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Identification of selenocompounds with promising properties to reverse cancer multidrug resistance

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ABSTRACT

In previous studies, 56 novel selenoesters and one cyclic selenoanhydride with chemopreventive, antiproliferative and cytotoxic activity were described. Herein, the selenoanhydride and selected selenoesters were evaluated for their ability to reverse the cancer multidrug resistance (MDR) using the ABCB1 efflux pump inhibition assay in mouse MDR T-lymphoma cells. Results showed that the selenoanhydride (**1**) and the selenoesters with ketone terminal fragments (**9**–**11**) exerted (1.7–3.6)-fold stronger efflux pump inhibitory action than the reference verapamil. In addition, those four derivatives triggered apoptotic events in more than 80% of the examined MDR mouse cells.

Selenium and its organic and inorganic derivatives fulfil several vital biological functions.[1] Interest in selenium has arisen since the discovery of the fact that its deficiency can cause clinical disorders, e.g., the diseases of Keshan and Kashin-Beck.[2] Afterwards, epidemiological studies reported by Li in China[3] and Clark in USA[4,5] proposed that the dietary supplementation with selenium reduced the incidence of lung, oesophagus[4] and prostate[5] cancer. The publication of those results initiated an intense and productive search of novel inorganic and organic selenocompounds with chemopreventive, antiproliferative and cytotoxic activity against cancer.[6–9] Among the inorganic selenium-containing salts, sodium selenite could be highlighted for its anticancer properties[10] whereas several of the most known organic selenocompounds with anticancer properties are methylselenol,[11,12] methylseleninic acid,[13,14] selenocyanates[15,16] and diphenyldiselenide.[17,18] Selenium nanoparticles are also being deeply studied, both in cancer field[19,20] and in cancer or bacterial multidrug resistance.[21,22]

Considering these antecedents, our previous studies concerned the design, synthesis and biological evaluation of selenium-containing anticancer agents, including 56 selenoesters and one selenoanhydride.[23,24] Those organoselenic derivatives displayed significant cytostatic action with IG_{50} values in nanomolar ranges, whereas the LD_{50} values of the most cytotoxic selenocompounds were in the micromolar range.[23,24] Various lines of evidence[25–29] indicate that selenocompounds enhance synergistically the activity of anticancer drugs when they are administered in combination. Thus, synergistic effects over the anticancer activity have been observed in the following selenocompounds and anticancer drug combinations: diphenylmethyl selenocyanate and cisplatin in a murine tumor model,[25] selenium nanoparticles and irinotecan in HCT-8 colon cancer cells,[26] selenite and imatinib in HCT116 colorectal cancer cells,[27] selenocysteine and auranofin in A549 lung cancer cells;[28] and methylseleninic acid and paclitaxel in a murine cancer model.[29] The synergistic effects observed suggest multidrug resistance (MDR) reversing activity for the selenocompounds. The MDR against the chemotherapeutic drugs action is a worrying problem in cancer treatment.[30,31] Among the different mechanisms involved in the cancer MDR processes, the increased activity of the efflux pumps is one of the most common.[32] The membrane transport proteins, especially the P-glycoprotein (P-gp, ABCB1

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protein), are able to recognize and expel various exogenous molecules out of the cell.[32,33] This is a natural protective mechanism of the cells against the action of toxic compounds that is stimulated in cancer cells.

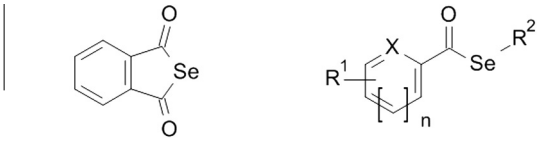
Thus, the design of inhibitors of the efflux pumps, especially of the ABCB1, is a promising strategy in cancer therapy. For the evaluation of the efflux-pump related resistance in cancer, cell lines with over-expressed efflux pump systems, such as ABCB1, are commonly used.[34]

In this context, we have selected the representative structures **1**–**11** (Table 1) among the selenocompounds previously reported in Refs. 23,24 to investigate their capacity to reverse the cancer MDR via the inhibition of the efflux pump ABCB1. The representative compounds contain a selenoanhydride (**1**) or a selenoester (**2**–**11**) functional group. The alkyl moiety bounded to the selenium atom in the selenoesters is a methyl group (**2**–**5**) or includes secondary functional groups, such as: amides (**6**), carboxylic esters (**7**–**8**) or ketones (**9**–**11**). The compounds were resynthesized in the amount required to perform the biological assays, using the methods described earlier.[23,24] They were examined on the following biological actions: (i) inhibition of ABCB1 via the fluorescent substrate retention assay, (ii) cytotoxicity in the MTT assay and (iii) their capacity to induce apoptotic processes.

The biological studies were performed in two cell lines, the parental L5178Y and the MDR-derived mouse T-lymphoma cells transfected with the human *MDR1* (*ABCB1*) gene that codes for the ABC transporter ABCB1.[35]

The efflux modulating effects of compounds **1**–**11** were investigated in the MDR cancer cells (mouse T-lymphoma) using the rhodamine 123 accumulation assays.[34–37] Rhodamine 123 is the dye–substrate for ABCB1. The percentage of mean fluorescence intensity was calculated for the treated MDR cells in comparison with the untreated cells. Results, expressed as the fluorescence activity ratio (FAR), have been compared to the action of the reference verapamil by the FAR quotient. The FAR and quotient values were computed according to the Eqs. 1 and 2, respectively (Table 2). Results indicated that the selenoesters **2**–**8** slightly affected the efflux activity of ABCB1, whereas the compounds **1** and **9**–**11** (Fig. 1) displayed a pronounced modulating action on this efflux pump in the MDR mouse T-lymphoma cells. It is noteworthy that derivatives **1** and **9** inhibited the ABCB1 pump (1.6–3.4)-fold stronger than the reference inhibitor (verapamil) at its 10-fold higher concentration. When evaluated at the same concentration than verapamil, compounds **1** and **9**–**11** inhibited this efflux pump (1.7–3.6)-fold stronger than this known inhibitor.

Table 1
Structure of the selenocompounds evaluated as MDR reversing agents



A. Cyclic selenoanhydride (**1**) **B**. Selenoesters (**2**-**11**)

Compd	Group	R^1	X	*n*	R^2
1	**A**	—	—	—	—
2	**B**	5-$COSeCH_3$	S	0	$-CH_3$
3	**B**	6-$COSeCH_3$	N	1	$-CH_3$
4	**B**	3-$COSeCH_3$	C	1	$-CH_3$
5	**B**	4-$COSeCH_3$	C	1	$-CH_3$
6	**B**	—H	C	1	$-CH_2CONH_2$
7	**B**	4-Cl	C	1	$-CH_2COOCH_3$
8	**B**	—H	C	1	$-CH_2COOPh$
9	**B**	4-Cl	C	1	$-CH_2COCH_3$
10	**B**	4-Cl	C	1	$-CH_2COC(CH_3)_3$
11	**B**	3,5-$DiOCH_3$	C	1	$-CH_2COC(CH_3)_3$

Compd: Compound.

Table 2
Effects of selenocompounds on rhodamine 123 retention by L5178Y multidrug resistant (MDR) mouse T-lymphoma cells

Sample	Concentration (μM)	FAR[a]	FAR quotient[b] (%)
Verapamil	20	27.4	100
1	2	43.14	157.4
1	20	97.61	356.2
2	2	0.99	3.61
2	20	1.07	3.19
3	2	1.13	3.98
3	20	1.49	5.44
4	2	0.93	2.78
4	20	0.98	3.39
5	2	3.97	18.64
5	20	3.09	14.49
6	2	1.02	3.04
6	20	1.05	3.72
7	2	6.45	30.28
7	20	8.53	23.54
8	2	0.65	3.05
8	20	5.68	2.37
9	2	94.20	343.79
9	20	84.64	308.91
10	2	9.28	33.87
10	20	48.67	177.63
11	2	12.56	45.84
11	20	46.35	169.16
DMSO	2%	0.76	2.77

[a] FAR: fluorescence activity ratio, calculated as follows:

$$\mathrm{FAR} = \frac{\mathrm{MDR}_{\mathrm{treated}}/\mathrm{MDR}_{\mathrm{control}}}{\mathrm{parental}_{\mathrm{treated}}/\mathrm{parental}_{\mathrm{control}}}. \quad (1)$$

[b] Calculated as follows:

$$\mathrm{Quotient} = \frac{\mathrm{FAR}_{\mathrm{compound}}}{\mathrm{FAR}_{\mathrm{verapamil}}} \times 100. \quad (2)$$

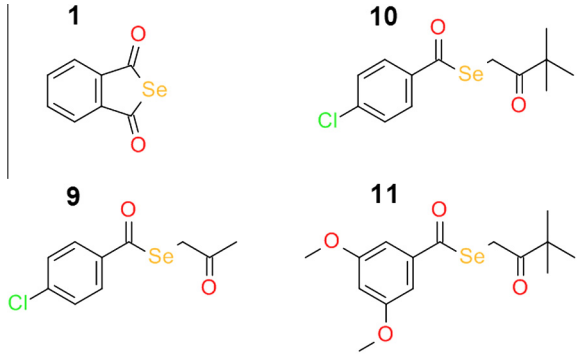


Figure 1. Structure of the most active selenocompounds.

The cytotoxic effects of the selenocompounds **1**–**11** were determined and compared with the action exerted by two reference drugs with moderate (thioridazine) or weak (verapamil) cytotoxicity. Results were expressed as IC_{50} values (Table 3). Six compounds (**2**, **4**–**8**) were less cytotoxic than both reference drugs. However, the most active compounds (**1** and **9**–**11**) showed a significantly higher cytotoxicity than thioridazine and verapamil in both cell lines. Besides, the selenoesters **9**–**11** exhibited their cytotoxic action in the nanomolar range at least in one of the two mouse T-lymphoma cell lines investigated. In the case of the most active one (**10**), the IC_{50} value was 430 nM in the MDR cells.

Table 3
Cytotoxic effects of selenocompounds on L5178Y parental (PAR) and multidrug resistant (MDR) mouse T-lymphoma cells

Compd	PAR mouse T-lymphoma cells		MDR mouse T-lymphoma cells	
	IC_{50} (μM)	SD±	IC_{50} (μM)	SD±
1	3.97	1.26	4.65	0.71
2	>100	—	>100	—
3	19.5	2.10	16.9	3.23
4	>100	—	>100	—
5	>100	—	>100	—
6	>100	—	36.4	9.91
7	>100	—	87.8	5.54
8	>100	—	>100	—
9	0.78	0.17	1.03	0.31
10	0.94	0.11	0.43	0.25
11	1.31	0.12	0.97	0.28
VP	>100	—	47.85	1.88
TZ	12.72	0.56	7.43	0.68

Compd: compound, VP: verapamil, TZ: thioridazine, DMSO: dimethyl-sulfoxide.

The capacity of the selenocompounds **1**–**11** to induce apoptosis in mouse MDR and PAR cells (Table 4) was measured at the concentration of 2 μM. The apoptosis inducer 12*H*-benzo[α]phenothiazine (M627) was used as positive control at the concentration of 20 μM.[37] In accordance with previous assays, only compounds **1** and **9**–**11** caused significant apoptotic events, whereas the remaining compounds (**2**–**8**) were inactive. The compounds **1**, **10**, and **11** proved to exert early apoptosis-inducing activity in ABCB1 over-expressing mouse MDR T-lymphoma cells, being the proportion of early apoptosis 32.2%, 15.9%, and 16.6%, respectively. In addition, they triggered late apoptosis or necrosis in the 45.0%, 21.9%, and 30.5 % of the treated cell population, respectively. Compound **9** induced late apoptosis in the 85.8% of the cell population. If we consider the early and late apoptosis/necrosis together, derivatives **1** and **9**–**11** induced apoptosis in the 77.2%, 90.6%, 47.8% and 47.1% of cells, respectively. Interestingly, it was demonstrated the same order of activity as that observed in the ABCB1 inhibition assay. The selenoesters (**2**–**8**) were significantly less pro-apoptotic as they triggered these cell death processes in less than 10% of cells studied. Thus, **1** was the most pro-apoptotic compound within the tested series. It demonstrated an apoptosis induction close to the positive control (M627; 93.6%) at a 10-fold lower concentration.

Alternatively, the selenium derivatives (**1** and **9**–**11**) induced early apoptosis events on the parental mouse PAR T-lymphoma cells in the range of 30% of the analyzed cells for **1**, **9** and **10**; and of 15% for **11** (Table 4). Late apoptosis and necrosis were triggered by these compounds in the range of 53–67% of the cells evaluated. In this cell line, the four active compounds (**1** and **9**–**11**) triggered apoptotic events in more than 80% of the cells evaluated, what was much higher than the apoptotic induction exerted by the positive control of 12*H*-benzo[α]phenothiazine (~57%) at a 10-fold higher concentration. The remaining selenoesters tested (**2**–**8**) induced apoptotic events in the range from 5% to 14% of the analyzed cells.

Seven out of the eleven selenocompounds evaluated were more pro-apoptotic in PAR cells than in MDR cells. This experimental fact seems to be related to the more intensive expelling of the compounds by the ABCB1 efflux pump in the case of the multidrug resistant cells. These pumps are over-expressed in the MDR cells, whereas their level is much lower in the PAR cells. Consequently, the parental cells expel out a minor percentage of the selenocompounds, allowing them to trigger a stronger pro-apoptotic action on the cell. The collaboration between apoptotic and ABCB1 modulating actions can be observed for the active compounds **10** and **11**. These two derivatives modulated weakly the ABCB1 pump at 2 μM (**10** and **11**, Table 2) and demonstrated an almost 2-fold decrease of the apoptotic effects in the MDR T-lymphoma cells. In contrast, the compounds that strongly modulated the efflux pump at a 2 μM concentration (**1** and **9**, Table 2) showed also a strong apoptosis induction in MDR cells, reducing thus the divergence of their total apoptotic action between parental and MDR cells to a percentage below 10% (Table 4). The observed differential action could be explaining considering that compounds **10** and **11** exerted a partial inhibition of the efflux pumps at the assayed concentration, enabling in this way the expelling out of the cell of a fraction of the apoptotic compound. This would reduce the concentration of the selenocompound inside the cell, thus reducing its apoptotic induction.

The results obtained within the different biological assays allowed us to perform a structure–activity relationship analysis for the tested series of selenocompounds (**1**–**11**). The active compounds are the same in all the assays: the selenoanhydride **1** and the selenoesters **9**–**11**. The investigated cytotoxic activity seems to depend on the chemical variation at the alkyl chain directly bounded to selenium atom. The *tert*-butyl (**10**, **11**) or methyl (**9**) ketones seem to be the most profitable, whereas ester (**7**, **8**), amide (**6**) or methyl (**4**, **6**) groups decrease significantly the cytotoxicity in both parental and MDR mouse T-lymphoma cells. These SAR are in concordance with the results reported in previous cytotoxicity studies in different cancer cell lines.[23] The observed variations of the activity with the change of the functional group can be related with the modulation of the polarity of the molecule exerted by this functional group at the alkyl moiety, as well as with the modulation of the hydrolysis rate of the selenoester; as hypothesized earlier.[23]

The role of the substituents at the aromatic ring seems to be less important for the cytotoxicity but it can be noticeable, too. In the case of the most active selenoesters (**9** and **10**), the aromatic phenyl ring has a chlorine atom at *para* position. The replacement of this 4-chlorophenyl moiety of **10** by the 3,5-dimethoxyphenyl one (**11**) caused an almost 2-fold reduction of the cytotoxic activity in both cell lines studied (Table 3).

The SAR analysis for the rhodamine 123 retention studies (Table 2) is in analogy with that for the cytotoxicity assay. Results of the efflux modulating effects in comparison with the structures of compounds **1**–**11** highlight the importance of the aforesaid structural features, including: the ketone in the *Se*-alkyl moiety (**9**–**11**) and the cyclic selenoanhydride (**1**). However, the compound containing a methyl ketone (**9**) was the most potent ABCB1 inhibitor, better than the selenoanhydride (**1**), and both of them affected more this efflux pump than the compounds with the *tert*-butylketone terminate fragment (**10**, **11**). In the case of the apoptosis-inducing activity assays, the SAR analysis is in high compatibility with that of the rhodamine 123 retention tests.

The beneficial role of the selenoanhydride (**1**) and methyl ketone selenoester moiety (**9**) is unquestionable on the basis of the most potent apoptotic effects, in the range of the reference M627 or higher, that can be noted for these structures in both PAR and MDR cell lines. The presence of *tert*-butylketone ends (**10**, **11**) seems to be especially profitable for the apoptotic effects in parental T-lymphoma cells, whereas a replacement of the ketone fragment with ester-, amide- or methyl one (**2**–**8**) causes a huge loss of the activity (Table 4).

In conclusion, these studies allowed us to identify four derivatives (**1**, **9**–**11**), out of the eleven tested selenocompounds, that demonstrated high potency to inhibit cancer MDR efflux pump ABCB1 with simultaneous cytotoxic- and strong pro-apoptotic activities in the mouse T-lymphoma cells. Results of the biological assays suggest a synergistic mechanism of the pro-apoptotic and the efflux pump inhibitory actions for the active compounds (**1**, **9**–**11**). The active agents found belong to two following families of selenocompounds: the benzoselenophene-diones (**1**) and the

Table 4
Apoptosis-inducing activity of selenocompounds in MDR and in PAR mouse T-lymphoma cells, after 1 h of incubation

	Concn (μM)	Gated events in mouse MDR T-lymphoma cells				Gated events in mouse PAR T-lymphoma cells			
		Early apoptosis (%)	Late apoptosis, necrosis (%)	Cell death (%)	Total apoptotic events	Early apoptosis (%)	Late apoptosis, necrosis (%)	Cell death (%)	Total apoptotic events
A− I−	—	0	0	0	0	0.217	0	0	0.217
A− I+	—	0.015	0.01	2.18	0.025	0.085	1.14	1.55	1.225
A+ I−	—	4.35	0	0	4.35	4.27	0.02	0	4.29
A+ I+	—	2.2	1.57	0.03	3.77	2.60	0.818	0.12	3.418
DMSO	2%	6.46	2.28	0.09	8.74	3.96	0.859	0.191	4.819
M627	20	47.9	45.7	0.12	93.6	51.2	5.58	0.279	56.78
1	2	32.2	45	0.47	77.2	31.5	53.9	0.767	85.4
2	2	5.07	2.34	0.30	7.41	6.64	6.93	0.352	13.57
3	2	5.38	3.28	0.24	8.66	5.97	7.18	0.202	13.15
4	2	4.35	2.8	0.45	7.15	4.47	3.79	0.076	8.26
5	2	3.01	3.25	0.37	6.26	3.61	2.01	0.110	5.62
6	2	3.01	2.91	0.33	5.92	2.46	2.24	0.081	4.70
7	2	2.69	1.47	0.03	4.16	5.09	2.52	0.497	7.61
8	2	3.90	2.26	0.16	6.16	3.00	2.11	0.240	5.11
9	2	3.86	85.8	7.66	90.6	30.8	52.7	0.874	83.5
10	2	15.9	21.9	1.21	47.8	36.1	56.1	0.255	92.1
11	2	16.6	30.5	0.51	47.1	15.7	66.6	0.167	82.3

A+: Annexin V-FITC staining, A−: without Annexin V-FITC, I+: propidium iodide staining, I−: without propidium iodide.
Concn: Concentration; M627: 12*H*-benzo[α]phenothiazine. 'Total apoptotic events' is the sum of 'early apoptosis' and 'late apoptosis, necrosis'.

2-oxoalkyl-benzoselenoesters (**9**–**11**). The SAR-analysis underlines that a ketone moiety, bounded to selenium in the selenoesters through a methylene bridge, plays a crucial role for the expected pro-apoptotic/ABCB1 inhibitory properties. The promising activity of derivatives **1** and **9**–**11** could be of future interest in oncology. The compounds benzo[*c*]selenophene-1,3-dione (**1**) and *Se*-2-oxopropyl 4-chlorobenzoselenoate (**9**) will be selected as two parallel lead-structures for further search of pro-apoptotic cancer MDR modulators among selenium containing compounds.

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Supplementary data

Supplementary data (the material and methods descriptions of the experiments) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.064. These data include MOL files and InChiKeys of the most important compounds described in this article.

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