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*Graphical Abstract (for review)

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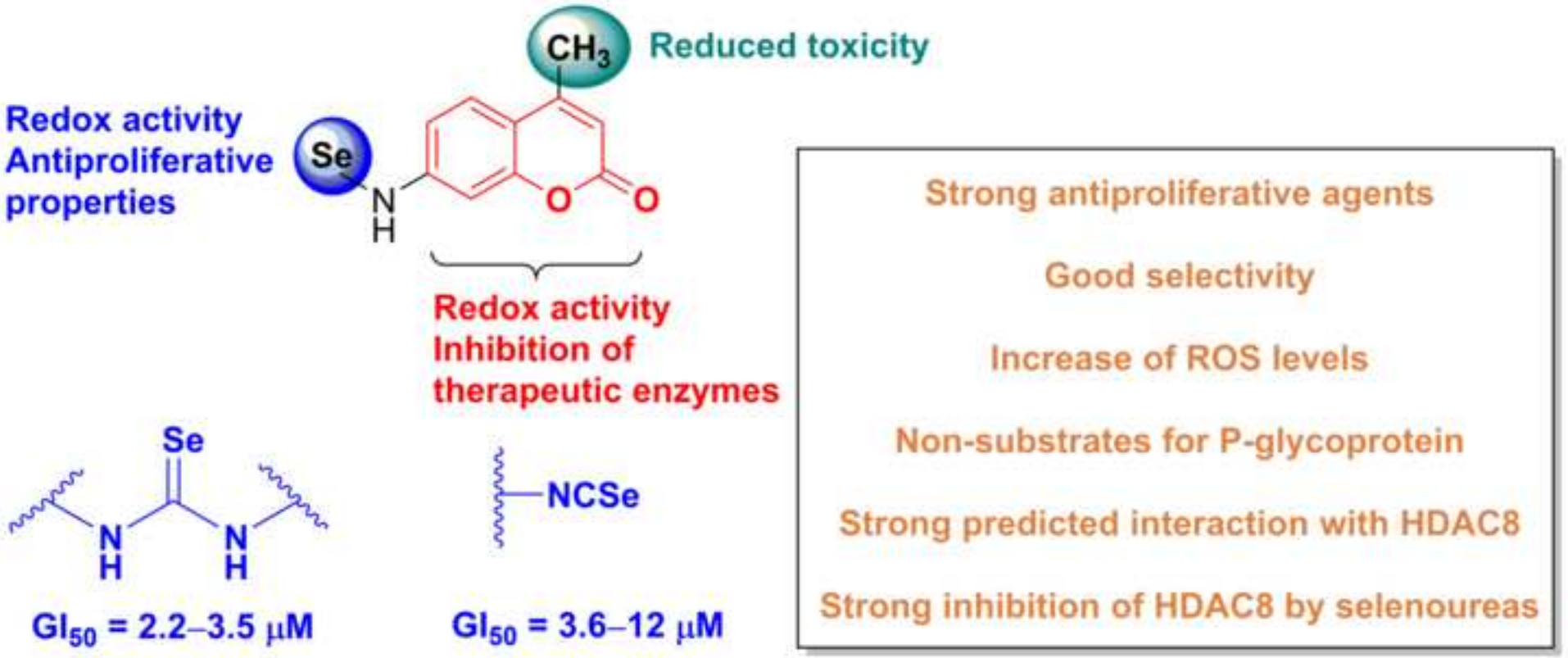

Selenocoumarins as new multitarget antiproliferative agents: synthesis, biological evaluation and *in silico* calculations

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Highlights

- A straightforward preparation of coumarin-based selenoderivatives was achieved
- Isoselenocyanate and selenoureas were strong and selective antiproliferative agents
- An increased level of ROS was detected in their mode of action
- Non-substrates for P-glycoprotein
- Coumarin-selenoderivatives proved inhibitors of HDAC8

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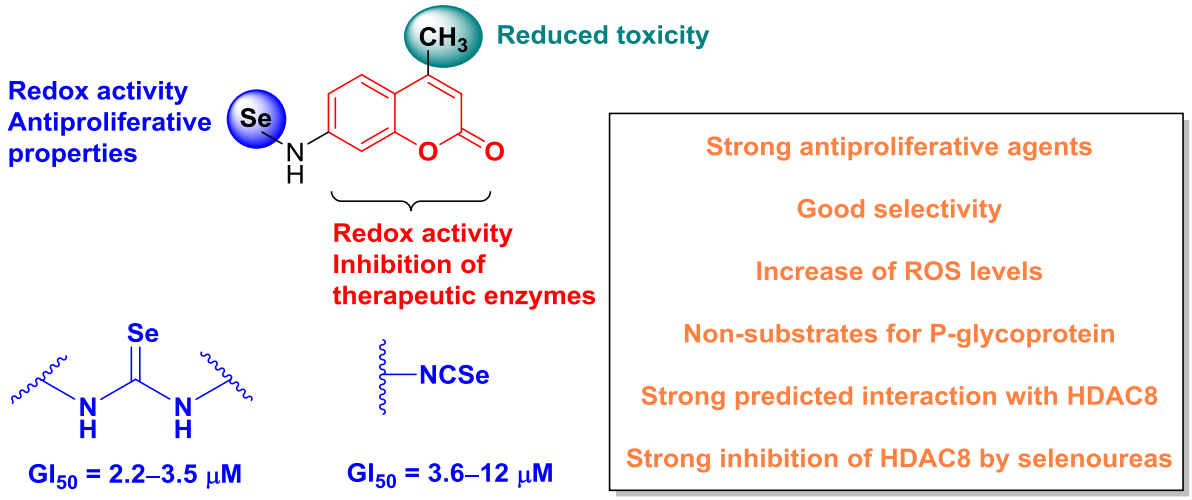
Graphical Abstract



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Abstract

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Two compounds showed good antiproliferative data and appear as plausible leads for further testings. The symmetrical coumarin **6** displays the best *in vitro* inhibition of HDAC8, but is affected by P-gp. In contrast, the *N*-butyl selenourea coumarin derivative **5a** escapes P-gp resistance but has lower HDAC8 inhibition activity.

Keywords

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1. Introduction

Coumarin (2*H*-chromen-2-one), whose name comes from *coumarou*, the vernacular name for tonka beans (*Dipteryx odorata* Wild.), from where it was isolated in 1820 [1], is an ubiquitous phytochemical [2] mainly found in plants, but also in bacteria and fungi. So far, over 1300 coumarins have been isolated from such sources [3]. Naturally-occurring or synthetic [4] coumarins are currently considered as privileged scaffolds [5], endowed with a plethora of biological activities [6]. Although one of the most known properties is their strong antioxidant character [7], a wide spectrum of other bioactivities has been reported for numerous coumarin-containing derivatives: anti-HIV and anti-HCV [8], antituberculosis [9], anti-leishmanial [10], anticancer [11], anti-inflammatory [12], and anti-Alzheimer's [13], among others.

The planar structure and the presence of a lactone scaffold in chromenones allows the establishment of non-covalent interactions, promoting the inhibition of key enzymes currently considered as therapeutic targets, such as cholinesterases (Alzheimer's) [14], glycosidases (type-2 diabetes, lysosomal storage disorders) [15] MAO (Alzheimer's, Parkinson) [16, 17], or carbonic anhydrase (cancer) [18].

The coumarin core skeleton has inspired the development of new therapeutic agents. In fact, there are some coumarin-based marketed drugs, among which, the anticoagulant agents warfarin and dicoumarol are relevant examples [19]. These vitamin K antagonists are effective in thrombosis prevention, but are also frequently implicated in adverse drug reactions, among which bleeding threatens the life of treated patients [20]. It has been demonstrated [21] that alkylation on C-3 and/or C-4 positions of coumarin strongly reduces the hepatoxicity of such template, probably by the diminished formation of the transient 3,4-epoxide in the metabolization of coumarin.

The preparation of hybrid structures derived from coumarins is currently an emerging area of interest within Medicinal Chemistry [22, 23]. Such hybrid structures have proved relevant properties against Alzheimer's disease [24] and cancer [25]. The high complexity associated to multifactorial diseases, among which cancer is a relevant example, has obliged to change the design of new drugs, shifting from the one-target one-drug approach to the multitarget paradigm. Classical drug cocktails required for tackling the diverse targets usually lead to severe side-effects or undesirable drug-drug interactions. In this scenario, multitarget drug approach emerged as a rational way of combatting several targets simultaneously upon combination of a series of pharmacophores within the drug structure [26].Accordingly, the 4-methyl coumarin scaffold seemed to be a good starting point in the synthetic strategy to reduce side-effects in the final compounds (Fig. 1).

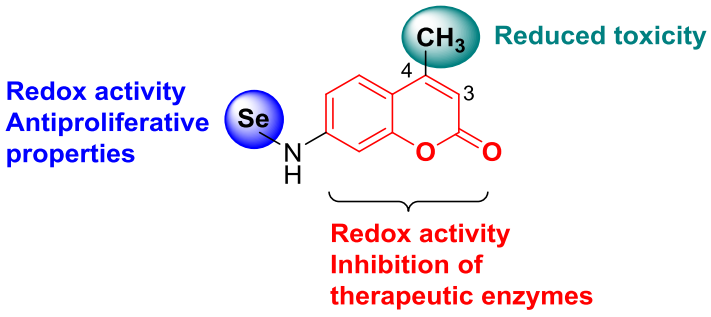


Figure 1. General bioactivity pattern for the selenium-containing coumarins.

Herein we report the preparation of novel multitarget coumarin-based derivatives by combination of the chromenone scaffold with an organoselenium motif. We have successfully accomplished the preparation of selenium-containing hybrid derivatives of iminosugars [27], steroids [28, 29], and polyphenols [30] as novel antiproliferative agents. The organoselenium motif can be useful for altering the tumor cell redox status [31] and probably induce cell death. Moreover, coumarin-containing derivatives have been found to exhibit inhibition of histone deacetylases (HDACs) [32, 33]. HDACs catalyze the removal of the acetyl group of ε-*N*-acetyl lysine on histone, achieving a more compact chromatin structure upon ionic interactions between histones and DNA, and thus controlling genetic transcription. HDAC inhibitors have been reported to act as anticancer agents [34].

To the best of our knowledge, the combination of selenium and coumarin scaffolds has rarely been described only in literature. A selenocyanato-containing umbelliferone has recently been reported to exert myeloprotection in therapies based on the administration of carboplatin, by diminishing the level of pro-oxidant species, and restoration of the glutathione redox pool [35], and at the same time, it potentiated the anti-tumor properties of the cytotoxic agent.

2. Results and discussion

2.1. Chemistry

Herein we present the design of three different families of coumarin-based selenoderivatives as potential novel antiproliferative agents: isoselenocyanate, selenocarbamates and selenoureas. Modification of the selenium-containing functional group, paired with different stereoelectronic effects of the substituents might modulate the biological properties of the target compounds, allowing the establishment of valuable structure-activity relationships for designing the lead compound.

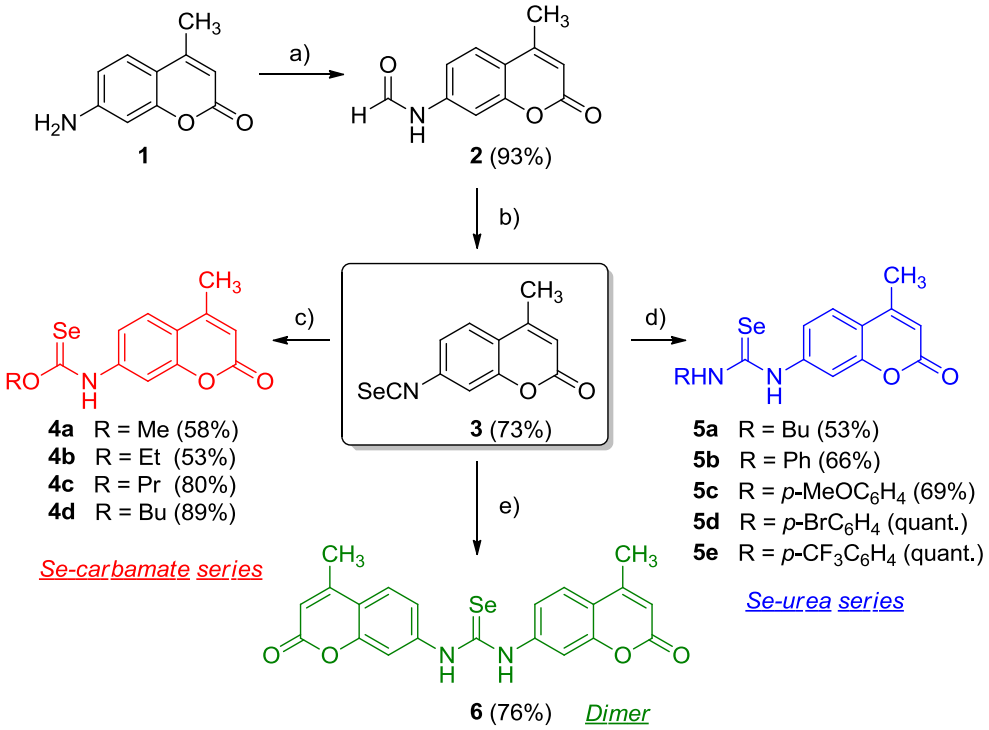
The synthesis of the selenocoumarins was started from commercially-available 7-amino-4-methylcoumarin (AMC, **1**). Derivative **1** was transformed into formamide **2** (Scheme 1) upon treatment with freshly-prepared acetic formic anhydride (AFA) [36]. Attempts to accomplish the synthesis of **2** using a biphasic medium (CH_2Cl_2–sat. aq. $NaHCO_3$), similar to what was reported for sugar-derived formamides [37] produced moderate yields (36%). Replacement of biphasic medium with pyridine produced **2** [38] with a 93% yield.

Next, formamide was transformed into the hitherto unknown coumarin-based isoselenocyanate **3** (Scheme 1) in a one-pot two-step procedure, which involves triphosgene-based dehydration of **2** into a transient non-isolated isocyanide, followed by addition of elemental selenium black (73% overall yield) [37, 39]. ^{13}C-NMR signals around 130 ppm confirmed the presence of the isoselenocyanate group.

Isoselenocyanates are quite versatile synthetic intermediates, due to the pronounced electrophilicity of the heterocumulene moiety [31]. This feature allowed us the straightforward transformation of **3** into selenocarbamates **4** (53–89%), *N*-alkyl(aryl) selenoureas **5**, and dimeric **6** (53–quant.) upon reaction with alcohols, amines, and AMC (**1**) (Scheme 1), respectively. ^{13}C-NMR resonances (190, 180 ppm) confirmed the presence of the selenocarbamate or selenoureido motif, respectively. Attempts to purify selenoureas **5** and **6**

using column chromatography (silica gel or alumina as the stationary phase) led to extensive decomposition. To our delight, precipitation of title compounds from the crude reaction mixture led to pure selenoureas **5** and **6** as stable crystalline solids. This observation strongly contrasts with selenocarbamates **4**, which could be purified by silica gel column chromatography without decomposition. Presumably, the higher +R effect of the NH group compared to oxygen leads to increased electronic density on selenium atom, and a longer C–Se bond, making selenoureas more prone to chemical transformations.

N-Phenyl selenourea **5b** was reported recently as a chromo-fluorogenic chemosensor for anion recognition. It was prepared in a modest yield (37%) by coupling phenyl isoselenocyanate with AMC (**1**) [40].



Scheme 1. a) HCO_2COCH_3, Py, rt, 24 h, 93%; b) i) Triphosgene, Et_3N, CH_2Cl_2, N_2, reflux, 2.5 h; ii) black Se, N_2, dark, 24 h, 73%; c) ROH, N_2, 65 ºC, dark, 53–89%; d) RNH_2, N_2, rt (40 ºC for **5e**), dark, 53%-quant.; e) **1**, THF, N_2, dark, 3 h, 60%.

2.2. Antiproliferative activity

The antiproliferative activity of selenocompounds reported in Scheme 1 was studied in five human solid tumor cell lines. In addition to precursor AMC (**1**), the standard anticancer drug cisplatin (CDDP) was used as reference compound. Besides tumor cells, active compounds in all cell lines were tested against the non-tumor cell line BJ-hTERT for selectivity. We followed the NCI protocol [41] and the GI_{50} vales are shown in Table 1.

Overall, the data on antiproliferative activity shows that most of the tested selenocompounds exhibit growth inhibition in all tumor cell lines, whilst AMC (**1**) is inactive (GI_{50} > 100 µM) against all cells. Based on the GI_{50} range (Fig. 2), the most active compounds of the series are selenoureas **5a** and **5d**, and dimer **6**. These three products show

GI_{50} values below 10 μM. The activity is comparable to that of CDDP, and they are more active against the drug resistant lines T-47D and WiDr (up to 6-fold improved activity). In contrast, only one selenocarbamate derivative is active (**4c**) and displayed modest GI_{50} values (23-42 μM), showing a strong correlation between the nature of the organoselenium motif and the antiproliferative behavior. Additionally, precursor isoselenocyanate **3** has a similar activity profile to selenoureas. The results do not allow inferring structure activity relationships between the alkoxy and amino groups (R, Scheme 1) studied in this work.

When considering selectivity toward non-tumor cells, selenoureas **5b-c** do not inhibit cell proliferation of fibroblasts. Although these compounds are not the most active of the series, the observed selectivity makes them potential candidates for further testing. Isoselenocyanate **3** also exhibited good selectivity towards cancer cell lines, with a selectivity index of up to 14.

Table 1. Antiproliferative activity (GI_{50}) of compounds against human solid tumor cell lines and human fibroblasts.[a]

Comp.	A549 (lung)	HBL-100 (breast)	HeLa (cervix)	T-47D (breast)	WiDr (colon)	BJ-hTERT (fibroblasts)
1	>100	>100	>100	>100	>100	
3	**3.6±0.5**	**5.0±0.2**	12±1	**3.6±0.4**	**4.8±0.3**	50±12
4a	>100	>100	78±14	>100	>100	
4b	>100	>100	>100	>100	>100	
4c	37±7	34±8	23±4	41±1	42±7	33±9
5a	**3.0±0.5**	**2.3±0.7**	**5.2±0.4**	**2.9±1.1**	**3.8±1.4**	3.1±0.4
5b	28±8	28±3	24±4	24±4	34±4	>100
5c	28±4	38±6	35±9	33±2	23±6	>100
5d	**2.7±0.9**	**2.2±0.2**	**2.2±0.6**	**2.8±0.3**	**3.5±0.4**	4.4±0.9
5e	**8.6±2.4**	13±4	21±1	**8.7±0.3**	**6.0±0.1**	32±2
6	**3.2±0.7**	**3.5±1.4**	**2.9±0.1**	**3.6±0.4**	**4.5±1.0**	4.9±0.1
CDDP	4.9±0.2	1.9±0.2	1.8±0.5	17±3	23±4	14±2

[a] GI_{50} values are given in μM. Standard deviation was calculated from at least two independent experiments. Cisplatin (CDDP) was used as reference drug. Compound **4d** was not tested due to poor solubility under protocol conditions. Values in bold face represent the best antiproliferative data against tumor cell lines (GI_{50} < 10 μM).

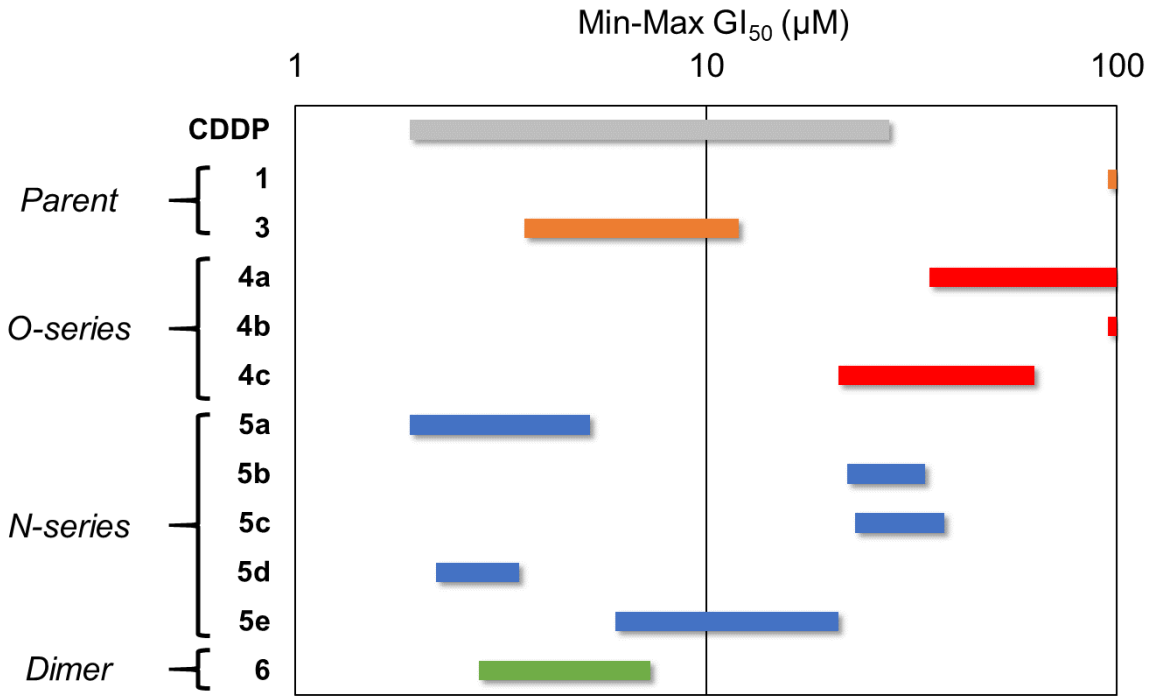


Figure 2. GI_{50} range plot of tested compounds.

2.3. P- glycoprotein assay

P-glycoprotein (P-gp) is a transporter from the ATP-binding cassette family that functions as a pump by extruding toxins and xenobiotics out of the cell. One of the resistance mechanisms to anticancer drugs involves the overexpression of P-gp by cancer cells. In drug discovery and development programs, it is relevant to know whether compounds are substrates for P-gp or not [42]. To test the effect of P-gp overexpression in our compounds, we used a cell line based assay. For this purpose, we use one wild type cell line (SW1573) and its P-gp overexpressing variant (SW1573/Pgp) [43]. Additionally, compounds are tested against both cell lines in the presence or absence of 10 μM verapamil (a known P-gp and CYP3A4/5 inhibitor) [44]. The standard microtubule-interacting drugs paclitaxel (PTX) and vinblastine (VB) are compounds used as reference in this assay. For better comparison of the data, we defined resistance factor (Rf) for a given compound as the ratio of GI_{50} values in the P-gp overexpressing and the wild type cell line.

From the set of coumarins described in this work, we tested all compounds that were active in the antiproliferative assay (Table 1). Table 2 shows experimental GI_{50} values obtained after 48 h exposure of compounds to wild type and P-gp-overexpressing SW1573 cells, and in the presence (+) or absence (-) of verapamil. Compounds **3**-**5** show low Rf values, denoting no effect of P-gp on their biological activity. However, dimer **6** was the most susceptible derivative to this test (Rf = 12). Overall, the results indicate that our selenocoumarins are not substrates for P-gp.

Table 2. Antiproliferative activity (GI_{50}) of selenocoumarins **3-6** in SW1573 and SW1573/P-gp cell lines.[a]

	- Verapamil			+ Verapamil		
	SW1573	SW1573/P-gp	Rf	SW1573	SW1573/P-gp	Rf
3	27±3.3	29±1.2	1.1	29±3.9	24±3.5	0.9
4c	16±2.3	32±2.4	2.0	12±0.9	35±9.6	3.0
5a	3.0±0.4	2.3±0.3	0.8	2.7±0.2	2.0±0.1	0.7
5b	23±5.3	21±1.1	0.9	25±6.3	21±2.0	0.8
5c	14±1.4	24±2.3	1.7	10±0.8	25±8.5	2.5
5d	11±4.0	9.6±0.3	0.9	16±4.4	16±0.6	1.0
5e	20±4.4	24±1.6	1.2	26±0.5	22±3.2	0.9
6	4.5±0.3	55±15	12	9.0±0.7	21±0.2	2.3
PTX	1.5±0.5	196±53	128	1.6±0.2	4.2±0.9	3
VB	0.9±0.3	2051±682	2388	0.8±0.2	1.0±0.5	1

[a] Paclitaxel (PTX) and vinblastine (VB) were used as reference drugs. GI_{50} values are given in μM for compounds **3-6**, and in nM for PTX and VB. Standard deviation was calculated from at least two independent experiments. Rf represents the ratio between GI_{50} values in SW1573/PGP and SW1573 cells.

2.4. Reactive oxygen species production

Oxidative stress as a therapeutic approach against cancer is still under debate [45,46]. Anticancer treatments may use molecules that prevent ROS formation or use drugs that promote a sudden increase of ROS to kill tumor cells. Despite this controversy, we studied the ability of our compounds to increase ROS production. Inside the cell, ROS are converted to H_2O_2 (the longest-lived ROS). Thus, an increase in H_2O_2 after treatment can reflect a general increase in the ROS level. For this purpose, a luminescence cell-based assay was used. This assay does not rely on horseradish peroxidase (HRP), which has been reported to cause a high number of false hits.

We exposed HeLa cells to selected compounds at 1 (**5a**, **5d**, **6**) or 10 μM (**3**, **4c**, **5b-c**, **5e**) for 48 h. The doses were selected based on the GI_{50} values against HeLa cells (Table 1). The results shown in Fig. 3 show an increase of ROS levels for all compounds. Compounds **3** and **5e**, with the highest ROS production, showed the best antiproliferative activity within the group of compounds tested at 10 μM concentration.

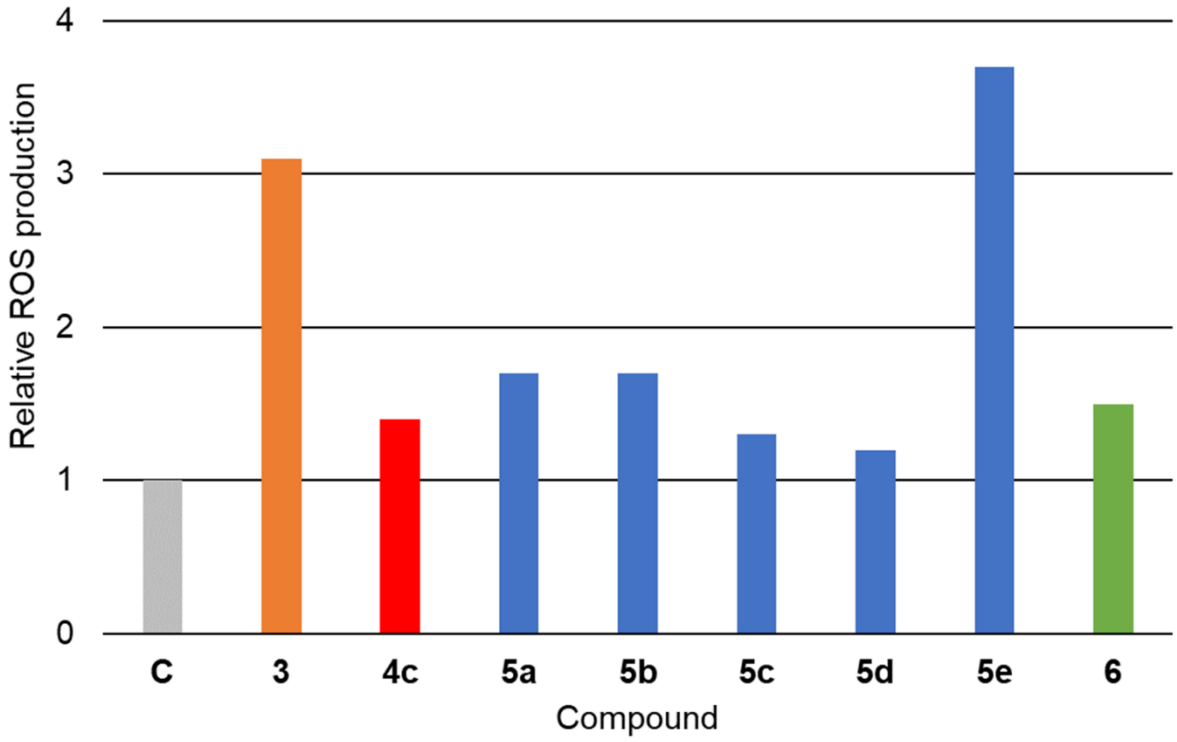


Figure 3. Level of ROS in HeLa cells after 48 h of incubation with selenocoumarins. C = control.

2.5. Docking studies

In order to predict targets of the selenocompounds, we looked at DrugBank, a web-enabled database containing information about drugs and their targets [47]. As aforementioned, our selenocompounds represent analogs of AMC (**1**, DrugBank ID: DB08168). Therefore, we run a structure search to look for reported targets of the parent compound AMC (**1**). The results pointed to three targets: peptidyl-prolyl *cis-trans* isomerase A (PPIase, *E. coli*), mitochondrial peptidyl-prolyl cis-trans isomerase F (PPIF, human) and histone deacetylase 8 (5FCW, human). Thus, we performed docking calculations for the selenocompounds **3**-**6** on the binding site of each of the three targets.

Table 3 shows the results expressed as docking score (representing the variation in free energy of binding). The software provided energy scores for each compound. This value predicts how favorable the interaction between the protein and the ligand is. Thus, lower docking scores (more negative energy) indicate better interaction. As seen in Table 3, all selenocompounds described in this work (**3**-**6**) display a remarkable better score against HDAC8 than the reference drug AMC (**1**). This effect is more noticeable for selenoureas **5a–e** and **6**. Besides the better score displayed in Table 3, the reference compound **1** has no directional interactions with HDAC8, as shown in Fig. 4. Compound **5a** has a better score than compound **1** and establishes π-π interactions with residues Phe152, His180 and Tyr306 and an electrostatic interaction with the Zn cation. Compound **6** has a better score than compound **1** and establishes π-π interactions with residue Phe152 and a H-bond interaction with Lys33.

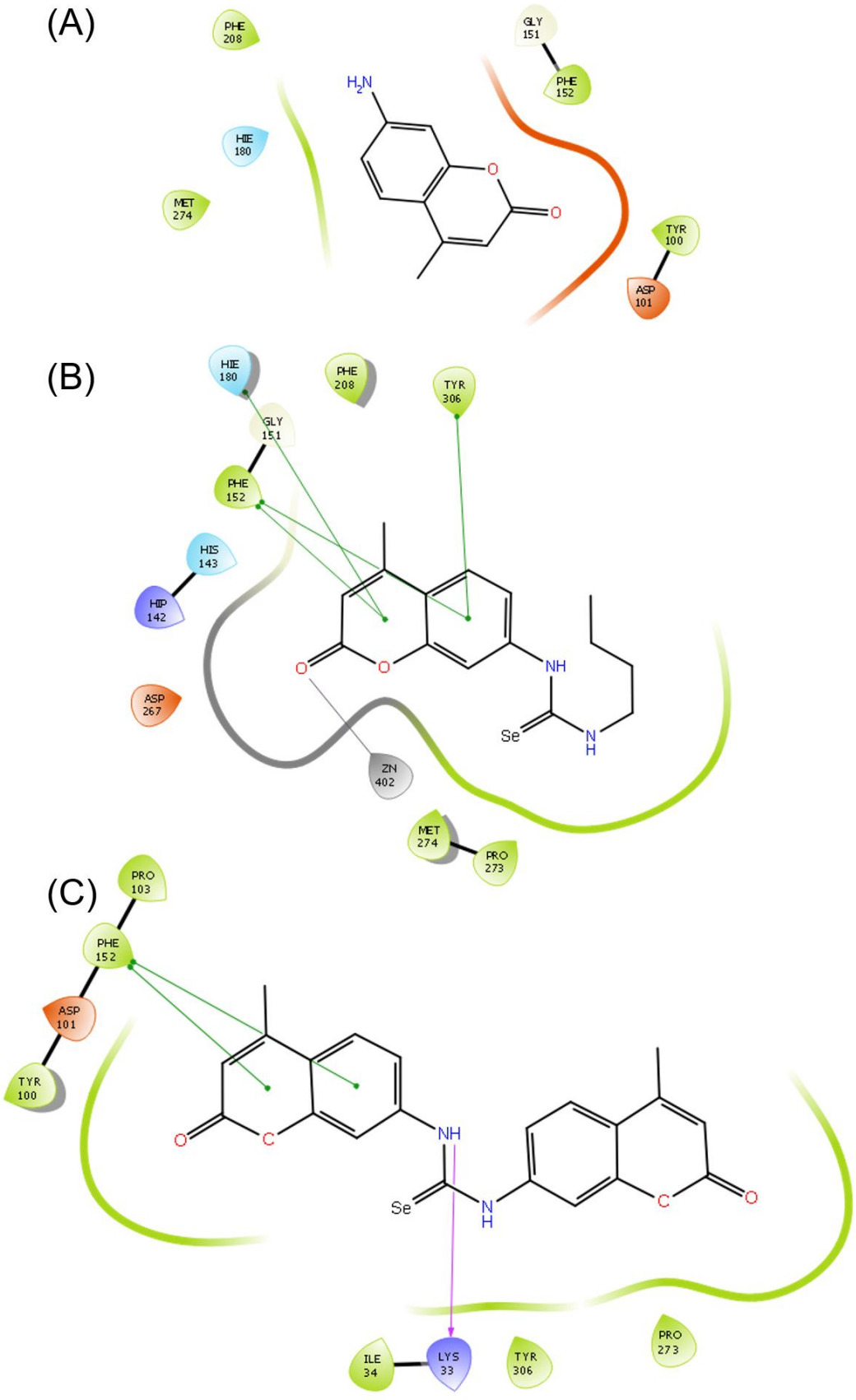


Figure 4. Predicted protein-ligand complex of compounds **1** (A), **5a** (B) and **6** (C) within the binding site of HDAC8 (PDB ID: 5FCW).

Table 3. Docking scores (kcal/mol) of compounds selenocompounds **3**-**6** against reported AMC targets.

Compound	Target (PDB ID)		
	PPIase (1VBS)	PPIF (3R49)	HDAC8 (5FCW)
1	-9.4	-7.3	-7.0
3	-12.6	-7.5	-15.8
4a	-5.0	-9.2	-14.6
4b	-9.1	-11.2	-14.6
4c	-9.4	-13.3	-19.3
4d	-0.4	-11.4	-20.3
5a	-3.4	-8.7	-20.7
5b	-5.4	-17.2	-22.9
5c	-13.6	-12.3	-24.7
5d	-10.5	-7.9	-22.6
5e	-12.7	-8.0	-23.7
6	-11.0	-11.3	-23.9

2.6. HDAC8 inhibition assay

In order to validate the computational results (Table 3), we tested the inhibition of HDAC8 *in vitro* using a commercially available kit. We selected for testing selenocompounds **5a**, **5d-e** and **6** based on their antiproliferative (Table 1) and docking results (Table 3). The inhibitory activity was compared with that obtained for the HDAC8 inhibitor trichostatin A (TSA), which was provided in the kit as a reference compound. According to manufacturer's recommendation, all compounds (selenocompounds and TSA) were tested at a single concentration of 10 μM. This allows for a direct comparison of the obtained data. The results (Fig. 5) show that dimer **6** exhibits more inhibitory activity (1.65 times) than the reference drug TSA. In contrast, selenoureas **5** resulted less potent than TSA.

HDAC8 is a Zn-depending enzyme, which is overexpressed in numerous cancers. This enzyme is currently considered a druggable target for both cancer and neurodegenerative diseases [48]. Taken as a whole, the results of the antiproliferative activity of selenocompounds **5a**, **5d-e** and **6** (Table 1) are consistent with those obtained for HDAC8 inhibitory activity (Fig. 5).

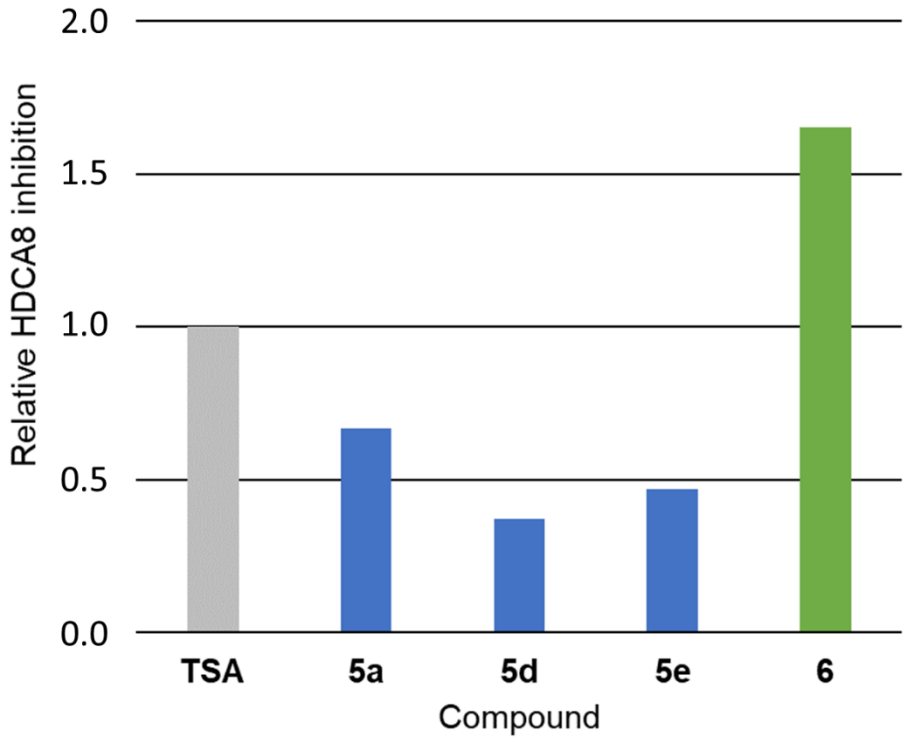


Figure 5. Inhibition of HDAC8 activity by selenocoumarins. TSA = trichostatin A.

3. Conclusions

AMC (**1**) was efficiently transformed into the corresponding 7-isoselenocyanato derivative **3**, which in turn reacted with alcohols and alkyl/aryl amines to produce coumarin-derived selenocarbamates **4** and selenoureas **5**-**6**. Strong activities, within the low micromolar range, were found for isoselenocyanate **3** and some selenoureas **5**-**6**, which were accompained with good selectivity towards cancer cells. High levels of ROS were detected after treatment of HeLa cells with selenocoumarins **5**-**6**, which might be involved in the antiproliferative mechanism. Furthermore, selenoderivatives did not act as P-glycoprotein substrates, thus avoiding the rise of drug resistance. Moreover, *in silico* calculations predicted that all selenoderivatives prepared herein had a more favorable interaction with HDAC8 (a key therapeutic target against cancer) than parent AMC (**1**). This interaction was confirmed in vitro using a HDAC8 inhibition assay.

Overall, two compounds could be selected as lead for further testing, namely selenoureas **5a** and **6**. Although both compounds show good antiproliferative profile, dimer **6** displays the best in vitro inhibition of HDAC8, but it can be extruded by P-gp. In contrast, the advantage of compound **5a** relies in avoiding P-gp resistance, but has lower HDAC8 inhibition activity.

4. Materials and methods

4.1. Materials

Melting points (uncorrected) were recorded on an Electrothermal apparatus. ^{1}H (300.1 and 500.1 MHz) and ^{13}C (75.5 and 125.7 MHz) NMR spectra were recorded at 25 °C on a Bruker

Avance 300//Avance III 500 MHz spectrometers ($CDCl_3$ and DMSO-d_6). The assignments of ^1H and ^{13}C signals were confirmed by COSY and HSQC bidimensional experiments. Mass spectra (ESI) were recorded on a QExactive mass spectrometer. TLCs were performed on aluminium pre-coated sheets (E. Merck Silica gel 60 F_{254}); spots were visualized by UV light, and by charring with 10% vanillin in EtOH containing 1% of H_2SO_4. Column chromatography was performed using E. Merck Silica Gel 60 (40–63 μm), using the eluant indicated in each case.

4.2. Experimental procedures

*4.2.1. 7-Isoselenocyanato-4-methyl-2*H*-chromen-2-one (**3**)*

To a solution of *N*-(4-methyl-2-oxo-2*H*-chromen-7-yl)formamide **2** (325.6 mg, 1.60 mmol), Et_3N (960 μL, 6.88 mmol, 4.3 equiv) and CH_2Cl_2 (4 mL) a solution of triphosgene (253.4 mg, 0.85 mmol, 0.53 mol. equiv.) in CH_2Cl_2 (4 mL). was slowly added. The resulting mixture was refluxed for 2.5 h in the dark and under inert atmosphere. Then, black selenium (254.3 mg, 3.22 mmol, 2.0 equiv) was added, and the mixture was refluxed for further 24 h. After that, the crude reaction was filtered through a Celite® pad and washed with cold CH_2Cl_2. The filtrate was concentrated to dryness and the residue was purified by column chromatography (CH_2Cl_2) to give **3** as a solid. Yield: 310.6 mg (74%). R_f 0.92 (20:1 CH_2Cl_2–MeOH); mp: 172 ºC (dec.); ^1H-NMR (300 MHz, $CDCl_3$) δ 7.59 (d, 1H, $J_{5,6}$ = 9.0 Hz, H-5), 7.23 (d, 1H, $J_{6,8}$= 2.0 Hz, H-8), 7.21 (dd, 1H, $J_{5,6}$ = 9.0 Hz, $J_{6,8}$= 2.0 Hz, H-6), 6.31 (q, 1H, J_{3,CH_3} = 1.2 Hz, H-3), 2.43 (d, 3H, J_{3,CH_3} = 1.2 Hz, Ar-C*H*$_3$) ppm; ^{13}C-NMR (125.7 MHz, $CDCl_3$) δ 159.9 (CO), 154.1 (C-4), 151.5 (C-8a), 133.7, 132.6 (C-7, NCSe), 126.0 (C-5), 122.4 (C-6), 119.7 (C-4a), 115.7 (C-3), 114.5 (C-8), 18.8 (Ar-*C*H$_3$) ppm; HREI-MS *m/z* calculated for $C_{11}H_7NO_2{}^{80}Se$ ($[M]^+$): 264.9637, found: 264.9644.

*4.2.2. General procedure for the preparation of selenocarbamates **4a–d***

A solution of isoselenocyanate **3** (142.6 mg, 0.54 mmol) in the corresponding alcohol (8 mL) was heated (refluxing conditions for **4a**, **4b**; 65 ºC for **4c**, **4d**) in the dark and under inert atmosphere during the time indicated below. After that, the crude reaction was concentrated *in vacuo* and the residue was purified as indicated in each case.

4.2.2.1. O-*Methyl*-N-*(4-methyl-2-oxo-2*H*-chromen-7-yl)selenocarbamate (**4a**)*

Reaction took place for 2 h, and the residue was purified by column chromatography (cyclohexane→5:1 cyclohexane–EtOAc) to give **4a** as a white solid. Yield: 92.5 mg (58%). R_f 0.79 (20:1 CH_2Cl_2–MeOH); mp: 167 ºC (dec.). ^1H-NMR (300 MHz, DMSO-d_6) δ: 12.0 (s, 1H, NH), 7.73 (d, 1H, $J_{5,6}$ = 8.6 Hz, H-5), 7.45 (m, 2H, H-6, H-8), 6.33 (brs, 1H, H-3), 4.18 (s, 3H, OCH_3), 2.40 (brd, 3H, J_{3,CH_3} = 0.7 Hz, Ar-C*H*$_3$) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 191.6 (CSe), 159.9 (CO), 153.3, 153.0 (C-4, C-8a), 140.3 (C-7), 126.0 (C-5), 117.4 (C-6), 116.4 (C-4a), 113.3 (C-3), 108.5 (C-8), 61.8 (OCH_3), 18.0 (Ar-*C*H$_3$) ppm; HRESI-MS *m/z* calculated for $C_{12}H_{12}NO_3{}^{80}Se$ $[M+H]^+$: 297.9977, found: 297.9977.

4.2.2.2. O-*Ethyl*-N-*(4-methyl-2-oxo-2*H*-chromen-7-yl)selenocarbamate (**4b**)*

Reaction took place for 2 h, and the residue was purified by column chromatography (CH_2Cl_2) to give **4b** as a yellow solid. Yield: 89.1 mg (53%). R_f 0.66 20:1 (CH_2Cl_2–MeOH); mp: 193 ºC (dec). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.94 (s, 1H, NH), 7.74 (d, 1H, $J_{5,6}$ = 8.7 Hz, H-5), 7.45 (m, 2H, H-6, H-8), 6.32 (brq, 1H, J_{3,CH_3} = 1.0 Hz, H-3), 4.68 (q, 2H, $J_{H,H}$ = 6.8 Hz, OCH_2), 2.40 (d, 3H, J_{3,CH_3} = 1.0 Hz, Ar-CH_3), 1.40 (t, 3H, $J_{H,H}$ = 6.8 Hz, OCH_2CH_3) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 190.1 (CSe), 159.8 (CO), 153.3, 152.9 (C-4, C-8a), 140.3 (C-7), 126.1 (C-5), 117.3 (C-6), 116.3 (C-4a), 113.2 (C-3), 108.4 (C-8), 71.7 (OCH_2CH_3), 18.0 (Ar-CH_3), 14.0 (OCH_2CH_3) ppm; HRESI-MS *m/z* calculated for $C_{13}H_{13}NNaO_3{}^{80}Se$ $[M+Na]^+$: 333.9953, found: 333.9951.

4.2.2.3. N-*(4-Methyl-2-oxo-2*H-*chromen-7-yl)*-O-*propylselenocarbamate (**4c**)*
Reaction took place for 3.5 h, and the residue was purified by column chromatography (10:1 cyclohexane–EtOAc) to give **4c** as a white solid. Yield: 140.0 mg (80%). R_f 0.54 (60:1 CH_2Cl_2–MeOH); mp: 167-169 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.96 (s, 1H, NH), 7.74 (d, 1H, $J_{5,6}$ = 8.7 Hz, H-5), 7.45 (m, 2H, H-6, H-8), 6.32 (d, 1H, J_{3,CH_3} = 1.1 Hz, H-3), 4.59 (t, 2H, $J_{H,H}$ = 6.3 Hz, OCH_2), 2.40 (d, 3H, J_{3,CH_3} = 1.1 Hz, Ar-CH_3), 1.81 (sext, 2H, $J_{H,H}$ = 7.1 Hz, OCH_2CH_2), 0.97 (t, 3H, $J_{H,H}$ = 7.1 Hz, $O(CH_2)_2CH_3$) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 190.3 (CSe), 159.8 (CO), 153.3 152.9 (C-4, C-8a), 140.4 (C-7), 126.0 (C-5), 117.3 (C-6), 116.4 (C-4a), 113.2 (C-3), 108.4 (C-8), 77.2 (OCH_2), 21.4 (OCH_2CH_2), 18.0 (Ar-CH_3), 10.3 ($O(CH_2)_2CH_3$) ppm; HRESI-MS *m/z* calculated for $C_{14}H_{15}NNaO_3{}^{80}Se$ $[M+Na]^+$: 348.0109, found: 348.0106.

4.2.2.4. O-*Butyl*-N-*(4-methyl-2-oxo-2*H-*chromen-7-yl)selenocarbamate (**4d**)*
Reaction took place for 3.5 h, and the residue was purified by crystallization (CH_2Cl_2–cyclohexane) to give **4d** as a yellow solid. Yield: 163.0 (89%). R_f 0.46 (60:1 CH_2Cl_2–MeOH); mp: 129-131ºC; ^{1}H-NMR (300 MHz, 90ºC, DMSO-d_6) δ: 11.96 (brs, 1H, NH), 7.75 (d, 1H, $J_{5,6}$ = 8.6 Hz, H-5), 7.42 (m, 2H, H-6, H-8), 6.33 (d, 1H, J_{3,CH_3} = 1.1 Hz, H-3), 4.64 (m, 2H, OCH_2), 2.41 (brd, 3H, J_{3,CH_3} = 1.1 Hz, Ar-CH_3), 1.76 (m, 2H, OCH_2CH_2), 1.43 (sext, 2H, $J_{H,H}$ = 7.4 Hz, CH_2CH_3), 0.94 (t, 3H, $J_{H,H}$ = 7.4 Hz, $O(CH_2)_3CH_3$) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 190.3 (CSe), 159.9 (CO), 153.5, 153.0 (C-4, C-8a), 140.5 (C-7), 126.2 (C-5), 118.6 (C-6), 116.4 (C-4a), 113.1 (C-3), 108.4 (C-8), 75.4 (OCH_2), 30.0 (OCH_2CH_2), 18.7, 18.0 (CH_2CH_3, Ar-CH_3), 13.6 ($O(CH_2)_3CH_3$) ppm; HRESI-MS *m/z* calculated for $C_{15}H_{17}NNaO_3{}^{80}Se$ $[M+Na]^+$: 362.0266, found: 362.0263.

*4.2.3. General procedure for the preparation of selenoureas **5a-e**, **6***
A solution of **3** (100.4 mg, 0.38 mmol) and the corresponding amine (1.0 equiv.) in anhydrous THF (4 mL) was stirred under N_2 and in the dark (temperature and time indicated in each case). After that, the crude reaction was concentrated to dryness and purified by precipitation.

4.2.3.1. N-*Butyl*-N'-*(4-methyl-2-oxo-2*H-*chromen-7-yl)selenourea (**5a**)*

Butylamine (38 µL, 0.38 mmol) was used, and the reaction was kept at rt for 30 min. After removal of the solvent under reduced pressure, the residue was purified by precipitation (CH_2Cl_2-cyclohexane) to give **5a** as a yellow-green solid. Yield: 68.5 mg (53%). R_f 0.69 (20:1 CH_2Cl_2–MeOH), mp: 165 ºC (dec). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.19 (s, 1H, NH'), 8.58 (t, 1H, $J_{NH,CH2}$ = 5.9 Hz, NH), 7.71 (d, 1H, $J_{5,6}$ = 8.6 Hz, H-5), 7.58 (d, 1H, $J_{6,8}$ = 2.1 Hz, H-8), 7.34 (brdd, 1H, $J_{5,6}$ = 8.6 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 6.30 (brs, 1H, H-3), 3.57 (q, 2H, $J_{H,H}$ = 6.2 Hz, NHCH_2), 2.41 (d, 3H, $J_{3,CH3}$ = 0.8 Hz, Ar-CH_3), 1.57 (quint., 2H, $J_{H,H}$ = 7.3 Hz, $NHCH_2CH_2$), 1.33 (sext, 2H, $J_{H,H}$ = 7.4 Hz, CH_2CH_3), 0.91 (t, 3H, $J_{H,H}$ = 7.4 Hz, $(CH_2)_3CH_3$) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 179.1 (CSe), 160.0 (CO), 153.4 (C-4), 153.1 (C-8a), 142.6 (C-7), 125.8 (C-5), 118.5 (C-6), 115.6 (C-4a), 112.5 (C-3), 109.0 (C-8), 46.6 ($NHCH_2$), 30.3 ($NHCH_2CH_2$), 19.6 (CH_2CH_3), 18.0 (Ar-CH_3), 13.7 (CH_2-CH_3) ppm; HRESI-MS *m/z* calculated for $C_{15}H_{19}N_2O_2{}^{80}Se$ $[M+H]^+$: 339.0606, found: 339.0602.

4.2.3.2. N-*(4-Methyl-2-oxo-2*H-*chromen-7-yl)*-N'-*phenylselenourea (**5b**)*
Aniline (35 µL, 0.38 mmol) was used, and the reaction was kept at rt for 2.5 h. After removal of the solvent under reduced pressure, the residue was purified by precipitation (CH_2Cl_2–cyclohexane) to give **5b** as a yellow-green solid. Yield: 89.0 mg (66%). R_f 0.75 (20:1 CH_2Cl_2–MeOH); mp: 164 ºC (dec.); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.49 (s, 2H, NH, NH'), 7.72 (d, 1H, $J_{5,6}$ = 8.7 Hz, H-5), 7.59 (d, 1H, $J_{6,8}$ = 2.1 Hz, H-8), 7.46 (dd, $J_{5,6}$ = 8.7 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 7.44-7.33 (m, 4H, Ar-H), 7.23-7.18 (m, 1H, Ar-H), 6.32 (d, 1H, J_{3,CH_3} = 1.2 Hz, H-3), 2.42 (d, 3H, J_{3,CH_3} = 1.2 Hz, Ar-CH_3) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 179.0 (CSe), 159.9 (CO), 153.2 (C-4, C-8a), 143.2 (C-7), 139.5 (Ar-C-*ipso*), 128.7 (Ar-C-*m*), 125.5, 125.4 (Ar-C-*p*, C-5), 124.6 (Ar-C-*o*), 119.8 (C-6), 116.1 (C-4a), 112.9 (C-3), 110.4 (C-8), 18.1 (Ar-CH_3) ppm; HRESI-MS *m/z* calculated for $C_{17}H_{15}N_2O_2{}^{80}Se$ $[M+H]^+$: 359.0293, found: 359.0290.

4.2.3.3. N-*(4-Methoxyphenyl)*- N'-*(4-Methyl-2-oxo-2*H-*chromen-7-yl)selenourea (**5c**)*
p-Anisidine (46.8 mg, 0.38 mmol) was used, and the reaction was kept at rt for 30 min and at 40 ºC for 1.5 h. After removal of the solvent under reduced pressure, the residue was purified by precipitation (CH_2Cl_2–cyclohexane) to give **5c** as a light green solid. Yield: 101.5 mg (69%). R_f 0.54 (CH_2Cl_2-MeOH 20:1), mp: 163 ºC (dec). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.36, 10.29 (2s, 1H each, NH, NH'), 7.73 (d, 1H, $J_{5,6}$ = 8.7 Hz, H-5), 7.59 (d, 1H, $J_{6,8}$ = 2.0 Hz, H-8), 7.45 (dd, 1H, $J_{5,6}$ = 8.7 Hz, $J_{6,8}$ = 2.0 Hz, H-6), 7.30 (m, 2H, Ar-H-*o*), 6.93 (m, 2H, Ar-H-*m*), 6.31 (d, 1H, J_{3,CH_3}= 1.1 Hz, H-3), 3.75 (s, 3H, OCH_3), 2.41 (d, 3H, J_{3,CH_3}= 1.1 Hz, Ar-CH_3) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 179.5 (CSe), 160.8 (CO), 157.8 (Ar-C-*p*), 154.0 (C-4), 153.4 (C-8a), 143.6 (C-7), 132.5 (Ar-C-*ipso*), 127.4 (Ar-C-*o*), 125.9 (C-5), 120.6 (C-6), 116.7 (C-4a), 114.5 (Ar-C-*m*), 113.3 (C-3), 111.2 (C-8), 55.8 (OCH_3), 18.5 (Ar-CH_3) ppm; HRESI-MS *m/z* calculated for $C_{18}H_{17}N_2O_3{}^{80}Se$ $[M+H]^+$: 389.0399, found: 389.0394.

4.2.3.4. N-*(4-Bromophenyl)*-N'-*(4-methyl-2-oxo-2*H-*chromen-7-yl)selenourea (**5d**)*
p-Bromoaniline (65.5 mg, 0.38 mmol) was used, and the reaction was kept at rt for 4 h. After removal of the solvent under reduced pressure, the residue was purified by precipitation

(cyclohexane) to give **5d** as a yellow solid. Yield: 165.8 mg (quant.). R_f 0.43 (20:1 CH_2Cl_2–MeOH), mp: 178 ºC. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.54 (brs, 2H, NH, NH'), 7.73 (d, 1H, $J_{5,6}$ = 8.7 Hz, H-5), 7.58 (d, 1H, $J_{6,8}$ = 2.1 Hz, H-8), 7.55 (m, 2H, Ar-H-*m*), 7.45 (dd, 1H, $J_{5,6}$ = 8.7 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 7.40 (m, 2H, Ar-H-*o*), 6.33 (m, 1H, H-3), 2.42 (d, 3H, J_{3,CH_3} = 1.1 Hz, CH_3) ppm; ^{13}C-NMR (125.7 MHz, DMSO-d_6) δ: 179.2 (CSe), 159.9 (CO), 153.3, 153.1 (C-4, C-8a), 143.0 (C-7), 139.0 (Ar-C-*ipso*), 131.5 (Ar-C-*m*), 126.7 (Ar-C-*o*), 125.5 (C-5), 120.5, 119.8 (Ar-C-*p*, C-6), 116.2 (C-4a), 112.9 (C-3), 110.5 (C-8), 18.5 (Ar-*C*H_3) ppm; HRESI-MS *m/z* calculated for $C_{17}H_{14}BrN_2O_2Se$ $[M+H]^+$: 436.9398, found: 436.9396.

4.2.3.5. N-*(4-Methyl-2-oxo-2*H*-chromen-7-yl)*-N'-*(4-trifluoromethyl)selenourea (**5e**)*
p-Trifluoromethylaniline (48 µL, 0.38 mmol) was used, and the reaction was kept at 40 ºC for 2 h. After removal of the solvent under reduced pressure, the residue was purified by precipitation (cyclohexane) to give **5e** as a yellow-green solid. Yield: 162.5 mg (quant.). R_f 0.44 (20:1 CH_2Cl_2–MeOH); mp: 186 ºC. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.74 (brs, 2H, 2 NH), 7.74 (d, $J_{5,6}$ = 8.6 Hz, 1H, H-5), 7.71 (m, 2H, Ar-H-*m*), 7.68 (m, 2H, Ar-H-*o*), 7.59 (d, 1H, $J_{8,6}$ = 2.1 Hz, H-8), 7.47 (dd, 1H, $J_{5,6}$ = 8.6 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 6.34 (d, 1H, J_{3,CH_3} = 1.1 Hz, H-3), 2.42 (d, 3H, J_{3,CH_3} = 1.1 Hz, Ar-CH_3) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6) δ: 179.6 (CSe), 159.8 (CO), 153.1, 153.0 (C-8a, C-4), 143.3, 142.8 (Ar-C-*ipso*, C-7), 125.0 (q, $^2J_{C,F}$ = 32.7 Hz, Ar-C-*p*), 125.5 (C-5), 124.3 (q, $^1J_{C,F}$ = 270.3 Hz, CF_3), 125.7 (c, $^3J_{C,F}$ = 3.5 Hz Ar-C-*m*), 124.3 (Ar-C*o*), 119.7 (C-6), 116.3 (C-4a), 113.2 (C-3), 110.5 (C-8), 18.0 (Ar-*C*H_3) ppm; HRESI-MS *m/z* calculated for $C_{18}H_{14}F_3N_2O_2{}^{80}Se$ $[M+H]^+$: 427.0167, found: 427.0167.

4.2.3.6. N,N'-*Bis(4-methyl-2-oxo-2*H*-chromen-7-yl)selenourea (**6**)*
AMC **1** (66.5 mg, 0.38 mmol) was used, and the reaction was kept at 45 ºC for 3 h, and at rt for further 18 h. After removal of the solvent under reduced pressure, the residue was washed with Et_2O, EtOAc and a 1:1 cyclohexane–EtOAc mixture to give **6** as a yellow solid. Yield: 126.3 mg (76%). R_f 0.26 (20:1 CH_2Cl_2–MeOH); mp: 199 ºC (dec). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.78 (brs, 2H, 2NH), 7.75 (d, 2H, $J_{5,6}$ = 8.7 Hz, H-5), 7.61 (d, 2H, $J_{6,8}$ = 2.1 Hz, H-8), 7.48 (dd, 2H, $J_{5,6}$ = 8.7 Hz, $J_{6,8}$ = 2.1 Hz, H-6), 6.33 (m, 2H, H-3), 2.42 (brd, 6H, J_{3,CH_3}= 1.0 Hz, Ar-CH_3) ppm; ^{13}C NMR (125.7 MHz, DMSO-d_6) δ: 179.2 (CSe), 159.8 (CO), 153.2, 153.1 (C-4, C-8a), 143.0 (C-7), 125.5 (C-5), 119.8 (C-6), 116.4 (C-4a), 113.0 (C-3), 110.5 (C-8), 18.1 (Ar-*C*H_3) ppm; HRESI-MS *m/z* calculated for $C_{21}H_{17}N_2O_4{}^{80}Se$ $[M+H]^+$: 441.0348, found: 441.0342.

4.3. Cell lines and growth conditions
Cell lines used in this study were kindly provided by Dr. Godefridus J. Peters (Cancer Center Amsterdam, Vrije Universiteit, Amsterdam, The Netherlands). All cells were grown in RPMI 1640 supplemented with 1 mM glutamine, 5% FBS and antibiotics. Cells were grown at 37 ºC in a humidified atmosphere of 5% CO_2 and maintained at low passage.

4.4. Antiproliferative activity

The antiproliferative activity was tested in vitro against human cancer cells using the protocol of the National Cancer Institute (NCI) of the USA [49] with minor modifications [41]. Samples for testing were dissolved initially in DMSO at 40 mM, i.e. 400 times the maximum test concentration. For each compound, the cells were exposed to serial decimal dilutions in the range of 0.001–100 μM for a period of 48 h.

4.5. ROS assay

To measure the level of ROS we used the commercially available kit ROS-GloTM H_2O_2 Assay (Promega Corporation, WF, USA). Cells were exposed to compounds at the indicated doses for 48 h, after which time the non-lytic assay was performed following manufacturer's indications. Luminescence was measured on a Synergy HTX multimode microplate reader (BioTek, VT, USA).

4.6. Molecular modeling

Peptidyl-prolyl *cis-trans* isomerase A (PDB ID: 1VBS), mitochondrial U Peptidyl-prolyl *cis-trans* isomerase F (PDB ID: 3R49) and U Histone deacetylase 8 (PDB ID: 5FCW) were downloaded from Protein Data Bank. We used HyperChem software (Hypercube Inc., FL, USA) to draw and optimize the molecular structures and OpenBabel application to convert the files into adequate format before exporting them to docking software Glide v8.2 (Schrödinger, MA, USA). For docking calculations, we removed ligands and non-essential water molecules present in the crystal structure and added polar hydrogen atoms to the protein chain before running any docking calculations. We used "Standard Precision" protocol in Glide with OPLS 2005 forcefield with post-docking minimization.

To assess docking reliability we performed redocking of co-crystallized ligands for all used structures. The RMSDs for the co-crystallized ligand of PPIase was 0.15Å, ligand of PPIF was 0.18Å and for the ligand of histone deacetylase 8 was 0.49Å which ensures that the docking protocol is valid.

4.7. HDAC8 inhibition assay

To study the inhibitory activity of selenocompounds against HDAC8 we used the commercially available kit Histone Deacetylase 8 (HDAC8) Inhibitor Screening Kit (Sigma-Aldrich, MO, USA). Compounds were tested at 10 μM in 96-well plates according to manufacturer's indications. Fluorescence was measured on a FLUOstar Omega microplate reader (BMG Labtech, Germany).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at _____

Declarations of interest: none

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