3,5-Dimethoxyphenyl	0	1	$CONH_2$	6.36	70.6	>100	5.76	36.0	>100	0.9
4	Phenyl	1	1	$CONH_2$	35.68	>100	>100	ND	ND	ND	ND
5	Anhydride	—	—	—	7.91	79.66	>100	28.9	>100	>100	3.7
6	Phenyl	0	1	$COOCH_3$	2.94	8.76	>100	4.97	16.3	88.3	1.7
7	4-Chlorophenyl	0	1	$COOCH_3$	9.10	73.99	>100	8.60	55.8	>100	0.9
8	2-Chlorophenyl	0	1	$COOCH_3$	4.18	9.06	>100	3.84	15.8	91.2	0.9
9	3,5-Dimethoxyphenyl	0	1	$COOCH_3$	4.29	9.07	>100	6.10	16.3	89.4	1.4
10	3,4,5-Trimethoxyphenyl	0	1	$COOCH_3$	5.61	57.31	>100	10.4	57.1	>100	1.9
11	2-Thienyl	0	1	$COOCH_3$	4.87	18.38	>100	5.62	14.11	76.6	1.2
12	Phenyl	1	1	$COOCH_3$	4.81	10	>100	3.06	35.7	99.2	0.6
13	Phenyl	1	2	$COOCH_3$	2.22	19.71	>100	0.88	4.85	9.55	0.4
14	Phenyl	0	1	$COOC(CH_3)_3$	45.22	>100	>100	ND	ND	ND	ND
15	4-Chlorophenyl	0	1	$COOC(CH_3)_3$	63.72	>100	>100	ND	ND	ND	ND
16	3,5-Dimethoxyphenyl	0	1	$COOC(CH_3)_3$	27.0	77.7	>100	ND	ND	ND	ND
17	Phenyl	1	1	$COOC(CH_3)_3$	43.56	>100	>100	ND	ND	ND	ND
18	Phenyl	0	1	$COOC_6H_5$	6.38	79.41	>100	7.16	46.3	>100	1.1
19	4-Chlorophenyl	0	1	$COOC_6H_5$	6.86	82.68	>100	7.54	>100	>100	1.1
20	3,5-Dimethoxyphenyl	0	1	$COOC_6H_5$	36.64	99.19	>100	ND	ND	ND	ND
21	4-Chlorophenyl	0	1	$COCH_3$	1.58	3.14	4.71	2.03	5.32	8.60	1.3
22	4-Chlorophenyl	0	1	$COC(CH_3)_3$	2.19	28.3	>100	2.29	5.60	8.91	1.0
23	3,5-Dimethoxyphenyl	0	1	$COC(CH_3)_3$	2.66	6.91	>100	3.34	6.30	9.25	1.3
24	Phenyl	0	0	CH_3	13.03	>100	>100	ND	ND	ND	ND
25	4-Chlorophenyl	0	0	CH_3	8.58	71.31	>100	27.8	>100	>100	3.2
26	4-Cyanophenyl	0	0	CH_3	36.30	77.36	>100	ND	ND	ND	ND
27	3,5-Dimethoxyphenyl	0	0	CH_3	14.41	71.28	>100	ND	ND	ND	ND
28	4-Methylselenoacylphenyl	0	0	CH_3	0.82	18.41	>100	5.24	39.4	>100	6.4
29	3-Methylselenoacylphenyl	0	0	CH_3	0.0570	23.69	>100	0.0244	0.975	65.0	0.4
30	6-Methylselenoacylpyridyl	0	0	CH_3	0.0084	1.69	>100	0.0264	3.01	67.2	3.1
31	5-Methylselenoacylthienyl	0	0	CH_3	0.0089	0.81	>100	0.0091	0.729	>100	1.0
	Etoposide				0.63	3.98	79.43	0.9	4.8	>100	1.4
	Cisplatin				5.01	50.12	>100	2.4	5.87	>100	0.4

[a] GI_{50}: growth inhibition 50%.
[b] TGI: total growth inhibition.
[c] LC_{50}: lethal concentration 50%.
[d] ND: not determined.
[e] SI: selectivity index (GI_{50} RWPE-1/GI_{50} PC-3).

anticancer properties [48–52]. Five of the compounds considered (*i.e.* **25**, **28**, **29**, **30** and **31**) exhibited GI_{50} values below 10 μM. Remarkably derivatives **28**–**31**, which possess a molecular symmetry in their structure, are the most potent amidst all compounds tested. Molecular symmetry is a structural property frequently presented in anticancer drugs [53,54] used in prostate cancer treatment [55,56]. Additionally, the effect of substituents on the phenyl ring of the tail as well as the length of the alkyl chains between the carbonyl groups has also been investigated (Fig. 1); although no apparent correlations were found between these parameters and the biological activity.

Compounds are classified as cytostatic or cytotoxic depending of the separation between the different cell survival/proliferation parameters obtained by the MTT assay (LC_{50}, TGI, GI_{50}). In this way, compounds whose three parameters reside more or less in the same order are considered as cytotoxic derivatives; whereas the ones with a noteworthy gap between the cytotoxic and the two-antiproliferative markers are denoted as cytostatic agents. For example, compounds **6** (GI_{50} = 2.94 μM, TGI = 8.76 μM and LC_{50} > 100 μM), **8** (GI_{50} = 4.18 μM, TGI = 9.06 μM and LC_{50} > 100 μM), **9** (GI_{50} = 4.29 μM, TGI = 9.07 μM and LC_{50} > 100 μM), **23** (GI_{50} = 2.66 μM, TGI = 6.91 μM and LC_{50} > 100 μM), **30** (GI_{50} = 0.0084 μM, TGI = 1.69 μM and LC_{50} > 100 μM) and **31** (GI_{50} = 0.0089 μM, TGI = 0.81 μM and LC_{50} > 100 μM) exhibited a very interesting pattern of selective cytostatic potency against the PC-3 cell line. In contrast, compound **21** (GI_{50} = 1.58 μM, TGI = 3.14 μM and LC_{50} = 4.71 μM) may be classified as cytotoxic agent for showing the highest overall potency in all three parameters, including the LC_{50} value.

The activity of the compounds that presented a good inhibitory activity (GI_{50} < 10 μM) in PC-3 cells has been determined in a non-tumour prostate cell line (RWPE-1), following the same experimental procedure, to obtain a rough measure of their selectivity between cancer cells and normal cells. In order to achieve this, the selectivity index (SI) was calculated as the ratio of the GI_{50} values determined for the non-tumour and the malignant cells. Compounds **5**, **25**, **28** and **30** were the most selective ones for exhibiting SI values of 3.6, 3.4, 6.4 and 3.1, respectively. In contrast, the remaining derivatives showed similar values in cancer and non-tumour cells, pointing towards a more general, unspecific cytotoxic/cytostatic activity.

Finally, taking into account that each cancer cell line presents a distinctive pattern of sensitivity, thirteen compounds (**6**, **8**, **9**, **11**, **12**, **13**, **21**, **22**, **23**, **28**, **29**, **30** and **31**) were selected for additional cytotoxicity assays in breast adenocarcinoma (MCF-7), human lung cancer (A-549) and colon carcinoma (HT-29) cell lines (Table 2). Here, having a GI_{50} value <5 μM in the PC-3 cell line was the arbitrary and demanding criterion considered for selecting the aforesaid derivatives. With the exception of compounds **8** and **11**, that exerted a modest antiproliferative activity in MCF-7, the remaining compounds tested demonstrated a potent cytostatic effect on all the cell lines tested in a dose-dependent manner (Fig. 2). Interestingly, all the selenoesters tested in these cell lines exhibited a higher activity than cisplatin in HT-29 cells, whereas nine and three of them improved the action of this reference drug in A-549 and MCF-7 cell lines, respectively. In addition, three derivatives (**29**, **30** and **31**) had nanomolar GI_{50} values in all the cell lines studied. It is quite remarkable that compounds **28** and **29** exhibited rather different activity profiles, with a marked cytostatic activity found for **29** – but not for **28** – in A-549 and HT-29 cells, in spite of their structural similarity.

In terms of cell line sensitivity, the higher activities generally were observed for A-549 > PC-3 > HT-29 > MCF-7 cell lines.

2.2.2. Redox modulating activity

Nowadays, there is an increased interest of using antioxidants for medical purposes. Thus, drugs possessing redox modulating activities, such as free radical scavenging and antioxidant properties, are considered for prevention and/or treatment of diseases directly related to a disturbed redox homeostasis present in the organisms. Moreover, recent literature reported the possible role of selenium in the etiology of carcinogenesis by modulating oxidative stress [25,57–59]. The aim of developing hybrid compounds through the combination of the antitumour and antioxidant activities in the same molecule may have a synergistic effect, resulting in a higher activity than the sum of the different components. Systems-oriented drug design, which considers network crosstalk and regulation instead of an individual target, has thus become a promising alternative approach in overcoming the problems of targeting a single preselected target. The results previously described prompted us to perform further biological studies in order to gain a preliminary insight into the mechanisms of action of selected compounds. Derivatives **28** and **30** were chosen because they were the most selective ones among the four compounds with nanomolar GI_{50} values in PC-3 cells. Compound **21** was selected for being the only one that exerted a cytotoxic action in prostate cancer cells. And finally, derivative **5** was chosen for being a selenoanhydride instead a selenoester: here, selenium anion formation should be favoured upon hydrolysis due to its higher reactivity.

Table 2
Average GI_{50}, TGI and LC_{50} values (μM) for selected compounds.

Compound	MCF-7			A-549			HT-29		
	GI_{50}[a]	TGI[b]	LC_{50}[c]	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
6	8.1	>100	>100	2.7	6.5	38.3	5.5	37.0	>100
8	10.9	>100	>100	4.1	7.1	38.6	3.6	9.2	>100
9	8.9	>100	>100	4.2	7.2	44.8	4.7	9.5	96.5
11	16.3	>100	>100	3.7	6.9	23.7	3.4	25.7	>100
12	9.5	74.9	>100	2.2	6.9	82.0	3.7	24.7	>100
13	7.8	>100	>100	1.3	5.8	51.8	3.3	7.3	>100
21	4.5	9.3	>100	1.7	7.1	87.0	2.6	5.8	9.1
22	5.3	>100	>100	1.2	5.6	>100	3.6	6.8	>100
23	6.3	>100	>100	6.01	27.5	92.5	5.0	8.9	94.2
28	8.9	>100	>100	2.9	42.7	>100	5.5	60.8	>100
29	0.52	>100	>100	0.061	0.680	>100	0.331	7.9	>100
30	0.29	>100	>100	0.062	4.2	>100	0.047	3.5	>100
31	0.32	>100	>100	0.079	0.710	>100	0.295	7.99	>100
Etoposide	19.95	>100	>100	4.46	>100	>100	31.62	>100	>100
Cisplatin	3.16	>100	>100	3.63	39.9	>100	7.95	>100	>100

[a] GI_{50}: growth inhibition 50%.
[b] TGI: total growth inhibition.
[c] LC_{50}: lethal concentration 50%.

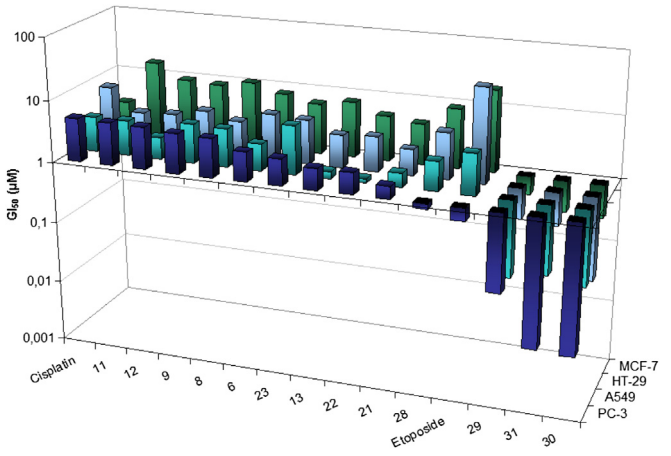


Fig. 2. Cytotoxicity of compounds, cisplatin and etoposide (GI_{50}) against MCF-7 (breast), HT-29 (colon), A-549 (lung) and PC-3 (prostate) human cancer cell lines, being the *z* axis logarithmic and cut by the basal *xy* plane at 1 μM to highlight the potency of the most cytostatic compounds.

Therefore, measurements of the electrochemical behaviour, the capacity to interact with hydrogen peroxide (GPx-like activity) and the capability to interact with a stable free radical (DPPH) may provide useful information about the redox modulating and/or antioxidant profile of the four selected active compounds.

In a first step we have employed cyclic voltammetry in order to evaluate whether the compounds are involved in redox reactions, using a mercury-drop or carbon electrode and aqueous, buffered electrolyte solutions (to represent certain aspects of biological conditions) [60]. Voltammograms of compounds **5**, **21** and **28** could be recorded, whilst it has been impossible to analyze compound **30** due to its lower solubility in aqueous media. The compounds examined showed peaks in mercury electrode, and two of them (**5** and **21**), also showed signals related to electrochemical processes at the carbon electrode (at compound concentrations of 5 mM). These signals can be interpreted as follows: in general terms, selenoesters showed a cathodic reduction peak E_{pc} in the potential interval between −550 and −700 mV and a corresponding anodic oxidation peak E_{pa} generally a couple of tens of millivolts at more positive potentials (see Table 3). Not unexpectedly, these oxidation and reduction potentials point to the presence of a readily reductable diselenide (R–SeSe–R′) and a readily oxidizable anionic selenium species, probably the corresponding selenolate (RSe^-). In contrast, the potentials of the selenoanhydride (**5**) are in the range between −250 and −400 mV, indicating a very different redox behaviour due to the distinct structural features of this compound.

Fig. 3 includes a representative voltammogram for the mercury electrode. Whilst it is fairly difficult to derive at a more detailed interpretation of the various oxidation (and reduction) potentials measured due to complications with adsorption, a few interesting observations can be made: first of all, the results confirm that the compounds studied are redox active within the physiologically relevant potential range. In turn, this activity may form the basis for the kind of redox modulating activity – and subsequent cytotoxic/cytostatic effects – observed in cell culture. Secondly, the redox couple at the heart of this activity seems to involve selenol(ate) and diselenide species, confirming that the compounds can become hydrolyzed and, as expected, liberate a redox active, anionic selenolate species (there are additional signals which may represent the non-hydrolyzed precursors and/or further decomposition products). Thirdly, whilst it is not always possible to calculate exact redox potentials (the redox couple in question is sometimes quasi-reversible and hence calculating $E_{1/2}$ is complicated), both, the generally observed reversibility and position of the potentials bodes well for a pronounced biological redox activity which, because of reversibility, may also include aspects of catalysis (see below).

Table 3
Electrochemical parameters obtained for selected selenoesters. Mercury-drop electrode. v = 200 mV/s; MeOH:H_2O (phosphate buffer pH 7.4) 1:4; t = 5 min. Please see text for further details.

Compound	E_{pc}	E_{pa}	ΔE_p	$E_{1/2}$
5	−393 mV	−274 mV	119 mV	−334 mV
21	−588 mV	−518 mV	70 mV	−553 mV
28	−696 mV	−639 mV	57 mV	−668 mV

The GPx-like catalytic activity for the four selected compounds (**5**, **21**, **28** and **30**) was investigated using a thiophenol–(PhSH) based assay [61], which measures the ability of the compounds to catalyse the reaction of hydrogen peroxide with a selected thiol *in vitro* (since this activity resembles the one of the selenium enzyme GPx, the assay is sometimes also referred to as a "GPx_assay"). The results are expressed as the ratio between the initial rates of the catalyzed reaction by the tested compound (Vcat) and the spontaneous reaction (Vsp). Diphenyldiselenide was used as positive control and all the compounds were studied at a concentration of 100 μM. The results obtained show that the activity of compound **30** was comparable to the one of the reference, whilst derivatives **5**, **21** and **28** presented a clearly lower activity, as shown in Table 4. It is therefore unlikely that these compounds *per se* are good antioxidants.

The DPPH radical has been widely used to test the ability of compounds to behave as free radical scavengers or hydrogen donors. Briefly, the assay measures the decrease in absorbance of the DPPH radicals at a characteristic wavelength after a half-hour incubation of the DPPH radical with the different concentrations (5, 15, 50, 150 and 500 μM) of the antioxidant compound. Hence we carried out experiments to explore the free radical scavenging ability of the novel selenoesters through their capacity for scavenging DPPH. The values obtained were then compared with the ones measured for the positive control, *i.e.* the ascorbic acid [62]. Results are expressed as the percentage of the DPPH free radical scavenging at five concentrations, as shown in Table 5 and Fig. 4. Each value is expressed as the average of three experiments per concentration ± SEM.

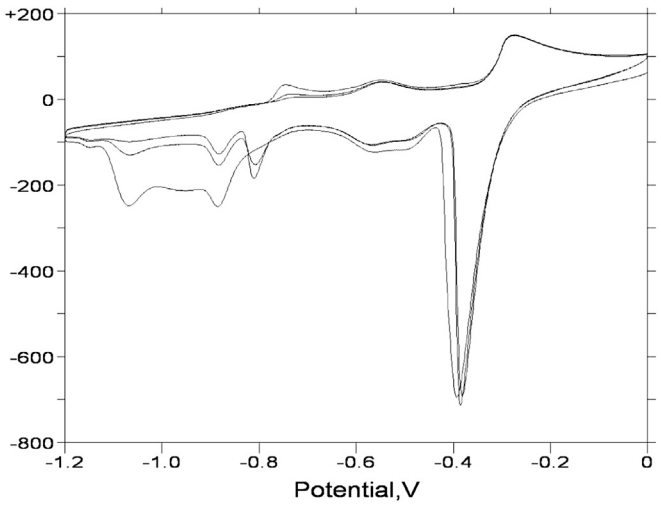


Fig. 3. Voltammogram corresponding to compound **5** in mercury electrode.

Table 4
The GPx-like catalytic activity determined for the four selected compounds (using diphenyldiselenide as benchmark).

Compound	Ratio Vcat/Vsp
5	4.0
21	2.4
28	4.9
30	11.5
Diphenyldiselenide	13.3

The tested compounds showed a weak to moderate capacity for scavenging the radical DPPH in comparison to the positive control (ascorbic acid). It was not possible to calculate an IC_{50} value for most of these compounds as only compound **5**, which was the one with the most promising antioxidant profile, has a DPPH scavenging over 50% at least one of the concentrations assayed; the IC_{50} of this compound is 74.1 μM. As for the thiophenol assay, the DPPH assay counts against a pronounced antioxidant activity of the compounds tested. Whilst these compounds are clearly redox active (see electrochemical results), their redox activity may result in a more pro-oxidant, redox modulating activity, rather than in a classical antioxidant activity. Despite being speculative at this time, such a pro-oxidant activity may indeed explain why these compounds do not protect and hence enhance the growth of cancer cells, but actually hinder proliferation and on occasion even exhibit outright cytotoxic properties.

3. Conclusion

In summary, we describe the synthesis and the cytotoxic and cytostatic evaluation of 31 new selenoesters against the prostate cancer cell line PC-3, for the most active compounds, a more detailed investigation of the biological activity against three further cancer cell lines (breast = MCF-7, lung = A-549 and colon = HT-29) and one non-tumour prostate cell line (RWPE-1).

The majority of the compounds exerted a remarkable cytostatic activity in PC-3 cancer cells. Compounds **29**, **30** and **31**, with GI_{50} values in the nanomolar order, were even more potent than etoposide, while 13 of them (**6**, **8**, **9**, **11**, **12**, **13**, **21**, **22**, **23**, **28**, **29**, **30** and **31**) were more active than cisplatin. The same compounds, with the exception of derivatives **8** and **11** in MCF-7, exhibited GI_{50} values below 10 μM against the other cancer cell lines tested. It was found that biological activity is often selective for the cancer cells (compared to the normal cells) and tends to increase when the polarity of the alkyl moiety bound to the selenium atom of the selenoester group is diminished; whereas the existence of symmetric elements in the molecular structure seems to enhance the biological activity. On the other hand, results enable us to confirm that the presence of methyl-seleno moiety substantially increase the antiproliferative activity of the compounds previously published by our group [33,36], being noteworthy the activity improvement observed as regards carboxymethylarylselenoates described [33] and used as starting compounds in the design of the

Table 5
Percentages of free radical scavenging activity (DPPH radical) obtained for the various compounds.

Compound	5 μM	15 μM	50 μM	150 μM	500 μM
5	14.1 ± 5.3	21.9 ± 0.9	48.0 ± 2.3	56.4 ± 2.4	46.4 ± 1.6
21	4.1 ± 0.7	5.7 ± 0.4	4.3 ± 3.4	5.2 ± 3.4	8.8 ± 1.2
28	4.1 ± 1.9	3.8 ± 1.6	8.9 ± 2.0	15.9 ± 1.6	43.1 ± 2.9
30	6.5 ± 2.6	4.9 ± 6.7	10.5 ± 5.3	9.7 ± 4.9	21.5 ± 3.5
Ascorbic acid	29.1 ± 2.1	87.5 ± 0.9	98.5 ± 1.2	98.5 ± 1.2	98.9 ± 1.6

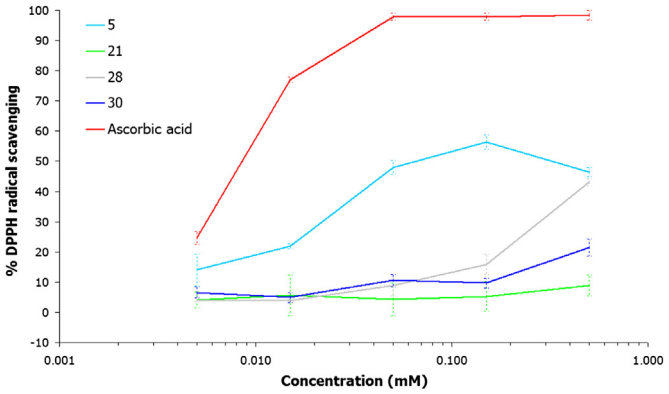


Fig. 4. Variation of DPPH radical scavenging activity *vs* concentration of test compounds.

derivatives included in this work. It is noteworthy to remark that the most active compounds presented in this work (**5**, **25**, **28** and **30**) have higher selectivity indexes than the reference drugs employed. Therefore, this fact justifies the realization of more in-depth studies to ascertain their mechanism of action; as well as they become a promising starting point to future modulations of their activity through the design, synthesis and evaluation of structural variants of these selenoesters. Due to the better activity and/or selectivity as cytostatic or cytotoxic agents, compounds **5**, **21**, **28** and **30** have been selected for subsequent screening procedures in order to assess their redox properties relevant to a biological, possibly redox modulating activity. Here, our results indicate that the compounds studied are all redox active within the physiological potential range, and that the key parameters of this redox activity point towards the presence of a possibly catalytically active selenolate/diselenide redox pair. While the mechanism of the aforesaid cytostatic/cytotoxic activity is therefore neither due to a significant peroxidase activity or free radical scavenging antioxidant properties, it is possible that these redox compounds modulate the intracellular redox state of the cancer cells affected and hence prevent proliferation and/or induce apoptosis. Indeed, such redox modulating, often pro-oxidant and pro-apoptotic properties have been observed by us and others for a wide range of selenium and tellurium compounds attacking the cellular thiolstat in the past. Additional experiments are obviously required in the future to investigate the precise mechanism or mechanisms responsible of the pronounced biological activity apparently associated with these selenocompounds. In any case, it can be stated that the compounds investigated show considerable antiproliferative and cytotoxic properties, which places them at the centre for future anticancer drug research.

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. Silica gel 60 (0.040–0.063 mm) 1.09385.2500 (Merck KGaA, 64271 Darmstadt, Germany) was used for Column Chromatography and 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 **1**–**4***

A solution of sodium borohydride (1.00 g, 26.4 mmol) in 12.5 mL of distilled water was added to a stirred suspension of grey selenium (1.00 g, 12.7 mmol) in 12.5 mL of distilled water at room temperature. The reaction mixture was stirred until an almost colourless solution of NaHSe was formed. The corresponding acyl chloride (12.7 mmol) was added in small portions and the reaction mixture was magnetically stirred at 50 °C for 1 h. The solid was filtered and after that, chloroacetamide (1.18 g, 12.7 mmol) was added to the filtrate. The mixture was heated at 50 °C for 2 h. The solid formed was solved in diethyl ether:methanol (9:1), to remove metallic impurities by filtration. Final compound is precipitated by addition of hexane to the filtrate.

*4.1.1.1. Carbamoylmethyl benzoselenoate (**1**).* From benzoyl chloride (1.78 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and chloroacetamide (1.18 g, 12.7 mmol). Yield 37% (1.444 g); mp: 114–115 °C. IR (KBr) cm^{-1}: 3383–3191 (m, N–H), 3055–2930 (w, C–H), 1654 (s, C=O ester), 1625 (s, C=O amide). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.73 (s, 2H, $SeCH_2CONH_2$); 7.15–7.65 (br s, 2H, $CONH_2$); 7.59 (t, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 7.9$ Hz, $J_{3\text{-}4,5\text{-}4} = 7.6$ Hz); 7.74 (t, 1H, H_4); 7.91 (dd, 2H, $H_2 + H_6$, $J_{2\text{-}4,6\text{-}4} = 1.0$ Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.0 ($SeCH_2$); 127.7 ($C_2 + C_6$); 130.3 ($C_3 + C_5$); 135.3 (C_4); 138.8 (C_1); 170.8 ($CONH_2$); 194.4 (COSe). MS (*m*/*z*% abundance): 243 (M^+, 1), 105 (100). Elemental Analysis for $C_9H_9NO_2Se$, calcd/found (%): C: 44.64/44.73; H: 3.75/3.88; N: 5.78/5.77.

*4.1.1.2. Carbamoylmethyl 4-chlorobenzoselenoate (**2**).* From 4-chlorobenzoyl chloride (2.22 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and chloroacetamide (1.18 g, 12.7 mmol). The solid was solved in ethyl ether (50 mL), washed with water (3 × 40 mL) and finally extracted with hexane (3 × 25 mL). The organic layer was dried and evaporated for obtaining a white solid (0.193 g, 6%); mp: 135–136 °C. IR (KBr) cm^{-1}: 3384–3192 (m, N–H), 3096–2923 (w, C–H), 1677 (s, C=O ester), 1657 (s, C=O amide). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.74 (s, 2H, $SeCH_2CONH_2$); 7.16 (br s, 2H, NH_2); 7.66 (d, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.6$ Hz); 7.91 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 28.6 ($SeCH_2$); 129.0 ($C_2 + C_6$); 130.5 ($C_3 + C_5$); 137.4 (C_1); 140.0 (C_4); 170.6 ($CONH_2$); 193.4 ($COSeCH_2$). MS (*m*/*z*% abundance): 277 (M^+, 1), 139/141 (100/34). Elemental Analysis for $C_9H_8ClNO_2Se$, calcd/found (%): C: 39.08/39.03; H: 2.92/2.96; N: 5.06/5.02.

*4.1.1.3. Carbamoylmethyl-3,5-dimethoxybenzoselenoate (**3**).* From 3,5-dimethoxybenzoyl chloride (1.27 g, 6.3 mmol), grey selenium (0.50 g, 6.3 mmol), sodium borohydride (0.50 g, 13.2 mmol) and chloroacetamide (0.59 g, 6.33 mmol). The solid was solved in ethyl ether/methanol (1:5, 50 mL), washed with water (3 × 40 mL) and finally extracted with hexane (3 × 25 mL). The organic layer was dried and evaporated for obtaining a white solid (0.380 g, 20%); mp: 121–124 °C. IR (KBr) cm^{-1}: 3374–3187 (s, N–H), 3096–2931 (w, C–H), 1686 (m, C=O ester), 1658 (s, C=O amide). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.67 (s, 2H, $SeCH_2CONH_2$); 3.86 (s, 6H, OCH_3); 5.47–6.29 (br s, 2H, NH_2); 6.73 (t, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 2.3$ Hz); 7.04 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 29.3 ($SeCH_2$); 56.5 (OCH_3); 105.2 ($C_2 + C_6$); 106.6 (C_4); 140.9 (C_1); 161.7 ($C_3 + C_5$); 170.7 ($CONH_2$); 194.3 ($COSeCH_2$). MS (*m*/*z*% abundance): 303 (M^+•, 2), 165 (100). Elemental Analysis for $C_{11}H_{13}NO_4Se$, calcd/found (%): C: 43.72/43.53; H: 4.34/4.44; N: 4.64/4.86.

*4.1.1.4. Carbamoylmethyl phenylethaneselenoate (**4**).* From phenylacetyl chloride (1.96 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and chloroacetamide (1.18 g, 12.7 mmol). The solid was solved in ethyl ether/methanol (9:1) and extracted with hexane (3 × 25 mL). The organic layer was dried and evaporated for obtained a white-grey solid (1.685 g, 52%); mp: 107–109 °C. IR (KBr) cm^{-1}: 3345–3173 (m, N–H), 3061–2798 (w, C–H), 1697 (s, C=O ester), 1659 (s, C=O amide). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.45 (s, 2H, $CH_2COSeCH_2CONH_2$); 3.99 (s, 2H, $ArCH_2COSe$); 7.03–7.49 (br s, 2H, NH_2); 7.33–7.36 (m, 5H, H_{Ar}). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.4 ($SeCH_2$); 53.9 ($ArCH_2COSe$); 128.6 (C_4); 129.2 ($C_3 + C_5$); 130.6 ($C_2 + C_6$); 132.4 (C_1); 172.8 ($CONH_2$); 201.9 (COSe). MS (*m*/*z*% abundance): 253/254/255/257/259 (M^+, 1/1/2/4/2), 91 (100). Elemental Analysis for $C_{10}H_{11}NO_2Se$, calcd/found (%): C: 46.89/46.78; H: 4.33/4.51; N: 5.47/5.43.

*4.1.2. Benzo[c]selenophen-1,3-dione (**5**)*

A 1 M solution of lithium aluminium hydride (7.00 mL, 7.00 mmol) was added to a stirred suspension of grey selenium (1.10 g, 13.9 mmol) in 15 mL of THF at room temperature. The reaction mixture was stirred until the end of the gas liberation. Phthaloyl chloride (1.41 g, 6.9 mmol) solved in dichloromethane (20 mL) was added in small portions and the reaction mixture was magnetically stirred at 50 °C for 1 h. After this time sulphuric acid (10 mL) was added during five minutes. The solid was filtered and was washed with chloroform (4 × 15 mL). The organic layer was dried and evaporated for obtaining a brown solid that was recrystallized from hexane to isolate an orange solid. Yield 95% (1.378 g); mp: 122–124 °C. IR (KBr) cm^{-1}: 1749 (C=O), 1685 (C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 7.95 (dd, 2H, $H_4 + H_5$, $J_{4\text{-}6,5\text{-}3} = 3.2$ Hz, $J_{4\text{-}3,5\text{-}6} = 5.7$ Hz); 7.98 (dd, 2H, $H_3 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 124.0 ($C_3 + C_6$); 135.3 ($C_1 + C_2$), 142.2 ($C_4 + C_5$), 194.5 ($COSeCO$). MS (*m*/*z*% abundance): 212 (M^+•, 11), 76 (100). Elemental Analysis for $C_8H_4O_2Se$, calcd/found (%): C: 45.48/45.33; H: 1.89/1.91.

*4.1.3. General procedure for compounds **6**–**13***

0.50 g of the corresponding selanylacetic acid is solved in methanol (20 mL) in presence of hydrochloric acid (0.2 mL). The mixture was heated by refluxing during 4 h. After this period, water (25 mL) was added and extracted with chloroform (2 × 30 mL). The organic phase was washed with water (3 × 20 mL), dried over Na_2SO_4 and concentrated to dryness. The resultant residue was purified by the appropriate treatment.

*4.1.3.1. Methoxycarbonylmethyl benzoselenoate (**6**).* From benzoylselanylacetic acid (0.50 g, 2.06 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The residue was stirred during 48 h with ethyl ether (100 mL), silica gel and activated carbon. The organic phase was filtered, dried and evaporated to dryness. A yellowish liquid was obtained. Yield 35% (0.184 g). IR (KBr) cm^{-1}: 3061–2850 (m, C–H), 1738 (s, C=O ester), 1678 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.77 (s, 3H, $COOCH_3$); 3.87 (s, 2H, $SeCH_2COOCH_3$); 7.49 (dt, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.1$ Hz, $J_{3\text{-}4,5\text{-}4} = 7.6$ Hz); 7.64 (m, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 1.3$ Hz); 7.92 (dd, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.5 ($SeCH_2$); 53.2 ($COOCH_3$); 127.8 ($C_2 + C_6$); 129.4 ($C_3 + C_5$); 134.5 (C_4); 138.8 (C_1); 171.1 (COO); 192.7 (COSe). MS (*m*/*z*% abundance): 258 (M^+, 1), 225/227 (1/2), 105 (100). Elemental Analysis for $C_{10}H_{10}O_3Se$, calcd/found (%): C: 46.71/46.98; H: 3.92/4.08.

*4.1.3.2. Methoxycarbonylmethyl 4-chlorobenzoselenoate (**7**).* From 4-chlorobenzoylselanylacetic acid (0.55 g, 1.26 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The residue was dried in vacuum to give a white solid. Yield 61% (0.230 g); mp: 46–47 °C. IR (KBr) cm^{-1}: 3001–2945 (w, C–H), 1737 (s, C=O ester); 1677 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.77 (s, 3H, $COOCH_3$); 3.87 (s, 2H, $SeCH_2COOCH_3$); 7.48 (d, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.7$ Hz); 7.86 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.7 ($SeCH_2$); 53.2 ($COOCH_3$); 56.1 ($ArOCH_3$), 105.4 ($C_2 + C_6$); 106.8 (C_4); 140.3 (C_1); 161.4 ($C_3 + C_5$); 171.1 (COO); 192.7 (COSe). MS (*m/z*% abundance): 139/141 (100/34), 111/113 (46/15). Elemental Analysis for $C_{10}H_9ClO_3Se$, calcd/found (%): C: 41.19/41.49; H: 3.11/3.16.

*4.1.3.3. Methoxycarbonylmethyl 2-chlorobenzoselenoate (**8**).* From 2-chlorobenzoylselanylacetic acid (0.46 g, 1.66 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The residue was stirred during 48 h with ethyl ether (100 mL), silica gel and activated carbon. The organic phase was filtered, dried and evaporated to dryness. A yellowish liquid was obtained. Yield 48% (0.230 g). IR (KBr) cm^{-1}: 3064–2846 (m, C–H), 1738 (s, C=O ester); 1692 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.77 (s, 3H, $COOCH_3$); 3.89 (s, 2H, $SeCH_2COO$); 7.35–7.39 (m, 1H, H_5); 7.46–7.48 (m, 2H, $H_4 + H_3$); 7.75 (dd, 1H, H_6, $J_{6\text{-}5} = 7.7$ Hz, $J_{6\text{-}4} = 2.0$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 26.9 ($SeCH_2$); 53.2 (OCH_3); 127.4 (C_5); 129.8 (C_3); 130.8 (C_2); 131.7 (C_6); 133.4 (C_4); 138.0 (C_1); 170.8 (COO); 192.1 (COSe). MS (*m/z*% abundance): 139/141 (100/34), 111/113 (38/11), 85/87 (5/1). Elemental Analysis for $C_{10}H_9ClO_3Se$, calcd/found (%): C: 41.19/40.83; H: 3.11/2.86.

*4.1.3.4. Methoxycarbonylmethyl 3,5-dimethoxybenzoselenoate (**9**).* From 3,5-dimethoxybenzoylselanylacetic acid (1.0 g, 3.30 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The residue was stirred during 48 h with ethyl ether (100 mL), silica gel and activated carbon. The organic phase was filtered, dried and evaporated to dryness. An orangish liquid was obtained which was purified by silica gel column chromatography (hexane/acetate 4:1) to give **9**. Yield 35% (0.371 g). IR (KBr) cm^{-1}: 3091–2840 (m, C–H), 1738 (s, C=O ester), 1668 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.77 (s, 3H, $COOCH_3$); 3.85 (s, 2H, $SeCH_2COOCH_3$); 3.85 (s, 6H, $ArOCH_3$); 6.71 (t, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 2.3$ Hz); 7.05 (d, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.7 ($SeCH_2$); 53.2 ($COOCH_3$); 56.1 ($ArOCH_3$), 105.4 ($C_2 + C_6$); 106.8 (C_4); 140.3 (C_1); 161.4 ($C_3 + C_5$); 171.1 (COO); 192.7 (COSe). MS (*m/z*% abundance): 165 (100). Elemental Analysis for $C_{12}H_{14}O_5Se$, calcd/found (%): C: 45.44/45.39; H: 4.45/4.42.

*4.1.3.5. Methoxycarbonylmethyl 3,4,5-trimethoxybenzoselenoate (**10**).* From 3,4,5-trimetoxybenzoylselanylacetic (0.40 g, 1.20 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The residue was dried in vacuum to give a white solid. Yield 49% (0.203 g); mp: 36–38 °C. IR (KBr) cm^{-1}: 3000–2837 (m, C–H), 1738 (m, C=O ester), 1680 (m, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.66 (s, 3H, $COOCH_3$); 3.77 (s, 3H, 4-OCH_3); 3.87 (s, 6H, 3,5-diOCH_3); 3.89 (s, 2H, $SeCH_2COOH$); 7.13 (2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.7 ($SeCH_2$); 53.2 ($COOCH_3$); 56.1 ($ArOCH_3$), 105.4 ($C_2 + C_6$); 106.8 (C_4); 140.3 (C_1); 161.4 ($C_3 + C_5$); 171.1 (COO); 192.7 (COSe). MS (*m/z*% abundance): 195 (100). Elemental Analysis for $C_{13}H_{16}O_6Se$, calcd/found (%): C: 44.97/44.60; H: 4.64/4.63.

*4.1.3.6. Methoxycarbonylmethyl 2-thiophenecarboselenoate (**11**).* From thieno-2-ylselanylacetic acid (0.50 g, 2.01 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The residue was stirred during 48 h with ethyl ether (100 mL), silica gel and activated carbon. The organic phase was filtered, dried and evaporated to dryness. A yellowish liquid was obtained. Yield 31% (0.164 g). IR (KBr) cm^{-1}: 3103–2847 (m, C–H), 1738 (s, C=O ester); 1668 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.76 (s, 3H, $COOCH_3$); 3.86 (s, 2H, $SeCH_2$); 7.17 (dd, 1H, H_4, $J_{4\text{-}5} = 3.9$ Hz, $J_{4\text{-}3} = 4.9$ Hz); 7.73 (dd, 1H, H_3, $J_{3\text{-}4} = 4.9$ Hz, $J_{3\text{-}5} = 1.1$ Hz); 7.83 (dd, 1H, H_5, $J_{5\text{-}4} = 3.9$, $J_{5\text{-}3} = 1.1$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.6 ($SeCH_2COOCH_3$); 53.2 ($COOCH_3$); 128.5 (C_4); 132.6 (C_5); 134.4 (C_3); 142.9 (C_2); 170.9 (CH_2COOCH_3); 182.8 ($COSeCH_2$). MS (*m/z*% abundance): 264 (M^+, 2), 111 (100). Elemental Analysis for $C_8H_8O_3SSe$, calcd/found (%): C: 36.51/36.87; H: 3.06/3.10.

*4.1.3.7. Methoxycarbonylmethyl phenylethaneselenoate (**12**).* From phenylacetoylselanylacetic acid (0.50 g, 1.94 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The residue was stirred during 48 h with ethyl ether (100 mL), silica gel and activated carbon. The organic phase was filtered, dried and evaporated to dryness. A yellowish liquid was obtained. Yield 25% (0.132 g); mp: 88–89 °C. IR (KBr) cm^{-1}: 3062–2846 (w, C–H), 1736 (m, C=O ester), 1704 (m, C=O selenoester). ^1H NMR (400 Hz, $CDCl_3$) δ: 3.62 (s, 2H, $SeCH_2COO$); 3.70 (s, 3H, $COOCH_3$); 3.89 (s, 2H, $ArCH_2COSe$); 7.30–7.34 (m, 2H, $H_2 + H_6$); 7.35–7.40 (m, 3H, $H_3 + H_4 + H_5$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.8 ($SeCH_2$); 41.6 ($ArCH_2CO$); 53.7 ($COOCH_3$); 128.5 (C_4); 129.2 ($C_3 + C_5$); 130.6 ($C_2 + C_6$); 132.6 (C_1); 171.0 (COO); 198.8 (COSe). MS (*m/z*% abundance): 272 ($M^{+\bullet}$, 1), 91 (100). Elemental Analysis for $C_{11}H_{12}O_3Se$, calcd/found (%): C: 48.72/48.90; H: 4.46/4.28.

*4.1.3.8. Methoxycarbonylethyl phenylethaneselenoate (**13**).* From 3-phenylacetoylselanyl propionic acid (0.50 g, 1.84 mmol), methanol (20 mL) and hydrochloric acid (0.2 mL). The propionic acid used as reagent was prepared according the following protocol. A solution of sodium borohydride (1.00 g, 26.4 mmol) in 12.5 mL of distilled water was added to a stirred suspension of grey selenium (1.00 g, 12.7 mmol) in 12.5 mmol of distilled water at room temperature. The reaction mixture was stirred until an almost colourless solution of NaHSe was formed. The 2-phenylacetyl chloride (1.96 g, 12.7 mmol) was added in small portions and the reaction mixture was magnetically stirred for 1 h. A yellow solution was formed and the 3-bromo propionic acid (1.94 g, 12.7 mmol) was added. Within 60 min at 40 °C a solid was formed. The product was filtered and purified by column chromatography (ethyl acetate) and recrystallized from carbon tetrachloride for obtaining a white solid. Yield 17% (0.569 g). IR (KBr) cm^{-1}: 3064–2565 (s, C–H); 1709 (s, C=O acid); 1689 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.80 (t, 2H, $SeCH_2CH_2CO$, $J_{CH(CO)-CH(Se)} = 6.9$ Hz); 3.07 (t, 2H, $SeCH_2CH_2CO$); 3.87 (s, 2H, $ArCH_2CO$); 7.30 (dd, 2H, $H_2 + H_6$, $J_{2\text{-}3,6\text{-}5} = 7.6$ Hz, $J_{2\text{-}4,6\text{-}4} = 1.8$ Hz); 7.34–7.38 (m, 3H, $H_3 + H_4 + H_5$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.6 ($SeCH_2CH_2$); 35.4 (CH_2CH_2CO); 54.5 ($ArCH_2$); 128.2 (C_4); 129.1 ($C_3 + C_5$); 130.5 ($C_2 + C_6$); 133.7 (C_1); 178.8 (COOH); 200.7 (COSe). MS (*m/z*% abundance): 268/269/270/272/274 ($M^{+\bullet}$, 1/1/2/4/1, Se), 91 (100). Elemental Analysis for $C_{11}H_{12}O_3Se$, calcd/found (%):C: 48.72/48.62; H: 4.46/4.28.

The residue was stirred during 48 h with ethyl ether (100 mL), silica gel and activated carbon. The organic phase was filtered, dried and evaporated to dryness. A yellowish liquid was obtained which was purified by silica gel column chromatography (dichloromethane, 100) to give **13**. Yield 26% (0.137 g). IR (KBr) cm^{-1}: 3062–2846 (m, C–H), 1737 (s, C=O ester); 1697 (s, C=O, selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.75 (t, 2H, $SeCH_2CH_2COOCH_3$, $J_{CH(CO)-CH(Se)} = 7.0$ Hz); 3.08 (t, 2H, $COSeCH_2CH_2COOCH_3$); 3.69 (s, 3H, $COOCH_3$); 3.86 (s, 2H, $ArCH_2COSe$); 7.30 (dd, 2H, $H_2 + H_6$, $J_{2\text{-}3,6\text{-}5} = 7.7$ Hz, $J_{2\text{-}4,6\text{-}4} = 1.8$ Hz); 7.34–7.39 (m, 3H, $H_3 + H_4 + H_5$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.1 ($SeCH_2CH_2CO$); 35.4 ($SeCH_2CH_2CO$); 52.2 (OCH_3); 54.5 ($ArCH_2COSe$); 128.2 (C_4); 129.0 ($C_3 + C_5$); 130.5 ($C_2 + C_6$); 133.2 (C_1); 173.0 ($COOCH_3$); 200.6 (COSe). MS (*m/z*% abundance): 284 ($M^{+\bullet} - 2$, 3), 91 (100). Elemental Analysis for $C_{12}H_{14}O_3Se$, calcd/found (%): C: 50.54/50.70; H: 4.95/4.86.

*4.1.4. General procedure for compounds **14**–**17***

A solution of sodium borohydride (1.00 g, 26.4 mmol) in 12.5 mL of distilled water was added to a stirred suspension of grey selenium (1.00 g, 12.7 mmol) in 12.5 mmol of distilled water at room temperature. The reaction mixture was stirred until an almost colourless solution of NaHSe was formed. The corresponding acyl chloride (12.7 mmol) was added in small portions and the reaction mixture was magnetically stirred at 50 °C for 1 h. The solid was filtered and *tert*-butyl bromoacetate (2.47 g, 12.7 mmol) was added upon the solution. The mixture was heated during 2 h at 50 °C. The mixture of the reaction was extracted with chloroform (3 × 40 mL), washed with water (3 × 40 mL), dried over Na_2SO_4 and concentrated to dryness. The residue was stirred with ethyl ether (100 mL), silica gel and active carbon during 24 h. The organic phase was filtered, dried and evaporated to dryness and the residue was purified by column chromatography.

*4.1.4.1. tert-Butoxycarbonylmethyl benzoselenoate (**14**).* From benzoyl chloride (1.78 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and *tert*-butyl bromoacetate (2.47 g, 12.7 mmol). Purified by column chromatography (toluene, 100%) to obtain **14** as a yellowish liquid. Yield 26% (0.987 g). IR (KBr) cm^{-1}: 3062–2932 (w, C–H), 1731 (s, C=O ester), 1677 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.49 (s, 9H, $O(C\underline{H}_3)_3$); 3.79 (s, 2H, $SeC\underline{H}_2CO$); 7.49 (t, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.1$ Hz, $J_{3\text{-}4,5\text{-}4} = 7.9$ Hz); 7.63 (m, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 1.3$ Hz); 7.92 (dd, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.6 ($Se\underline{C}H_2CO$); 29.0 ($OC(\underline{C}H_3)_3$); 82.3 ($O\underline{C}(CH_3)_3$); 127.7 ($C_2 + C_6$); 129.3 ($C_3 + C_5$); 134.4 (C_4); 138.7 (C_1); 169.7 ($\underline{C}OO$); 193.1 ($\underline{C}OSe$). MS (*m*/*z*% abundance): 240/241/242/244/246 (1/1/2/4/1), 223/224/225/227/229 (1/1/2/4/1).

*4.1.4.2. tert-Butoxycarbonylmethyl 4-chlorobenzoselenoate (**15**).* From 4-chlorobenzoyl chloride (2.22 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and *tert*-butyl bromoacetate (2.47 g, 12.7 mmol). Purified by column chromatography (toluene, 100%) to obtain **15** as a yellowish liquid. Yield 8% (0.328 g). IR (KBr) cm^{-1}: 3090–2932 (s, C–H), 1731 (s, C=O ester), 1678 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.49 (s, 9H, $C(CH_3)_3$); 3.79 (s, 2H, $SeCH_2$); 7.47 (d, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.7$ Hz); 7.86 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.8 ($SeCH_2$); 29.2 ($C(\underline{C}H_3)_3$); 82.5 ($\underline{C}(CH_3)_3$); 129.0 ($C_2 + C_6$); 130.2 ($C_3 + C_5$); 136.7 (C_4); 140.6 (C_1); 169.4 (COO); 191.9 (COSe). MS (*m*/*z*% abundance): 276 (3), 139/141 (20/7, Cl), 111/113 (13/4, Cl), 57 (100). Elemental Analysis for $C_{13}H_{15}ClO_3Se$, calcd/found (%): C: 46.80/46.52; H: 4.53/4.67.

*4.1.4.3. tert-Butoxycarbonylmethyl 3,5-dimethoxybenzoselenoate (**16**).* From 3,5-dimethoxybenzoyl chloride (2.54 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and *tert*-butyl bromoacetate (2.47 g, 12.7 mmol). Purified by column chromatography (hexane/acetate, 4:1) to obtain **16** as a white solid; mp: 65–66 °C. Yield 21% (0.952 g). IR (KBr) cm^{-1}: 3004–2844 (m, C–H), 1735 (s, C=O ester), 1671 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.49 (s, 9H, $C(CH_3)_3$); 3.77 (s, 2H, $SeCH_2$); 3.85 (s, 6H, OCH_3); 6.70 (t, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 2.2$ Hz); 7.05 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.8 ($C(\underline{C}H_3)_3$); 29.2 ($Se\underline{C}H_2$); 56.1 ($O\underline{C}H_3$); 82.4 ($\underline{C}(CH_3)_3$); 104.5 (C_4); 106.0 ($C_2 + C_6$); 140.6 (C_1); 161.4 ($C_3 + C_5$); 169.6 ($\underline{C}OO$); 193.0 ($\underline{C}OSe$). MS (*m*/*z*% abundance): 274/275/276/278/280 (2/1/3/3/1, Se), 57 (100). Elemental Analysis for $C_{15}H_{20}O_5Se$, calcd/found (%): C: 50.15/50.00; H: 5.61/5.48.

*4.1.4.4. tert-Butoxycarbonylmethyl phenylethaneselenoate (**17**).* From phenylacetyl chloride (0.978 g, 6.3 mmol), grey selenium (0.50 g, 6.3 mmol), sodium borohydride (0.50 g, 13.2 mmol) and *tert*-butyl bromoacetate (1.235 g, 6.3 mmol). Purified by column chromatography (toluene, 100%) to obtain **17** as a clear yellowish liquid. Yield 24% (0.472 g). IR (KBr) cm^{-1}: 3064–2932 (w, C–H); 1728 (s, C=O ester), 1714 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.43 (s, 3H, $C(CH_3)_3$); 3.55 (s, 2H, $SeCH_2$); 3.89 (s, 2H, $ArCH_2$); 7.33–7.36 (m, 5H, H_{Ar}). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.8 ($Se\underline{C}H_2$); 28.3 ($C(\underline{C}H_3)_3$); 53.9 ($Ar\underline{C}H_2$); 82.2 ($\underline{C}(CH_3)_3$); 128.4 (C_4); 129.2 ($C_3 + C_5$); 130.5 ($C_2 + C_6$); 132.8 (C_1); 169.6 ($\underline{C}OO$); 198.9 ($\underline{C}OSe$). MS (*m*/*z*% abundance): 316 ($M^{+\bullet} + 2$, 1), 91 (100). Elemental Analysis for $C_{14}H_{18}O_3Se$, calcd/found (%): C: 53.68/53.49; H: 5.79/6.02.

*4.1.5. General procedure for compounds **18**–**20***

A solution of sodium borohydride (0.50 g, 13.2 mmol) in 12.5 mL of distilled water was added to a stirred suspension of grey selenium (0.50 g, 6.3 mmol) in 12.5 mmol of distilled water at room temperature. The reaction mixture was stirred until an almost colourless solution of NaHSe was formed. The corresponding acyl chloride (6.3 mmol) was added in small portions and the reaction mixture was magnetically stirred at 50 °C for 1 h. The solid was filtered and phenyl bromoacetate (6.3 mmol) was added upon the solution. The mixture was heated during 2 h at 50 °C. After this period, the reaction was filtered and the solid extracted with dichloromethane (3 × 40 mL), washed with water (3 × 40 mL), dried over Na_2SO_4 and concentrated to dryness. The residue was purified.

*4.1.5.1. Phenoxycarbonylmethyl benzoselenoate (**18**).* From benzoyl chloride (1.245 g, 8.9 mmol), grey selenium (0.70 g, 8.5 mmol), sodium borohydride (0.70 g, 18.5 mmol) and phenyl bromoacetate (1.36 g, 6.3 mmol). The residue was purified by stirring in dichloromethane (100 mL), and filtered; mp: 40–41 °C. Yield 53% (1.070 g). IR (KBr) cm^{-1}: 3062–2940 (w, C–H), 1739 (s, C=O ester), 1679 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.08 (s, 2H, $SeCH_2$); 7.15 (dd, 2H, $H_{2'} + H_{6'}$, $J_{2'\text{-}3',6'\text{-}5'} = 8.3$ Hz, $J_{2'\text{-}4',6'\text{-}4'} = 1.2$ Hz); 7.25 (m, 1H, $H_{4'}$, $J_{4'\text{-}3',4'\text{-}5'} = 7.7$ Hz); 7.40 (dt, 2H, $H_{3'} + H_{5'}$); 7.52 (dt, 2H, $H_3 + H_5$, $J_{3\text{-}4,5\text{-}4} = 8.2$ Hz, $J_{3\text{-}2,5\text{-}6} = 8.0$ Hz); 7.66 (m, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 1.0$ Hz); 7.95 (dd, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.7 ($Se\underline{C}H_2$); 121.8 ($C_{2'} + C_{6'}$); 126.4 ($C_{4'}$); 127.8 ($C_2 + C_6$); 129.5 ($C_3 + C_5$); 129.8 ($C_{3'} + C_{5'}$); 134.6 (C_4); 138.3 (C_1); 151.2 ($C_{1'}$); 169.3 ($\underline{C}OO$); 192.4 ($\underline{C}OSe$). MS (*m*/*z*% abundance): 223/224/225/227/229 (3/3/8/16/4, Se), 195/197/199/201 (1/1/2/2, Se), 105 (100). Elemental Analysis for $C_{15}H_{12}O_3Se$, calcd/found (%): C: 56.44/56.15; H: 3.79/3.86.

*4.1.5.2. Phenoxycarbonylmethyl 4-chlorobenzoselenoate (**19**).* From 4-chlorobenzoyl chloride (1.11 g, 6.33 mmol), grey selenium (0.50 g, 6.3 mmol), sodium borohydride (0.50 g, 13.2 mmol) and phenyl bromoacetate (1.36 g, 6.3 mmol). The residue was purified by column chromatography (hexane/toluene, 11:9) to obtain **19** as a white solid; mp: 67–68 °C. Yield 24% (0.547 g). IR (KBr) cm^{-1}: 3067–2854 (s, C–H), 1752 (s, C=O ester), 1676 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 4.08 (s, 2H, $SeC\underline{H}_2COOPh$); 7.14 (dd, 2H, $H_{2'} + H_{6'}$, $J_{2'\text{-}3',6'\text{-}5'} = 7.6$ Hz, $J_{2'\text{-}4',6'\text{-}4'} = 1.0$ Hz); 7.26 (dt, 1H, $H_{4'}$, $J_{4'\text{-}3',4'\text{-}5'} = 7.7$ Hz); 7.40 (t, 2H, $H_{3'} + H_{5'}$); 7.49 (dd, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.7$ Hz); 7.89 (dd, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 26.0 ($Se\underline{C}H_2COOPh$); 121.7 ($C_{2'} + C_{6'}$); 126.5 ($C_{4'}$); 129.1 ($C_2 + C_6$); 129.8 ($C_3 + C_5$); 129.9 ($C_{3'} + C_{5'}$); 136.6 (C_4); 141.1 (C_1); 151.1 ($C_{1'}$); 169.1 ($COSeCH_2\underline{C}OOPh$); 191.3 ($\underline{C}OSeCH_2$-COOPh). MS (*m*/*z*% abundance): 300/302/303/304/306/308 (3/7/4/11/12/4), 255/257/258/259/260/261/263/265 (1/3/2/6/1/12/5/1), 156/158 (5/3), 139/141 (100/34), 111/113 (28/10). Elemental Analysis for $C_{15}H_{11}ClO_3Se$, calcd/found (%): C: 50.94/51.26; H: 3.14/3.27.

*4.1.5.3. Phenoxycarbonylmethyl 3,5-dimethoxybenzoselenoate (**20**).* From 3,5-dimethoxybenzoyl (1.27 g, 6.33 mmol), grey selenium (0.50 g, 6.3 mmol), sodium borohydride (0.50 g, 13.2 mmol) and phenyl bromoacetate (1.36 g, 6.3 mmol). The residue was purified by column chromatography (hexane/ethyl acetate, 4:1) to obtain **20** as a yellow liquid. Yield 26% (0.625 g). IR (KBr) cm^{-1}: 3071–2839 (m, C–H), 1752 (s, C=O ester), 1680 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.87 (s, 6H, 3,5-diOCH_3); 4.06 (s, 2H, $SeCH_2$-COOPh); 6.73 (t, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 2.3$ Hz); 7.08 (d, 2H, $H_2 + H_6$); 7.15 (dd, 2H, $H_{2'} + H_{6'}$, $J_{2'\text{-}3',6'\text{-}5'} = 8.6$ Hz, $J_{2'\text{-}4',6'\text{-}4'} = 1.1$ Hz); 7.25 (m, 1H, $H_{4'}$, $J_{4'\text{-}3',4'\text{-}5'} = 7.4$ Hz); 7.40 (m, 2H, $H_{3'} + H_{5'}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.9 ($SeCH_2COOPh$); 56.1 (OCH_3); 105.5 ($C_2 + C_6$); 106.8 (C_4); 121.8 ($C_{2'} + C_{6'}$); 126.4 ($C_{4'}$); 129.8 ($C_{3'} + C_{5'}$); 140.2 (C_1); 151.1 ($C_{1'}$); 161.5 ($C_3 + C_5$); 169.2 ($COSeCH_2COOPh$); 192.4 ($COSeCH_2$-COOPh). MS (*m/z*% abundance): 258 (3), 165 (100). Elemental Analysis for $C_{17}H_{16}O_5Se$, calcd/found (%): C: 53.84/53.74; H: 4.25/4.37.

*4.1.6. General procedure for compounds **21**–**23***

A solution of sodium borohydride (0.50 g, 13.2 mmol) in 12.5 mL of distilled water was added to a stirred suspension of grey selenium (0.50 g, 6.3 mmol) in 12.5 mL of distilled water at room temperature. The reaction mixture was stirred until an almost colourless solution of NaHSe was formed. The corresponding acyl chloride (6.33 mmol) was added in small portions and the reaction mixture was magnetically stirred at 50 °C for 1 h. The solid was filtered and the adequate α-haloketone (6.3 mmol) was added upon the solution. The mixture was heated during 2 h at 50 °C. The solid resultant was filtered, washed with appropriate solvents and purified.

*4.1.6.1. 2-Oxopropyl 4-chlorobenzoselenoate (**21**).* From 4-chlorobenzoyl chloride (1.11 g, 6.33 mmol), grey selenium (0.50 g, 6.3 mmol), sodium borohydride (0.50 g, 13.2 mmol) and 1-chloro-2-propanone (0.59 g, 6.3 mmol). The solid was solved in dichloromethane (50 mL) and was washed with water (3 × 15 mL). The organic layer was dried and evaporated and the white solid was identified as 21; mp: 41–42 °C. Yield 54% (0.945 g). IR (KBr) cm^{-1}: 3078–2921 (w, C–H), 1709 (s, C=O ketone), 1660 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.37 (s, 3H, $COCH_3$); 3.94 (s, 2H, $SeCH_2COCH_3$); 7.48 (d, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.7$ Hz); 7.87 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.1 ($SeCH_2$); 35.0 ($COCH_3$); 129.1 ($C_2 + C_6$); 129.7 ($C_3 + C_5$); 136.7 (C_4); 141.1 (C_1); 192.0 ($COSe$); 203.7 ($COCH_3$). MS (*m/z*% abundance): 139/141 (100/33), 111/113 (46/15), 85/87 (4/1). Elemental Analysis for $C_{10}H_9ClO_2Se$, calcd/found (%): C: 43.58/43.30; H: 3.29/3.27.

4.1.6.2. 3,3-Dimethyl-2-oxobutyl 4-chlorobenzoselenoate (22). From 4-chlorobenzoyl chloride (1.11 g, 6.33 mmol), grey selenium (0.50 g, 6.3 mmol), sodium borohydride (0.50 g, 13.2 mmol) and 1-chloropinacolone (0.85 g, 6.3 mmol). The solid was solved in dichloromethane (50 mL) and washed with water (2 × 40 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness for obtaining **22** as a white solid; mp: 119–120 °C. Yield 59% (1.184 g). IR (KBr) cm^{-1}: 3092–2871 (w, C–H), 1718 (s, C=O ketone), 1665 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.29 (s, 9H, $SeCH_2COC(CH_3)_3$); 4.19 (s, 2H, $SeCH_2COC(CH_3)_3$); 7.46 (d, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.5$ Hz); 7.86 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.1 ($SeCH_2COC(CH_3)_3$); 32.0 ($SeCH_2COC(CH_3)_3$); 45.2 ($COSeCH_2COC(CH_3)_3$); 128.9 ($C_2 + C_6$); 129.6 ($C_3 + C_5$); 137.2 (C_4); 140.7 (C_1); 192.8 ($COSeCH_2COCH_3$); 210.4 ($COSeCH_2COC(CH_3)$). MS (*m/z*% abundance): 195 (4), 139/141 (100/31), 111/113 (40/13). Elemental Analysis for $C_{13}H_{15}ClO_2Se$, calcd/found (%): C: 49.15/49.08; H: 4.76/4.82.

*4.1.6.3. 3,3-Dimethyl-2-oxobutyl 3,5-dimethoxybenzoselenoate (**23**).* From 3,5-dimethoxybenzoyl chloride (1.27 g, 6.33 mmol), grey selenium (0.50 g, 6.3 mmol), sodium borohydride (0.50 g, 13.2 mmol) and 1-chloropinacolone (0.85 g, 6.3 mmol). The solid was solved in dichloromethane (50 mL) and washed with water (2 × 40 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness for obtaining **23** as a white solid; mp: 67–70 °C. Yield 57% (1.239 g). IR (KBr) cm^{-1}: 3091–2838 (m, C–H), 1713 (s, C=O ketone), 1668 (s, C=O selenoester). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.29 (s, 9H, $SeCH_2COC(CH_3)_3$); 3.85 (s, 6H, $ArOCH_3$); 4.16 (s, 2H, $SeCH_2$-$COC(CH_3)_3$); 6.70 (t, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 2.3$ Hz); 7.05 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.7 ($SeCH_2COC(CH_3)_3$); 31.8 ($SeCH_2COC(CH_3)_3$); 45.2 ($COC(CH_3)_3$); 56.6 (OCH_3); 104.5 ($C_2 + C_6$); 106.0 (C_4); 140.7 (C_1); 161.4 ($C_3 + C_5$); 193.9 ($COSeCH_2COOPh$); 210.7 ($COSeCH_2COC(CH_3)_3$). MS (*m/z*% abundance): 165 (100). Elemental Analysis for $C_{15}H_{20}O_4Se$, calcd/found (%): C: 52.48/52.37; H: 5.87/5.92.

*4.1.7. General procedure for compounds **24**–**31***

A solution of sodium borohydride (1.00 g, 26.4 mmol) in 12.5 mL of distilled water was added to a stirred suspension of grey selenium (1.00 g, 12.7 mmol) in 12.5 mL of distilled water at room temperature. The reaction mixture was stirred until an almost colourless solution of NaHSe was formed. The corresponding acyl chloride (12.7 mmol for monofunctionalized chlorides and 6.3 for dichlorides) was added in small portions and the reaction mixture was magnetically stirred at 50 °C for 1 h. The solid was filtered and methyl iodide (1.5 mL) was added upon the solution. The mixture was heated during 1 h at 50 °C. The final product is obtained by filtration in the case of difunctionalized derivatives. In the case of the monofunctionalized ones, the solid is solved in ether:-dichloromethane (1:1) to remove metallic impurities by filtration, being eliminated the solvent in vacuum afterwards.

*4.1.7.1. Methyl benzoselenoate (**24**).* From benzoyl chloride (1.78 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and methyl iodide (1.5 mL, excess). Yellowish liquid, isolated after reaction by decantation instead of by filtration. Yield 52% (1.316 g). IR (KBr) cm^{-1}: 3060–2932 (w, C–H), 1673 (s, C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.42 (s, 3H, $SeCH_3$); 7.48 (m, 2H, $H_3 + H_5$, $J_{3\text{-}4,5\text{-}4} = 7.7$ Hz, $J_{3\text{-}2,5\text{-}6} = 7.6$ Hz); 7.61 (m, 1H, H_4, $J_{4\text{-}2,4\text{-}6} = 1.2$ Hz); 7.93 (dd, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 5.5 ($SeCH_3$); 127.5 ($C_2 + C_6$); 129.2 ($C_3 + C_5$); 133.9 (C_4); 139.4 (C_1); 195.3 ($ArCOSeCH_3$). MS (*m/z*% abundance): 196/197/198/200 ($M^{+\bullet}$, 1/1/1/3), 105 (100).

*4.1.7.2. Methyl 4-chlorobenzoselenoate (**25**).* From 4-chlorobenzoyl chloride (2.22 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and methyl iodide (1.5 mL, excess). Yellowish solid; mp: 46–47 °C. Yield: 79% (2.341 g). IR (KBr) cm^{-1}: 3093–2928 (w, C–H), 1668 (s, C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.42 (s, 3H, $SeCH_3$); 7.45 (d, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}6} = 8.6$ Hz); 7.87 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 5.8 ($SeCH_3$); 128.8 ($C_2 + C_6$); 129.5 ($C_3 + C_5$); 137.7 (C_4); 140.3 (C_1); 194.1 ($ArCOSeCH_3$). MS (*m/z*% abundance): 232/234/236 ($M^{+\bullet}$, 1/2/1), 156/158 (9/3), 139/141 (100/34), 111/113 (39/13), 85 (7), 75 (12), 50 (6). Elemental Analysis for C_8H_7ClOSe, calcd/found (%): C: 41.14/40.98; H: 3.02/2.92.

*4.1.7.3. Methyl 4-cyanobenzoselenoate (**26**).* From 4-cyanobenzoyl chloride (2.06 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and methyl iodide (1.5 mL, excess). Red solid; mp: 104–108 °C. Yield: 71% (2.020 g). IR (KBr) cm^{-1}: 3091–2938 (m, C–H), 2229 (s, CN), 1665 (s, C=O). ^1H NMR (400 MHz, $CDCl_3$) δ: 2.47 (s, 3H, $SeCH_3$); 7.79 (d, 2H, $H_3 + H_5$, $J_{3\text{-}2,5\text{-}}$

$_6$ = 8.7 Hz); 8.01 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 6.2 (Se$\underline{C}H_3$); 117.1 ($\underline{C}N$); 118.2 (C_4); 127.8 ($C_2 + C_6$); 133.1 ($C_3 + C_5$); 142.3 (C_1); 194.4 (Ar$\underline{C}OSeCH_3$). MS (*m*/*z*% abundance): 221/222/223/225/227 ($M^{+\bullet}$, 1/1/1/2/1), 130 (100), Elemental Analysis for C_9H_7NOSe, calcd/found (%): C: 48.23/47.90; H: 3.15/3.18; N: 6.25/6.15.

*4.1.7.4. Methyl 3,5-dimethoxybenzoselenoate (**27**).* From 3,5-dimethoxybenzoyl chloride (2.54 g, 12.7 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and methyl iodide (1.5 mL, excess). White solid; mp: 54–55 °C. Yield: 72% (2.374 g). IR (KBr) cm^{-1}: 3105–2836 (m, C–H), 1672 (s, C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.40 (s, 3H, Se$\underline{C}H_3$); 3.86 (s, 6H, 3,5-di-O$\underline{C}H_3$); 6.69 (t, 1H, H_4, $J_{4\text{-}2,4\text{-}6}$ = 2.3 Hz); 7.07 (d, 2H, $H_2 + H_6$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 5.7 (Se$\underline{C}H_3$); 56.0 (O$\underline{C}H_3$); 105.2 ($C_2 + C_6$); 106.2 (C_4); 141.4 (C_1); 161.3 ($C_3 + C_5$); 195.3 (Ar$\underline{C}OSeCH_3$). MS (*m*/*z*% abundance): 260 ($M^{+\bullet}$, 2), 165 (100). Elemental Analysis for $C_{10}H_{12}O_3Se$, calcd/found (%): C: 46.35/46.32; H: 4.67/4.67.

*4.1.7.5. Dimethyl benzene-1,4-dicarboselenoate (**28**).* From terephthaloyl chloride (1.29 g, 6.3 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and methyl iodide (1.5 mL, excess). Yellow solid; mp: 111–112 °C. Yield 75% (1.511 g). IR (KBr) cm^{-1}: 3085–2928 (w, C–H), 1655 (s, C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.45 (s, 6H, Se$\underline{C}H_3$); 7.99 (s, 4H, H_{Ar}). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 6.0 (Se$\underline{C}H_3$); 127.8 ($C_2 + C_3 + C_5 + C_6$); 142.8 ($C_1 + C_4$); 194.8 ($\underline{C}OSeCH_3$). MS (*m*/*z*% abundance): 318/319/320/322/324 ($M^{+\bullet}$, 1/1/2/2/1), 221/223/224/225/*227*/229 (2/20/19/52/*100*/18), 213 (1), 193/195/196/197/199/201 (1/4/4/10/21/4). Elemental Analysis for $C_{10}H_{10}O_2Se_2$, calcd/found (%): C: 37.52/37.36; H: 3.15/2.95.

*4.1.7.6. Dimethyl benzene-1,3-dicarboselenoate (**29**).* From isophthaloyl chloride (1.29 g, 6.3 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and methyl iodide (1.5 mL, excess). White solid; mp: 59–61 °C. Yield: 84% (1.710 g). IR (KBr) cm^{-1}: 3059–2932 (w, C–H); 1653 (s, C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.45 (s, 6H, Se$\underline{C}H_3$); 7.59 (t, 1H, H_5, $J_{5\text{-}4,5\text{-}6}$ = 7.8 Hz); 8.12 (dd, 2H, $H_4 + H_6$, $J_{4\text{-}2,6\text{-}2}$ = 1.8 Hz); 8.42 (t, 1H, H_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 6.0 (Se$\underline{C}H_3$); 125.9 (C_5); 129.8 (C_2); 131.9 ($C_4 + C_6$); 140.0 ($C_1 + C_3$); 194.5 ($\underline{C}OSeCH_3$). MS (*m*/*z*% abundance): 318/319/320/322/324 ($M^{+\bullet}$, 1/1/2/2/1), 221/223/224/225/*227*/229 (2/20/19/52/*100*/18), 213 (1), 193/195/196/197/199/201 (1/4/4/10/*21*/4). Elemental Analysis for $C_{10}H_{10}O_2Se_2$, calcd/found (%): C: 37.52/37.16; H: 3.15/3.15.

*4.1.7.7. Dimethyl pyridine-2,6-dicarboselenoate (**30**).* From pyridine-2,6-dicarbonyl dichloride (1.29 g, 6.3 mmol), grey selenium (1.00 g, 12.7 mmol), sodium borohydride (1.00 g, 26.4 mmol) and methyl iodide (1.5 mL, excess). Yellow solid; mp: 135–136 °C. Yield 44% (0.894 g). IR (KBr) cm^{-1}: 3102–2926 (d, C–H), 1655 (s, C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.38 (s, 6H, Se$\underline{C}H_3$); 8.03 (ddd, 1H, H_4, $J_{4\text{-}3,4\text{-}5}$ = 8.4 Hz); 2.09 (dd, 2H, $H_3 + H_5$, $J_{3\text{-}5,5\text{-}3}$ = 1.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 5.6 (Se$\underline{C}H_3$); 122.8 ($C_3 + C_5$); 139.6 (C_4); 152.5 ($C_2 + C_6$); 197.9 ($\underline{C}OSeCH_3$). MS (*m*/*z*% abundance): 319/321/323/325 ($M^{+\bullet}$ + 1, 2/4/4/1), 255/256/257/259/261 (1/1/3/5/1), 224/225/226/228/230 (2/2/4/8/2), 196/197/198/200/202 (2/2/4/8/2), 164 (30), 137 (100). Elemental Analysis for $C_9H_9NO_2Se_2$, calcd/found (%): C: 33.67/33.29; H: 2.83/2.74; N: 4.36/4.27.

*4.1.7.8. Dimethyl thiophene-2,5-dicarboselenoate (**31**).* From thiophene-2,5-dicarbonyl dichloride (0.97 g, 4.6 mmol), grey selenium (0.73 g, 9.3 mmol), sodium borohydride (0.73 g, 19.3 mmol) and methyl iodide (1.5 mL, excess). Yellow solid; mp: 99–101 °C. Yield: 69% (1.042 g). IR (KBr) cm^{-1}: 3076–2928 (w, C–H), 1654 (s, C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.46 (s, 6H, Se$\underline{C}H_3$); 7.75 (s, 2H, $H_3 + H_4$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 6.2 ($\underline{C}OSeCH_3$); 130.9 ($C_3 + C_4$); 148.9 ($C_2 + C_5$); 186.2 ($\underline{C}OSeCH_3$). MS (*m*/*z*% abundance): 319/321/323/325 ($M^{+\bullet}$ + 1, 2/4/4/1), 255/256/257/259/261 (1/1/3/5/1), 224/225/226/228/230 (2/2/4/8/2), 196/197/198/200/202 (2/2/4/8/2), 137 (100). Elemental Analysis for $C_8H_8O_2SSe_2$, calcd/found (%): C: 29.46/29.55; H: 2.47/2.79.

4.2. Cytotoxic and antiproliferative activities

Cell culture materials were obtained from BD Bioscience. Stock solutions of the different compounds were prepared as follows: all compounds were dissolved in DMSO at a concentration between 0.01 and 0.005 M. The sterile filtration of the compounds was achieved using 0.2 μm filter disks. Serial dilutions with supplemented medium were prepared daily to a final concentration of less than 2% DMSO in cell culture.

The cytotoxic effect of each substance was determined according to the protocol of Denizot and Lang [47].

Briefly, 2×10^3 cells (PC-3, MCF-7, HT-29, A-549 or RWPE-1) in 50 μL of supplemented media, 10% fetal bovine serum (Hyclone) and 1% penicillin–streptomycin (Lonza) were plate onto each well of 96-well plates. After 12 h incubation, 50 μL of the different compounds at dilutions between 0.01 μM and 100 μM were added and incubated for 72 h at 37 °C and 5% CO_2. Cells were then incubated for 4 h in the presence of 10 μl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and analyzed for their ability to generate a purple formazan dye. Absorbance was measured at a wavelength of 590 nm and the ratio of viable cells was calculated. 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.

4.3. Cyclic voltammetry

Assays were performed with a CGME (Controlled-Growth Mercury Electrode) BAS potentiostat and data was processed with an Electrochemical Analyzer BAS 100B. Several preliminary assays were performed to determine the most suitable conditions for the cyclic voltammetry assays of the selenoesters presented in this work. Optimal conditions were found to include: working electrode: mercury-drop electrode, although most of the active compounds give also signals at a glassy-carbon electrode. Scan rate: 200 mV/s. Concentration: 0.5 mM. Solvent: aqueous phosphate buffer, pH 7.4, with 20% of methanol to ease solubilization of compounds. Time: 5 min after addition compound to the voltammetric cell in order to allow equilibration (and, where applicable, a sufficient degree of hydrolysis to detect the hydrolyzed (selenolate) products).

4.4. GPx-like activity

The evaluation of the GPx-like activity of the selenoesters is performed following a revised procedure based in the one described by Iwaoka and Tomoda [61]. Briefly, the initial rates of reduction of hydrogen peroxide catalyzed by the compounds in the presence of thiophenol are determined at 305 nm using a four-channel Cary 50 Bio UV–vis spectrophotometer. The GPx-like activity of the compounds is expressed as the ratio of the catalyzed over the non-catalyzed, spontaneous reaction involving thiophenol and hydrogen peroxide. Prior to the measurements, the spectrophotometer is blanked with a thiophenol solution. Then,

compound and hydrogen peroxide are added and measurements are taken each 30 s during the first 10 min. So, initial rates are calculated by a kinetics program as the initial, linear slope of the absorbance *vs* time plot during the initial reaction stages.

4.5. DPPH assay

The procedure of the DPPH-radical scavenging assay is based in an adaptation of the one described by Sharma and Bhat [62]. A blank and a triplicate of five concentrations of compounds and ascorbic acid are sieved in a 96-well plate (100 μL of compound, control or blank are added, respectively). Afterwards, a DPPH solution is added to each well and the plate is stored in dark for 30 min prior the spectrophotometric measurement at a wavelength of 550 nM. Finally, the absorbance data is used to determine the percentage of the radical scavenged. The final concentrations of DPPH and compounds are 50 μM and 5, 15, 50, 150 and 500 μM, respectively.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.11.034.

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