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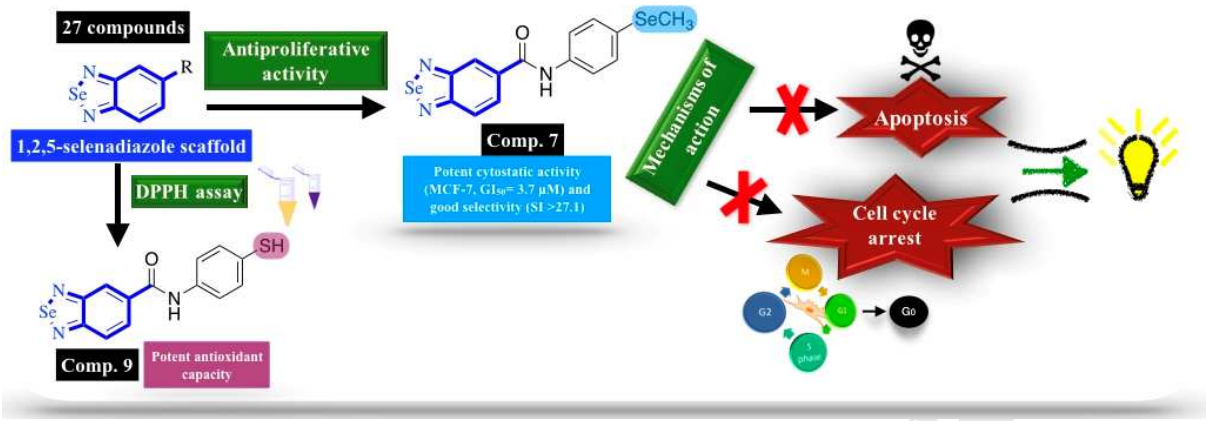


Highlights

- 27 novel BSCA derivatives were designed and synthesized
- Compound **7** potently inhibited cancer cell viability and showed high selectivity.
- Compound **7** neither induced apoptosis nor had effect on cell cycle progression.
- Compound **9** showed significant high radical scavenging activity.

Novel selenadiazole derivatives as selective antitumor and radical scavenging agents

Ana Carolina Ruberte[1,2], Daniel Plano[1,2,3], Ignacio Encío[4], Carlos Aydillo[1,2], Arun K. Sharma[3] and Carmen Sanmartín[1,2*]

[1]Department of Organic and Pharmaceutical Chemistry, University of Navarra, Irunlarrea 1, E-31008 Pamplona, Spain.

[2]Instituto de Investigación Sanitaria de Navarra (IdiSNA), Irunlarrea 3, E-31008 Pamplona, Spain.

[3]Department of Pharmacology; Penn State Cancer Institute, CH72; Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, USA.

[4]Department of Health Sciences, Public University of Navarra, Avda. Barañain s/n, E-31008 Pamplona, Spain.

* Prof. Carmen Sanmartín
Department of Organic and Pharmaceutical Chemistry
University of Navarra
Irunlarrea, 1, E-31008 Pamplona
SPAIN
+34 948 425 600 (Telephone)
+34 948 425 649 (Fax)
e-mail: sanmartin@unav.es

Abstract

Twenty-seven novel benzo[*c*][1,2,5]selenadiazole-5-carboxylic acid (BSCA) derivatives were designed and synthesized. Anti-proliferative activity of these structures was tested *in vitro* against a panel of five human cancer cell lines, including prostate (PC-3), colon (HT-29), leukemia (CCRF-CEM), lung (HTB-54) and breast (MCF-7). Four compounds (**5**, **6**, **7** and **19**) showed potent inhibitory activity with GI_{50} values below 10 µM in at least one of the cancer cell lines. The selectivity of these compounds was further examined in two non-malignant cell lines derived from breast (184B5) and lung (BEAS-2B). Compound **7** exhibited promising anti-proliferative activity (GI_{50} = 3.7 µM) in MCF-7 cells, together with high selectivity index (SI>27.1).

The induction of cell death by compound **7** was independent of the apoptotic process and it did not affect cell cycle progression either. Likewise, radical scavenging properties of the new selenadiazole derivatives were confirmed by testing their ability to scavenge DPPH radicals. Four compounds (**1**, **2**, **8** and **9**) showed potent radical scavenging activity, compound **9** being the most effective. Overall, while compound **7** was identified as the most cell growth inhibitory agent and selectively toxic to cancer cells, compound **9** proved to be the most potent antioxidant among the selenadiazole derivatives synthesized. This series of compounds can serve as an excellent scaffold to achieve new and potent antioxidant compounds useful for several diseases, i.e. cancer, neurodegenerative, heart diseases and leishmaniasis, considering the high radical scavenging activity and low toxicity showed by most of the compounds.

Keywords: Anti-proliferative activity, radical scavenging, selenadiazole, selenium.

1. Introduction

Cancer is a major public health problem worldwide due to its high incidence, morbidity and mortality [1]. According to the American Cancer Society, 1,735,350 new cancer cases are projected to occur in the United States in 2018 [2], the most prevalent being prostate, breast, lung, and colorectal cancers [3].

There are many different types of cancer treatments, including surgery, radiation therapy, and/or systemic therapy (e.g., chemotherapy, immunotherapy). Chemotherapy is an effective treatment against cancer but is often associated with undesired side effects, making the seeking of new chemotherapies a priority. Over the last decade, selenium (Se) compounds have been demonstrated to have inhibitory effects on cancer cell growth and proliferation. Many mechanisms of action have been identified, including the induction of apoptosis, the modulation of the activity of some kinases, antioxidant effects mainly through selenoproteins [4], or a combination of these and other mechanisms [5, 6]. Several chemical entities have been identified: methylseleno derivatives such as methylseleninic acid and selenomethionine, isoselenocyanates, selenosemicarbazones, selenourea scaffolds, selenocyanates and diselenides, or sugar-conjugated Se analogues [7-9] as attractive scaffolds for the development of new anticancer agents.

Recently, fused selenoheteroaryl derivatives have received great attention due to their possible usefulness as therapeutic drugs for cancer treatment. Some representative examples include 2-Phenyl-1,2-benzisoselenazol-3[2*H*]-one (ebselen, EBS) [10], 2,2'-(1,2-Ethanediyl)bis(1,2-benzoselenazol-3[2*H*]-one) (ethaselen, BBSKE) [11] and 1,2,5-selenadiazole derivatives [12] (**Figure 1**). EBS shows high antioxidant capacity through the catalysis of several essential reactions for the protection of cellular components

from oxidative and free radical damage, acting as a mimetic of glutathione peroxidase [13]. Besides, it is noteworthy for its excellent pharmacological profile as well as for its therapeutic safety as demonstrated by outcomes of Phase II clinical trials for prevention of noise-induced hearing loss [14].

On the other hand, BBSKE has demonstrated antioxidant and anti-inflammatory activities, as well as important antitumoral effects in lung [11] and colon cancer models, alone or in combination with cisplatin or sunitinib, through apoptosis induction [15, 16]. Furthermore, it inhibited the proliferation of PC-3 and DU-145 prostate cancer cell lines by cell cycle arrest at the S phase and the induction of apoptosis [17]. In addition, it presents an excellent pharmacological profile, as shown in Phase I clinical trials [18].

Other well documented seleno heteroaryl derivatives are 1,2,5-selenadiazole derivatives (**Figure 1**), that have been identified as novel agents with anti-proliferative effect against human cancer cells through the induction of apoptosis or cell cycle arrest with the involvement of oxidative stress. Among them, 4-(benzo[*c*][1,2,5]selenadiazol-6-yl)-benzene-1,2-diamine (**SD-1** in **Figure 1**) is a potent apoptosis inducer in glioma acting through the mitochondrial pathway [19]. Moreover, these 1,2,5-selenadiazole compounds have shown to be less toxic to non-tumoral cells [12, 20, 21]. There have been many reports related to structural modifications in this chemical scaffold, particularly at position "5". So, Xie *et al*. [22] described novel 5-susbstituted selenadiazoles (**SD-2** in **Figure 1**) as apoptosis inducers in A375 human melanoma cells, with some of them (**SD-3** in **Figure 1**) acting as intracellular redox balance disruptors [20]. In addition, the introduction of simple groups, i.e. 5-methyl or 5-nitro (**SD-4** in **Figure 1**), enhanced the anticancer activity alleviating the toxicity to the main organs [21]. On the other hand, some of these 5-functionalized selenadiazole derivatives (**SD-3** in **Figure 1**) have demonstrated better anti-proliferative activity and higher

stability than the reference drug mitomycin, a clinically used anti-bladder cancer drug [12].

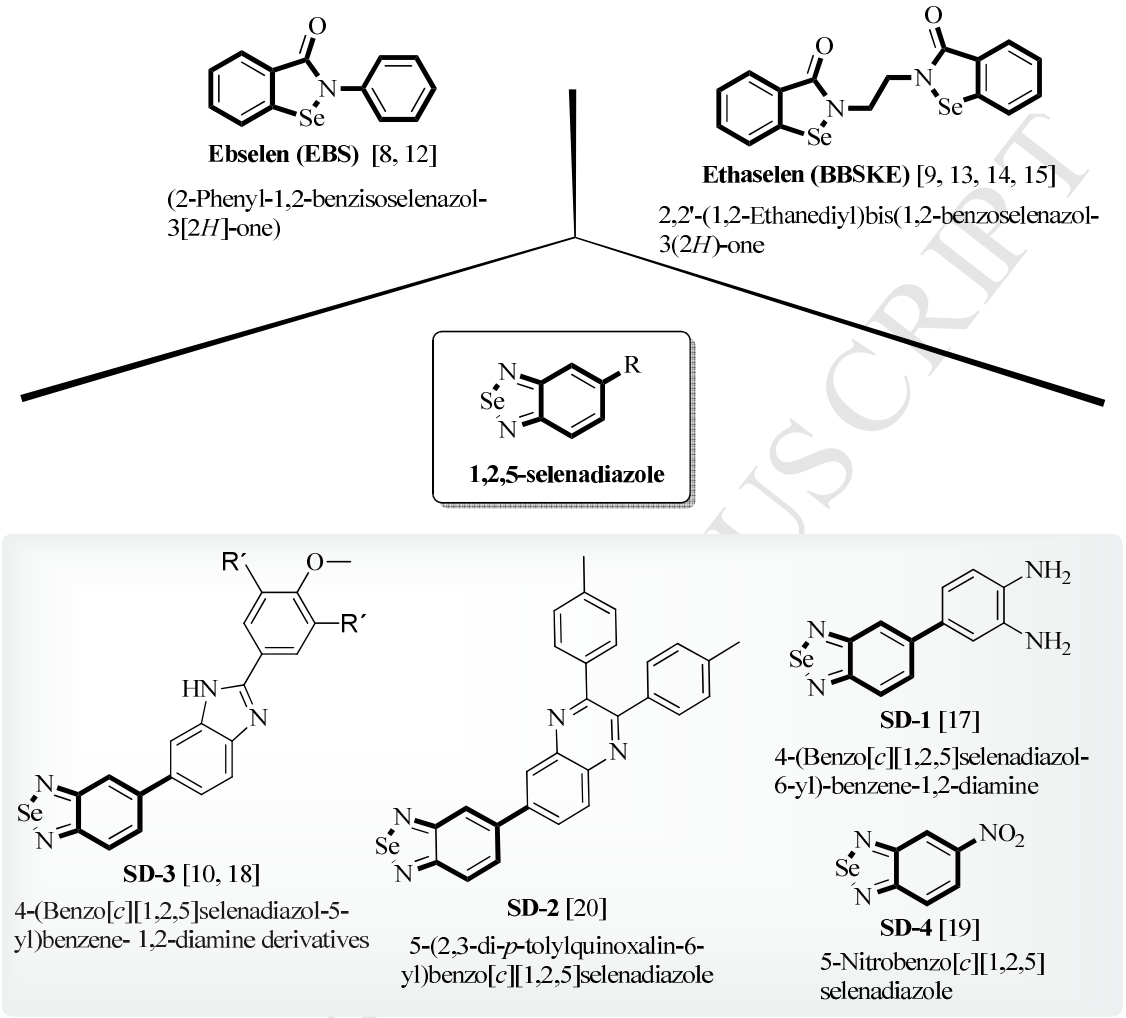


Figure 1. Structures of EBS, BBSKE and 5-substituted 1,2,5-selenadiazole.

Based on the above facts and as a continuation of our previous work [23], we optimized the bioactive entity benzo[*c*][1,2,5]selenadiazol-5-carboxylic acid (BSCA) by manipulating the "5" position with a wide variety of moieties (compounds **1-27**, **Figure 2**) using an amide linkage. The amide linkage was chosen because of it plays a major role in biological systems, including peptides and proteins [24]. Likewise, this functionality is widely used in medicinal chemistry and it is present in numerous anticancer drugs such as flutamide, bicalutamide and sorafenib [25, 26].

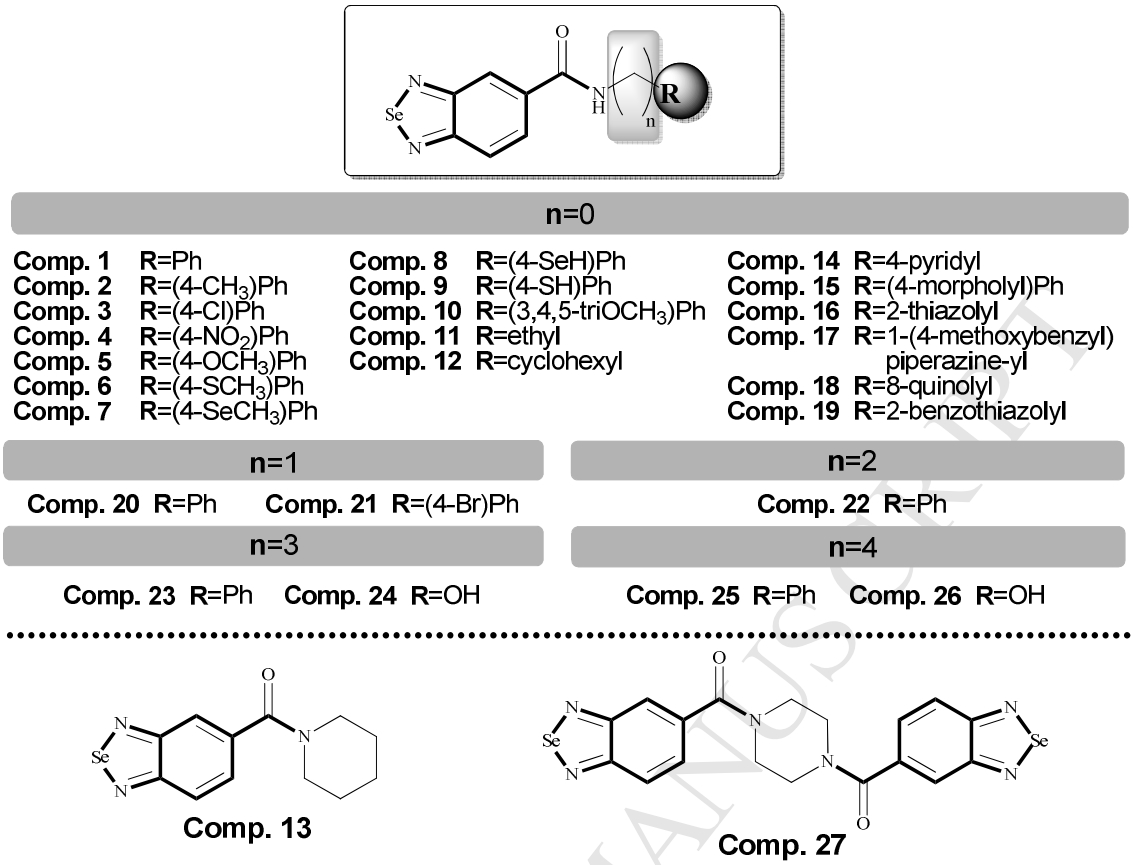


Figure 2. General structure of the novel selenadiazole compounds.

All the synthesized compounds were tested *in vitro* against a panel of five human tumor cell lines and two non-malignant cell lines in order to determine their anti-proliferative activity and their selectivity. Likewise, the capability of the synthesized compounds to interact with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was examined. The most active and selective compound (compound **7**) was further evaluated for its apoptotic effects and cell cycle distribution. The structure of this compound was confirmed by X-ray diffraction.

2. Results and discussion

2.1. Design

The novel BSCA analogs were designed by optimizing the parent scaffold by manipulating the "5" position with a variety of moieties using the following criteria:

a) Several electron-withdrawing or electron-donating groups were substituted in the phenyl ring in order to modulate the electronic distribution. Moreover, several chemical Se forms, with proven antitumoral effects, e. g. selenol [27] and methylseleno [28] were also incorporated in "para" position of the phenyl ring.

b) Modulation of the length of the linker between the amide and R groups, from 0 to 4 methylenes (carbon atoms), with the aim of finding the optimal chain length required for activity.

c) Several heterocycles (mono- and bi-cyclic) with proven cytotoxic effects, such as 2-aminothiazole [29, 30], aminoquinoline [31-33] and 2-aminobenzothiazole [34, 35], which also have antioxidant capacity [36].

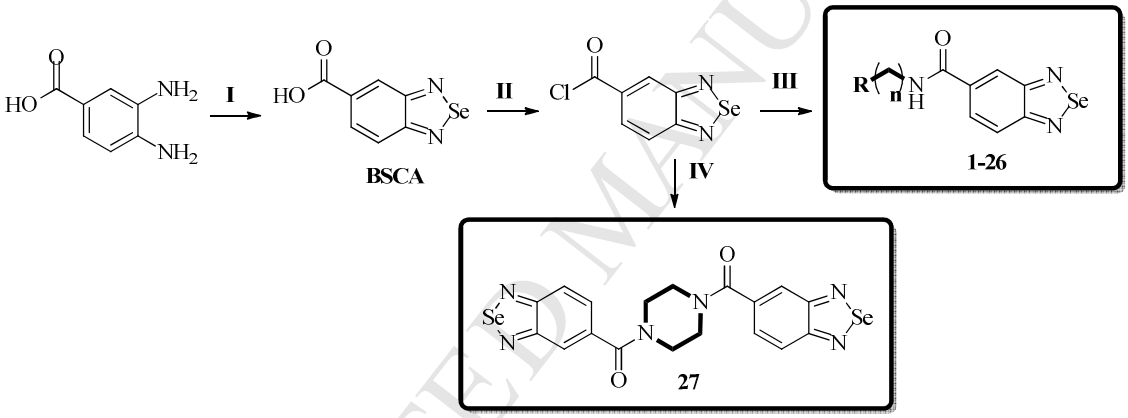
d) Alkyl, cycloalkyl and hydroxyalkyl chains in order to modulate the lipophilicity along with the entropy of the molecules.

e) Finally, a symmetric bi-functionalized amine was used to obtain compound **27**, in order to evaluate whether the presence of a second selenadiazole ring would impact on the antitumor activity.

2.2. Chemistry

The synthetic procedures for the preparation of the targeted compounds (**1-27**) are summarized in **Schemes 1** and **2**. The benzo[*c*][1,2,5]selenadiazole-5-carboxylic acid (BSCA) was obtained as previously reported by us [23]. Chlorination of this acid with thionyl chloride, followed by reaction with the corresponding amines at room

temperature in dry chloroform and 1 equivalent of triethylamine have been used to prepare compounds **1-27**. The starting amines that are not commercially available, such as 4-(methylselanyl)aniline (**7i**) and 4-aminobenzeneselenol (**8i**), were synthesized as shown in **Scheme 2**. The amine **7i** was synthesized by treating a methanolic solution of 4-Aminophenylselenocyanate and sodium hydroxide (0.4 N) with iodomethane in a 1:1.5 molar ratio at room temperature for 40 min. 4-Aminophenylselenocyanate was prepared following a previously described synthetic procedure [23]. The synthetic route for amine **8i** was based on the reaction between 4-bromoaniline and selenourea in a 1:1.1 molar ratio at reflux in absolute ethanol as solvent for 2 h.



Reagents: (**I**) SeO_2; fusion (260 ºC) **(II)** $SOCl_2$; reflux **(III)** $CHCl_3$ / TEA; 1 eq. **R**-$(CH_2)_n$-NH_2 **(IV)** $CHCl_3$ / TEA; 0.5 eq. piperazine.

Scheme 1. General schematic for the synthesis of novel BSCA derivatives (**1-27**).

Reagents: (**I**) NaOH/MeOH; 1.5 eq. CH_3I **(II)** EtOH; 1.1 eq. selenourea

Scheme 2. Schematic for the synthesis of amines **7i** and **8i**.

The structures of all the compounds were confirmed using spectroscopic methods (IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analyses, as described in the Experimental Section.

In general, the IR spectra of the compounds displayed absorption bands around 3395-3093 cm^{-1} originated from the N-H stretching vibration. The strong bands between 1689 and 1620 cm^{-1} corresponded to carbonyl bonds stretching. Inspection of ^{1}H-NMR spectra revealed one sharp peak in the range of 12.96-8.18 ppm due to the presence of the secondary amide proton. The presence of methylene as linker between the amide group and phenyl ring led to a decrease in the chemical shift of this sharp peak from 10.56 ppm (compound **1**) to 9.37, 8.90, 8.82 and 8.78 ppm (compounds **20**, **22**, **23** and **25**, respectively). The proton signals for the heteroaryl ring containing Se appear as a singlet between 8.69 and 5.64 ppm, and two multiplets from 7.36 to 8.72 ppm. The chemical shift of the methyl group attached to the chalcogen elements (oxygen, sulfur or Se) appear at 3.76 (compounds **5**), 2.48 (compound **6**) and 2.34 ppm (compound **7**), respectively. For the methylene linker (-CH_2-) directly attached to oxygen and nitrogen atoms, the proton signals show in the range of 3.51-3.29 ppm, and the rest of protons for other methylene linkers appear at higher fields (1.76-1.48 ppm). Similarly, in the ^{13}C-NMR spectra, the chemical shifts appeared between 122.72 and 37.71 ppm while methylene groups signals were observed in 33.16-26.59 ppm region.

2.3. Biological evaluation

2.3.1. Anti-proliferative activity

All the newly synthesized compounds were screened for their anti-proliferative activity against a panel of five human tumor cell lines: lymphocytic leukemia (CCRF-CEM), lung carcinoma (HTB-54), colon carcinoma (HT-29), prostate adenocarcinoma (PC-3) and breast adenocarcinoma (MCF-7). The *in vitro* inhibition of cell viability was

analyzed by a colorimetric microassay based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to a method previously described [37]. The five cell lines were treated with each compound for 72 h at two concentrations (100 μM and 10 μM). Compound **21** was not tested at 100 μM due to solubility problems. The data are expressed as percentage of cell growth ± SEM at least 3 independent experiments performed in quadruplicates (**Table S1**). Results at 10 μM concentration are summarized in **Figure 3**.

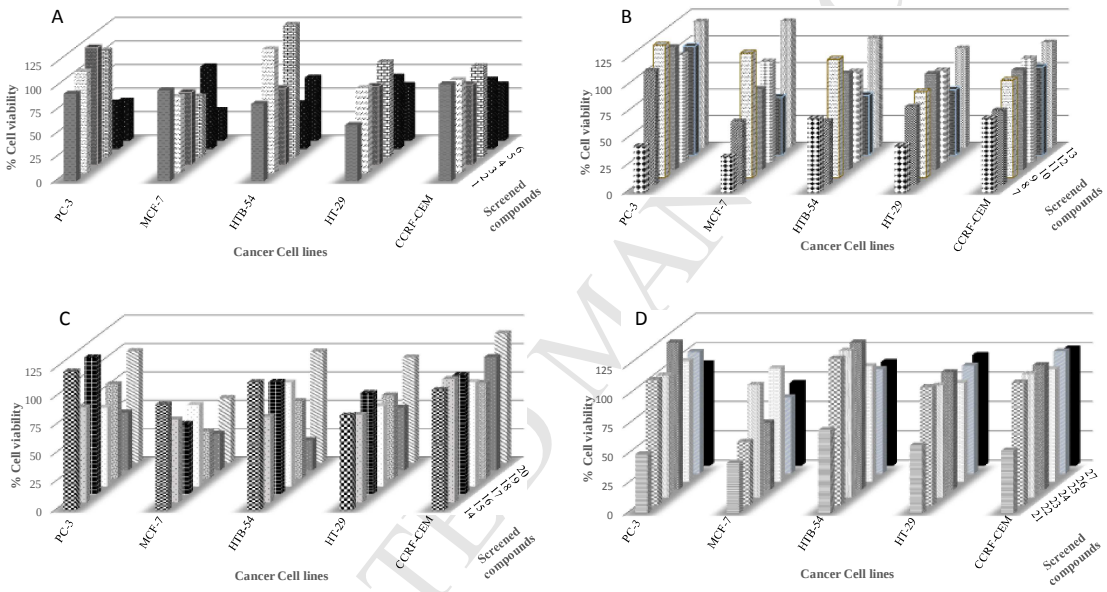


Figure 3. Anti-proliferative effect of synthesized compounds **1 – 6 (A), 7 – 13 (B), 14 – 20 (C)** and **21 – 27 (D)** on five human cancer cell lines after 72 h of treatment with a dose of 10 μM for each compound.

As shown in **Figure 3**, some compounds were active under our experimental conditions, MCF-7 cells being the most sensitive cells. These results allowed us to determine some preliminary structure-activity relationships, since:

a) The length of the linker between the phenyl ring and the amide group seems not to be important for inhibition of cell viability, as no significant

differences were observed with the increase of the linker length (compounds **1**, **20**, **22, 23** and **25**).

b) Among the different substituents on the phenyl ring, methylchalcogen (methoxy, methylthio, methylseleno) groups seemed to be the most active ones (compounds **5**, **6** and **7** respectively). Nevertheless, the presence of three methoxy groups leads to a lower anti-proliferative effect (compound **10**).

c) Great differences in the cell viability can be observed among mono- and bi-cyclic heterocycles with proven cytotoxic effects. Thus, compound **19**, with a benzothiazole substituent, inhibited viability of MCF-7 and HTB-54 cells, while compound **16**, with a thiazole group, showed negligible effect against all tested cell lines.

d) The incorporation of nitrogen atom into a six-member cycle (piperidyl or piperazinyl in compounds **13** and **27**, respectively) in the "5" position of the selenadiazolic core seems to decrease the inhibitory effect of these derivatives as compared to the analog carbocyclic substituent (cyclohexyl in compound **12**).

As shown in **Figure 3,** compounds **5**, **6**, **7** and **19** were found to be the most active, with reduction of the cell growth by >50%, after 72 h of treatment at 10 μM, in at least two cell lines. They were further tested at four different concentrations (1, 10, 50 and 100 μM), in order to establish their dose-response curve. As a guide with regard to selectivity, these compounds were further examined for toxicity in two non-malignant cell lines, one established from normal breast tissue (184B5) and another established from normal bronchial epithelium (BEAS-2B). Results are expressed as GI_{50}, TGI,

LC_{50}, and selectivity index (SI) that was calculated as the ratio of the GI_{50} values determined for the non-malignant and the tumoral cells (GI_{50} (184B5)/ GI_{50} (MCF-7) and GI_{50} (BEAS-2B) / GI_{50} (HTB-54)). The obtained results are shown in **Tables 1** and **2**. EBS and BBSKE were used as reference drugs, given that both are seleno heterocyclic compounds that are currently under clinical trials. BBSKE has been synthesized according to a previously reported synthetic route [38], with minor modifications described in the supplementary material. Furthermore, doxorubicin [39] and etoposide [40, 41] were used as positive controls due to the promising results shown by these compounds *in vitro* and *in vivo* against different tumor types.

Table 1. Anti-proliferative activity (average GI_{50}, TGI and LC_{50} values expressed in µM) of selenadiazoles **5**, **6**, **7** and **19** and EBS, BBSKE, doxorubicin and etoposide.

	Cell lines								
	HT-29			**PC-3**			**CCRF-CEM**		
Comp.	**GI_{50}[a]**	**TGI[b]**	**LC_{50}[c]**	**GI_{50}[a]**	**TGI[b]**	**LC_{50}[c]**	**GI_{50}[a]**	**TGI[b]**	**LC_{50}[c]**
5	40.3	>100	>100	**9.8**	>100	>100	83.9	>100	>100
6	53.4	>100	>100	**7.4**	>100	>100	62.4	>100	>100
7	**8.8**	>100	>100	**8.8**	>100	>100	85.6	>100	>100
19	22.3	>100	>100	10.9	>100	>100	>100	>100	>100
EBS	>100	>100	>100	>100	>100	>100	57.1	73.8	90.5
BBSKE	20.3	33.2	46.0	24.8	39.1	61.0	5.0	8.5	25.4
doxorubicin	0.1	4.0	25.1	$1x10^{-2}$	1.2	6.6	$3x10^{-2}$	$7x10^{-2}$	0.3
etoposide[d]	31.6	>100	>100	0.6	4.0	79.4	1.6	50.5	89.9

[a] GI_{50}: concentration that reduces by 50% the growth of treated cells with respect to untreated controls.
[b] TGI: concentration that completely inhibits the cell growth.
[c] LC_{50}: concentration that kills 50% of the initial cells.
[d] NCI data (http://dtp.nci.nih.gov).
[e] n.d., not determined.

Table 2. Anti-proliferative activity (average GI_{50}, TGI and LC_{50} values expressed in μM) and selectivity (calculated SI values) of selenadiazoles **5**, **6**, **7** and **19** and EBS, BBSKE, doxorubicin and etoposide.

	Cell lines												
	MCF-7			184B5				HTB-54			BEAS-2B			
Comp.	**GI_{50}[a]**	**TGI[b]**	**LC_{50}[c]**	**GI_{50}[a]**	**TGI[b]**	**LC_{50}[c]**	**SI[d]**	**GI_{50}[a]**	**TGI[b]**	**LC_{50}[c]**	**GI_{50}[a]**	**TGI[b]**	**LC_{50}[c]**	**SI[d]**
5	77.1	>100	>100	>100	>100	>100	**>1.3**	18.7	>100	>100	>100	>100	>100	**>5.4**
6	**6.2**	>100	>100	82.2	>100	>100	**13.2**	18.0	>100	>100	96.4	>100	>100	**5.4**
7	**3.7**	86.9	>100	>100	>100	>100	**>27.1**	**6.3**	>100	>100	>100	>100	>100	**>15.9**
19	13.7	72.7	>100	95.6	>100	>100	**7.0**	**6.5**	>100	>100	>100	>100	>100	**>15.4**
EBS	58.8	>100	>100	30.1	>100	>100	**0.51**	68.6	83.7	98.7	49.5	76.9	>100	**0.72**
BBSKE	24.2	40.7	72.8	19.2	31.5	43.8	0.79	19.7	30.3	40.9	20.6	30.3	40.0	**1.04**
doxorubicin	$2x10^{-3}$	0.3	7.8	1.2	4.6	8.1	574	$<1x10^{-2}$	1.3	3.5	0.1	1.5	1.8	**>13**
etoposide[e]	19.9	>100	>100	n.d.[f]	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	

[a] GI_{50}: concentration that reduces by 50% the growth of treated cells with respect to untreated controls.
[b] TGI: concentration that completely inhibits the cell growth.
[c] LC_{50}: concentration that kills 50% of the initial cells.
[d] SI: Selectivity index was calculated as the ratio of the GI_{50} values determined for the non-malignant and the tumoral cells (GI_{50} (184B5)/ GI_{50} (MCF-7) and GI_{50} (BEAS-2B) / GI_{50} (HTB-54)).
[e] NCI data (http://dtp.nci.nih.gov).
[f] n.d.: not determined.

As shown in **Tables 1** and **2**, the selected cancer cell lines present different sensitivity profiles to the action of these derivatives. Thus, three compounds (**6**, **7** and **19**) effectively inhibited cell viability with GI_{50} values ranging from 3.7 μM to 18 μM on solid tumors (MCF-7, HTB-54 and PC-3 cell lines). However, these compounds were inactive against liquid tumors (CCRF-CEM cell line).

Interestingly, these selenadiazoles exhibited greater anti-proliferative activity than EBS in solid tumors, along with higher selectivity indexes (**Tables 1** and **2**). For instance, compounds **6** (GI_{50} = 6.2 μM) and **7** (GI_{50} = 3.7 μM) in MCF-7 cells were 9.5 and 15.9 times more active, respectively, than standard EBS (GI_{50} = 58.8 μM) and they also

showed at least 25.9 and 53.1 times higher selectivity index, respectively. Besides, we have improved significantly the selectivity indexes of BBSKE and the potency of starting compound, BSCA [23].

Moreover, compound **7**, with two Se atoms in the structure and having GI_{50} value of 3.7 μM and SI > 27.1 in MCF-7 cell line, emerged as the most active and selective derivative. Taking into account the effects on the cell viability in the four cancer cell lines tested, together with the selectivity (**Tables 1** and **2**), derivative **7** was selected as the lead compound for further biological evaluation.

2.3.2. Evaluation of cell cycle progression and apoptosis induction

Various scientific pieces of evidence show that Se compounds are potent antitumor agents owing to the induction of apoptosis and the inhibition of cell proliferation [6, 42]. Compound **7** presented notable inhibitory activity in MCF-7 cells. Therefore, we decided to evaluate whether this activity was related to its ability to induce apoptosis and / or its effect on the cell cycle progression in MCF-7 cells. This study was conducted using the Apo Direct™ kit (BD Pharmigen) [43] based on the TUNEL technique with 50 μM of the compound and 72 h of treatment. Camptothecin was employed as a positive control.

As shown in **Figure 4**, the obtained results demonstrated that the inhibition of cell growth was independent both of the apoptotic process and effect on cell cycle progression.

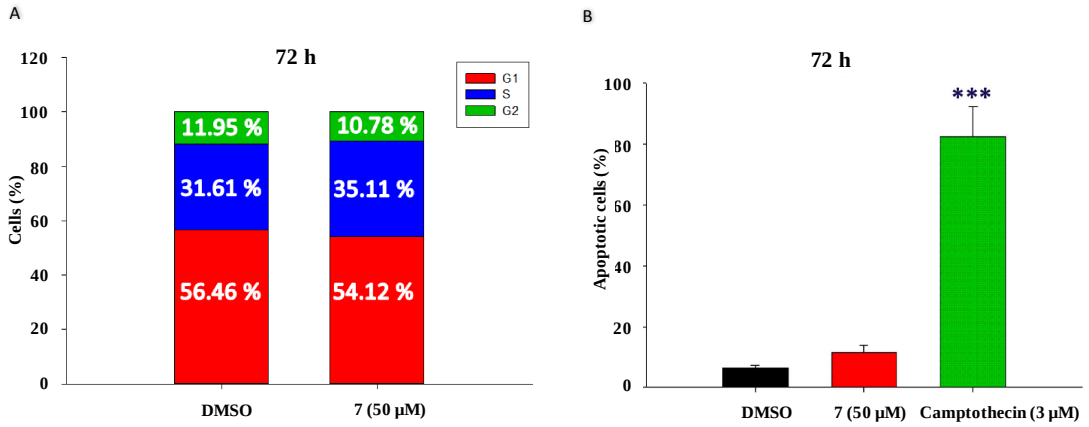


Figure 4. The activity of compound **7** was independent of apoptotic process and it did not modulate cell cycle progression. (A) Bar chart representing the distribution of cells in different phases of the cell cycle. (B) Percentage of apoptotic cells were calculated. Results are expressed as mean ± SD of at least three independent experiments performed in duplicate. *p< 0.05, **p<0.01 and ***p<0.001 with respect the control.

2.4. X-ray crystallography of *N*-(4-(methylselanyl)phenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (compound 7)

The structure of the lead compound **7** was further confirmed by X-ray diffraction after developing a single crystal. Structure and crystal packing of the compound are presented in **Figures 5** and **6**, respectively, and selected interatomic distances and angles for this structure are collected in **Table S2** and **S3** in the Supplementary material. The crystal data and structure refinement are listed in **Table S4**. Hydrogen bonding interactions in the crystal structure are given in **Table S4**.

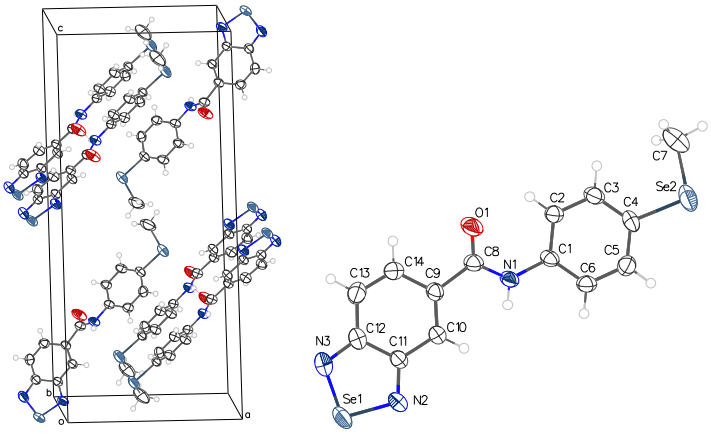


Figure 5. ORTEP diagram of compound **7** with displacement ellipsoids drawn at 50% probability level.

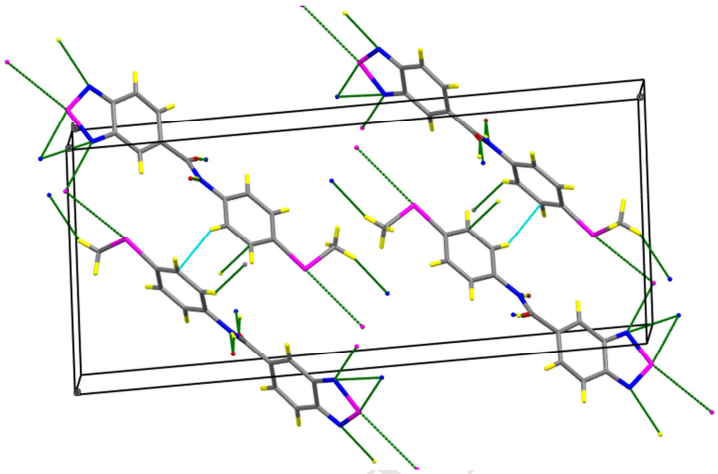


Figure 6. Crystal packing of compound **7**; some hydrogen atoms were omitted for clarity.

2.5. DPPH free radical-scavenging activity.

Se compounds, including EBS, show potential chemopreventive activity, which has been related to their strong antioxidant activity and their ability to interact with redox status of the cell [44]. We decided to evaluate the antioxidant capacity of the selenadiazole derivatives using the DPPH method [45].

Determinations were performed at five different concentrations of all the synthesized compounds ranging from 3.13 x 10^{-5} to 0.25 mg/mL and were recorded at different time

points (30´, 90´, 180´). The ascorbic acid was used as the positive control and the EBS was used as a reference compound.

The results (**Figure 7**) demonstrated that only four compounds (**1**, **2**, **8** and **9**) were able to scavenge the DPPH activity, with values above 20% of inhibition at 0.25 mg/mL. Compound **9** presented DPPH scavenging values greater than 40% at 0.25 mg/mL, displaying greater radical scavenging than EBS. Finally, as shown in **Figure 7**, these compounds exhibited concentration-dependent but not time-dependent radical scavenging activity *in vitro*.

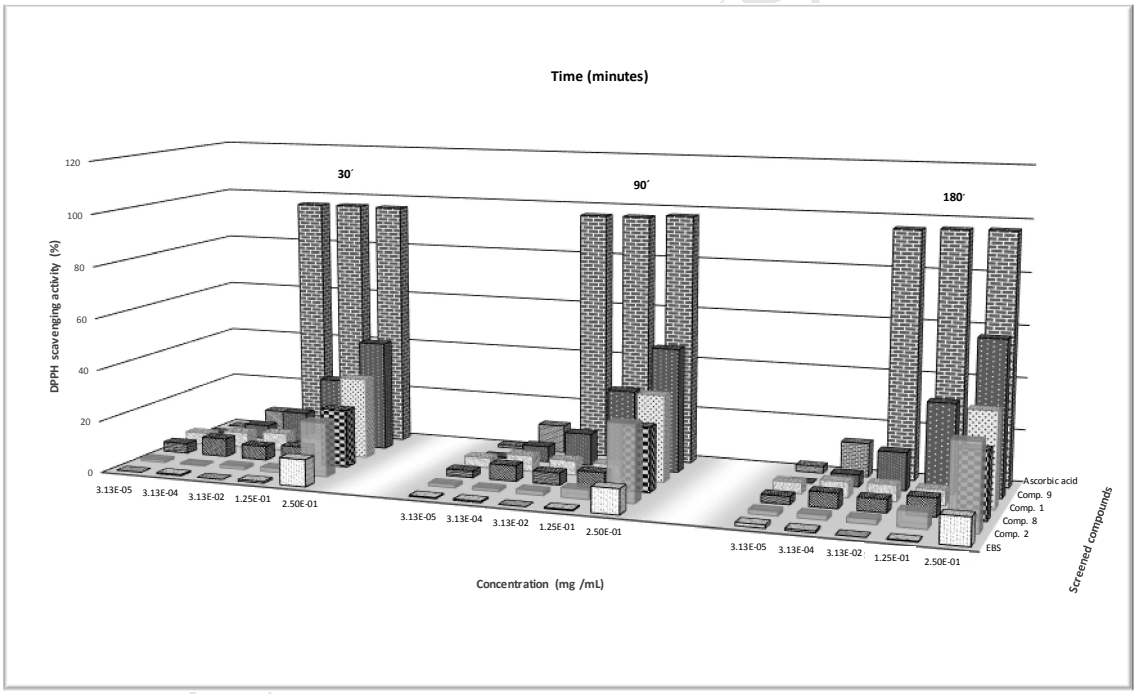


Figure 7. Analysis of DPPH radical scavenging activity for compounds **1**, **2**, **8** and **9** at different concentrations and different time points.

3. Conclusion

In this study a series of twenty-seven selenadiazole derivatives were synthesized and their anti-proliferative effect against five human tumor cell lines by standard MTT assay, and antioxidant activity using DPPH test were evaluated. Results demonstrated

that "5" position is a favorable site to carry out modifications, given that many analogs formed by decorating several substituent over this position through an amide linkage displayed better anti-proliferative effects than the parent structure (BSCA). Compounds **5**, **6**, **7** and **19** were the most potent in inhibiting viability of at least one cancer cell line with GI_{50} values ranging from 3.7 to 9.8 µM. These activities were 10-fold more potent than the reference EBS and the selectivity indexes were greater than EBS and BBSKE. Furthermore, the radical scavenging capacity of **1**, **2**, **8** and **9** was greater than EBS.

Taking together all the results, compound **7** emerged as the most promising and suggest the importance of methylphenylselane group for BSCA scaffold. It is interesting to note that contrary to the parent compound (BSCA) and EBS, compound **7** induced cell growth inhibition independent of the apoptotic process and without affecting the cell cycle progression. This behavior is quite unique and warrants further investigation of the in-depth mechanism by which this compound is potently exerting inhibition of viability of breast cancer cells while sparing the non-tumoral 184B5 cells. It is clear that compound **7**, with GI_{50} greater than 100 µM in normal cells and promising anti-proliferative activity in MCF-7 cells (GI_{50} = 3.7 µM) is highly promising as a lead for developing effective new chemotherapeutics, particularly for breast cancer.

Compound **9** on the other hand having better radical scavenging activity than EBS may prove to be potent antioxidant. These data suggest that appropriate manipulation of EBS structure can lead to a set of promising small drug-like anti-cancer molecules with diverse mechanisms of action. Given the greater selectivity and moderate antioxidant activity, these compounds can serve as excellent scaffolds to achieve new and potent antioxidant compounds useful for several diseases such as cancer, neurodegenerative, heart and leishmaniasis diseases.

4. Experimental

4.1. Chemistry

4.1.1. Material and methods

Melting points (mp) were determined with a Mettler FP82 + FP80 apparatus (Greifensee, Switzerland). The proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker 400 Ultrashield™ spectrometer (Rheinstetten, Germany) using DMSO-d_6 as solvent. The IR spectra were obtained on a Thermo Nicolet FT- IR Nexus spectrophotometer with KBr pellets. Purity of all final compounds was 95% or higher and was dwtermined by elemental microanalyses carried out on vacuum-dried samples using a LECO CHN-900 Elemental Analyzer. Furthermore, Column Chromatography using Silica gel 60 (0.040 - 0.063 mm) (Merck KGaA, Darmstadt, Germany) and Thin Layer Chromatography (TLC) assays were carried out in Alugram® SIL G/UV$_{254}$ sheets (Layer: 0.2 mm) (Macherey-Nagel, Düren, Germany). Chemicals were purchased from E. Merck (Darmstadt, Germany), 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).

4.1.2. *Benzo[c][1,2,5]selenadiazole-5-carboxylic acid* (**BSCA**)

This compound was prepared according to a previously published procedure [23]. Briefly, a mixture of 3,4-diaminobenzoic acid and SeO_2 was heated at 260 ºC. The crude product was washed with water and recrystallized from dioxane.

4.1.3. General procedure for the synthesis of compounds (**1-26**)

A mixture of BSCA (0.5 g, 2 mmol) and thionyl chloride (20 mL) was stirred at reflux for 2 h. The reaction was monitored by IR spectroscopy. Formation of the acyl chloride was confirmed following the wavenumber position in infrared spectroscopy peaks: carbonyl group showed up around: 1682 cm^{-1} and the simple bond of OH group was detected around 3448 cm^{-1} in the starting acid whereas the carbonyl in the acyl chloride arose around 1750 cm^{-1}. The resulting acyl chloride was isolated by rotatory evaporation of the thionyl chloride under vacuum and the excess of thionyl chloride was removed with 3 fractions of toluene (40 mL). The resulting acyl chloride was used without further purification. A solution of the corresponding amine (2 mmol) in dry chloroform (15 mL) was added to a mixture of acyl chloride (0.5 g, 2 mmol) and triethylamine (0.28 mL, 2 mmol) in dry chloroform (40 mL). The mixture was stirred at room temperature for 12-48 h. The product was isolated by filtration or by rotatory evaporation of the solvent under vacuum and washed with water (3 x 50 mL). Final product was purified by washing, recrystallization, or column chromatography.

4.1.3.1. *N-Phenylbenzo[c][1,2,5]selenadiazole-5-carboxamide* (**1**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and aniline. The resulting product, *N*-phenylbenzo[*c*][1,2,5]selenadiazole-5-carboxamide (**1**), did not require further purification. A brown powder was obtained. Yield: 40 %; mp: 245-246 ºC. IR (KBr): $\tilde{\nu}$ 3276 (N-H); 1650 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.56 (s, 1H, N**H**), 8.52 (s, 1H, $\mathbf{H_4}$), 8.01 (dd, $J_{6\text{-}7}$ = 9.3; $J_{6\text{-}4}$ = 1.7 Hz, 1H, $\mathbf{H_6}$), 7.96 (dd, $J_{7\text{-}4}$ = 0.7, 1H, $\mathbf{H_7}$), 7.82 (dd, 2H, $J_{2'\text{-}3'} = J_{6'\text{-}5'}$ = 8.5; $J_{2'\text{-}4'} = J_{6'\text{-}4'}$ = 0.9 Hz, $\mathbf{H_{2'}}$ + $\mathbf{H_{6'}}$), 7.41 - 7.36 (dd, $J_{3'\text{-}4'} = J_{5'\text{-}4'}$ = 7.6 Hz, 2H, $\mathbf{H_{3'}}$+ $\mathbf{H_{5'}}$), 7.14 (tt, 1H, $\mathbf{H_{4'}}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.6 (**C**=O), 161.2 + 160.0 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 139.8 + 135.9 ($\mathbf{C_5}$ + $\mathbf{C_{1'}}$), 129.6 + 128.9 ($\mathbf{C_{3'}}$ + $\mathbf{C_6}$ +$\mathbf{C_{5'}}$), 124.9 + 124.0 + 123.5 + 121.3 ($\mathbf{C_4}$ + $\mathbf{C_7}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{4'}}$ + $\mathbf{C_{6'}}$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1561.1. Elemental analysis calculated

(%) for $C_{13}H_9N_3SeO \cdot 1/2\ H_2O$: C: 50.17, H: 3.22, N: 13.51; found: C: 50.27, H: 3.34, N: 13.41.

4.1.3.2. *N-(p-Tolyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (***2***)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and *p*-toluidine. The resulting product, *N*-(*p*-tolyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**2**), did not require further purification. A brown powder was obtained. Yield: 81 %; mp: 237-238.5 ºC. IR (KBr): $\tilde{\nu}$ 3284 (N-H), 1645 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.49 (s, 1H, N**H**), 8.51 (s, 1H, $\mathbf{H_4}$), 8.01 (dd, $J_{6\text{-}7}$ = 9.3; $J_{6\text{-}4}$ = 1.7 Hz, 1H, $\mathbf{H_6}$), 7.95 (dd, $J_{7\text{-}4}$ = 0.5 Hz, 1H, $\mathbf{H_7}$), 7.70 (d, $J_{3'\text{-}2'} = J_{5'\text{-}6'}$ = 8.4 Hz, 2H, $\mathbf{H_{3'}} + \mathbf{H_{5'}}$), 7.18 (d, 2H, $\mathbf{H_{2'}} + \mathbf{H_{6'}}$), 2.29 (s, 3H, -C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.4 (**C**=O), 161.2 + 160.0 ($\mathbf{C_3} + \mathbf{C_8}$), 137.3 + 136.0 + 133.8 ($\mathbf{C_{1'}} + \mathbf{C_{4'}} + \mathbf{C_5}$), 129.9 + 128.9 ($\mathbf{C_{3'}} + \mathbf{C_{5'}} + \mathbf{C_6}$), 123.9 +123.4 + 121.3 ($\mathbf{C_4}$+ $\mathbf{C_7}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{6'}}$), 21.4 (-**C**H_3). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1560.5. Elemental analysis calculated (%) for $C_{14}H_{11}N_3OSe \cdot 1/2H_2O$: C: 51.70, H: 3.69, N: 12.92; found: C: 52.14, H: 3.98, N: 12.94.

4.1.3.3. *N-(4-Chlorophenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**3**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-chloroaniline. The resulting product, *N*-(4-chlorophenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**3**), did not require further purification. A brown powder was obtained. Yield: 63 %; mp: 233-235 ºC. IR (KBr): $\tilde{\nu}$ 3273 (N-H), 1648 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.69 (s, 1H, N**H**), 8.53 (s, 1H, $\mathbf{H_4}$), 7.99 (dd, $J_{6\text{-}7}$ = 9.3; $J_{6\text{-}4}$ = 1.7 Hz, 1H, $\mathbf{H_6}$), 7.97 (dd, $J_{7\text{-}4}$ = 0.7 Hz, 1H, $\mathbf{H_7}$), 7.86 (d, $J_{2'\text{-}3'} = J_{6'\text{-}5'}$ = 8.8 Hz, 2H, $\mathbf{H_{2'}} + \mathbf{H_{6'}}$), 7.45 (d, 2H, $\mathbf{H_{3'}} + \mathbf{H_{5'}}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7 (**C**=O), 161.2 + 160.0 ($\mathbf{C_3} + \mathbf{C_8}$), 138.8 + 135.6 ($\mathbf{C_{1'}} + \mathbf{C_{4'}}$), 129.5 + 128.7 + 128.4 ($\mathbf{C_5} + \mathbf{C_6} + \mathbf{C_{3'}} + \mathbf{C_{5'}}$), 124.0 +123.6 +122.8 ($\mathbf{C_4} + \mathbf{C_7} + \mathbf{C_{2'}} + \mathbf{C_{6'}}$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1562.2.

Elemental analysis calculated (%) for $C_{12}H_8ClN_3OSe$: C: 46.37, H: 2.38, N: 12.48; found: C: 46.07, H: 2.74, N: 12.45.

4.1.3.4. *N-(4-Nitrophenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**4**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-nitroaniline. The product was purified by recrystallization from ethanol. A yellow powder was obtained. Yield: 9 %; mp: 245-256 ºC. IR (KBr): $\tilde{\nu}$ 3395 (N-H), 1665 (C=O), 1334 cm^{-1} (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.08 (s, 1H, N**H**), 8.57 (s, 1H, $\mathbf{H_4}$), 8.29 (d, $J_{3'-2'} = J_{5'-6'}$ = 9.1 Hz, 2H, $\mathbf{H_{3'}} + \mathbf{H_{5'}}$), 8.09 (d, 2H, $\mathbf{H_{2'}} + \mathbf{H_{6'}}$), 8.00 (dd, J_{6-7} = 9.4; J_{6-4} = 1.08 Hz, 1H, $\mathbf{H_6}$), 7.97 (d, 1H, $\mathbf{H_7}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.3 (**C**=O), 161.2 + 159.8 ($\mathbf{C_3} + \mathbf{C_8}$), 146.0 + 143.5 ($\mathbf{C_{4'}} + \mathbf{C_{1'}}$), 135.0 + 128.6 ($\mathbf{C_5} + \mathbf{C_6}$), 125.6 + 124.1 +120.8 ($\mathbf{C_4} + \mathbf{C_7} + \mathbf{C_{2'}} + \mathbf{C_{3'}} + \mathbf{C_{5'}} + \mathbf{C_{6'}}$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1565.2. Elemental analysis calculated (%) for $C_{13}H_8N_4O_3Se \cdot H_2O$: C: 42.74, H: 2.74, N: 15.34; found: C: 42.30, H: 3.06, N: 14.90.

4.1.3.5. *N-(4-Methoxyphenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**5**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and *p*-anisidine. The resulting product *N*-(4-methoxyphenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**5**), did not require further purification. A yellow powder was obtained. Yield: 68 %; mp: 233-234 ºC. IR (KBr): $\tilde{\nu}$ 3284 (N-H), 1640 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.44 (s, 1H, N**H**), 8.50 (s, 1H, $\mathbf{H_4}$), 8.01 (d, J_{6-7} = 9.3 Hz, 1H, $\mathbf{H_6}$), 7.95 (d, 1H, $\mathbf{H_7}$), 7.72 (d, $J_{2'-3'} = J_{6'-5'}$ = 8 Hz, 2H, $\mathbf{H_{2'}} + \mathbf{H_{6'}}$), 6.96 (d, 2H, $\mathbf{H_{3'}} + \mathbf{H_{5'}}$), 3.76 (s, 3H, O-C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.1 (**C**=O), 161.2 + 160.1 ($\mathbf{C_3} + \mathbf{C_8}$),156.6 ($\mathbf{C_{4'}}$), 136.0 + 132.9 ($\mathbf{C_5} + \mathbf{C_{1'}}$), 128.9 ($\mathbf{C_6}$), 123.9 + 123.3 + 122.9 ($\mathbf{C_4} + \mathbf{C_7} + \mathbf{C_{2'}} + \mathbf{C_{6'}}$), 114.7 ($\mathbf{C_{3'}} + \mathbf{C_{5'}}$), 56.1 (O-**C**$H_3$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1560.0. Elemental analysis calculated (%) for $C_{14}H_{11}N_3O_2Se \cdot 1/2H_2O$: C: 48.01, H:

3.70, N: 12.00; found: C: 48.09, H: 3.63, N: 11.74.

4.1.3.6. *N-(4-(Methylthio)phenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**6**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-(methylthio)aniline. The product was purified by recrystallization from ethanol. A yellow powder was obtained. Yield: 21 %; mp: 260-261 ºC. IR (KBr): $\tilde{\nu}$ 3093 (N-H), 1647 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.55 (s, 1H, N**H**), 8.51 (s, 1H, **H$_4$**), 8.00 (d, $J_{6\text{-}7}$ = 8 Hz, 1H, **H$_6$**), 7.95 (d, 1H, **H$_7$**), 7.78 (d, $J_{2'\text{-}3'} = J_{6'\text{-}5'}$ = 8 Hz, 2H, H$_{2'}$ + **H$_{6'}$**), 7.30 (d, 2H, **H$_{3'}$** + **H$_{5'}$**), 2.48 (s, 3H, S-C**H$_3$**). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.4 (**C**=O), 161.2 + 160.0 (**C$_3$** + **C$_8$**), 137.2 + 135.7 + 133.6 (**C$_5$** + **C$_{1'}$** + **C$_{4'}$**), 128.8 + 127.7 (**C$_6$** + **C$_{3'}$** + **C$_{5'}$**), 124.0 + 123.5 + 121.9 (**C$_4$** + **C$_7$** + **C$_{2'}$** + **C$_{6'}$**), 16.2 (S-**C**H$_3$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1561.3. Elemental analysis calculated (%) for $C_{14}H_{11}N_3O_2SSe$: C: 48.27, H: 3.16, N: 12.07; found: C: 47.77, H: 3.64, N: 11.85.

4.1.3.7. *4-(Methylselenyl)aniline* (**7i**)

1.5 mmol of the iodomethane was added to a methanolic solution of 1 mmol of 4-Aminophenylselenocyanate and 43 mL of sodium hydroxide (0.4 N) and stirred at room temperature for 40 min. The reaction medium was then poured into water and the product was isolated by filtration and extraction with dichloromethane (3 x 50 mL). The product was purified by column chromatography on silica gel using dichloromethane as eluent. 4-Aminophenylselenocyanate was prepared following the synthetic procedure previously described [23]. Yield: 22 %. IR (KBr): $\tilde{\nu}$ 3447-3355 (N-H$_2$), 3213 (=C-H), 2924 (Se-CH$_3$), 1619-1492 cm^{-1} (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.32 (d, $J_{3\text{-}2} = J_{5\text{-}6}$ = 8.0 Hz, 2H, **H$_3$** + **H$_5$**), 6.63 (d, 2H, **H$_2$** + **H$_6$**), 2.29 (s, 3H, Se-**C**H$_3$).

4.1.3.8. *N-(4-(Methylselanyl)phenyl)benzo[c][1,2,5]selenadiazole-5-*

carboxamide (**7**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-(methylselenyl)aniline (**7i**). The crude product was purified by washing with chloroform (3 x 15 mL). A yellow powder was obtained. Yield: 38 %; mp: 258-260 ºC. IR (KBr): $\tilde{\nu}$ 3296 (N-H), 1647 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.56 (s, 1H, N**H**), 8.51 (s, 1H, $\mathbf{H_4}$), 8.00 (d, $J_{6\text{-}7}$ = 8.9 Hz, 1H, $\mathbf{H_6}$), 7.94 (d, 1H, $\mathbf{H_7}$), 7.76 (d, $J_{2'\text{-}3'} = J_{6'\text{-}5'}$ = 7.6 Hz, 2H, $\mathbf{H_{2'}}$ + $\mathbf{H_{6'}}$), 7.42 (d, 2H, $\mathbf{H_{3'}}$ + $\mathbf{H_{5'}}$), 2.34 (s, 3H, Se-C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.9 (**C**=O), 161.2 + 160.0 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 138.1 + 135.7 ($\mathbf{C_5}$ + $\mathbf{C_{1'}}$), 131.1 + 128.8 + 126.8 ($\mathbf{C_6}$ + $\mathbf{C_{3'}}$ + $\mathbf{C_{4'}}$ + $\mathbf{C_{5'}}$), 124.0 + 123.5 + 122.0 ($\mathbf{C_4}$ + $\mathbf{C_7}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{6'}}$), 7.8 (Se-**C**H_3). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1561.5, 189.3. Elemental analysis calculated (%) for $C_{14}H_{11}N_3O_2Se_2 \cdot 1/2HCl$: C: 40.65, H: 2.66, N: 10.16; found: C: 41.06, H: 3.09, N: 10.45.

4.1.3.9. *4-Aminobenzeneselenol* (**8i**)

12 mmol of the 4-bromoaniline was added to a solution of 13.2 mmol of selenourea in 15 mL of absolute ethanol and stirred at reflux for 2 h. The product was isolated by filtration and washed with water (50 mL). The resulting product, 4-aminobenzeneselenol (**8i**), did not require further purification. Yield: 20 %. IR (KBr): $\tilde{\nu}$ 3428-3322 (N-H_2), 3232 (=CH-), 2681 (SeH), 1618-1486 cm^{-1} (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.11 (d, $J_{3\text{-}2} = J_{5\text{-}6}$ = 8 Hz, 2H, $\mathbf{H_3}$ + $\mathbf{H_5}$), 6.50 (d, 2H, $\mathbf{H_2}$ + $\mathbf{H_6}$), 5.22 (s, 2H, -N$\mathbf{H_2}$), 3.85 (s, 1H, -Se**H**). Elemental analysis calculated (%) for $C_6H_7NSe \cdot 1/2H_2O$: C: 39.79, H: 4.42, N: 7.74; found: C: 40.81, H: 3.89, N: 7.94.

4.1.3.10. *N-(4-Hydroselenophenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**8**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-aminobenzeneselenol (**8i**).

The resulting product, *N*-(4-hydroselenophenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**8**), did not require further purification. A brown powder was obtained. Yield: 80 %; mp: 253.4-255.8 ºC. IR (KBr): $\tilde{\nu}$ 3273 (N-H), 1647 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.66 (s, 1H, N**H**), 8.52 (s, 1H, **H$_4$**), 7.99 (dd, J_{6-7} = 9.3; J_{6-4} = 1.5 Hz, 1H, **H$_6$**), 7.97 (dd, J_{7-6} = 0.7 Hz, 1H, **H$_7$**), 7.81 (d, $J_{2'-3'} = J_{6'-5'}$ = 8 Hz, 2H, **H$_{2'}$** + **H$_{6'}$**), 7.57 (d, 2H, **H$_{3'}$** + **H$_{5'}$**). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7 (**C**=O), 161.2 + 160.0 (**C$_3$** + **C$_8$**), 139.2 +135.6 (**C$_{1'}$** + **C$_5$**), 132.4 + 128.7 (**C$_6$** + **C$_{3'}$** + **C$_{5'}$**), 124.0 +123.6 + 123.1 + 116.5 (**C$_7$** + **C$_4$** + **C$_{2'}$** + **C$_{4'}$** + **C$_{6'}$**). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1562.4. Elemental analysis calculated (%) for $C_{13}H_9N_3OSe_2 \cdot 1/2H_2O$: C: 40.01, H: 2.56, N: 10.77; found: C: 39.82, H: 2.29, N: 10.97.

4.1.3.11. *N-(p-Mercaptophenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**9**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-aminothiophenol. The resulting product, *N*-(*p*-mercaptophenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**9**), did not require further purification. An orange powder was obtained. Yield: 51 %; mp: 189.0-188.6 ºC. IR (KBr): $\tilde{\nu}$ 3399 (NH), 1654 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.49 (s, 1H, **H$_4$**), 7.99 (d, J_{7-6} = 9.3 Hz, 1H, **H$_7$**), 7.91 (dd, J_{6-4} = 1.7 Hz, 1H, **H$_6$**), 7.15 (d, $J_{2'-3'} = J_{6'-5'}$ = 8.5 Hz, 2H, **H$_{2'}$** + **H$_{6'}$**), 6.66 (d, 2H, **H$_{3'}$** + **H$_{5'}$**), 5.64 (s, 1H, S**H**). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 191.9 (**C**=O), 161.6 + 159.7 (**C$_3$** + **C$_8$**), 151.5 (**C$_{1'}$**) 137.1 + 136.9 (**C$_{3'}$** +**C$_{4'}$** + **C$_{5'}$**), 126.6 + 125.0 + 124.0 (**C$_5$** + **C$_6$** + C$_7$) 115.3 + 110.1 (**C$_4$** + **C$_{2'}$** + **C$_{6'}$**). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1573.7. Elemental analysis calculated (%) for $C_{13}H_9N_3OSSe$: C: 46.71, H: 2.71, N: 12.57; found: C: 46.51, H: 3.07, N:12.40.

4.1.3.12. *N-(3,4,5-Trimethoxyphenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**10**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 3,4,5-trimethoxianiline. The resulting product, *N*-(3,4,5-trimethoxyphenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**10**), did not require further purification. A yellow powder was obtained. Yield: 50 %; mp: 207.2-208.3 ºC. IR (KBr): $\tilde{\nu}$ 3272 (N-H), 1651 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.45 (s, 1H, N**H**), 8.53 (s, 1H, $\mathbf{H_4}$), 8.02 (d, $J_{6\text{-}7}$ = 9.3 Hz, 1H, $\mathbf{H_6}$), 7.95 (d, 1H, $\mathbf{H_7}$), 7.28 (s, 2H, $\mathbf{H_{2'}}$ + $\mathbf{H_{6'}}$), 3.80 (s, 6H, 2OC$\mathbf{H_3}$), 3.66 (s, 3H, OC$\mathbf{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.3 (**C**=O), 161.2 + 160.0 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 153.5 ($\mathbf{C_{3'}}$ + $\mathbf{C_{5'}}$), 136.0 + 135.8 + 134.8 + 128.7 ($\mathbf{C_5}$ + $\mathbf{C_6}$ + $\mathbf{C_{1'}}$ + $\mathbf{C_{4'}}$), 124.0 + 123.4 ($\mathbf{C_7}$ + $\mathbf{C_4}$), 99.0 ($\mathbf{C_{2'}}$ + $\mathbf{C_{6'}}$), 61.0 (O**C**H_3), 56.6 (2O**C**H_3). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1561.6. Elemental analysis calculated (%) for $C_{16}H_{15}N_3O_4Se \cdot 1/2H_2O$: C: 47.89, H: 3.99, N: 10.47; found: C: 47.25, H: 4.40, N: 10.15.

4.1.3.13. *N-Ethylbenzo[c][1,2,5]selenadiazole-5-carboxamide* (**11**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and ethylamine solution. The mixture was filtered and the product was isolated by rotatory evaporation of the solvent and purified by successive washing with water (50 mL) and hexane (15 mL). A brown powder was obtained. Yield: 5 %; mp: 161-164 ºC. IR (KBr): $\tilde{\nu}$ 3282 (N-H), 2982 (C sp^2), 1637 cm^{-1} (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.18 (s, 1H, $\mathbf{H_4}$), 7.92 (dd, $J_{6\text{-}7}$ = 9.3; $J_{6\text{-}4}$ = 1.2 Hz, 1H, $\mathbf{H_6}$), 7.90 (d, 1H, $\mathbf{H_7}$), 6.34 (s, 1H, N**H**), 3.62-3.53 (m, 2H, -C$\mathbf{H_2}$-), 1.32 (t, $J_{CH3\text{-}CH2}$= 7.3 Hz, 3H, -C$\mathbf{H_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.0 (**C**=O), 161.1 + 160.2 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 135.6 + 128.7 ($\mathbf{C_5}$ + $\mathbf{C_6}$), 123.8 +122.7 ($\mathbf{C_4}$ + $\mathbf{C_7}$), 35.2 (-**C**H_2-), 15.5 (-**C**H_3). Elemental "analysis calculated (%) for $C_9H_9N_3OSe \cdot 1/2H_2O$: C: 41.04, H: 3.80, N: 15.96; found: C: 41.50, H: 3.76, N: 15.23.

4.1.3.14. *N-Cyclohexylbenzo[c][1,2,5]selenadiazole-5-carboxamide* (**12**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and cyclohexylamine. The resulting

product, *N*-cyclohexylbenzo[*c*][1,2,5]selenadiazole-5-carboxamide (**12**), did not require further purification. A white powder was obtained. Yield: 63 %; mp: 242.6-246.4 ºC. IR (KBr): $\tilde{\nu}$ 3303 (N-H), 2934-2854 (cyclohexane, C sp^2), 1631 cm^{-1} (C=O). ^{1}H NMR (400 MHz, TFA) δ (ppm): 11.00 (s, 1H, N**H**), 7.79 (s, 1H, $\mathbf{H_4}$), 7.41 (bs, 2H, $\mathbf{H_6}$ + $\mathbf{H_7}$), 3.42-3.41 (bs, 1H, $\mathbf{H_{1'}}$), 1.55-1.37 (bs, 2H, $\mathbf{H_{2a'}}$ + $\mathbf{H_{2b'}}$), 1.22-1.21 (bs, 2H, $\mathbf{H_{6a'}}$ + $\mathbf{H_{6b'}}$), 1.14-1.00 (bs, 1H, $\mathbf{H_{4a'}}$), 0.89-0.81 (bs, 4H, $\mathbf{H_{3a'}}$ +$\mathbf{H_{3b'}}$ +$\mathbf{H_{5a'}}$ +$\mathbf{H_{5b'}}$), 0.65-0.61 (bs, 1H, $\mathbf{H_{4b'}}$). Elemental analysis calculated (%) for $C_{13}H_{14}N_3OSe \cdot 1/2H_2O$: C: 49.37, H: 4.75, N: 13.29; found: C: 49.92, H: 5.19, N: 13.27.

4.1.3.15. *N-(Piperidin-1-yl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**13**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and piperidine. The resulting product, *N*-(piperidin-1-yl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**13**), did not require further purification. A brown powder was obtained. Yield: 56 %; mp: 103.3-101.9 ºC. IR (KBr): $\tilde{\nu}$ 2935-2855 (C sp^2), 1621 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.90 (d, $J_{7\text{-}6}$ = 9.2 Hz, 1H, $\mathbf{H_7}$), 7.82 (s, 1H, $\mathbf{H_4}$), 7.49 (dd, $J_{6\text{-}4}$=1.5 Hz, 1H, $\mathbf{H_6}$), 3.61 (bs, 2H, 2$\mathbf{H_{1'}}$), 3.33 (bs, 2H, 2$\mathbf{H_{5'}}$), 1.47 + 1.60 (bs+ bs, 6H, 2$\mathbf{H_{2'}}$ + 2$\mathbf{H_{3'}}$ + 2$\mathbf{H_{4'}}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3 (**C**=O), 160.2 + 159.8 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 137.8 ($\mathbf{C_5}$) + 129.0 ($\mathbf{C_6}$), 124.5 + 121.5 ($\mathbf{C_4}$ + $\mathbf{C_7}$), 48.9 + 43.2 ($\mathbf{C_{1'}}$ + $\mathbf{C_{5'}}$), 26.8 + 26.1 + 24.9 ($\mathbf{C_{2'}}$ + $\mathbf{C_{3'}}$ + $\mathbf{C_{4'}}$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1548.0. Elemental analysis calculated (%) for $C_{12}H_{13}N_3OSe$: C: 48.99, H: 4.45, N: 14.28; found: C: 49.29, H: 4.96, N: 14.44.

4.1.3.16. *N-(Pyridin-4-yl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**14**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-aminopyridine. The resulting product, *N*-(pyridin-4-yl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**14**), did not require further purification. A brown powder was obtained. Yield: 83 %; mp: 277.4-

280.1 ºC. IR (KBr): ῦ 3266 (N-H), 1689 cm^{-1}(C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.88 (s, 1H, N**H**), 8.55 (s, 1H, **H_4**), 8.50 (d, $J_{3'-2'}$ = $J_{5'-6'}$ = 5.8 Hz, 2H, **$H_{3'}$** + **$H_{5'}$**), 7.97 (dd, J_{6-7} = 9.3; J_{6-4} = 1.4 Hz, 1H, **H_6**), 7.94 (d, 1H, **H_7**), 7.81 (d, 2H, **$H_{2'}$** + **$H_{6'}$**). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.6 (**C**=O), 161.2 + 159.8 (**C_3** + **C_8**), 151.2 + 146.6 (**$C_{3'}$** + **$C_{5'}$** + **$C_{1'}$**), 135.0 + 128.6 (**C_5** + **C_6**), 124.1 + 124.0 (**C_4** + **C_7**), 114.9 (**$C_{2'}$** + **$C_{6'}$**). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1565.0. Elemental analysis calculated (%) for $C_{12}H_8N_4OSe \cdot 1/2HCl$: C: 44.83, H: 2.65, N: 17.43; found: C: 45.47, H: 2.72, N: 17.46.

4.1.3.17. *N-(4-Morpholinophenyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**15**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-morpholinoaniline. The resulting product, *N*-(4-morpholinophenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**16**), did not require further purification. A yellow powder was obtained. Yield: 88 %; mp: 278.4-279.8 ºC. IR (KBr): ῦ 3273 (N-H), 2965-2824 (C sp^2), 1644 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.41 (s, 1H, N**H**), 8.48 (s, 1H, **H_4**), 8.00 (dd, J_{6-7} = 9.3; J_{6-4} = 1.7 Hz, 1H, **H_6**), 7.94 (dd, J_{7-4} = 0.6 Hz, 1H, **H_7**), 7.67 (d, $J_{2'-3'}$ = $J_{6'-5'}$ = 9.0 Hz, 2H, **$H_{2'}$** + **$H_{6'}$**), 6.96 (d, 2H, **$H_{3'}$** + **$H_{5'}$**), 3.74 (t, $J_{CH2-CH2}$= 4.7 Hz, 4H, 2 -C**H_2**-O-), 3.08 (t, 4H, 2 -C**H_2**-N-). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.0 (**C**=O), 161.1 + 160.1 (**C_3** + **C_8**), 148.6 + 136.0 (**$C_{4'}$** + **$C_{1'}$**), 131.9 + 128.9 (**C_5** + **C_6**), 123.9 + 123.2 +122.4 (**C_4** + **C_7** + **$C_{2'}$** + **$C_{6'}$**), 116.1 (**$C_{3'}$** + **$C_{5'}$**), 67.0 (2 -**C**H_2-O-), 49.6 (2 -**C**H_2-N-). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1559.5. Elemental analysis calculated (%) for $C_{17}H_{16}N_4\ O_2Se \cdot 1/2H_2O$: C: 51.38, H: 4.28, N: 14.10; found: C: 51.26, H: 4.41, N: 13.89.

4.1.3.18. *N-(Thiazol-2-yl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**16**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 2-aminothiazol. The resulting product, *N*-(thiazol-2-yl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**16**), did not require further purification. A yellow powder was obtained. Yield: 70 %; mp: 284.2-285.1 ºC. IR (KBr): $\tilde{\nu}$ 3276 (N-H), 1662 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.96 (s, 1H, N**H**), 8.69 (s, 1H, $\mathbf{H_4}$), 8.07 (d, $J_{6\text{-}7}$ = 8.7 Hz, 1H, $\mathbf{H_6}$), 7.94 (d, 1H, $\mathbf{H_7}$), 7.59 (s, 1H, $\mathbf{H_{3'}}$), 7.31 (s, 1H, $\mathbf{H_{4'}}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.6 (**C**=O), 161.3+ 159.9 ($\mathbf{C_3}$ + $\mathbf{C_8}$ + $\mathbf{C_{1'}}$), 138.2 + 133.3 ($\mathbf{C_5}$ + $\mathbf{C_{3'}}$), 128.4 ($\mathbf{C_6}$), 124.8 + 124.1 ($\mathbf{C_4}$ + $\mathbf{C_7}$), 114.9 ($\mathbf{C_{4'}}$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1565.0. Elemental analysis calculated (%) for $C_{10}H_6N_4OSSe$: C: 38.84, H: 1.96, N: 18.12; found: C: 38.53, H: 2.24, N: 17.82.

4.1.3.19. *N-(4-(p-Methoxybenzyl)piperazin-1-yl)benzo[c][1,2,5]selenadiazol-5-carboxamide* (**17**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 1-(4-methoxybencyl)piperazine. The reaction mixture was then poured into water and the product was isolated by extraction with dichloromethane (3 x 50 mL). The resulting product, *N*-(4-(*p*-methoxybenzyl)piperazin-1-yl)benzo[*c*][1,2,5]selenadiazol-5-carboxamide (**17**), did not require further purification. A brown powder was obtained. Yield: 45 %; mp: 93.5-94.3 ºC. IR (KBr): $\tilde{\nu}$ 2804-2763 (C sp^2), 1622 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.90 (d, $J_{7\text{-}6}$ = 9.1 Hz, 1H, $\mathbf{H_7}$), 7.83 (s, 1H, $\mathbf{H_4}$), 7.49 (dd, $J_{6\text{-}4}$ = 1.3 Hz, 1H, $\mathbf{H_6}$), 7.21 (d, $J_{2'\text{-}3'} = J_{6'\text{-}5'}$ = 8.5 Hz, 2H, $\mathbf{H_{2'}}$ + $\mathbf{H_{6'}}$), 6.87 (d, 2H, $\mathbf{H_{3'}}$ + $\mathbf{H_{5'}}$), 3.72 (s, 3H, O-C$\mathbf{H_3}$), 3.65 (s, 2H, -C$\mathbf{H_2}$-benzyl), 3.43 - 3.42 (bs, 4H, 2 C$\mathbf{H_2}$-N_1), 2.33 + 2.37 (bs + bs, 4H, 2 C$\mathbf{H_2}$ -N_4).^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.4 (**C**=O), 160.3 + 159.7 + 159.2 ($\mathbf{C_3}$ + $\mathbf{C_8}$ + $\mathbf{C_{4'}}$), 137.2 ($\mathbf{C_5}$), 131.1 + 130.3 + 129.1 ($\mathbf{C_6}$ + $\mathbf{C_{1'}}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{6'}}$), 124.5 +121.9 ($\mathbf{C_4}$ + $\mathbf{C_7}$), 114.4 ($\mathbf{C_{3'}}$ + $\mathbf{C_{5'}}$), 62.0 (-**C**H_2-benzyl), 55.8 (O-**C**H_3). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1549.3. Elemental analysis calculated (%) for

$C_{19}H_{20}N_4O_2Se$: C: 54.94, H: 4.85, N: 13.49; found: C: 54.90, H: 5.23, N: 13.44.

4.1.3.20. *N-(Quinolin-8-yl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**18**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-aminoquinoline. Final product was purified by washing with a mixture of *N*,*N*-dimethylformamide/ethanol (5:5; v:v). A green powder was obtained. Yield: 23 %; mp: 188.9-191.1 ºC. IR (KBr): $\tilde{\nu}$ 3332 (N-H), 1660 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 10.83 (s, 1H, N**H**), 9.01 (d, $J_{4'-5'}$ = 3.5 Hz, 1H, $\mathbf{H_{4'}}$), 8.71 (d, $J_{6'-5'}$= 7.5 Hz, 1H, $\mathbf{H_{6'}}$), 8.55 (s, 1H, $\mathbf{H_4}$), 8.47 (d, $J_{10'-9'}$ = 8.2 Hz, 1H, $\mathbf{H_{10'}}$), 8.05 (d, J_{6-7} = 9.4 Hz, 1H, $\mathbf{H_6}$), 8.02 (d, 1H, $\mathbf{H_7}$), 7.78 (d, 1H, $\mathbf{H_{9'}}$), 7.70 (d, $J_{8'-9'}$=3.9 Hz, 1H, $\mathbf{H_{8'}}$), 7.70 (d, 1H, $\mathbf{H_{5'}}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.6 (**C**=O), 160.8 + 159.6 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 149.8 ($\mathbf{C_{4'}}$), 139.1 + 137.3 + 135.3 +134.4 ($\mathbf{C_5}$ + $\mathbf{C_{1'}}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{6'}}$), 128.4 +127.6 + 127.5 ($\mathbf{C_6}$ + $\mathbf{C_{7'}}$ + $\mathbf{C_{9'}}$), 124.2 + 123.3 + 122.9 ($\mathbf{C_4}$ + $\mathbf{C_7}$ + $\mathbf{C_{5'}}$ + $\mathbf{C_{10'}}$), 117.9 ($\mathbf{C_{8'}}$). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1564.0. Elemental analysis calculated (%) for $C_{16}H_{10}N_4OSe\cdot 1/2H_2O$: C: 53.00, H: 2.76, N: 15.46; found: C: 53.49, H: 3.14, N: 15.40.

4.1.3.21. *N-(Benzo[d]thiazol-2-yl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**19**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-aminobenzothiazol. The resulting product, *N*-(benzo[*d*]thiazol-2-yl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**20**), did not require further purification. A yellow powder was obtained. Yield: 50 %; mp: >300 ºC. IR (KBr): $\tilde{\nu}$ 3348 (N-H), 1631 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.75 (s, 1H, $\mathbf{H_4}$), 8.13 (dd, J_{6-7}= 9.4; J_{6-4}= 1.3 Hz, 1H, $\mathbf{H_6}$), 8.04 (d, $J_{4'-5'}$= 7.6 Hz, 1H, $\mathbf{H_{4'}}$), 7.99 (d, 1H, $\mathbf{H_7}$), 7.81 (d, J_{6-7} = 7.6 Hz, 1H, $\mathbf{H_{7'}}$), 7.49 (t, $J_{6'-5'}$= 7.6 Hz, 1H, $\mathbf{H_{6'}}$),7.37 (t, 1H, $\mathbf{H_{5'}}$). Elemental analysis calculated (%) for $C_{14}H_8N_4OSSe\cdot 1/2H_2O$: C: 45.62, H: 2.17, N: 15.21; found: C: 45.22, H: 2.50, N: 14.96.

4.1.3.22. *N-Benzylbenzo[c][1,2,5]selenadiazole-5-carboxamide* (**20**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 2-benzylamine. The product was purified by recrystallization from isopropanol. A yellow powder was obtained. Yield: 46 %; mp: 177.5-179ºC. IR (KBr): $\tilde{\nu}$ 3272 (N-H), 3026 (C sp^{-2}), 1631 (C=O), 1600-1400 cm^{-1} (-HC=CH-). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.37 (t, $J_{NH\text{-}CH2}$ = 5.6 Hz, 1H, N**H**), 8.41 (s, 1H, $\mathbf{H_4}$), 7.98 (dd, $J_{6\text{-}7}$ = 9.3; $J_{6\text{-}4}$ = 1.2 Hz, 1H, $\mathbf{H_6}$), 7.91 (d, 1H, $\mathbf{H_7}$), 7.39-7.31 (m, 4H, $\mathbf{H_{2'}}$ + $\mathbf{H_{3'}}$ + $\mathbf{H_{5'}}$ + $\mathbf{H_{6'}}$), 7.26 (t, $J_{4'\text{-}3'}$ = $J_{4'\text{-}5'}$ = 6.4 Hz, 1H, $\mathbf{H_{4'}}$), 4.53 (d, 2H, -C$\mathbf{H_2}$-). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.4 (**C**=O), 161.2 + 160.2 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 140.2 + 135.3 ($\mathbf{C_{1'}}$ + $\mathbf{C_5}$), 129.2 + 128.7 + 128.2 + 127.7 ($\mathbf{C_6}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{3'}}$ + $\mathbf{C_{4'}}$+ $\mathbf{C_{5'}}$+ $\mathbf{C_{6'}}$), 123.9 + 123.0 ($\mathbf{C_4}$ + $\mathbf{C_7}$), 43.8 (-**C**H_2-). Elemental analysis calculated (%) for $C_{14}H_{11}N_3OSe$: C: 53.16, H: 3.48, N: 13.29; found: C: 52.60, H: 3.79, N: 13.13.

4.1.3.23. *N-(4-Bromobenzyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**21**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-bromobenzylamine. The resulting product, *N*-(4-bromobenzyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**21**), did not require further purification. A white powder was obtained. Yield: 74 %; mp: 187.8-190 ºC. IR (KBr): $\tilde{\nu}$ 3273 (N-H), 1625 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.38 (t, $J_{NH\text{-}CH2}$ = 5.7 Hz, 1H, N**H**), 8.41 (s, 1H, $\mathbf{H_4}$), 7.96 (dd, $J_{6\text{-}7}$ = 9.3; $J_{6\text{-}4}$ = 1.4 Hz, 1H, $\mathbf{H_6}$), 7.93 (d, 1H, $\mathbf{H_7}$), 7.54 (d, $J_{3'\text{-}2'}$ = $J_{5'\text{-}6'}$ = 8.3 Hz, 2H, $\mathbf{H_{3'}}$ + $\mathbf{H_{5'}}$), 7.33 (d, 2H, $\mathbf{H_{2'}}$ + $\mathbf{H_{6'}}$), 4.50 (d, 2H, -C$\mathbf{H_2}$-).^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.4 (**C**=O), 161.2 + 160.1 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 139.7 + 135.2 ($\mathbf{C_5}$ + $\mathbf{C_{1'}}$), 132.1 + 130.4 + 128.6 ($\mathbf{C_6}$ + $\mathbf{C_{3'}}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{5'}}$ + $\mathbf{C_{6'}}$), 123.9 + 123.07 + 120.7 ($\mathbf{C_4}$ + $\mathbf{C_7}$ + $\mathbf{C_{4'}}$), 43.2 (-**C**H_2-). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1559.5. Elemental analysis calculated (%) for $C_{14}H_{10}BrN_3OSe \cdot 1/2H_2O$: C: 41.58, H: 2.72, N: 10.40; found: C: 41.98, H: 2.88, N:

10.89.

4.1.3.24. *N-Phenethylbenzo[c][1,2,5]selenadiazole-5-carboxamide* (**22**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 2-phenylethylamine. The product was purified by recrystallization from isopropanol. A yellow powder was obtained. Yield: 33 %; mp: 194.1-195 ºC. IR (KBr): $\tilde{\nu}$ 3294 (N-H), 3061-3028 (C sp^{-2}), 1634 (C=O), 1600-1400 cm^{-1} (-HC=CH-). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.90 (t, J_{NH-CH2} = 5.3 Hz, 1H, N**H**), 8.30 (s, 1H, $\mathbf{H_4}$), 7.91 (dd, J_{6-7} = 9.3; J_{6-4} = 1.5 Hz, 1H, $\mathbf{H_6}$), 7.90 (dd, J_{7-4} = 0.8 Hz, 1H, $\mathbf{H_7}$), 7.34 - 7.26 (m, 4H, $\mathbf{H_{2'}}$ + $\mathbf{H_{3'}}$ + $\mathbf{H_{5'}}$ +$\mathbf{H_{6'}}$), 7.21 (tt, $J_{4'-3'} = J_{4'-5'}$ = 6.8; $J_{4'-2'} = J_{4'-6'}$ = 1.6 Hz, 1H, $\mathbf{H_{4'}}$), 3.54 (q, $J_{CH2-CH2} = J_{CH2-NH}$ = 7.4 Hz, 2H, -C$\mathbf{H_2}$-NH), 2.89 (t, 2H, -C$\mathbf{H_2}$-Ph). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.3 (**C**=O), 161.1 + 160.2 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 140.3 + 135.6 ($\mathbf{C_5}$ + $\mathbf{C_{1'}}$), 129.6 +129.2 + 128.7 + 127.0 ($\mathbf{C_6}$ + $\mathbf{C_{2'}}$ + $\mathbf{C_{3'}}$ + $\mathbf{C_{4'}}$ + $\mathbf{C_{5'}}$ + $\mathbf{C_{6'}}$), 123.8 + 122.8 ($\mathbf{C_4}$ + $\mathbf{C_7}$), 41.9 (-**C**H_2-NH), 35.8 (-**C**H_2-Ph). Elemental analysis calculated (%) for $C_{15}H_{13}N_3OSe$: C: 54.55, H: 3.94, N: 12.73; found: C: 54.30, H: 4.32, N: 12.74.

4.1.3.25. *N-(3-Phenylpropyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**23**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 3-phenylpropylamine. The resulting product, *N*-(3-phenylpropyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**23**), did not require further purification. A brown powder was obtained. Yield: 86 %; mp: 147-148 ºC. IR (KBr): $\tilde{\nu}$ 3288 (N-H), 3024 (C sp^{-2}), 1632 (C=O), 1600-1400 cm^{-1} (-HC=CH-). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.82 (t, J_{NH-CH2} = 4.9 Hz, 1H, N**H**), 8.31 (s, 1H, $\mathbf{H_4}$), 7.92 (d, J_{6-7} = 9.3 Hz, 1H, $\mathbf{H_6}$), 7.88 (d, 1H, $\mathbf{H_7}$), 7.31-7.21 (m, 4H, $\mathbf{H_{2'}}$ + $\mathbf{H_{3'}}$ + $\mathbf{H_{5'}}$ +$\mathbf{H_{6'}}$), 7.17(t, $J_{4'-3'} = J_{4'-5'}$ = 7.0 Hz, 1H, $\mathbf{H_{4'}}$), 3.33 (q, $J_{CH2-CH2}$ = 7.4 Hz, 2H, -C$\mathbf{H_2}$-NH), 2.65 (t, 2H, -C$\mathbf{H_2}$-Ph), 1.86 (m, 2H, -CH_2-C$\mathbf{H_2}$-CH_2-).^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.3 (**C**=O), 161.1 + 160.2 ($\mathbf{C_3}$ + $\mathbf{C_8}$), 142.6 + 135.7 ($\mathbf{C_{1'}}$ + $\mathbf{C_5}$),

129.2 + 129.1 +128.8 + 126.6 (**C$_6$** + **C$_{2'}$** + **C$_{3'}$** + **C$_{4'}$** + **C$_{5'}$** + **C$_{6'}$**), 123.8 + 122.8 (**C$_4$** + **C$_7$**), 33.5 + 31.6 (-**C**H$_2$-NH + -CH$_2$-**C**H$_2$-CH$_2$- + -**C**H$_2$-Ph). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1557.4. Elemental analysis calculated (%) for $C_{16}H_{15}N_3OSe\cdot 1/2\ H_2O$: C: 54.40, H: 4.53, N: 11.90; found: C: 54.90, H: 4.38, N: 11.80.

4.1.3.26. *N-(3-Hydroxypropyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**24**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 3-amino-1-propanol. The product was purified by recrystallization from diclorometh ane. Yield: 18 %; mp: 158-160 ºC. IR (KBr): $\tilde{\nu}$ 3295 (N-H), 2948 (C, sp^2), 1637 (C=O), 1600-1400 cm^{-1} (-HC=CH-). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.76 (t, $J_{NH\text{-}CH2}$ = 5.2 Hz, 1H, N**H**), 8.33 (s, 1H, **H$_4$**), 7.94 (dd, $J_{6\text{-}7}$ = 9.3; $J_{6\text{-}4}$ = 1.5 Hz, 1H, **H$_6$**), 7.89 (d, 1H, **H$_7$**), 3.49 (t, $J_{CH2\text{-}CH2}$ =5.9 Hz, 2H, -C**H$_2$**-OH-), 3.36 (q, $J_{CH2\text{-}CH2}$ = 6.7 Hz, 2H, -C**H$_2$**-NH-), 1.76-1.69 (m, 2H, -CH$_2$-C**H$_2$**-CH$_2$-).^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.3 (**C**=O), 161.1 + 160.2 (**C$_3$** + **C$_8$**), 135.7 (**C$_5$**), 128.7 (**C$_6$**), 123.8 + 122.8 (**C$_4$** + **C$_7$**), 59.5 (-**C**H$_2$-OH-), 37.7 + 33.2 (-**C**H$_2$-NH- + -CH$_2$-**C**H$_2$-CH$_2$-). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1557.6. Elemental analysis calculated (%) for $C_{10}H_{11}N_3O_2Se\cdot H_2O$: C: 39.74, H: 3.64, N: 13.91; found: C: 39.87, H: 3.74, N: 13.68.

4.1.3.27. *N-(4-Phenylbutyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**25**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-phenylbutylamine. The resulting product, *N*-(4-phenylbutyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide (**25**), did not require further purification. A brown powder was obtained. Yield: 78 %; mp: 170.6-171.1 ºC. IR (KBr): $\tilde{\nu}$ 3299 (N-H), 3026 (C, sp^{-2}), 1632 (C=O), 1600-1400 cm^{-1} (-HC=CH-). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.78 (t, $J_{NH\text{-}CH2}$ = 5.2 Hz, 1H, N**H**), 8.32 (s, 1H, **H$_4$**), 7.93 (d, $J_{6\text{-}7}$ = 9.3 Hz, 1H, **H$_6$**), 7.89 (d, 1H, **H$_7$**), 7.27(t, $J_{2'\text{-}3'}$=$J_{6'\text{-}5'}$=7.5 Hz, 2H, **H$_{2'}$**+ **H$_6$**), 7.21 (d, 2H, **H$_{3'}$**+ **H$_{3'}$**), 7.16(t, $J_{4'\text{-}3'}$ = $J_{4'\text{-}5'}$=7.1 Hz, 1H, **H$_{4'}$**), 3.34 (q,

$J_{CH2\text{-}CH2}$ = 6.10 Hz 2H, -C**H**$_2$- NH), 2.62 (t, $J_{CH2\text{-}CH2}$ =*7.17*, 2H, -C**H**$_2$-Ph), 1.61 (m, 4H, -CH_2-C**H**$_2$-C**H**$_2$-CH_2-). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.2 (**C**=O), 161.1 + 160.2 (**C**$_3$ + **C**$_8$), 143.0 + 135.7 (**C**$_5$ + **C**$_{1'}$), 129.2 + 129.1 + 128.7 + 126.5 (**C**$_6$ **+ C**$_{2'}$ **+C**$_{3'}$ + **C**$_{4'}$ + **C**$_{5'}$ + **C**$_{6'}$), 123.8 + 122.7 (**C**$_4$ + **C**$_7$), 35.7 +29.5 + 29.4 (-**C**H_2- NH + NH-CH_2-**C**H_2- + -**C**H_2-CH_2-Ph + -**C**H_2-Ph). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1557.6. Elemental analysis calculated (%) for $C_{17}H_{17}N_3OSe$: C: 56.99, H: 4.75, N: 11.73; found: C: 56.95, H: 5.04, N: 11.49.

4.1.3.28. *N-(4-Hydroxybutyl)benzo[c][1,2,5]selenadiazole-5-carboxamide* (**26**)

From benzo[*c*][1,2,5]selenadiazole-5-acyl chloride and 4-amino-1-butanol. The product was purified by recrystallization from THF. A white powder was obtained. Yield: 61 %; mp: 160-162 ºC. IR (KBr): $\tilde{\nu}$ 3293 (N-H), 2943-2867 (C, sp^2), 1630 (C=O), 1600-1400 cm^{-1} (-HC=CH-). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.76 (t, $J_{NH\text{-}CH2}$ = 5.0 Hz, 1H, N**H**), 8.34 (s, 1H, **H**$_4$), 7.94 (d, $J_{6\text{-}7}$ = 9.2 Hz, 1H, **H**$_6$), 7.89 (d, 1H, **H**$_7$), 3.46-3.42 (m, 2H, -C**H**$_2$-OH-), 3.34-3.29 (q, $J_{CH2\text{-}CH2}$ = 6.1 Hz, 2H, -C**H**$_2$- NH-), 1.62-1.56 (m, 2H, -CH_2-C**H**$_2$-CH_2-OH), 1.53-1.48 (m, 2H, -NH-CH_2-C**H**$_2$-CH_2-).^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.2 (**C**=O), 161.1 + 160.2 (**C**$_3$ + **C**$_8$), 135.7 (**C**$_5$), + 128.7 (**C**$_6$), 123.75 + 122.7 (**C**$_7$ + -**C**H_2-OH-), 61.3 (-**C**H_2-NH-), 30.9 (-CH_2-**C**H_2-CH_2-OH), 26.6 (-NH-CH_2-**C**H_2-CH_2-). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1557.5. Elemental analysis calculated (%) for $C_{11}H_{13}N_3O_2Se$: C: 44.29, H: 4.36, N: 14.09; found: C:44.19, H: 4.33, N: 13.61.

4.1.4. Synthesis of *Piperazine-1,4-diylbis(benzo[c][1,2,5]selenadiazol-5-ylmethanone* (**27**)

A mixture of BSCA (0.5 g, 2 mmol) and thionyl chloride (20 mL) was stirred at reflux for 2 h. The reaction was monitored by IR spectroscopy. The resulting acyl chloride was

isolated by rotatory evaporation of the thionyl chloride under vacuum and the excess of thionyl chloride was removed with 3 fractions of toluene (40 mL). The resulting acyl chloride was used without further purification. A solution of the piperazine (0.09 g, 1 mmol) in dry chloroform (15 mL) was added to a mixture of the resulting acyl chloride (0.5 g, 2 mmol) and triethylamine (0.28 mL, 2 mmol) in dry chloroform (40 mL). The mixture was stirred at room temperature for 72 h. The product was isolated by filtration and washed with water (3x 50 mL). The resulting product, piperazine-1,4-diylbis(benzo[*c*][1,2,5]selenadiazol-5-ylmethanone (**27**), did not require further purification. A brown powder was obtained. Yield: 99 %; mp: 229.4-231.3 ºC. IR (KBr): $\tilde{\nu}$ 1620 cm^{-1} (C=O). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.91 (d, 4H, 2**H$_6$** + 2**H$_7$**), 7.55 (s, 2H, **H$_4$**), 3.69-3.57 (bs, 8H, 2 C**H$_2$**-C**H$_2$**). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.8 (2**C**=O), 160.3 + 159.7 (2**C$_3$** + 2**C$_8$**), 136.8 (2**C$_5$**), 129.1 (2**C$_6$**), 124.6 + 122.2 (2**C$_4$** + 2**C$_7$**), 40.31 (2 **C**H_2-**C**H_2). ^{77}Se NMR (76 MHz, DMSO-d_6) δ (ppm): 1550.3. Elemental analysis calculated (%) for $C_{18}H_{14}N_6O_2Se_2 \cdot 1/2H_2O$: C: 42.09, H: 2.92, N: 16.37; found: C: 42.32, H: 3.14, N: 16.03.

4.2. Biological evaluation

4.2.1. Anti-proliferative activity

The cell lines were obtained from the American Type Culture Collection (ATCC). Five tumor cell lines (MCF-7, PC-3, HT-29, HTB-54 and CCRF-CEM) were grown in RPMI medium (Gibco), supplemented with 10 % fetal bovine serum (FBS; Gibco) and 1 % antibiotics (10.00 units/mL penicillin and 10.00 µg /mL streptomycin; Gibco). 184B5 cells were grown in DMEM / F12 (1 : 1) (1X) + GlutaMAX^{TM} medium (Gibco) supplemented with 5 % FBS, 1 % antibiotics and a supplement cocktail containing 1 mL of hydrocortisone (100 nm; Aldrich), 1 x ITS (Lonza), 10 mL of sodium pyruvate

(2 mM; Lonza), 10 µL of EGF (20 ng /mL; Aldrich) and 150 µL of trans-retinoic acid (0.3 nM; Aldrich). BEAS-2B were grown in DMEM medium (Gibco), supplemented with 10 % FBS and 1 % antibiotics. Cells were preserved in tissue culture flasks at 37 ºC and 5% CO_2. Culture medium was replaced every three days.

The effect of each compound on cell viability was tested using the MTT assay [46]. Each compound was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 0.01 M. Sterile filtration of the compounds was achieved using 0.2 µM filter disk. Serial dilutions were prepared with non-supplemented culture medium. The inhibition of cell viability was determined at four different concentration ranging from 1 to 100 µM. Cells were seeded at 1 x 10^4 per well in the case of MCF-7, PC-3, HT-29, HTB-54, 184B5 and BEAS-2B cells onto flat-bottomed 96-well culture plates and 4 x 10^4 per well in the case of CCRF-CEM cells onto round-bottomed 96-well culture plates. They were treated with either DMSO or increasing concentration of the corresponding compound for 72 h. Then, they were incubated with 50 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (2 mg /mL stock; Aldrich) for 4 h and analyzed for their ability to generate a purple formazan dye. These formazan crystals were dissolved in 150 µL of DMSO. The absorbance was measured at a wavelength of 550 nm and the ratio of viable cells was calculated. Results are expressed as GI_{50}, concentration that reduces by 50% the cell growth with respect to untreated controls; TGI, concentration that completely inhibits cell growth of treated cells with respect to untreated controls; and LC_{50}, concentration that kills 50% of the initial cells. Furthermore, the selectivity index (SI) was calculated as the ratio of the GI_{50} values determined for the non-malignant and the tumoral cells (GI_{50} (184B5)/GI_{50} (MCF-7) and GI_{50} (BEAS-2B)/GI_{50} (HTB-54)). Data were obtained from at least three independent experiments performed in quadruplicates.

4.2.2. Evaluation of cell cycle progression and apoptosis induction

For the MCF-7 cells, the apoptosis evaluation and cell cycle analysis of the cells were determined using the Apo Direct™ kit (BD Pharmingen), based on the TUNEL technique, according to the procedure described by the manufacturer. To fix the cells, they were suspended in 1% paraformaldehyde in PBS (pH = 7.4) at a concentration of $1x10^6$ cells/mL and placed on ice for 60 min. Then, cells were centrifuged, washed, adjusted to $1x10^6$ cells/mL in 70% ethanol and incubated on ice for 30 min. After fixation, the cells were stained. Briefly, the cells were centrifuged, washed and resuspended in FITC dUTP-DNA labelling solution and incubated for 60 min at 37 ºC. Finally, at the end of the incubation time, the cells were rinsed with 1 mL of the Rinse Buffer, and the cells were resuspended in PI/RNase Staining Buffer, incubated for 30 min at RT, in the dark. These cells were analyzed by flow cytometer (Coulter Epics XL, Beckmam Coulter).

4.3. X-ray crystallography of compound 7

Single crystals of *N*-(4-(methylselanyl)phenyl)benzo[*c*][1,2,5]selenadiazole-5-carboxamide, (compound **7**) were grown from solution of DMSO by slow evaporation. A suitable crystal was selected and mounted on a nylon loop using paratone oil, for data collection on a 'Bruker SMART APEX CCD' diffractometer. The crystal was kept at room temperature (~25 ºC) during data collection. Using Olex2 [47], the structure was solved with the XS [48] structure solution program using Direct Methods and refined with the XL [48] refinement package using Least Squares minimization.

4.4. DPPH free radical- scavenging assay

The DPPH method is one of the most efficient methods for evaluating the radical-scavenging action by a chain-breaking mechanism [49]. This bioassay is a standard to

estimate the antioxidant property of chemicals like novel selenadiazole compounds, previously described by Svinyarov [45]. This is based on the reduction of a stable free radical, DPPH with the presence of antioxidants, by the donation of a hydrogen atom. Then, this reduction of DPPH causes the decrease in its absorbance at 517 nm and we can determine the corresponding DPPH radical- scavenging activity.

A methanolic solution (0.04 mg/mL) of DPPH (Aldrich) was prepared daily and protected from light. Besides, each compound was initially dissolved in DMSO at a concentration of 1.25 mg/mL and serial dilutions in absolute methanol were prepared (0.25, 0.0625, 0.000625, 0.0000625 mg/mL). The blank of colourless sample was prepared with DMSO mixed with absolute methanol at the same concentrations of DMSO in samples. Nevertheless, for coloured samples, like compound **9**, the most concentrated dilution was used as a blank. 750 µL of each sample were dissolved in 750 µL of solution of DPPH, and the control was prepared dissolving 750 µL of each blank in 750 µL of solution of DPPH. After 30, 90 and 180 min of incubation at 37 ºC in the dark, the absorbance was read against a blank at 517 nm. The ascorbic acid (vitamin C) was used as the positive control and the EBS was used as a reference compound. All the measurements were carried out in triplicate. Results are expressed as the percentage of the radical scavenger, calculated using the following formula:

a) For colourless samples:

$$\%\ \text{DPPH radical scavenging} = \frac{(A_{control} - A_{blank}) - (A_{sample} - A_{blank})}{(A_{control} - A_{blank})}$$

b) For coloured samples:

$$\%\ \text{DPPH radical scavenging} = \frac{(A_{control} - A_{blank}) - (A_{sample} - A_{sample\ blank})}{(A_{control} - A_{blank})}$$

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