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Original article

Sulfur and selenium derivatives of quinazoline and pyrido[2,3-*d*]pyrimidine: Synthesis and study of their potential cytotoxic activity *in vitro*

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ABSTRACT

The synthesis, cytotoxic activities and selectivities of 35 derivatives related to quinazoline and pyrido[2,3-*d*]pyrimidine are described. The synthesized compounds were screened *in vitro* against four tumoral cell lines – leukemia (CCRF-CEM), colon (HT-29), lung (HTB-54) and breast (MCF-7) – and two cell lines derived from non-malignant cell lines, one mammary (184B5) and one from bronchial epithelium (BEAS-2B). MCF-7 and HTB-54 were the most sensitive cell lines with GI_{50} values below 10 μM for eleven and ten compounds, respectively. Two compounds (**2o** and **3a**) were identified that evoked a marked cytotoxic effect in all cell lines tested and one compound, **7h**, was potent and selective against MCF-7. A preliminary study into the mechanism of the potent derivatives **2o**, **3a** and **7h** indicated that the cytotoxic activities of these compounds might be mediated by inducing cell death without affecting cell cycle phases.

1. Introduction

The disease of cancer has been ranked as a major health burden. At present, a wide range of cytotoxic drugs with different mechanisms of action are used to treat human cancer, either alone or in combination. Numerous compounds are also in different phases of clinical trials. The main drawback of these cytotoxic drugs is that they do not discriminate between cancerous and normal cell types and are accompanied by toxic side effects that are often cumulative and dose limiting [1].

Growth factor signaling pathways have been a main focus of research for novel targeted anticancer agents because of their fundamental role in regulating key cellular functions, including cell-proliferation, differentiation, metastasis and survival [2].

Quinazoline derivatives have attracted attention due to their broad range of pharmacological activities, which include, among others, antifungal [3], antimalarial [4], anti-inflammatory [5], anticonvulsant [6], antibacterial [7] and antihypertensive [8], and their anticancer activity [9–17] is one of the most promising aspects as they act through multiple targets. Several of these compounds are remarkable dihydrofolate reductase [18] and tyrosine kinase [19–21] inhibitors such as gefitinib, lapatinib and pazopanib. Some quinazolines interact with tubulin [22] and interfere with its polymerization. Others act by modulating aurora kinase activity [23] or have an effect in critical phases in the cell cycle [11,24] or act as apoptosis inducers [16,17,25–27] (Fig. 1).

There are also numerous examples in the literature of compounds with very diverse biological profiles shown by the pyrido[2,3-*d*]pyrimidine nucleus. The family based on pyrido[2,3-*d*]pyrimidines has been developed and these compounds show great specificity for individual subgroups of receptor tyrosine kinases [28,29]. Other members of this family inhibit non-receptor tyrosine kinases such as Abl [30,31], Akt [32] or cyclin kinases [33]. Another target for these derivatives is dihydrofolate reductase inhibition, e.g. piritrexim [34], as well as cell cycle modulation [35] or as yet undetermined mechanisms of action [36] (Fig. 1).

Based on the observations outlined above, these nuclei have emerged as versatile templates for a diverse range of mechanisms of anticancer activity and, considering our experience with these heteroaromatic rings [26,37–41], we describe here the discovery of a novel series of 2,4-disubstituted quinazoline and pyrido[2,3-*d*]pyrimidine derivatives.

The different chemical groups selected for attachment to the central nucleus in the new structures are based on the diverse

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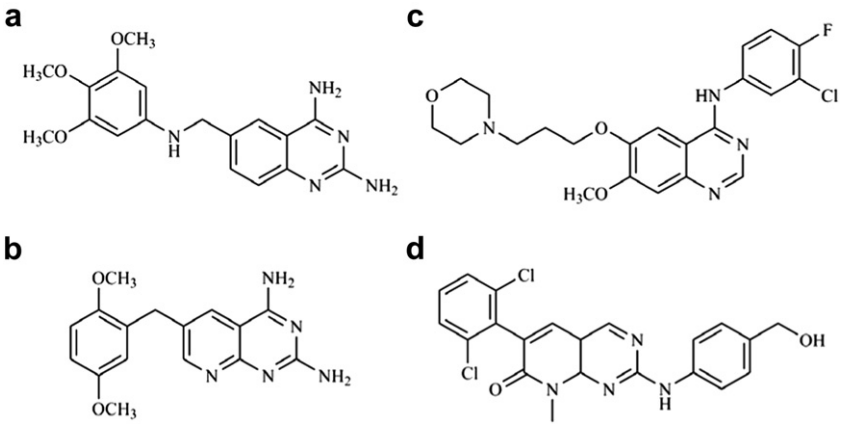


Fig. 1. Chemical structures for some quinazolines and pyrido[2,3-*d*]pyrimidines with anticancer activity. (a) Trimetrexate; (b) Piritrexim; (c) Gefitinib; (d) PD166326.

reference literature, which was reviewed in our previous works in order to optimize the substituents with respect to *in vitro* biological activity. With this aim in mind, the following structural modifications were considered (Fig. 2): quinazoline and pyridopyrimidine were selected in order to explore the influence of the total number of nitrogen atoms present in the central fragment [42], with the subsequent variation of the number of centers potentially involved in hydrogen bond formation; the influence of parameters like electronic environment, which would affect the lipophilicity and hence the activity of the target molecules [43], the length of the chains and positions in the annular system [44]. Various linkers were designed and incorporated between the central nucleus scaffold and the flexible aryl moiety in order to investigate their effects on the biological activity. The linkages investigated were (–NH–), which was inserted in position 4, and a sulfur [45] or selenium atom, which were mainly introduced in the 2-position. The choice of the latter linker was based on our research in recent years, which has involved the design and synthesis of structurally modified compounds containing selenium – many of which have demonstrated potent antitumoral activity [46–50]. Recently, we reported the positive effect on the cytotoxic properties achieved by replacing sulfur with selenium [46,50] and it seemed reasonable to assess the bioactivity of related compounds with this element instead of sulfur.

We report here the synthesis of 35 2-thio-, 2-seleno-4-amino- and 2,4-diseleno-derivatives of quinazoline and pyrido[2,3-*d*] pyrimidines and the evaluation of their biological activity as potential antitumoral agents.

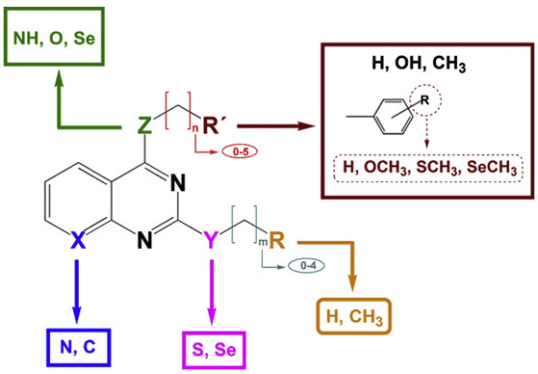


Fig. 2. Structural modifications carried out for the synthesized compounds.

2. Results and discussion

2.1. Chemistry

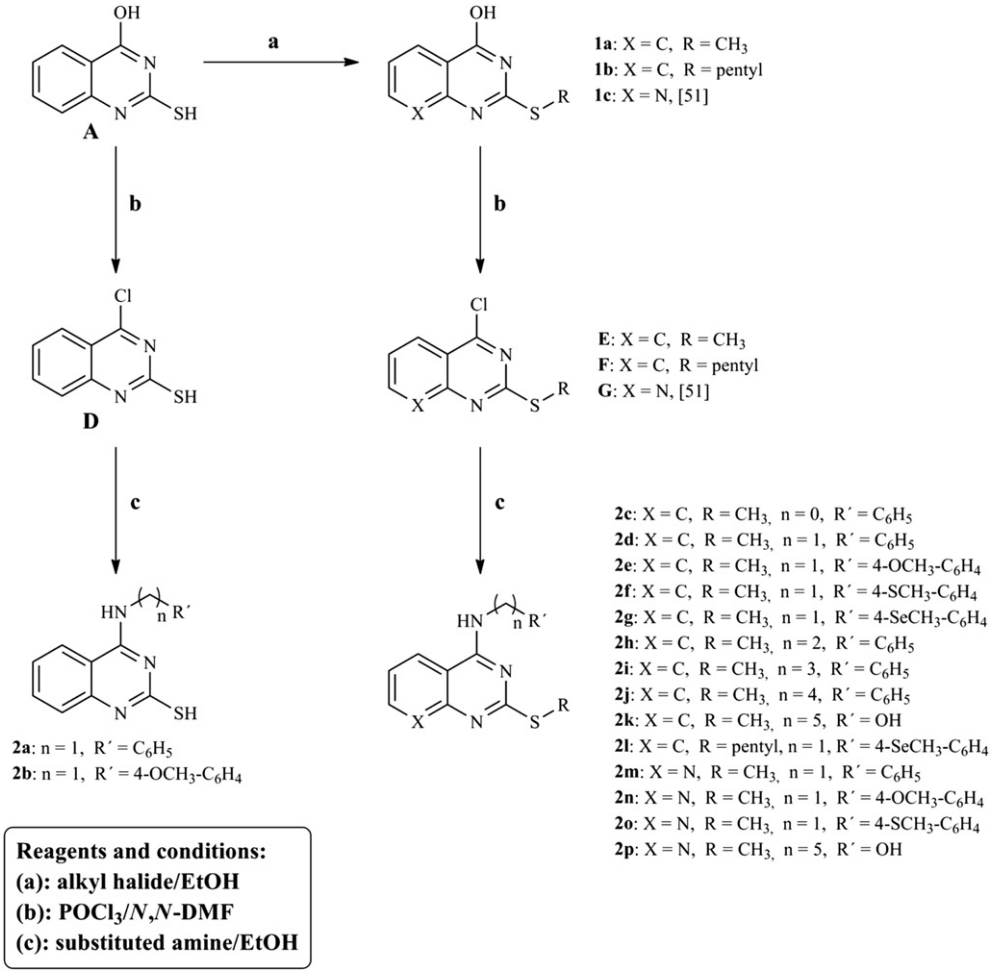
The synthesis of the 35 compounds described here was carried out according to Schemes 1–3.

In order to prepare these molecules, the most appropriate starting materials were the commercially available 2-mercapto-4(3*H*)quinazolinone **A** (Aldrich, 13906-09-7) (Scheme 1), 2,4(1*H*,3*H*)-quinazolinedione **B** (Aldrich, 86-96-4) (Scheme 2), pyrido[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **C** (Shanghai Richem International Co., Ltd) and a compound that was prepared previously by us, **1c** [51].

Compounds **1a** and **1b** (Scheme 1) were synthesized from **A** by reaction with methyl or pentyl iodide in basic media. The reaction of **A**, **1a**, **1b** or **1c** with $POCl_3$ and DMF (added as a catalyst) gave the corresponding halide intermediates (**D**–**G**), which were used without purification. The derivatives containing a chloro-substituent in the 4-position proved to be very versatile intermediates, since the chlorine could be displaced with a variety of nucleophiles. The nucleophilic displacement of the chloro-substituent in the key intermediates with the appropriate amine was achieved by heating an alcoholic solution under reflux to generate the desired final products 2-mercapto/2-alkylthio-4-substituted quinazolines and the corresponding pyridopyrimidines (**2a**–**p**) (Scheme 1), with yields ranging from 4% to 94%.

Chlorination of **B** or **C** with phosphoryl chloride, according to the method previously reported by us [51] (Scheme 2), afforded the corresponding dihalides. Nucleophilic displacement of the chloro group(s) with selenourea or ammonia gave the target compounds **3a**, **3b** and 4-amino-2-chloroquinazoline or pyrido[2,3-*d*]pyrimidine, respectively. Different methods were used to effect these displacements depending on the type of nucleophile. For example, **3a** and **3b** were obtained using ethanol as the solvent and the other derivatives were obtained with aqueous ammonia. The resulting compounds also served as intermediates to prepare other members of the series. Thus, **3a** and **3b** were converted to **3c**, **3d**, **3e** and **3f** by reaction with the appropriate alkyl iodide in ethanol under reflux. Treatment of 4-amino-2-chloroquinazoline or pyrido[2,3-*d*] pyrimidine with selenourea in ethanol provided **4a** and **4b** and these were alkylated to obtain the desired compounds **4c**, **4d**, **4e** and **4f**.

The key isoselenocyanates **6a**, **6b** and **6c** were synthesized in two steps [52,53] (Scheme 3). The first step involved formylation of phenylalkylamines with ethyl formate (compounds **5a**, **5b** and **5c**) followed by treatment with triphosgene and selenium powder in



Scheme 1. Synthesis route of the compounds of series **1** and **2**.

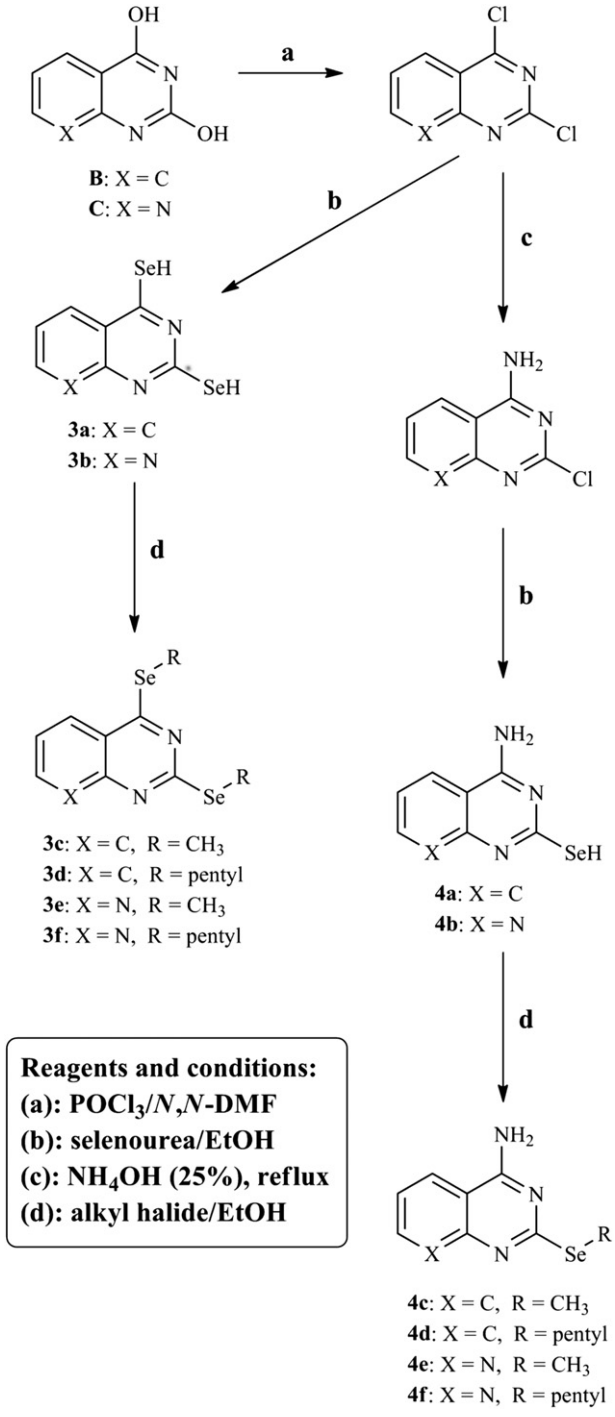
the presence of triethylamine to furnish the desired phenylalkyl isoselenocyanates in moderate yields. Compounds were purified by silica gel column chromatography using *n*-hexane/ethyl acetate as the eluent and they were characterized by ^{1}H NMR spectroscopy. Treatment of isoselenocyanates with *o*-aminobenzonitrile or 2-amino-3-cyanopyridine in the presence of dry pyridine under reflux gave the corresponding 4-phenylalkylamino-2-selenoquinazoline or 4-phenylalkylamino-2-selenopyrido[2,3-*d*] pyrimidine, which tautomerize to give **7a**, **7b**, **7c** and **7d** (Scheme 3). The latter compound was obtained in very modest yield. Alkylation of compounds **7a**, **7b** and **7c** with methyl iodide or pentyl iodide was performed in ethanol and compounds **7e**, **7f**, **7g**, **7h** and **7i** (Scheme 3) were obtained with variable yields. It is important to emphasize that under the same conditions the analogous reaction utilizing the pyridopyrimidine **7d** failed to give any alkylated product. Moreover, subsequent modification of the conditions (temperature, solvents) resulted in decomposition and complex reaction mixtures were obtained. On the whole, hydroseleno group is alkylated with alkyl iodides due to the nucleophilic character of selenium, nevertheless, this character is very sensitive to electronic effects of neighboring groups. For example, rings without electron-donating groups or π-deficient rings cannot react. In light of these findings, the corresponding alkylated pyridopyrimidines were not obtained and the importance of this structural change on the biological activity could not be evaluated.

The purity of all products was determined by thin layer chromatography using several solvent systems of different polarity. All compounds were pure and stable. The compounds were characterized by infrared, ^{1}H and ^{13}C nuclear magnetic resonance, mass spectrometry and CHN microanalysis.

2.2. Biological evaluation

2.2.1. Cytotoxicity

All of the synthesized compounds were screened for their cytotoxic and antiproliferative activities against a panel of four human tumor cell lines: lung carcinoma (HTB-54), colon carcinoma (HT-29), lymphocytic leukemia (CCRF-CEM) and breast adenocarcinoma (MCF-7) as well as two non-malignant cell lines – mammary gland (184B5) and bronchial epithelium cell (BEAS-2B). Cytotoxicity assays were based on the reactivity of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] as described by Mosmann [54]. Results are expressed as GI_{50}, i.e. the concentration that reduces by 50% the growth of treated cells with respect to untreated controls, TGI, the concentration that completely inhibits cell growth, and LC_{50}, the concentration that kills 50% of the cells. The cytotoxic effect of each substance was tested at five different concentrations between 0.01 and 100 μM. The GI_{50}, TGI and LC_{50} results are reported in Table 1. As a positive control we used cisplatin and etoposide at the same concentration.



Scheme 2. Synthesis route of the compounds of series **3** and **4**.

As guidance with regard to selectivity, all of the compounds were further examined for toxicity in a mammary gland cell line derived from non-malignant cells (184B5) and another derived from non-malignant bronchial epithelium cells (BEAS-2B) (Table 2). The analyses were all carried out with a minimum of three independent experiments and values were calculated after 72 h exposure.

In terms of cell line sensitivity, the highest activities were observed in MCF-7 and HTB-54 cell lines with GI_{50} values below 10 μM for eleven and ten compounds, respectively. Different profiles were observed for the compounds attending to cytostatic (mean GI_{50} and mean TGI) and cytotoxic parameters (mean LC_{50}). So, compounds **3a**, **4a** and **7h** displayed a very interesting pattern of selective cytostatic effect against these cell lines. On the other hand, compounds **2o** and **3a** can be classified as cytotoxic agents owing to their narrow difference between cytostatic and cytotoxic parameters, with LC_{50} values below 10 μM.

In this primary screening we included isoselenocyanate derivatives (**6a**–**c**), used as synthetic intermediates, because they have been described as effective antitumor agents for many types of cancer [53].

The following preliminary structure–activity relationships can be drawn for the screened compounds:

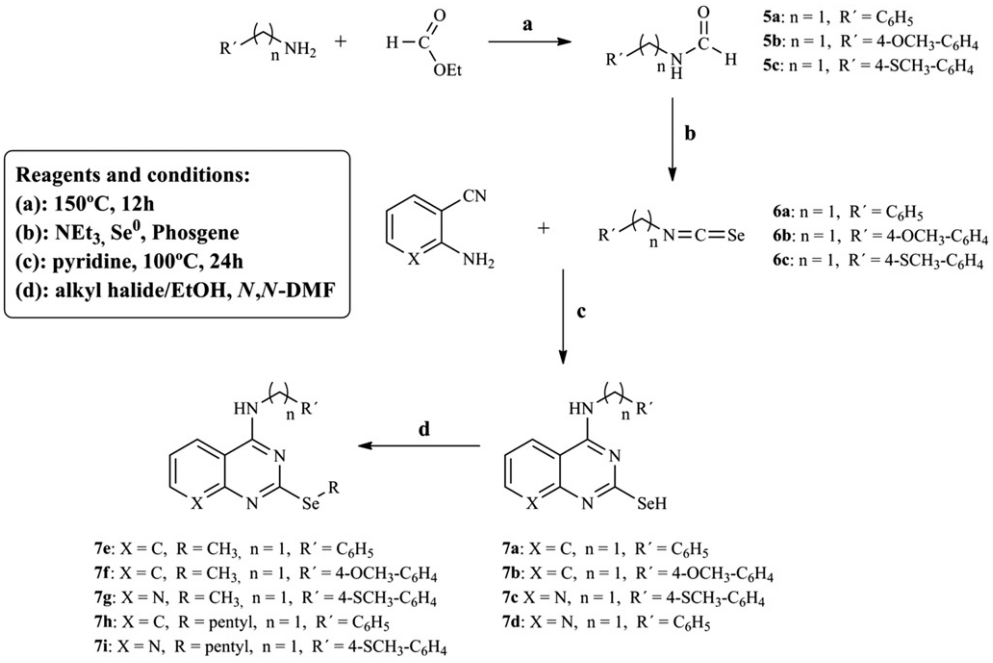
a) As a general trend, the pyridopyrimidines and quinazolines with a SeH group in the positions 2 and 4 usually have better antiproliferative activity than the corresponding analogs with selenoalkyl functionality (e.g. **3a** versus **3c** and **3d**; **3b** versus **3e** and **3f**).
b) In order to evaluate the importance of the long linear hydrophobic chain between the central nucleus and the phenyl ring, we synthesized compounds **2c** ($n = 0$), **2d** ($n = 1$), **2h** ($n = 2$), **2i** ($n = 3$) and **2j** ($n = 4$). The results did not show any correlation between the chain length and the activity of compounds, except for HT-29 − for which an increase in antiproliferative effect was observed from $n = 0$ ($GI_{50} = 65.84$ μM) to $n = 3$ ($GI_{50} = 19.60$ μM).
c) In some cases, replacement of the sulfur unit in derivatives **2** by a selenium fragment in derivatives **7** did not significantly affect the biological activity. Surprisingly, compounds **2a**–**b** showed better activities than their isosteric selenium analogs in several cancer cell lines.

Compared to the activities of cisplatin and etoposide, the antiproliferative effects of our derivatives are cell-line dependent. Among the thirty eight analogs synthesized and examined, thirteen compounds showed GI_{50} values below that of etoposide ($GI_{50} = 19.95$ μM) and six below that of cisplatin ($GI_{50} = 3.16$ μM) in MCF-7. If we consider the HT-29 cell line, twelve were more active than etoposide ($GI_{50} = 31.62$ μM) and five had values below that of cisplatin ($GI_{50} = 7.94$ μM). Finally, compounds **2b**, **2o** and **3a** exhibited better activity than etoposide ($GI_{50} = 12.59$ μM) in CCRF-CEM cell line.

Compounds **2o** and **3a** were the most active, with $LC_{50} < 10$ μM in all the cell lines tested, while the rest of them only exhibited these values in one or two cell lines (Table 1). With regard to selectivity, the cytotoxicity of all compounds described in this work was assayed against two nontumoral lines, one mammary gland cell line (184B5) and one bronchial epithelium line (BEAS-2B). The therapeutic index was calculated from the ratio of GI_{50} on normal cells and tumoral cells. Ratios between 3 and 6 denote moderate selectivity, ratios greater than 6 indicate high selectivity, while compounds that do not fulfill either of these criteria are rated as non-selective [55]. It is important to point out that derivatives **1b**, **3c** and **7h** were highly selective in MCF-7 cells, as evidenced by the ratios of their GI_{50} values ($GI_{50\ 184B5}/GI_{50\ MCF\text{-}7}$ ranged from >12 for **1b**, 12 for **3c** and 1418 for **7h**). The rest of the derivatives showed similar parameters in tumoral and normal cells. On the basis of these results we can conclude that **7h**, due to its potency and selectivity, and **2o** and **3a**, due to their potent cytotoxic effect in all the cell lines, are promising candidates to provide some insight into the molecular mechanisms involved in the antiproliferative activity.

2.2.2. Cell death and effects on cell cycle progression

In the search for a possible mechanism of action for the antitumor activity, we investigated the ability of selected compounds (**2o**, **3a** and **7h**) to induce internucleosomal degradation of genomic DNA (a hallmark of apoptosis) and their effects on the cell cycle. In this



Scheme 3. Synthesis route of the compounds of series **5**, **6** and **7**.

investigation the MCF-7 cell line, i.e. the most sensitive to the tested compounds, was used. The internucleosomal status of the cells was investigated after 24 h of treatment with 1, 5, 10, 15 and 20 μM of the corresponding compound using the *Apo-Direct* kit (BD Pharmingen) [56] based on the TUNEL technique. Camptothecin was used as a positive control. The results obtained are shown in Fig. 3. Regarding cell death, all of the compounds studied, and **3a** in particular, displayed an increase in their capacity to induce DNA degradation and the formation of oligonucleosomal fragments relative to the control (DMSO control) at 5, 10, 15 and 20 μM. These values for **3a** were 1.7-fold at 5 μM to 9-fold at 20 μM higher than those for the control. In order to ascertain whether this effect is time-dependent we checked the induction of cell death for these derivatives at 4, 12, 24 and 48 h with the drug concentration at 10 μM. Cells treated with the selected compounds evidence cell death at 12 h. These compounds induced a time-dependent cell death (Fig. 4). For **3a**, the cell death increased in all time tested although compounds **2o** and **7h** showed a slightly decrease in cell death at 48 h.

Flow cytometry analysis of cell cycle distribution was carried out after treatment of MCF-7 cells for 4, 12 and 24 h with the corresponding compounds (**2o**, **3a** and **7h**) at 10 μM (Table 3). DNA flow cytometric analysis indicated that treatment of the cells with these derivatives did not induce any specific phase arrest of the cell cycle relative to the control.

2.2.3. ADME prediction

As a part of our study, the compliance of compounds to Lipinski's rule of five was evaluated [57]. Although the cytotoxic effects of lead compounds are thought to be primarily due to their ability to modulate cell death, other factors such as solubility, stability and/or efflux properties within the cell may also contribute. The freely accessible program OSIRIS Explorer Properties [58] was used to analyze the results given in Table 4 and it was found that the selected active compounds comply with these rules with the exception of **7h**, which did not comply for *c* Log *P*. However, the reference drug for pharmacological testing (etoposide, *c* Log *P* = 0.53; MW = 588; *n*-OHNH = 3; *n*-ON = 13) did not fulfill two of Lipinski's rules when analyzed with this program.

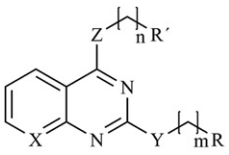
In addition, it is well known that numerous drug candidates have failed during clinical tests because of problems related to ADME (absorption, distribution, metabolism and excretion) properties. A very preliminary computational study designed to predict the absorption properties of our active and selective compounds found for each specific bioassay was performed (PreADMET program). The results are presented in Table 4. Human Intestinal Absorption (HIA) and Caco-2 permeability are good indicators of drug absorbance in the intestine and Caco-2 monolayer penetration, respectively.

Human Intestinal Absorption data are the sum of bioavailability and absorption evaluated from the ratio of excretion or cumulative excretion in urine, bile and feces [59]. The predicted percentages of intestinal absorption are excellent for all of the compounds tested, with values above 98% in all cases. The compounds present good permeability values in Caco-2 cells, ranging from 44 to 56 [60]. Hence, theoretically, all of these compounds should present good passive oral absorption and differences in their bioactivity cannot be attributed to this property.

3. Conclusions

In conclusion, we have synthesized a series of 35 sulfur and selenium quinazoline and pyrido[2,3-*d*]pyrimidine compounds and investigated their abilities to inhibit *in vitro* the proliferation of leukemia (CCRF-CEM), colon (HT-29), lung (HTB-54) and breast (MCF-7) cancer cell lines as well as their effect on two derived from non-malignant cell lines, one mammary (184B5) and one from bronchial epithelium (BEAS-2B). Some derivatives exhibited good antiproliferative activity and in some cases this was higher than that for reference drugs etoposide and cisplatin, mainly in MCF-7. The most promising compounds in these series were **2o** and **3a**, which showed stronger antiproliferative activities against all of cell lines tested, and **7h**, which exhibited good activity against MCF-7 and also displayed weak cytotoxicity to non-malignant cells. In order to elucidate the mechanisms of action, the cytotoxicity cell death status and cell cycle distribution were examined in MCF-7. The three compounds all induced cell death in a time and dose-dependent manner without involving cell cycle perturbation.

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



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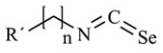


Series 6

Ref.	X	Y	*m*	R	Z	*n*	R′	CCRF-CEM[a]			HT-29[b]			HTB-54[c]			MCF-7[d]		
								GI_{50}[e]	TGI[f]	LC_{50}[g]	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
1b	C	S	4	CH_3	O	0	H	52.13	85.97	>100	74.59	>100	>100	30.40	>100	>100	7.82	81.44	>100
2a	C	S	0	H	NH	1	C_6H_5	82.41	>100	>100	10.14	63.50	>100	15.23	>100	>100	27.23	>100	>100
2b	C	S	0	H	NH	1	4-OCH_3–C_6H_4	10.38	41.01	71.63	6.49	28.63	63.91	13.49	45.53	77.57	7.08	36.47	78.39
2c	C	S	0	CH_3	NH	0	C_6H_5	74.60	>100	>100	65.84	>100	>100	55.80	>100	>100	78.35	>100	>100
2d	C	S	0	CH_3	NH	1	C_6H_5	87.90	>100	>100	36.53	>100	>100	37.87	82.30	>100	41.52	82.81	>100
2e	C	S	0	CH_3	NH	1	4-OCH_3–C_6H_4	93.68	>100	>100	70.18	>100	>100	93.68	>100	>100	30.92	85.14	>100
2f	C	S	0	CH_3	NH	1	4-SCH_3–C_6H_4	>100	>100	>100	34.66	>100	>100	7.22	44.49	89.13	47.29	>100	>100
2g	C	S	0	CH_3	NH	1	4-$SeCH_3$–C_6H_4	63.31	>100	>100	>100	>100	>100	57.01	>100	>100	36.41	>100	>100
2h	C	S	0	CH_3	NH	2	C_6H_5	32.87	72.78	>100	29.17	87.37	>100	50.55	>100	>100	80.29	>100	>100
2i	C	S	0	CH_3	NH	3	C_6H_5	39.49	87.82	>100	19.60	89.55	>100	50.64	>100	>100	77.21	>100	>100
2j	C	S	0	CH_3	NH	4	C_6H_5	46.99	>100	>100	>100	>100	>100	>100	>100	>100	32.03	63.96	95.88
2k	C	S	0	CH_3	NH	5	OH	>100	>100	>100	>100	>100	>100	31.37	71.44	>100	61.95	>100	>100
2l	C	S	4	CH_3	NH	1	4-$SeCH_3$–C_6H_4	>100	>100	>100	14.42	>100	>100	19.79	49.98	80.16	13.04	75.20	>100
2m	N	S	0	CH_3	NH	1	C_6H_5	56.27	98.55	>100	87.14	>100	>100	5.08	51.58	>100	39.42	>100	>100
2n	N	S	0	CH_3	NH	1	4-OCH_3–C_6H_4	>100	>100	>100	>100	>100	>100	>100	>100	>100	48.07	>100	>100
2o	N	S	0	CH_3	NH	1	4-SCH_3–C_6H_4	5.35	16.84	59.03	4.32	7.03	8.75	3.28	14.81	63.57	0.05	4.37	9.13
2p	C	S	0	CH_3	NH	5	OH	>100	>100	>100	63.87	>100	>100	>100	>100	>100	48.92	>100	>100
3a	C	Se	0	H	Se	0	H	2.99	6.01	9.03	8.98	45.22	87.94	5.98	65.88	>100	2.92	22.88	>100
3b	N	Se	0	H	Se	0	H	ND[h]	ND	ND	ND	ND	ND	4.03	7.93	68.35	9.83	46.14	82.74
3c	C	Se	0	CH_3	Se	0	CH_3	31.22	54.85	78.48	7.46	33.76	76.78	26.55	84.24	>100	5.97	50.73	>100
3d	C	Se	4	CH_3	Se	4	CH_3	65.70	>100	>100	>100	>100	>100	>100	>100	>100	38.35	95.13	>100
3e	N	Se	0	CH_3	Se	0	CH_3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3f	N	Se	4	CH_3	Se	4	CH_3	>100	>100	>100	>100	>100	>100	15.23	60.54	>100	40.84	67.72	94.60
4a	C	Se	0	H	NH	0	H	25.66	52.39	79.11	52.19	>100	>100	0.04	3.63	>100	0.15	>100	>100
4b	N	Se	0	H	NH	0	H	30.89	61.47	92.06	36.30	89.58	>100	44.28	96.56	>100	37.27	>100	>100
4c	C	Se	0	CH_3	NH	0	H	65.13	>100	>100	27.63	74.81	>100	17.98	88.69	>100	46.48	>100	>100
4d	C	Se	4	CH_3	NH	0	H	34.77	67.97	>100	40.30	>100	>100	34.62	84.04	>100	28.57	59.47	90.38
4e	N	Se	0	CH_3	NH	0	H	68.44	>100	>100	44.14	77.28	>100	78.21	>100	>100	42.99	>100	>100
4f	N	Se	4	CH_3	NH	0	H	12.93	42.75	72.57	5.91	19.98	62.08	8.78	50.99	98.46	22.67	53.84	85.01
6a								28.72	57.70	86.67	20.96	44.54	68.13	5.98	33.41	66.09	27.89	61.67	95.45
6c								39.30	60.87	82.44	57.49	>100	>100	37.20	>100	>100	0.62	7.55	81.17
6e								25.53	53.83	82.13	48.77	>100	>100	5.32	9.98	76.26	5.77	59.33	>100
7a	C	Se	0	H	NH	1	C_6H_5	>100	>100	>100	62.20	>100	>100	31.22	>100	>100	>100	>100	>100
7b	C	Se	0	H	NH	1	4-OCH_3–C_6H_4	>100	>100	>100	71.64	>100	>100	7.11	60.87	>100	42.73	94.00	>100
7c	C	Se	0	H	NH	1	4-SCH_3–C_6H_4	71.90	>100	>100	42.89	>100	>100	45.52	>100	>100	48.12	>100	>100
7d	N	Se	0	H	NH	1	C_6H_5	30.51	68.93	>100	6.35	33.11	>100	>100	>100	>100	0.03	>100	>100
7e	C	Se	0	CH_3	NH	1	C_6H_5	39.75	61.63	83.50	42.83	80.96	>100	74.58	>100	>100	15.80	67.01	>100C
7f	C	Se	0	CH_3	NH	1	4-OCH_3–C_6H_4	>100	>100	>100	73.63	>100	>100	33.04	>100	>100	42.24	98.61	>100
7g	C	Se	0	CH_3	NH	1	4-SCH_3–C_6H_4	48.71	86.88	>100	72.66	>100	>100	37.88	72.44	>100	54.05	>100	>100
7h	C	Se	4	CH_3	NH	1	C_6H_5	34.48	72.46	>100	72.68	>100	>100	37.73	81.11	>100	0.05	47.13	99.40
7i	C	Se	4	CH_3	NH	1	4-SCH_3–C_6H_4	35.02	83.90	>100	83.59	>100	>100	40.35	81.28	>100	34.73	65.03	95.33
Cisplatin								1.00	79.43	>100	7.94	>100	>100	ND	ND	ND	3.16	>100	>100
Etoposide								12.59	50.12	>100	31.62	>100	>100	ND	ND	ND	19.95	>100	>100

[a] Lymphocytic leukemia.
[b] Colon carcinoma.
[c] Lung carcinoma.
[d] Breast adenocarcinoma.
[e] Concentration that inhibits 50% of cell growth.
[f] Concentration that inhibits 100% of cell growth.
[g] Concentration that kills 50% of cells.
[h] ND: No data.

4. Experimental protocols

4.1. Chemistry

Melting points were determined with a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and have not been corrected. The ^{1}H NMR spectra were recorded on a Bruker 400 Ultrashield™ spectrometer (Rheinstetten, Germany) using TMS as the internal standard. The IR spectra were obtained on a Thermo Nicolet FT–IR Nexus spectrophotometer with KBr pellets. Elemental microanalyses were carried out on vacuum-dried samples using a LECO CHN-900 Elemental Analyzer. Silica gel 60 (0.040–0.063 mm) 1.09385.2500 (Merck KGaA, 64271 Darmstadt, Germany) was used for Column Chromatography and Alugram® SIL G/UV_{254} (Layer:

Table 2
Average GI_{50}, TGI and LC_{50} values (μM) for compounds in 184B5 and BEAS-2B cell lines.

Compound	184B5[a]			BEAS-2B[b]		
	GI_{50}[c] (μM)	TGI[d] (μM)	LC_{50}[e] (μM)	GI_{50}[c] (μM)	TGI[d] (μM)	LC_{50}[e] (μM)
1b	>100	>100	>100	51.43	>100	>100
2a	49.59	>100	>100	66.33	>100	>100
2b	7.83	36.55	69.23	6.13	32.50	70.18
2c	>100	>100	>100	54.75	>100	>100
2d	28.93	>100	>100	38.88	88.24	>100
2e	85.59	>100	>100	21.74	96.24	>100
2f	21.97	>100	>100	22.69	59.11	95.93
2g	70.27	>100	>100	73.47	>100	>100
2h	8.30	46.88	88.58	41.92	94.80	>100
2i	6.77	>100	>100	73.39	>100	>100
2j	28.49	53.55	78.60	6.81	>100	>100
2k	49.86	>100	>100	39.26	99.35	>100
2l	72.96	>100	>100	31.21	>100	>100
2m	49.03	>100	>100	25.88	61.70	97.51
2n	34.23	69.77	>100	58.51	>100	>100
2o	0.19	1.14	5.64	0.60	3.77	8.47
2p	>100	>100	>100	>100	>100	>100
3a	0.49	2.50	6.63	ND[f]	ND	ND
3b	0.71	8.26	67.50	ND	ND	ND
3c	71.48	>100	>100	ND	ND	ND
3d	71.30	>100	>100	>100	>100	>100
3e	ND	ND	ND	ND	ND	ND
3f	52.76	>100	>100	>100	>100	>100
4a	0.69	36.65	80.37	0.08	>100	>100
4b	5.20	31.05	74.98	5.89	87.73	>100
4c	24.40	59.04	93.68	23.61	54.56	85.52
4d	15.34	43.60	71.85	52.25	>100	>100
4e	45.53	87.15	>100	39.68	90.81	>100
4f	10.32	40.23	70.14	16.30	44.89	73.48
6a	15.45	45.15	74.86	4.10	32.75	69.59
6c	35.66	66.30	90.94	11.28	45.39	79.49
6e	5.84	33.97	67.32	7.78	42.34	75.60
7a	20.81	63.63	>100	3.54	>100	>100
7b	71.25	>100	>100	37.39	>100	>100
7c	6.84	86.27	>100	0.41	58.21	>100
7d	3.70	9.13	>100	8.40	>100	>100
7e	3.72	12.41	73.66	0.57	36.96	78.30
7f	75.59	>100	>100	>100	>100	>100
7g	37.12	>100	>100	35.86	78.54	>100
7g	70.90	>100	>100	44.05	>100	>100
7h	57.36	>100	>100	34.72	88.21	>100
Cisplatin	ND	ND	ND	ND	ND	ND
Etoposide	ND	ND	ND	ND	ND	ND

[a] Mammary gland cell culture derived from non-malignant cells.
[b] Bronchial epithelium cell culture derived from non-malignant cells.
[c] Concentration that inhibits 50% of cell growth.
[d] Concentration that inhibits 100% of cell growth.
[e] Concentration that kills 50% of cells.
[f] ND: No data.

0.2 mm) (Macherey–Nagel GmbH & Co. KG. Postfach 101352, D-52313 Düren, Germany) was used for Thin Layer Chromatography. Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma–Aldrich Química, S.A. (Alcobendas, Madrid, Spain), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, Belgium) and Lancaster (Bischheim–-Strasbourg, France). The 4-hydroxy-2-methylthioquinazoline **1a** was prepared according to the literature procedure. Yield: 88%; mp 183–184 °C. IR (KBr) cm^{-1}: 3163 (m, C–H); 3050–2845 (m, C–H); 1700 (m, C=O); 1618 (m, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 3H, SCH_3); 3.38 (br s, 1H, OH); 7.41 (dd, 1H, H_6, $J_{6-5} = 8.2$); 7.53 (d, 1H, H_5), 7.75 (dd, 1H, H_7, $J_{7-8} = 8.0$); 8.03 (d, 1H, H_8). MS (*m*/*z* % abundance): 192 (M^+, 100); 119 (43); 90 (38). Elemental Analysis for $C_9H_8N_2OS$ Calcd/Found (%): C: 56.25/56.14; H: 4.16/4.10; N: 16.66/16.81.

4.1.1. 4-Hydroxy-2-pentylthioquinazoline (**1b**)

The synthetic route previously published by Kane et al. was followed with a slight modification. The corresponding 4-hydroxy-2-mercaptoquinazoline (11.2 mmol) was dissolved in 0.4 N aqueous NaOH (50 mL). A solution of $C_5H_{11}I$ (13.4 mmol) in EtOH (25 mL) was added. The mixture was stirred for 18 h at 70 °C and then neutralized with acetic acid. The resulting precipitate was collected and was used without purification. Yield: 89%; mp 161–162 °C. IR (KBr) cm^{-1}: 3159 (m, C–H); 2954–2862 (m, C–H); 1675 (m, C=O); 1549 (m, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.87 (t, 3H, $(CH_2)_4$–CH_3); 1.35 (m, 4H, $(CH_2)_2$–$(CH_2)_2$–CH_3); 1.69 (q, 2H, CH_2–CH_2–$(CH_2)_2$–CH_3); 3.20 (t, 2H, CH_2–$(CH_2)_3$–CH_3); 7.41 (t, 1H, H_6, $J_{6-5} = 7.7$); 7.50 (d, 1H, H_5); 7.74 (t, 1H, H_7, $J_{7-6} = J_{7-8} = 7.7$); 8.02 (d, 1H, H_8); 12.53 (s, 1H, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.1 (S–$(CH_2)_4$–CH_3); 22.4 (S–$(CH_2)_3$–CH_2–CH_3); 29.2 (S–CH_2–CH_2–$(CH_2)_2$–CH_3); 29.8 (S–$(CH_2)_2$–CH_2–CH_2–CH_3); 31.2 (S–CH_2–$(CH_2)_3$–CH_3); 120.8 (C_9); 125.8 (C_8); 126.9 (C_5); 127.4 (C_7); 135.3 (C_6); 149.3 (C_{10}); 156.9 (C_2); 162.3 (C_4). MS (*m*/*z* % abundance): 248 (M^+, 21); 215 (20); 201 (49); 191 (33); 178 (100); 162 (33); 145 (20); 121 (44); 91 (26). Elemental Analysis for $C_{15}H_{16}N_2OS$ Calcd/Found (%): C: 62.90/62.58; H: 6.45/6.33; N: 11.29/11.21.

4.1.2. General procedure for compounds **2a**–**p**

To a suspension of **A**, **1a**, **1b** or **1c** in $POCl_3$ (20 mL) was added *N*,*N*-dimethylformamide (drops). The mixture was stirred and heated under reflux for 2 h and then concentrated. The solution was then poured into ice water and the aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, concentrated, and the chloro-derivatives were used without purification.

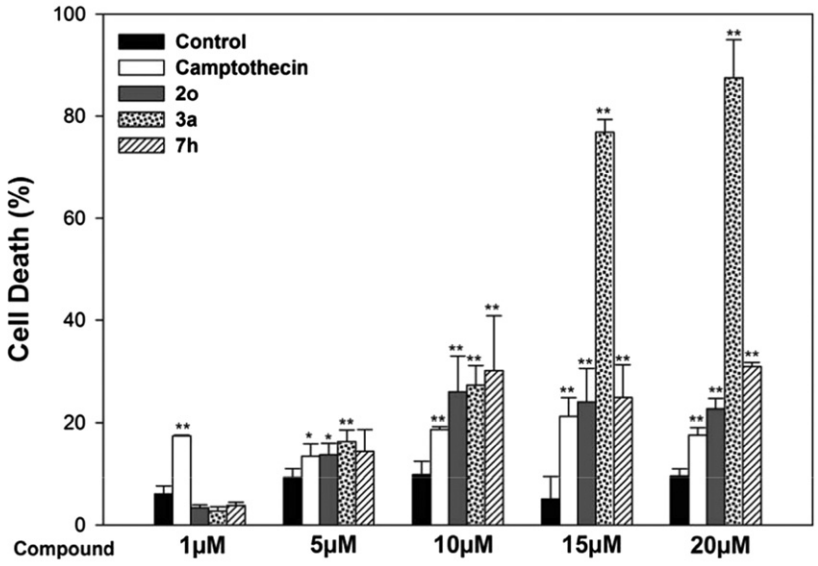
A solution of the corresponding 2-alkylthio/2-mercapto-4-chloroquinazoline or the corresponding pyrido[2,3-*d*]pyrimidine (4.0 mmol) in ethanol (25 mL) was cooled to 4 °C and the corresponding amine (4.4 mmol) was added. The mixture was stirred at room temperature for 5–6 h, heated at 75 °C during 15 h and was concentrated. The residue was treated with water and the precipitate was collected by filtration, washed with Et_2O (3 × 15 mL) and recrystallized from the appropriate solvent.

4.1.3. 4-Benzylamino-2-mercapto-quinazoline (**2a**)

From 4-chloro-2-mercaptoquinazoline and benzylamine. Yield: 6%; mp 208–209 °C. IR (KBr) cm^{-1}: 3204 (m, N–H); 3096 (m, C–H); 2921 (m, C–H); 1615 (m, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.56 (d, 2H, CH_2–NH; $J_{CH2-NH} = 5.6$ Hz); 6.68 (t, 1H, N*H*–CH_2); 7.10 (dt, 1H, H_6, $J_{6-5} = 7.9$ Hz, $J_{6-7} = 7.2$ Hz, $J_{6-8} = 1.1$ Hz); 7.26 (m, 2H, $H_{2'} + H_{6'}$); 7.36 (m, 4H, $H_5 + H_{3'} + H_{4'} + H_{5'}$); 7.56 (dt, 1H, H_7, $J_{7-8} = 8.0$ Hz, $J_{7-5} = 1.6$ Hz); 7.89 (dd, 1H, H_8); 10.92 (s, 1H, S*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 44.8 (CH_2); 118.9 (C_9); 126.8 (C_8); 127.2 (C_5); 127.9 (C_7); 128.5 ($C_{2'} + C_{6'}$); 128.9 ($C_{4'}$); 129.7 ($C_{3'} + C_{5'}$); 134.9 (C_6); 140.1 ($C_{1'}$); 151.3 (C_{10}); 155.3 (C_4); 162.8 (C_2). MS (*m*/*z* % abundance): 267 (M^+, 11); 251 (100); 234 (11); 178 (29); 119 (18); 106 (42); 91 (46). Elemental Analysis for $C_{15}H_{13}N_3S$ Calcd/Found (%): C: 67.41/67.78; H: 4.87/4.97; N: 15.73/15.60.

4.1.4. 2-Mercapto-4-(4′-methoxybenzyl)aminoquinazoline (**2b**)

From 4-chloro-2-mercaptoquinazoline and 4-methoxybenzylamine. Yield: 13%; mp 222–223 °C. IR (KBr) cm^{-1}: 3220 (m, N–H); 3124 (m, C–H); 2962–2833 (m, C–H); 1604 (m, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.75 (m, 4H, $H_2O + NH + OCH_3$); 4.67 (s, 2H, CH_2–NH); 6.85 (d, 2H, $H_{3'} + H_{5'}$, $J_{3'-2'} = J_{5'-6'} = 7.5$ Hz); 7.27 (d, 2H, $H_{2'} + H_{6'}$); 7.68 (m, 1H, H_6); 7.96 (m, 2H, $H_7 + H_5$); 8.54 (d, 1H, H_8, $J_{8-7} = 8.4$ Hz); 10.36 (s, 1H, S*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 44.6 (CH_2); 56.2 (OCH_3); 114.2 ($C_{3'} + C_{5'}$); 116.9 (C_9); 125.5 (C_8); 126.4 (C_5); 126.9 (C_7); 128.9 ($C_{2'} + C_{6'}$); 129.5 (C_6); 135.8 ($C_{1'}$); 151.7 (C_{10}); 156.5 (C_4); 159.3 ($C_{4'}$); 162.9 (C_2). MS (*m*/*z* % abundance): 296



Compound	1μM	5μM	10μM	15μM	20μM
Control	6.12 ± 1.61	9.28 ± 1.71	9.82 ± 2.62	5.13 ± 4.32	9.56 ± 1.38
Camptothecin	17.42** ± 0.15	13.42* ± 2.47	18.67** ± 0.56	21.21** ± 3.62	17.59** ± 1.46
2o	3.40 ± 0.58	13.70* ± 2.30	25.95** ± 7.03	23.95** ± 6.64	22.63** ± 2.04
3a	2.76 ± 0.85	16.32** ± 2.28	27.29** ± 3.86	76.82** ± 2.52	87.55** ± 7.39
7h	3.84 ± 0.65	14.37 ± 4.30	30.13** ± 1.72	24.88** ± 6.41	30.95** ± 0.78

Fig. 3. Effects of **2o**, **3a** and **7h** on cell death in MCF-7 cells at a concentration of 1, 5, 10, 15 and 20 μM for 24 h using the *Apo-Direct* kit (BD Pharmingen) based on the TUNEL technique. The results are presented as the mean ± SD of three independent experiments (duplicate wells). ** = difference statistically very significant with respect to the control ($p < 0.01$).

(M^+, 38); 191 (76); 175 (57); 163 (37); 136 (38); 121 (100); 91 (21); 77 (18). Elemental Analysis for $C_{16}H_{15}N_3OS \cdot 0.3$ HCl Calcd/Found (%): C: 62.34/62.07; H: 4.87/4.82; N: 13.64/13.35.

*4.1.5. 2-Methylthio-4-phenylquinazoline (**2c**)*

From 4-chloro-2-methylthioquinazoline and aniline. Yield: 73%; mp 79–80 °C. IR (KBr) cm^{-1}: 3329 (m, N–H); 2931 (m, C–H); 1563, (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.52 (s, 3H, SCH_3); 7.22 (t, 1H, $H_{4'}$); 7.44 (t, 2H, $H_{3'} + H_{5'}$); 7.58 (t, 1H, H_6, $J_{6-5} = J_{6-7} = 8.0$ Hz); 7.68 (d, 1H, H_5); 7.81 (d, 2H, $H_{2'} + H_{6'}$); 7.87 (t, 1H, H_7, $J_{7-8} = 8.0$ Hz); 8.62 (d, 1H, H_8); 10.51 (br s, 1H, N*H*–Ph). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.3 (*S*CH_3); 113.3 ($C_9 + C_{2'} + C_{6'}$); 118.2 ($C_{4'}$); 124.2 (C_8); 126.3 (C_5); 126.8 (C_7); 129.7 ($C_{3'} + C_{5'}$); 135.3 (C_6); 138.9 ($C_{1'}$); 147.2 (C_{10}); 157.8 (C_4); 167.1 (C_2). MS (*m*/*z* % abundance): 267 (M^+, 100); 220 (74); 129 (9); 77 (12). Elemental Analysis for $C_{15}H_{13}N_3S \cdot 1.6$ HCl Calcd/Found (%): C: 54.70/54.91; H: 4.49/4.62; N: 12.91/12.63.

*4.1.6. 4-Benzylamino-2-methylthioquinazoline (**2d**)*

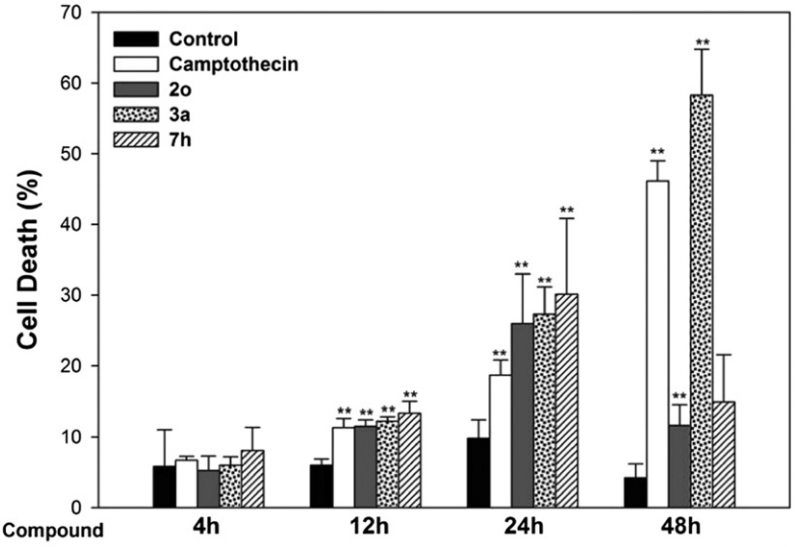
From 4-chloro-2-methylthioquinazoline and benzylamine. Yield: 44%; mp 139–140 °C. IR (KBr) cm^{-1}: 3210 (m, N–H); 3059 (m, C–H); 2973, (m, C–H); 1615–1567 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.49 (s, 3H, SCH_3); 4.79 (d, 2H, CH_2–NH, $J_{-CH2-NH} = 5.8$ Hz); 7.25 (t, 1H, $H_{4'}$, $J_{4'-3} = J_{4'-5} = 7.2$ Hz); 7.33 (t, 2H, $H_{3'} + H_{5'}$, $J_{3'-2'} = J_{5'-6'} = 7.6$ Hz); 7.39 (d, 2H, $H_{2'} + H_{6'}$); 7.46 (dd, 1H, H_6, $J_{6-5} = 8.0$ Hz, $J_{6-7} = 7.1$ Hz); 7.57 (d, 1H, H_5); 7.76 (dd, 1H, H_7, $J_{7-8} = 8.4$ Hz); 8.31 (d, 1H, H_8); 9.42, (br s 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.5 (*S*CH_3); 44.7 (*C*H_2); 117.9 (C_9); 125.7 (C_8); 126.5 (C_5); 127.4 (C_7); 128.3 ($C_{2'} + C_{6'}$); 128.9 ($C_{4'}$); 129.8 ($C_{3'} + C_{5'}$); 134.7 (C_6); 140.5 ($C_{1'}$); 151.2 (C_{10}); 155.4 (C_4); 163.1 (C_2). MS (*m*/*z* % abundance): 281 (M^+, 100); 235 (18); 191 (38); 106 (20); 91 (29); 77 (10). Elemental Analysis for $C_{16}H_{15}N_3S \cdot 0.45$ HCl Calcd/Found (%): C: 64.55/64.77; H: 5.04/5.35; N: 14.12/13.91.

*4.1.7. 4-(4′-Methoxybenzyl)amino-2-methylthioquinazoline (**2e**)*

From 4-chloro-2-methylthioquinazoline and 4-methoxybenzylamine. Yield: 7%; mp 109–110 °C. IR (KBr) cm^{-1}: 3283 (m, N–H); 2929 (m, C–H); 1612, (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.53 (s, 3H, SCH_3); 3.72 (s, 3H, OCH_3); 4.71 (d, 2H, CH_2–NH, $J_{-CH2-NH} = 5.8$ Hz); 6.89 (d, 2H, $H_{3'} + H_{5'}$, $J_{3'-2'} = J_{5'-6'} = 8.6$ Hz); 7.32 (d, 2H, $H_{2'} + H_{6'}$); 7.43 (dd, 1H, H_6, $J_{6-5} = 7.9$ Hz, $J_{6-7} = 7$ Hz); 7.55 (d, 1H, H_5); 7.74 (dd, 1H, H_7, $J_{7-8} = 8.4$ Hz); 8.26 (d, 1H, H_8); 9.24, (br s, 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.3 (*S*CH_3); 44.3 (*C*H_2); 56.5 (OCH_3); 113.3 (C_9); 113.9 ($C_{3'} + C_{5'}$); 124.9 (C_8); 125.8 (C_5); 127.3 (C_7); 129.3 ($C_{2'} + C_{6'}$); 132.2 (C_6); 134.6 ($C_{1'}$); 148.1 (C_{10}); 159.2 ($C_4 + C_{4'}$); 167.5 (C_2). MS (*m*/*z* % abundance): 311 (M^+, 26); 191 (18); 136 (24); 121 (100); 91 (28); 77 (44). Elemental Analysis for $C_{17}H_{17}N_3OS \cdot 0.35$ HCl Calcd/Found (%): C: 63.00/63.09; H: 5.25/5.37; N: 12.97/12.88.

*4.1.8. 4-(4′-Methylthiobenzyl)amino-2-methylthioquinazoline (**2f**)*

From 4-chloro-2-methylthioquinazoline and 4-methylthiobenzylamine. Yield: 38%; mp 119–120 °C. IR (KBr) cm^{-1}: 3226–2922 (m, N–H); 1625–1573 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3H, Ph–SCH_3); 2.54 (s, 3H, SCH_3); 4.71 (d, 2H, CH_2–NH, $J_{CH2-NH} = 5.8$ Hz); 7.23 (d, 2H, $H_{2'} + H_{6'}$, $J_{2'-6'} = J_{3'-5'} = 8.4$ Hz); 7.3 (d, 2H, $H_{3'} + H_{5'}$); 7.51 (dd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 6.7$ Hz); 7.6, (d, 1H, H_5); 7.82 (dd, 1H, H_7, $J_{7-8} = 8.2$ Hz); 8.37, (d, 1H, H_8); 9.87, (br s, 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.3 (het–SCH_3); 15.7 (Ph–SCH_3); 44.7 (*C*H_2); 112.9 (C_9); 123.0 (C_8); 124.7 (C_5); 126.4 (C_7); 126.9 ($C_{3'} + C_{5'}$); 129.1 ($C_{2'} + C_{6'}$); 131.2 (C_6); 136.0 ($C_{1'}$); 137.5



Compound	4h	12h	24h	48h
Control	5.85 ± 5.21	6.02 ± 0.85	9.82 ± 2.62	4.23 ± 1.99
Camptothecin	6.71 ± 0.56	11.36** ± 1.29	18.67** ± 0.56	46.12** ± 2.87
2o	5.26 ± 2.04	11.51** ± 0.92	25.95** ± 7.03	11.66** ± 2.86
3a	6.01 ± 1.20	12.26** ± 0.62	27.29** ± 3.86	58.31** ± 6.47
7h	8.11 ± 3.28	13.39** ± 1.62	30.13** ± 1.72	14.91** ± 6.65

Fig. 4. Compounds **2o**, **3a** and **7h** induce cell death on MCF-7 cells. MCF-7 cells were incubated either with 10 μM of the indicated compound or vehicle (control cells) for 4, 12, 24 and 48 h. The results are presented as the mean ± SD of three independent experiments (duplicate wells). ** = difference statistically very significant with respect to the control ($p < 0.01$).

($C_{4'}$); 146.5 (C_{10}); 159.2 (C_4); 166.9 (C_2). MS (*m*/*z* % abundance): 327 (M^+, 51); 190 (34); 175 (15); 152 (24); 137 (100); 121 (46); 103 (24); 91 (16); 77 (16). Elemental Analysis for $C_{17}H_{17}N_3S_2 \cdot 0.85$ HCl Calcd/Found (%): C: 56.98/56.81; H: 4.77/5.24; N: 11.73/11.71.

Table 3
Effects of **2o**, **3a** and **7h** on cell cycle distribution in MCF-7 cells. Exponentially growing cells were treated with 15 μM of the indicated compound or vehicle (control) for 24 h. The changes of cell cycle phase distribution were assessed by DNA flow cytometric analysis. Results are expressed as percentages of total cell counts. Camptothecin was used as a positive control. Results are presented as the mean ± SEM of three independent experiments (duplicate wells). $^{**}p < 0.01$ with respect the control.

Compound	Time	SubG$_1$	G$_0$/G$_1$	S	G$_2$/M
Control	4 h	5.90 ± 1.36	49.38 ± 4.74	19.09 ± 4.54	21.60 ± 5.37
	12 h	2.17 ± 0.24	57.34 ± 1.64	17.07 ± 4.00	22.11 ± 3.86
	24 h	2.93 ± 0.54	51.83 ± 5.64	12.39 ± 1.54	32.37 ± 7.73
2o	4 h	3.88 ± 1.75	50.42 ± 3.14	18.02 ± 1.88	23.88 ± 4.93
	12 h	2.24 ± 0.19	55.42 ± 2.22	19.35 ± 1.22	22.99 ± 3.06
	24 h	2.97 ± 0.27	52.91 ± 7.36	11.04 ± 1.32	30.20 ± 2.14
3a	4 h	3.98 ± 0.51	52.09 ± 4.47	23.29 ± 4.33	21.21 ± 5.58
	12 h	2.37 ± 0.53	56.21 ± 1.31	18.36 ± 1.39	25.63 ± 1.76
	24 h	3.46 ± 1.80	47.77 ± 3.69	15.75 ± 1.29	34.67 ± 5.42
7h	4 h	4.42 ± 0.19	46.24 ± 2.72	16.66 ± 1.33	28.50 ± 2.78
	12 h	2.13 ± 0.25	55.30 ± 1.93	17.01 ± 1.77	25.83 ± 5.28
	24 h	3.28 ± 0.18	46.97 ± 8.28	12.20 ± 0.82	34.79 ± 2.14
Camptothecin	4 h	5.39 ± 0.36	47.24 ± 2.81	18.36 ± 0.91	23.38 ± 5.00
	12 h	3.06** ± 0.19	59.50 ± 1.14	16.24 ± 2.14	20.21 ± 5.19
	24 h	3.05** ± 0.12	58.52 ± 1.15	15.27 ± 2.28	20.25 ± 5.45

4.1.9. 2-Methylthio-4-(4′-methylselenobenzyl)aminoquinazoline (**2g**)

From 4-chloro-2-methylthioquinazoline and 4-methylselenobenzylamine. Yield: 26%; mp 110–112 °C. IR (KBr) cm^{-1}: 3204 (m, N–H); 3096 (m, C–H); 2921 (m, C–H); 1615 (m, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.31 (s, 3H, Se–C*H*$_3$); 2.46 (d, 3H, S–C*H*$_3$; $J_{SCH3-CH2} = 5.7$ Hz); 4.71 (dd, 2H, C*H*$_2$–Ph; $J_{CH2-NH} = 12.0$ Hz); 7.29 (dt, 1H, H$_6$); 7.40 (m, 4H, CH$_2$–*Ph*); 7.53 (dd, 1H, H$_5$); 7.70 (dt, 1H, H$_7$); 8.22 (dd, 1H, H$_8$); 8.94 (m, 1H, N*H*–CH$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 7.5 (SeCH$_3$); 14.3 (SCH$_3$); 44.2 (CH$_2$); 113.7 (C$_9$); 123.8 (C$_8$); 125.6 (C$_5$); 126.9 (C$_7$); 129.6 (C$_{3'}$ + C$_{5'}$); 130.4 (C$_{2'}$ + C$_{6'}$); 131.0 (C$_6$); 135.0 (C$_{1'}$); 139.2 (C$_{4'}$); 150.6 (C$_{10}$); 159.3 (C$_4$); 167.7 (C$_2$). MS (*m*/*z* % abundance): 375 (M^+, 34); 281 (10); 220 (34); 200 (35); 185 (100); 176 (46); 162 (43); 146 (34); 129 (20); 121 (96); 91 (64); 77 (26). Elemental Analysis for $C_{17}H_{17}N_3SSe$ Calcd/Found (%): C: 54.54/55.00; H: 4.54/4.51; N: 11.23/10.91.

Table 4
Physico-chemical and absorption properties for the most active compounds.

Compounds	*c* Log *P*	MW	*n*-OHNH donors	*n*-ON acceptors	Lipinski's violations	Absorption	
						HIA (%)	Caco-2 (nm/sec)
2o	3.34	328	1	6	0	99.12	44.04
3a	0.12	290	2	4	0	100	46.72
7h	6.2	396	1	4	1	98.47	56.03

c Log *P*, logarithm of compound partition coefficient between *n*-octanol and water; MW, molecular weight; *n*-OHNH, number of hydrogen bond donors; *n*-ON, number of hydrogen bond acceptors; HIA, Human Intestinal Absorption; Caco-2, cells derived from human colon adenocarcinoma.

4.1.9.1. Procedure for the preparation of 4-methylselenobenzylamine

4.1.9.1.1. Preparation of 4-methylselenobenzaldehyde. A solution of $NaBH_4$ (22.20 mmol) in ethanol (30 mL) was added to a mixture of dimethyl diselenide (10.60 mmol) and ethanol (30 mL) under an N_2 atmosphere and the mixture was stirred at room temperature for 20 min. Benzaldehyde (20.14 mmol) was added and the mixture was heated at 150 °C during 2 h. The reaction mixture was evaporated and extracted with dichloromethane (3 × 20 mL), the organic phase was washed with water and dried over Na_2SO_4 and concentrated to dryness. The residue was used without purification. A yellowish liquid (2.84 g, 95%) was obtained. IR (KBr) cm^{-1}: 3052 (m, C–H), 1694 (s, C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.45 (s, 3H, SeCH_3); 7.51 (d, 2H, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.3$ Hz); 7.76 (dd, 2H, $H_2 + H_6$, $J_{2\text{-COH}} = J_{6\text{-COH}} = 0.4$ Hz); 9.96 (s, 1H, CO*H*). Elemental Analysis for C_8H_8OSe Calcd/Found (%): C: 48.24/48.12; H: 4.02/3.89.

4.1.9.1.2. Preparation of (E)-4-(methylseleno)benzaldehyde oxime. To a mixture of 4-methylselenobenzaldehyde (2.84 g, 14.27 mmol) and ethanol (30 mL) was added Na_2CO_3 (1.74 g, 4.16 mmol) in water (10 mL). Hydroxylamine hydrochloride (1.50 g, 2.14 mmol) in water (15 mL) was added slowly to the mixture and the solution was stirred at room temperature for 24 h. The reaction mixture was evaporated and washed with water, filtered and dried. The residue was recrystallized from ethanol to give a white solid (2.56 g). Yield: 83.2%; mp 185–186 °C. IR (KBr) cm^{-1}: 3285 (s, O–H); 2972–2929 (m, C–H); 1590 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.37 (s, 3H, SeCH_3); 7.41 (dd, 2H, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 6.8$ Hz, $J_{3\text{-Se}CH3} = J_{5\text{-Se}CH3} = 1.7$ Hz); 7.49 (dd, 2H, $H_2 + H_6$, $J_{2\text{-}CHN} = J_{6\text{-}CHN} = 1.4$ Hz); 8.09 (s, 1H, C*H*=N); 11.25 (s, 1H, O*H*). Elemental Analysis for C_8H_8ONSe Calcd/Found (%): C: 44.86/44.65; H: 4.20/4.24; N: 6.54/6.42.

4.1.9.1.3. 4-Methylselenobenzylamine. To a mixture of (*E*)-4-(methylseleno)benzaldehyde oxime (0.52 g, 2.43 mmol) in ethanol (50 mL) was added zinc (1.75 g, 27.00 mmol) in water (10 mL). 10% Hydrochloric acid (10 mL) was added slowly with vigorous agitation and the mixture was heated under reflux for 12 h. The mixture was cooled to room temperature and 20% NaOH (10 mL) was added and the product was extracted with dichloromethane (3 × 20 mL). The organic phase was washed with water, dried over Na_2SO_4 and concentrated to dryness. The residue was used without purification. A yellowish liquid (0.24 g, 49%) was obtained. IR (KBr) cm^{-1}: 3272 (m, N–H); 3003 (m, C–H); 2923 (m, C–H); 1589 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.32 (s, 3H, SeCH_3); 3.44 (br s, 2H, $H_2O + NH_2$); 3.68 (s, 2H, CH_2–NH_2); 7.25 (d, 2H, $H_3 + H_5$; $J_{3-2} = J_{5-6} = 8.0$ Hz); 7.35 (d, 2H, $H_2 + H_6$); Elemental Analysis for $C_8H_{11}NSe$ Calcd/Found (%): C: 48.00/48.25; H: 5.50/5.34; N: 7.00/6.79.

*4.1.10. 2-Methylthio-4-(2-phenylethyl)aminoquinazoline (**2h**)*

From 4-chloro-2-methylthioquinazoline and 2-phenylethylamine. Yield: 43%; mp 60–61 °C. IR (KBr) cm^{-1}: 3248 (m, N–H); 3063–3024 (m, C–H); 2925 (m, C–H); 1563 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.59 (s, 3H, SCH_3); 2.99 (t, 2H, CH_2–CH_2–Ph); 3.79 (m, 2H, CH_2–CH_2–Ph); 7.26 (m, 5H, Ph); 7.46, (t, 1H, H_6, $J_{6-5} = J_{6-7} = 8.0$ Hz); 7.57 (d, 1H, H_5); 7.77 (t, 1H, H_7, $J_{7-8} = 8.0$ Hz); 8.27 (d, 1H, H_8); 9.21, (br s, 1H, N*H*–CH_2–CH_2–Ph). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.9 (SCH_3); 35.0 (NH–CH_2–CH_2–Ph); 43.7 (NH–CH_2–CH_2–Ph); 113.1 (C_9); 124.3 (C_8); 124.9 (C_5); 126.3 (C_7); 129.0 ($C_{4'}$); 129.5 ($C_{2'} + C_{6'}$); 129.9 ($C_{3'} + C_{5'}$); 134.9 (C_6); 140.0 ($C_{1'}$); 147.7 (C_{10}); 159.2 (C_4); 167.2 (C_2). MS (*m*/*z* % abundance): 295 (M^+, 37); 204 (45); 191 (100); 175 (27); 160 (18); 145 (27); 129 (14); 91 (17). Elemental Analysis for $C_{17}H_{17}N_3S \cdot 0.8$ HCl Calcd/Found (%): C: 62.89/62.92; H: 5.83/5.59; N: 12.80/12.95.

*4.1.11. 2-Methylthio-4-(3-phenylpropyl)aminoquinazoline (**2i**)*

From 4-chloro-2-methylthioquinazoline and 3-phenylpropylamine. Yield: 73%; mp 117–118 °C. IR (KBr) cm^{-1}: 3234 (m, N–H); 3054 (m, C–H); 2928 (m, C–H); 1568, (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 1.96 (m, 2H, NH–CH_2–CH_2–CH_2–Ph); 2.47 (s, 3H, S–CH_3); 2.68 (m, 2H, NH–$(CH_2)_2$–CH_2–Ph); 3.53 (m, 2H, NH–CH_2–$(CH_2)_2$–Ph); 7.23 (m, 5H, Ph); 7.38 (t, 1H, H_6, $J_{6-5} = J_{6-7} = 7.7$ Hz); 7.52 (d, 1H, H_5); 7.69 (t, 1H, H_7, $J_{7-8} = 7.7$ Hz); 8.18 (d, 1H, H_8); 8.43 (br s, 1H, N*H*–$(CH_2)_3$–Ph). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.2 (SCH_3); 30.9 (NH–CH_2–CH_2–CH_2–Ph); 33.5 (NH–$(CH_2)_2$–CH_2–Ph); 41.2 (NH–CH_2–$(CH_2)_2$–Ph); 113.7 (C_9); 123.8 (C_8); 124.7 (C_5); 126.9 (C_7); 128.3 ($C_{4'}$); 128.7 ($C_{2'} + C_{6'}$); 129.6 ($C_{3'} + C_{5'}$); 134.9 (C_6); 140.5 ($C_{1'}$); 148.2 (C_{10}); 159.4 (C_4); 167.7 (C_2). MS (*m*/*z* % abundance): 309 (M^+, 43); 218 (20); 205 (100); 191 (21); 159 (26); 91 (25). Elemental Analysis for $C_{18}H_{19}N_3S \cdot 0.1$ HCl Calcd/Found (%): C: 69.08/68.93; H: 6.12/6.48; N: 13.43/13.15.

*4.1.12. 2-Methylthio-4-(4-phenylbutyl)aminoquinazoline (**2j**)*

From 4-chloro-2-methylthioquinazoline and 4-phenylbutylamine. Yield: 60%; mp 80–81 °C. IR (KBr) cm^{-1}: 3206 (m, N–H); 3080 (m, C–H); 2929 (m, C–H); 1569 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 1.70 (m, 4H, NH–CH_2–$(CH_2)_2$–CH_2–Ph); 2.49 (m, 2H, NH–$(CH_2)_3$–CH_2–Ph); 2.65 (s, 3H, SCH_3); 3.73 (m, 2H, NH–CH_2–$(CH_2)_3$–Ph); 7.21 (m, 5H, Ph); 7.60 (t, 1H, H_6, $J_{6-5} = J_{6-7} = 8.0$ Hz); 7.71 (d, 1H, H_5); 7.91 (t, 1H, H_7, $J_{7-8} = 8.0$ Hz); 8.53 (d, 1H, H_8); 10.39 (br s, 1H, N*H*–$(CH_2)_4$–Ph). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.1 (SCH_3); 28.3 (NH–$(CH_2)_2$–CH_2–CH_2–Ph); 29.2 (NH–CH_2–CH_2–$(CH_2)_2$–Ph); 35.6 (NH–$(CH_2)_3$–CH_2–Ph); 42.4 (NH–CH_2–$(CH_2)_3$–Ph); 112.0 (C_9); 123.3 (C_8); 124.9 (C_5); 127.2 (C_7); 127.7 ($C_{4'}$); 128.6 ($C_{2'} + C_{6'}$); 129.6 ($C_{3'} + C_{5'}$); 135.2 (C_6); 140.1 ($C_{1'}$); 148.8 (C_{10}); 158.8 (C_4); 165.8 (C_2). MS (*m*/*z* % abundance): 323 (M^+, 100); 276 (30); 218 (91); 205 (32); 191 (41); 175 (23); 145 (29); 91 (38). Elemental Analysis for $C_{19}H_{21}N_3S \cdot 1.65$ HCl Calcd/Found (%): C: 59.49/59.40; H: 6.91/6.30; N: 10.95/10.50.

*4.1.13. 4-(5-Hydroxypentyl)amino-2-methylthioquinazoline (**2k**)*

From 4-chloro-2-methylthioquinazoline and 5-hydroxy-1-pentylamino. Yield: 33%; mp 140–141 °C. IR (KBr) cm^{-1}: 3348 (br s, O–H); 2979–2929 (m, C–H); 1625–1579 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 1.37–1.41 (m, 2H, NH–CH_2–CH_2–CH_2); 1.44–1.49 (m, 2H, NH–CH_2–CH_2–CH_2–CH_2); 1.61–1.66 (m, 2H, –CH_2–CH_2–OH); 2.50 (s, 3H, SCH_3,); 3.38–3.42 (m, 2H, NH–CH_2, $J_{NH-CH2} = 5.8$ Hz); 3.48–3.52 (m, 2H, CH_2OH); 4.38 (t, 1H, CH_2O*H*); 7.37 (t, 1H, H_6); 7.51, (d, 1H, H_5); 7.68 (t, 1H, H_7); 8.16 (d, 1H, H_8); 8.31, (t, 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.2 (SCH_3); 24.0 (NH–$(CH_2)_2$–CH_2–$(CH_2)_2$–OH); 29.2 (NH–CH_2–CH_2–$(CH_2)_3$–OH); 33.1 (NH–$(CH_2)_3$–CH_2–CH_2–OH); 41.4 (NH–CH_2–$(CH_2)_4$–OH); 61.5 (NH–$(CH_2)_4$–CH_2–OH); 113.8 (C_9); 123.8 (C_8); 124.4 (C_5); 126.9 (C_7); 134.6 (C_6); 150.5 (C_{10}); 159.4 (C_4); 167.8 (C_2). MS (*m*/*z* % abundance): 205 (81); 191 (100); 175 (56); 160 (64); 145 (68); 129 (37); 103 (40); 91 (18); 77 (10). Elemental Analysis for $C_{14}H_{19}N_3SO \cdot 0.1$ HCl Calcd/Found (%): C: 59.86/59.78; H: 6.77/6.60; N: 14.96/14.84.

*4.1.14. 2-Pentylthio-4-(4′-methylselenobenzyl)aminoquinazoline (**2l**)*

From 4-chloro-2-pentylthioquinazoline and 4-methylselenobenzylamine. Yield: 17%; mp 179–180 °C. IR (KBr) cm^{-1}: 3287 (m, N–H); 3111 (m, C–H); 2967–2899 (m, C–H); 1595 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.82 (t, 3H, $(CH_2)_4$–CH_3); 1.22 (m, 4H, $(CH_2)_2$–$(CH_2)_2$–CH_3); 1.56 (q, 2H, CH_2–CH_2–$(CH_2)_2$–CH_3); 2.32 (s, 3H, SeCH_3); 3.11 (t, 2H, CH_2–$(CH_2)_3$–CH_3); 4.82 (br s, 2H, CH_2– Ph); 7.28 (d, 2H, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.1$ Hz); 7.37 (d, 2H, $H_2 + H_6$); 7.54 (t, 1H, H_6, $J_{6-5} = J_{6-7} = 7.9$ Hz); 7.62 (d, 1H, H_5); 7.84 (t, 1H, H_7,

$J_{7-8} = 7.9$ Hz); 8.46 (d, 1H, H_8); 10.11 (s, 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 7.3 (Se*C*H_3); 14.5 (S–(CH_2)$_4$–*C*H_3); 22.5 (S–(CH_2)$_3$–*C*H_2–CH_3); 29.2 (S–CH_2–*C*H_2–(CH_2)$_2$–CH_3); 29.9 (S–(CH_2)$_2$–*C*H_2–CH_2–CH_3); 31.2 (S–*C*H_2–(CH_2)$_3$–CH_3); 44.1 (*C*H_2); 112.8 (C_9); 124.8 (C_8); 125.5 (C_5); 126.9 (C_7); 129.3 ($C_{3'} + C_{5'}$); 130.8 ($C_{2'} + C_{6'}$); 135.4 (C_6); 136.7 ($C_{1'}$); 138.2 ($C_{4'}$); 147.5 (C_{10}); 159.3 (C_4); 166.4 (C_2). MS (*m*/*z* % abundance): 430 (M^+, 100); 384 (26); 361 (57); 200 (11); 185 (76); 170 (31); 91 (12). Elemental Analysis for $C_{21}H_{25}N_3SSe \cdot 0.3$ HI Calcd/Found (%): C: 53.80/53.75; H: 5.34/4.76; N: 8.60/8.40.

4.1.15. 4-Benzylamino-2-methylthiopyrido[2,3-d]pyrimidine (**2m**)

From 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and benzylamine. Yield: 94%; mp 194–195 °C. IR (KBr) cm^{-1}: 3289 (m, N–H); 2922 (m, C–H); 1568 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.46 (s, 3H, S*C*H_3); 4.76 (d, 2H, *C*H_2–NH, $J_{CH2-NH} = 5.1$ Hz); 7.25–7.39 (m, 5H, Ph); 7.43 (dd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 4.4$ Hz); 8.66 (dd, 1H, H_5, $J_{5-7} = 1.8$ Hz); 8.88 (dd, 1H, H_7); 9.21, (t, 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.4 (S*C*H_3); 44.9 (*C*H_2); 108.5 (C_{10}); 120.5 (C_6); 128.2 ($C_{4'}$); 128.6 ($C_{2'} + C_{6'}$); 129.6 ($C_{3'} + C_{5'}$); 133.6 (C_5); 139.6 ($C_{1'}$); 156.3 (C_7); 159.1 (C_9); 160.4 (C_4); 172.0 (C_2). MS (*m*/*z* % abundance): 282 (M^+, 39); 235 (11); 191 (40); 145 (19%); 103 (68%); 91 (100%); 77 (36%). Elemental Analysis for $C_{15}H_{14}N_4S$ Calcd/Found (%): C: 63.83/63.52; H: 4.96/5.15; N: 19.86/19.68.

4.1.16. 4-(4′-Methoxybenzyl)amino-2-methylthiopyrido[2,3-d]pyrimidine (**2n**)

From 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and 4-methoxybenzylamine. Yield: 30%; mp 169–170 °C. IR (KBr) cm^{-1}: 3284 (m, N–H); 2929 (C–H); 1575 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, S*C*H_3); 3.72 (s, 3H, O*C*H_3); 4.67 (d, 2H, *C*H_2–NH, $J_{CH2-NH} = 5.7$ Hz); 6.90 (d, 2H, $H_{3'} + H_{5'}$, $J_{3'-2'} = J_{5'-6'} = 8.7$ Hz); 7.31 (d, 2H, $H_{2'} + H_{6'}$); 7.41 (dd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 4.4$ Hz); 8.65 (dd, 1H, H_5, $J_{5-7} = 1.9$ Hz); 8.85, (dd, 1H, H_7); 9.11 (t, 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.4 (S*C*H_3); 44.3 (*C*H_2); 55.9 (O*C*H_3); 108.5 (C_{10}); 114.6 ($C_{3'} + C_{5'}$); 120.9 (C_6); 129.7 ($C_{2'} + C_{6'}$); 131.5 ($C_{1'}$); 133.5 (C_5); 156.3 (C_7); 159.2 ($C_4 + C_9$); 160.3 ($C_{4'}$); 172.0 (C_2). MS (*m*/*z* % abundance): 312 (M^+, 13); 191 (21); 136 (22); 121 (100); 103 (58); 91 (31); 77 (36). Elemental Analysis for $C_{16}H_{16}N_4OS$ Calcd/Found (%): C: 61.54/61.31; H: 5.13/5.43; N: 17.94/18.35.

4.1.17. 4-(4′-Methylthiobenzyl)amino-2-methylthiopyrido[2,3-d]pyrimidine (**2o**)

From 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and 4-methylthiobenzylamine. Yield: 4%; mp 157–158 °C. IR (KBr) cm^{-1}: 3229 (m, N–H); 2921 (m, C–H); 1579 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3H, Ph–S*C*H_3); 2.48 (s, 3H, het–S*C*H_3); 4.71 (d, 2H, *C*H_2–NH, $J_{CH2-NH} = 5.4$ Hz); 7.18 (d, 2H, $H_{3'} + H_{5'}$, $J_{3'-2'} = J_{5'-6'} = 8.6$ Hz); 7.33 (d, 2H, $H_{2'} + H_{6'}$); 7.46 (dd, 1H, H_6, $J_{6-5} = 4.4$ Hz, $J_{6-7} = 8.3$ Hz); 8.68 (dd, 1H, H_5, $J_{5-7} = 1.8$ Hz); 8.78 (dd, 1H, H_7); 9.25 (t, 1H, N*H*–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.9 (het–S*C*H_3); 16.2 (Ph–S*C*H_3); 44.6 (*C*H_2); 103.4 (C_{10}); 121.9 (C_6); 126.3 ($C_{3'} + C_{5'}$); 127.5 ($C_{2'} + C_{6'}$); 131.1 ($C_{1'}$); 133.4 (C_5); 138.6 ($C_{4'}$); 156.6 (C_7); 158.8 (C_9); 159.5 (C_4); 171.8 (C_2). MS (*m*/*z* % abundance): 326 (M^+, 39); 282 (26); 191 (49); 137 (64); 121 (89); 103 (100); 91 (86); 77 (54). Elemental Analysis for $C_{16}H_{16}N_4S_2 \cdot 0.45$ HCl Calcd/Found (%): C: 55.64/55.45; H: 4.64/4.99; N: 16.26/16.50.

4.1.18. 4-(5-Hydroxypentyl)amino-2-methylthiopyrido[2,3-d]pyrimidine (**2p**)

From 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and 5-hydroxy-1-pentylamine. Yield: 14%; mp 135–136 °C. IR (KBr) cm^{-1}: 3302 (m, N–H); 2933 (m, C–H); 1600–1577 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.36–1.38 (m, 2H, NH–CH_2–C*H*$_2$–CH_2); 1.41–1.49 (m, 2H, NH–CH_2–CH_2–C*H*$_2$–CH_2); 1.60–1.68 (m, 2H, C*H*$_2$–CH_2–OH); 2.51 (s, 3H, SC*H*$_3$); 3.38–3.42 (m, 2H, NH–C*H*$_2$); 3.49–3.56 (m, 2H, C*H*$_2$OH); 4.36 (t, 1H, CH_2–N*H*, $J_{CH2-NH} = 5.1$ Hz); 7.39 (dd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 4.4$ Hz); 8.57 (t, 1H, CH_2O*H*, $J_{CH2-OH} = 5.3$ Hz); 8.61 (dd, 1H, H_5, $J_{5-7} = 1.9$ Hz); 8.85 (dd, 1H, H_7). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.7 (S*C*H_3); 24.2 (NH–(CH_2)$_2$–*C*H_2–(CH_2)$_2$–OH); 29.8 (NH–CH_2–*C*H_2–(CH_2)$_3$–OH); 32.9 (NH–(CH_2)$_3$–*C*H_2–CH_2–OH); 42.3 (NH–*C*H_2–(CH_2)$_4$–OH); 62.3 (NH–(CH_2)$_4$–*C*H_2–OH); 102.4 (C_{10}); 121.6 (C_6); 132.9 (C_5); 156.1 (C_7); 158.8 (C_9); 159.6 (C_4); 171.2 (C_2). MS (*m*/*z* % abundance): 281 (M^+, 51); 191 (31); 160 (30); 103 (50); 91 (100); 77 (44). Elemental Analysis for $C_{13}H_8N_4OS$ Calcd/Found (%): C: 56.11/56.66; H: 6.47/6.63; N: 20.14/20.03.

4.1.19. General procedure for the preparation **3a** and **b**

To a solution of 2,4-dichloroquinazoline or 2,4-dichloropyrido[2,3-*d*]pyrimidine (3.7 mmol) in ethanol (50 mL) was added selenourea (9.85 mmol). The reaction mixture was heated under reflux for 5 h. The solid was filtered off, washed with water (3 × 15 mL) and recrystallized from the appropriate solvent.

4.1.19.1. 2,4-Dihydroselenoquinazoline (**3a**).

From 2,4-dichloroquinazoline and selenourea. Yield: 75%; mp >300 °C. IR (KBr) cm^{-1}: 3104 (m, C–H); 2941 (m, C–H); 1611 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.35–7.42 (m, 2H, $H_5 + H_6$); 7.80 (t, 1H, H_7, $J_{7-8} = J_{7-6} = 7.9$ Hz); 8.31 (d, 1H, H_8); 13.75 (s, 1H, $SeH_{(4)}$); 14.55 (s, 1H, $SeH_{(2)}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 124.6 (C_9); 125.3 (C_8); 127.4 (C_5); 128.9 (C_7); 135.4 (C_6); 149.3 (C_{10}); 164.5 (C_4); 175.8 (C_2). MS (*m*/*z* % abundance): 289 (M^+, 48); 209 (47); 160 (52); 129 (100); 103 (38); 80 (32). Elemental Analysis for $C_8H_6N_2Se_2$ Calcd/Found (%): C: 42.86/43.12; H: 3.12/3.42; N: 18.75/18.94.

4.1.19.2. 2,4-Dihydroselenopyrido[2,3-d]pyrimidine (**3b**).

From 2,4-dichloropyrido[2,3-*d*]pyrimidine and selenourea. Yield: 46%; mp >300 °C. IR (KBr) cm^{-1}: 3044 (m, C–H); 1545 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.39 (ddd, 1H, H_6, $J_{6-5} = 8.0$ Hz, $J_{6-7} = 4.7$ Hz, $J_{6\text{-}SeH(4)} = 0.5$); 8.55 (ddd, 1H, H_5, $J_{5-7} = 1.9$ Hz, $J_{5\text{-}SeH(4)} = 0.6$ Hz); 8.83 (ddd,1H, H_7, $J_{7\text{-}SeH(4)} = 0.6$ Hz); 14.18 (s, 1H, $SeH_{(4)}$); 14.69 (s, 1H, $SeH_{(2)}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.7 (C_{10}); 121.8 (C_6); 131.4 (C_5); 147.6 (C_7); 155.7 (C_9); 156.3 (C_4); 163.6 (C_2). MS (*m*/*z* % abundance): 290 (M^+, 24); 210 (26); 160 (48); 130 (52); 103 (100); 91 (33); 77 (67); 59 (37); 50 (32). Elemental Analysis for $C_7H_5N_3Se_2 \cdot 1.35$ HCl Calcd/Found (%): C: 24.79/24.42; H: 1.87/1.59; N: 12.39/12.38.

4.1.20. General procedure for the preparation **3c–f**

To a solution of the corresponding 2,4-dihydroselenoquinazoline or 2,4-dihydroselenopyrido[2,3-*d*]pyrimidine (2.8 mmol) in 0.4 N NaOH (40 mL) was added the corresponding alkyl iodide (6.11 mmol) and the mixture was heated under reflux for 24 h. The reaction mixture was cooled and acetic acid was added to give pH 5–6. The precipitate was filtered off and recrystallized.

4.1.20.1. 2,4-Dimethylselenoquinazoline (**3c**).

From 2,4-dihydroselenoquinazoline and methyl iodide. Yield: 61%; mp 61–63 °C. IR (KBr) cm^{-1}: 3160 (m, C–H); 2982–2858 (m, C–H); 1608 (s, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.53 (s, 3H, $SeCH_{3(4)}$); 2.56 (s, 3H, $SeCH_{3(2)}$); 7.61 (ddd, 1H, H_7, $J_{7-8} = 8.2$ Hz, $J_{7-6} = 7.0$ Hz, $J_{7-5} = 1.2$ Hz); 7.77 (dd, 1H, H_8, $J_{8-6} = 1.2$ Hz); 7.93 (ddd, 1H, H_6, $J_{6-5} = 7.9$ Hz); 7.95 (dd, 1H, H_5). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 6.7 ($SeCH_{3(4)}$); 7.6 ($SeCH_{3(2)}$); 124.2 (C_9); 126.1 (C_8); 127.9 (C_5); 129.4 (C_7); 135.7 (C_6); 148.7 (C_{10}); 164.6 (C_4); 170.8 (C_2). MS (*m*/*z* % abundance): 318 (M^+, 100); 303 (32); 238 (36); 223 (79); 208 (44); 143 (67); 129 (58); 121 (21); 103 (47); 95 (36); 77 (20); 51 (8).

Elemental Analysis for $C_{10}H_{10}N_2Se_2$ Calcd/Found (%): C: 37.97/37.86; H: 3.16/3.03; N: 8.86/8.72.

*4.1.20.2. 2,4-Dipentylselenoquinazoline (**3d**).* From 2,4-dihydroselenoquinazoline and pentyl iodide. Yield: 45%; oil. IR (KBr) cm^{-1}: 3156 (m, C–H); 2987–2862 (m, C–H); 1638 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.88 (t, 6H, $(CH_2)_4$–$CH_{3(2,4)}$); 1.38 (m, 8H, $(CH_2)_2$–$(CH_2)_2$–$CH_{3(2,4)}$); 1.79 (q, 4H, CH_2–CH_2–$(CH_2)_2$–$CH_{3(2,4)}$); 3.24 (t, 2H, CH_2–$(CH_2)_3$–$CH_{3(2,4)}$); 3.34 (t, 2H, CH_2–$(CH_2)_3$–$CH_{3(2,4)}$); 7.60 (ddd, 1H, H_6, $J_{6-5} = 8.1$ Hz, $J_{6-7} = 7.0$ Hz, $J_{6-8} = 1.2$ Hz); 7.74 (dd, 1H, H_5, $J_{5-7} = 1.2$ Hz); 7.89–7.93, (m, 2H, $H_7 + H_8$). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.1 (Se–$(CH_2)_4$–$CH_{3(2,4)}$); 22.5 (Se–$(CH_2)_3$–CH_2–$CH_{3(2,4)}$); 26.6 (Se–CH_2–CH_2–$(CH_2)_2$–$CH_{3(2,4)}$); 30.2 (Se–CH_2–$(CH_2)_3$–$CH_{3(2,4)}$); 32.3 (Se–$(CH_2)_2$–CH_2–CH_2–$CH_{3(2,4)}$); 124.3 (C_9); 126.1 (C_8); 127.8 (C_5); 129.7 (C_7); 135.7 (C_6); 149.0 (C_{10}); 164.6 (C_4); 171.8 (C_2). MS (*m/z* % abundance): 429 (M^+, 16); 360 (26); 290 (33); 209 (61); 145 (25); 129 (100); 103 (36); 69 (39); 55 (28). Elemental Analysis for $C_{18}H_{26}N_2Se_2$ Calcd/Found (%): C: 50.47/50.42; H: 6.07/6.28; N: 6.54/6.19.

*4.1.20.3. 2,4-Dimethylselenopyrido[2,3-d]pyrimidine (**3e**).* From 2,4-dihydroselenopyrido[2,3-*d*]pyrimidine and methyl iodide. Yield: 23%; mp 298–299 °C. IR (KBr) cm^{-1}: 1592 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.55 (s, 3H, Se$CH_{3(4)}$); 2.59 (s, 3H, S$CH_{3(2)}$); 7.61 (dd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 4.4$ Hz); 8.44 (dd, 1H, H_5); 9.11 (dd, 1H, H_7). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 9.1 (Se**C**$H_{3(4)}$); 9.6 (Se$CH_{3(2)}$); 121.1 (C_{10}); 121.9 (C_6); 131.0 (C_5); 148.5 (C_7); 155.0 (C_9); 156.4 (C_4); 157.8 (C_2). MS (*m/z* % abundance): 324 (M^+, 16); 254 (50); 179 (30); 156 (32); 140 (100); 127 (51); 116 (75); 76 (44); 50 (23). Elemental Analysis for $C_9H_9N_3Se_2 \cdot 0.05$ HI Calcd/Found (%): C: 33.39/33.08; H: 2.80/2.74; N: 12.99/13.05.

*4.1.20.4. 2,4-Dipentylselenopyrido[2,3-d]pyrimidine (**3f**).* From 2,4-dihydroselenopyrido[2,3-*d*]pyrimidine and pentyl iodide. Yield: 19%; oil. IR (KBr) cm^{-1}: 1594 (m, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.86 (t, 6H, $(CH_2)_4$–$CH_{3(2,4)}$); 1.35 (m, 8H, $(CH_2)_2$–$(CH_2)_2$–$CH_{3(2,4)}$); 1.70 (q, 4H, CH_2–CH_2–$(CH_2)_2$–$CH_{3(2,4)}$); 3.19 (t, 2H, CH_2–$(CH_2)_3$–$CH_{3(4)}$); 3.38 (t, 2H, CH_2–$(CH_2)_3$–$CH_{3(2)}$); 6.57 (dd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 4.2$ Hz); 7.76 (dd, 1H, H_5, $J_{5-7} = 1.7$ Hz); 8.40 (dd, 1H, H_7). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.2 (Se–$(CH_2)_4$–$CH_{3(2,4)}$); 22.5 (Se–$(CH_2)_3$–CH_2–$CH_{3(2,4)}$); 27.0 (Se–CH_2–CH_2–$(CH_2)_2$–$CH_{3(2,4)}$); 30.3 (Se–CH_2–$(CH_2)_3$–$CH_{3(2,4)}$); 32.1 (Se–$(CH_2)_2$–CH_2–CH_2–$CH_{3(2,4)}$); 121.4 (C_{10}); 122.3 (C_6); 131.4 (C_5); 148.4 (C_7); 155.4 (C_9); 156.9 (C_4); 160.2 (C_2). MS (*m/z* % abundance): 430 (M^+, 34); 287 (100); 179 (45); 156 (32); 103 (23); 77 (31). Elemental Analysis for $C_{17}H_{25}N_3Se_2$ Calcd/Found (%): C: 47.55/47.62; H: 5.83/5.62; N: 9.79/9.85.

*4.1.21. General procedure for compounds **4a** and **b***

A suspension of 2,4-dichloroquinazoline or 2,4-dichloropyrido[2,3-*d*]pyrimidine (5 mmol) in 25% ammonia (30 mL) was heated under reflux for 1.5 h. The resulting precipitate was filtered off and washed with water (4 × 15 mL). The corresponding intermediate 4-amino-2-chloroquinazoline or pyrido[2,3-*d*]pyrimidine was used without purification and was treated with selenourea in ethanol (20 mL) in a stoichiometric ratio of 1:1.2, respectively. The mixture was heated during 4 h and then cooled. The resulting precipitate was filtered off and recrystallized.

*4.1.21.1. 4-Amino-2-hydroselenoquinazoline (**4a**).* From 4-amino-2-chloroquinazoline and selenourea. Yield: 16%; mp 280–282 °C. IR (KBr) cm^{-1}: 3345 (m, N–H); 3141 (m, C–H); 1669 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.69 (ddd, 1H, H_6); 8.00 (m, 2H, $H_5 + H_6$); 8.44 (d, 1H, H_8); 9.48 (s, 1H, NH_2); 9.28 (s, 1H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 116.6 (C_9); 123.8 (C_8); 126.7 (C_5); 128.9 (C_7); 133.8 (C_6); 149.1 (C_{10}); 161.8 (C_4); 173.1 (C_2). MS (*m/z* % abundance): 225 (M^+, 15); 191 (24); 137 (79); 121 (83); 103 (34); 91 (100); 77 (53); 69 (58); 57 (84). Elemental Analysis for $C_8H_7N_3Se$ Calcd/Found (%): C: 42.86/43.12; H: 3.12/3.42; N: 18.75/18.94.

*4.1.21.2. 4-Amino-2-hydroselenopyrido[2,3-d]pyrimidine (**4b**).* From 4-amino-2-chloropyrido[2,3-*d*]pyrimidine and selenourea. Yield: 68%; mp >300 °C. IR (KBr) cm^{-1}: 3337–3278 (m, N–H); 3152 (m, C–H); 1647 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.40 (m, 1H, H_6); 8.51 (d, 1H, H_5); 8.73 (m, 3H, $H_7 + NH_2$); 13.17 (s, 1H, Se*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 106.4 (C_{10}); 121.1 (C_6); 134.8 (C_5); 153.2 (C_7); 156.2 (C_9); 159.0 (C_4); 181.7 (C_2). MS (*m/z* % abundance): 235 (M^+, 14); 191 (27); 137 (88); 121 (77); 103 (38); 91 (100); 77 (54); 65 (43); 55 (59). Elemental Analysis for $C_7H_6N_4Se \cdot 0.25$ HCl Calcd/Found (%): C: 35.84/35.80; H: 2.56/2.97; N: 23.90/24.15.

*4.1.22. General procedure for compounds **4c–f***

To a solution of the corresponding **4a** or **4b** (4 mmol) in ethanol (25 mL) was added the corresponding alkyl iodide (6 mmol) and the mixture was heated under reflux for 1.5 h. The solvent was removed in vacuo. The residue was treated with water (30 mL) and extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The corresponding solids were purified in the appropriate solvent.

*4.1.22.1. 4-Amino-2-methylselenoquinazoline (**4c**).* From **4a** and methyl iodide. Yield: 4%; mp 213–214 °C. IR (KBr) cm^{-1}: 3386–3306 (m, N–H); 3125 (m, C–H); 2927 (m, C–H); 1646 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.40 (s, 3H, SeCH_3); 7.40 (ddd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 7.0$ Hz, $J_{6-8} = 1.3$ Hz); 7.52 (dd, 1H, H_5, $J_{5-7} = 1.2$ Hz); 7.71 (ddd, 1H, H_7, $J_{7-8} = 8.2$ Hz); 7.89 (s, 2H, NH_2); 8.14 (dd, 1H, H_8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 8.1 (SeCH_3); 116.3 (C_9); 122.3 (C_8); 126.4 (C_5); 128.5 (C_7); 134.1 (C_6); 149.6 (C_{10}); 161.3 (C_4); 163.1 (C_2). MS (*m/z* % abundance): 239 (M^+, 64); 159 (100); 145 (64); 129 (30); 117 (36); 91 (31). Elemental Analysis for $C_9H_9N_3Se$ Calcd/Found (%): C: 45.38/45.60; H: 3.78/3.78; N: 17.65/17.39.

*4.1.22.2. 4-Amino-2-pentylselenoquinazoline (**4d**).* From **4a** and pentyl iodide. Yield: 4%; mp 92–94 °C. IR (KBr) cm^{-1}: 3312 (m, N–H); 3135 (m, C–H); 2949–2862 (m, C–H); 1646 (f, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.88 (t, 3H, $(CH_2)_4$–CH_3); 1.36 (m, 4H, $(CH_2)_2$–$(CH_2)_2$–CH_3); 1.76 (q, 2H, CH_2–CH_2–$(CH_2)_2$–CH_3); 3.13 (t, 2H, CH_2–$(CH_2)_3$–CH_3); 7.38 (ddd, 1H, H_6, $J_{6-5} = 8.2$ Hz, $J_{6-7} = 7.0$ Hz, $J_{6-8} = 0.8$ Hz); 7.49 (dd, 1H, H_5, $J_{5-7} = 1.0$ Hz); 7.70, (ddd, 1H, H_7, $J_{7-8} = 8.0$ Hz); 7.83 (br s, 2H, NH_2); 8.12 (dd, 1H, H_8). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.5 (Se–$(CH_2)_4$–CH_3); 21.7 (Se–$(CH_2)_3$–CH_2–CH_3); 26.6 (Se–CH_2–CH_2–$(CH_2)_2$–CH_3); 30.4 (Se–CH_2–$(CH_2)_3$–CH_3); 32.5 (Se–$(CH_2)_2$–CH_2–CH_2–CH_3); 113.7 (C_9); 124.7 (C_8); 127.5 (C_5); 129.8 (C_7); 133.9 (C_6); 150.9 (C_{10}); 161.5 (C_4); 165.3 (C_2). MS (*m/z* % abundance): 295 (M^+, 27); 252 (24); 239 (44); 225 (90); 214 (49); 159 (58); 145 (100); 129 (28); 121 (81); 103 (24); 91 (37). Elemental Analysis for $C_{13}H_{17}N_3Se$ Calcd/Found (%): C: 53.07/53.50; H: 5.78/5.71; N: 14.28/13.88.

*4.1.22.3. 4-Amino-2-methylselenopyrido[2,3-d]pyrimidine (**4e**).* From **4b** and methyl iodide. Yield: 47%; mp 279–280 °C. IR (KBr) cm^{-1}: 3347–3304 (m, N–H); 3120 (m, C–H); 2928 (m, C–H); 1647 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.49 (s, 3H, SeCH_3); 7.58 (dd, 1H, H_6, $J_{6-5} = 8.0$ Hz, $J_{6-7} = 4.5$ Hz); 8.71 (dd, 1H, H_5, $J_{5-7} = 1.5$ Hz); 8.90 (br s, 2H, NH_2); 8.92 (dd, 1H, H_7). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 6.7 (SeCH_3); 108.4 (C_{10}); 121.0 (C_6); 134.3 (C_5); 156.7 (C_7); 159.3 (C_9); 161.4 (C_4); 169.9 (C_2). MS (*m/z* % abundance): 253 (M^+, 6); 240 (45); 191 (24); 160 (100); 145 (42);

137 (29); 121 (49); 103 (60); 91 (64); 76 (53); 64 (29); 55 (42). Elemental Analysis for $C_8H_8N_4Se \cdot 0.1$ HI Calcd/Found (%): C: 38.14/38.33; H: 3.18/3.27; N: 22.25/22.40.

*4.1.22.4. 4-Amino-2-pentylselenopyrido[2,3-d]pyrimidine (**4f**).* From **4b** and pentyl iodide. Yield: 73%; mp 183–184 °C. IR (KBr) cm^{-1}: 2946–2917 (m, C–H); 1610 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.87 (t, 3H, $(CH_2)_4-CH_3$); 1.35 (m, 4H, $(CH_2)_2-(CH_2)_2-CH_3$); 1.77 (q, 2H, $CH_2-CH_2-(CH_2)_2-CH_3$); 3.16 (t, 2H, $CH_2-(CH_2)_3-CH_3$); 7.75 (dd, 1H, H_6, $J_{6-5} = 8.0$ Hz, $J_{6-7} = 4.2$ Hz); 8.23 (br s, 2H, NH_2); 8.60 (dd, 1H, H_5, $J_{5-7} = 1.2$ Hz); 8.87 (dd, 1H, H_7). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.7 ($Se-(CH_2)_4-CH_3$); 22.6 ($Se-(CH_2)_3-CH_2-CH_3$); 27.2 ($Se-CH_2-CH_2-(CH_2)_2-CH_3$); 30.4 ($Se-CH_2-(CH_2)_3-CH_3$); 32.0 ($Se-(CH_2)_2-CH_2-CH_2-CH_3$); 108.3 ($C_{10}$); 121.2 ($C_6$); 135.0 ($C_5$); 156.0 ($C_7$); 159.3 ($C_9$); 162.4 ($C_4$); 170.9 ($C_2$). MS (*m*/*z* % abundance): 296 (M^+, 26); 226 (100); 215 (48); 146 (49); 121 (79); 103 (47). Elemental Analysis for $C_{12}H_{16}N_4Se \cdot 0.25$ HI Calcd/Found (%): C: 44.04/44.39; H: 4.89/5.09; N: 17.12/16.85.

*4.1.23. Preparation of 4-methoxybenzylformamide **5b***

To a stirred solution of 4-methoxybenzylamine (3.93 mL, 30 mmol) was added ethyl formate (2.45 mL, 24.2 mmol). The reaction mixture was stirred and heated at 150 °C for 12 h. The reaction mixture was cooled to room temperature and the precipitate was collected by filtration, dried and washed with ethyl ether to give **5b** as a white solid (4.4 g). Yield: 88%; mp 79–80 °C; IR (KBr) cm^{-1}: 3286 (s, N–H); 3012 (m, C–H); 2943–2834 (m, C–H); 1645 (s, C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.73 (s, 3H, OCH_3); 4.22 (d, 2H, CH_2-NH); 6.89 (d, 2H, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.5$ Hz); 7.19 (d, 2H, $H_2 + H_6$); 8.1 (s, 1H, $H-C=O$); 8.43 (s, 1H, CH_2-NH); Elemental Analysis for $C_9H_{11}NO_2$ Calcd/Found (%): C: 65.45/65.07; H: 6.66/6.37; N: 8.48/8.29.

*4.1.24. Preparation of 4-methylthiobenzylformamide **5c***

To a stirred solution of 4-methylthiobenzylamine (4.23 g, 27.60 mmol) was added dropwise ethyl formate (2.15 mL, 29.00 mmol). The reaction mixture was stirred at 150 oC for 12 h. The reaction mixture was cooled to room temperature and the precipitate was collected by filtration, dried and washed with ethyl ether to give **5c** as a white solid (4.4 g). Yield: 85%; mp 87–89 °C; IR (KBr) cm^{-1}: 3282 (s, N–H); 3068 (m, C–H); 2918–2884 (m, C–H); 1652 (s, C=O). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.45 (s, 3H, SCH_3); 4.25 (d, 2H, CH_2-NH); 7.25 (m, 4H, $H_2 + H_3 + H_5 + H_6$); 8.12 (s, 1H, $H-C=O$); 8.49 (s, 1H, CH_2-NH); Elemental Analysis for $C_9H_{11}NOS$ Calcd/Found (%): C: 59.7/59.83; H: 6.08/5.81; N: 7.73/7.60.

*4.1.25. Preparation of benzylisoselenocyanate **6a***

To a cold mixture of **5a** (1.35 g, 10.0 mmol) and triethylamine (5.56 mL, 40.00 mmol) in dry toluene (50 mL) was added selenium powder (1.18 g, 15.00 mmol). A solution of phosgene (1.57 mL, 15.00 mmol) in toluene at 25% (15 mL) was added dropwise over a period of 1 h. After the addition was complete, the mixture was heated under reflux for 24 h. The mixture was cooled and filtered, and the solvent was evaporated to yield the crude product, which was purified by silica gel column chromatography (hexane 100%) to afford 0.23 g (12%) of **6a** as a viscous oil. IR (KBr) cm^{-1}: 2142 (s, N=C=Se). ^1H NMR (400 MHz, DMSO-d_6) δ: 5.08 (s, 2H, CH_2-Ph); 7.34 (m, 3H, $H_3 + H_4 + H_5$); 7.43 (d, 2H, $H_2 + H_6$, $J_{2-3} = J_{6-5} = 6.8$ Hz); Elemental Analysis for C_8H_7NSe Calcd/Found (%): C: 48.98/49.33; H: 3.57/3.96; N: 7.14/7.00.

*4.1.26. Preparation of 4-methoxybenzylisoselenocyanate **6b***

To a mixture of **5b** (4.40 g, 26.00 mmol) and triethylamine (14.84 mL, 104.00 mmol) in dry toluene (50 mL) was added selenium powder (3.16 g, 40.00 mmol). A solution of phosgene (4.2 mL, 42.00 mmol) in toluene at 25% (15 mL) was added dropwise over a period of 1 h. After the addition was complete, the mixture was heated under reflux and worked up as described above for **6a**. The crude residue was purified by silica gel column chromatography (EtOAc/hexane 5:95) to give 0.85 g (15%) of **6b** as an oil. IR (KBr) cm^{-1}: 2927 (m, C–H); 2139 (s, –N=C=Se); ^1H NMR (400 MHz, DMSO-d_6) δ: 3.73 (s, 3H, OCH_3); 3.88 (s, 2H, CH_2-Ph); 6.88 (d, 2H, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.7$ Hz); 7.17 (d, 2H, $H_2 + H_6$); Elemental Analysis for C_9H_9NOSe Calcd/Found (%): C: 47.79/47.51; H: 3.98/4.22; N: 6.19/6.09.

*4.1.27. Preparation of 4-methylthiobenzylisoselenocyanate **6c***

To a mixture of **5c** (4.25 g, 23.50 mmol) and triethylamine (13.10 mL, 94.00 mmol) in dry toluene (50 mL) was added selenium powder (2.78 g, 35.00 mmol). A solution of phosgene (3.70 mL, 35.00 mmol) in toluene at 25% (15 mL) was added dropwise over a period of 1 h. After the addition was complete, the mixture was heated under reflux and worked up as described above for **6a**. The crude residue was purified by silica gel column chromatography (EtOAc/hexane 5:95) to give 0.85 g (15%) of **6c** as an oil. IR (KBr) cm^{-1}: 2927 (m, C–H); 2139 (s, N=C=Se); ^1H NMR (400 MHz, DMSO-d_6) δ: 3.73 (s, 3H, OCH_3); 3.88 (s, 2H, CH_2-Ph); 6.88 (d, 2H, $H_3 + H_5$, $J_{3-2} = J_{5-6} = 8.7$ Hz); 7.17 (d, 2H, $H_2 + H_6$); Elemental Analysis for C_9H_9NOSe Calcd/Found (%): C: 47.79/47.51; H: 3.98/4.22; N: 6.19/6.09.

*4.1.28. General procedure for compounds **7a–d***

The corresponding isoselenocyanate (2.40 mmol) was added over 30 min to a stirred solution of 2-aminobenzonitrile or 2-amino-3-cyanopyridine (2.40 mmol) in dry pyridine (30 mL). After the addition was complete, the solution was stirred for 24 h at 100 oC. The solution was filtered in order to remove the selenium powder and then poured onto crushed ice and extracted with dichloromethane (4×15 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The corresponding solids were purified from the appropriate solvent.

*4.1.28.1. 4-Benzylamino-2-hydroselenoquinazoline (**7a**).* From 2-aminobenzonitrile and **6a**. Yield: 31%; mp 193–194 °C. IR (KBr) cm^{-1}: 3259 (m, N–H); 3172–3120 (m, C–H); 2934 (m, C–H); 1615 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 5.98 (br s, 2H, CH_2-NH); 7.21 (t, 1H, H_6); 7.29 (m, 5H, Ph); 7.40 (d, 1H, H_5); 7.60 (t, 1H, H_7); 8.09 (d, 1H, H_8,); 9.62 (s, 1H, $NH-CH_2$); 12.68 (br s, 1H, Se*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 53.9 (CH_2); 116.1 (C_9); 124.8 (C_8); 125.9 (C_5); 126.4 (C_7); 127.7 ($C_{4'}$); 128.1 ($C_{2'} + C_{6'}$); 129.3 ($C_{3'} + C_{5'}$); 134.2 (C_6); 137.6 ($C_{1'}$); 149.7 (C_{10}); 155.4 (C_4); 175.2 (C_2). MS (*m*/*z* % abundance): 315 (M^+, 13); 235 (30); 145 (25); 129 (34); 117 (23); 103 (36); 91 (100); 77 (29); 65 (34); 51 (24). Elemental Analysis for $C_{15}H_{13}N_3Se$ Calcd/Found (%): C: 57.32/56.88; H: 4.14/4.15; N: 13.37/12.96.

*4.1.28.2. 2-Hydroseleno-4-(4′-methoxybenzyl)aminoquinazoline (**7b**).* From 2-aminobenzonitrile and **6b**. Yield: 8%; mp 197–198 °C. IR (KBr) cm^{-1}: 3263 (m, N–H); 3172–3128 (m, C–H); 2924 (m, C–H); 1605 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 3.70 (s, 3H, OCH_3); 5.93 (br s, 2H, CH_2-NH); 6.84 (d, 2H, $H_{3'} + H_{5'}$, $J_{3'-2'} = J_{5'-6'} = 8.4$ Hz); 7.29 (t, 2H, H_6, $J_{6-5} = J_{6-7} = 8.1$ Hz); 7.33 (d, 2H, $H_{2'} + H_{6'}$); 7.38 (d, 1H, H_5); 7.58 (t, 1H, H_7, $J_{7-8} = 8.0$ Hz); 8.08 (d, 1H, H_8); 9.63 (s, 1H, $NH-CH_2$); 12.66, (s, 1H, Se*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 53.1 (CH_2); 55.6 (OCH_3); 114.1 ($C_{3'} + C_{5'}$); 116.2 (C_9); 123.5 (C_8); 124.9 (C_5); 125.8 (C_7); 129.7 ($C_{2'} + C_{6'}$); 134.5 (C_6); 136.1 ($C_{1'}$); 149.2 (C_{10}); 153.4 (C_4); 159.3 ($C_{4'}$); 175.2 (C_2). MS (*m*/*z* % abundance): 345 (M^+, 7); 264 (14); 121 (100); 91 (26); 77 (24). Elemental Analysis for $C_{16}H_{15}N_3OSe$ Calcd/Found (%): C: 55.81/55.67; H: 4.36/4.36; N: 12.21/12.32.

*4.1.28.3. 2-Hydroseleno-4-(4′-methylthiobenzyl)aminoquinazoline (**7c**).* From 2-aminobenzonitrile and **6c**. Yield: 63%; mp 170–172 °C. IR (KBr) cm^{-1}: 3250 (m, N–H); 3016 (C–H); 2963 (m, C–H); 1617 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.48 (s, 3H, SCH_3); 5.90 (br s, 2H, CH_2–NH); 7.19 (d, 2H, $H_{2'} + H_{6'}$); 7.3 (m, 3H, $H_6 + H_{3'} + H_{5'}$); 7.4 (d, 1H, H_5, $J_{5-6} = 8.1$ Hz); 7.6 (t, 1H, H_7, $J_{7-6} = J_{7-8} = 7.8$ Hz); 8.14 (d, 1H, H_8); 9.63 (s, 1H, NH–CH_2); 12.65, (s, 1H, SeH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 15.8 (SCH_3); 53.2 (CH_2); 116.1 (C_9); 126.2 (C_8); 126.9 (C_5); 127.3 (C_7); 128.3 ($C_{3'} + C_{5'}$); 129.5 ($C_{2'} + C_{6'}$); 134.4 (C_6); 135.6 ($C_{1'}$); 136.3 ($C_{4'}$); 149.3 (C_{10}); 154.9 (C_4); 175.3 (C_2). MS (*m*/*z* % abundance): 360 (M^+, 4); 281 (59); 235 (17); 191 (39); 175 (21); 161 (24); 137 (47); 106 (48); 121 (100); 91 (100); 77 (38). Elemental Analysis for $C_{16}H_{15}N_3SSe$ Calcd/Found (%): C: 42.10/42.20; H: 3.29/3.56; N: 9.21/9.20.

*4.1.28.4. 4-Benzylamino-2-hydroselenopyrido[2,3-d]pyrimidine (**7d**).* From 2-amino-3-cyanopyridine and **6a**. Yield: 7%; mp 225–227 °C. IR (KBr) cm^{-1}: 3339 (m, N–H); 3080–3012 (m, C–H); 2940 (m, C–H); 1637 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 6.00 (br s, 2H, CH_2–NH); 7.20–7.38 (m, 6H, H_6 + Ph); 8.53 (d, 1H, H_5); 8.58 (d, 1H, H_7); 9.90 (s, 1H, NH–CH_2); 13.10 (s, 1H, SeH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 53.3 (CH_2); 108.9 (C_{10}); 121.9 (C_6); 126.6 ($C_{4'}$); 127.1 ($C_{2'} + C_{6'}$); 128.6 ($C_{3'} + C_{5'}$); 131.0 ($C_{1'}$); 134.6 (C_5); 153.7 (C_7); 156.8 (C_9); 157.3 (C_4); 175.0 (C_2). MS (*m*/*z* % abundance): 327 (M^+, 9); 236 (27); 137 (23); 103 (31); 91 (100); 77 (30). Elemental Analysis for $C_{14}H_{12}N_4Se$ Calcd/Found (%): C: 53.23/52.91; H: 3.81/4.02; N: 17.71/17.54.

*4.1.29. General procedure for compounds **7e–i***

The appropriate alkyl iodide was added during 25 min to a solution of the corresponding 4-benzylamino-2-hydroseleno-quinazoline derivative **7a–c** (1.27 mmol) in ethanol (30 mL) and DMF (4 mL). The mixture was heated at 70 °C during 1.5 h. The solvent was removed in vacuo. The resulting residue was washed with water (3 × 25 mL) and recrystallized from the appropriate solvent.

*4.1.29.1. 4-Benzylamino-2-methylselenoquinazoline (**7e**).* From **7a** and methyl iodide. Yield: 31%; mp 195–196 °C. IR (KBr) cm^{-1}: 3309 (m, N–H); 3186–3038 (m, C–H); 1651 (m, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.57 (s, 3H, SeCH_3); 5.58 (br s, 2H, CH_2–NH); 7.35 (m, 5H, Ph); 7.74 (t, 1H, H_7); 7.79 (d, 1H, H_8); 8.06, (t, 1H, H_6); 8.53 (d, 1H, H_5); 10.06 (s, 1H, NH–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.3 (SeCH_3); 53.5 (CH_2); 113.8 (C_9); 125.8 (C_8); 126.4 (C_5); 127.1 (C_7); 127.9 ($C_{4'}$); 128.4 ($C_{2'} + C_{6'}$); 128.9 ($C_{3'} + C_{5'}$); 134.8 (C_6); 138.7 ($C_{1'}$); 147.2 (C_{10}); 155.6 (C_4); 157.8 (C_2). MS (*m*/*z* % abundance): 327 (M^+, 25); 314 (23); 234 (27); 207 (21); 191 (20); 137 (53); 121 (24); 103 (44); 91 (100); 77 (37). Elemental Analysis for $C_{16}H_{15}N_3Se \cdot HI$ Calcd/Found (%): C: 42.10/42.20; H: 3.29/3.56; N: 9.21/9.20.

*4.1.29.2. 4-(4′-Methoxybenzyl)amino-2-methylselenoquinazoline (**7f**).* From **7b** and methyl iodide. Yield: 31%; mp 190–191 °C. IR (KBr) cm^{-1}: 3329 (m, N–H); 3175–3058 (m, C–H); 1658 (m, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.58 (s, 3H, SeCH_3); 3.72 (s, 3H, OCH_3); 5.94 (br s, 2H, CH_2–NH); 6.80 (d, 2H, $H_{3'} + H_{5'}$, $J_{3'-2'} = J_{5'-6'} = 8.4$ Hz); 7.23 (t, 1H, H_7, $J_{7-8} = J_{7-6} = 8.0$ Hz); 7.31 (d, 2H, $H_{2'} + H_{6'}$); 7.42 (d, 1H, H_8); 7.61 (t, 1H, H_6, $J_{6-5} = 8.0$ Hz); 8.18, (d, 1H, H_5); 9.68 (s, 1H, NH–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.1 (Se–CH_3); 52.8 (CH_2); 55.8 (OCH_3); 114.2 ($C_{3'} + C_{5'}$); 116.2 (C_9); 124.9 (C_8); 125.8 (C_5); 126.4 (C_7); 129.4 ($C_{2'} + C_{6'}$); 134.7 (C_6); 135.7 ($C_{1'}$); 149.9 (C_{10}); 152.9 (C_4); 158.8 ($C_{4'}$); 161.2 (C_2). MS (*m*/*z* % abundance): 359 (M^+, 45); 191 (20); 91 (100); 77 (47). Elemental Analysis for $C_{17}H_{17}N_3OSe$ Calcd/Found (%): C: 56.98/56.82; H: 4.75/4.87; N: 11.73/11.52.

*4.1.29.3. 4-(4′-Methylthiobenzyl)amino-2-methylselenoquinazoline (**7g**).* From **7c** and methyl iodide. Yield: 50%; mp 160–161 °C. IR (KBr) cm^{-1}: 3245 (m, N–H); 3045 (C–H); 2960 (m, C–H); 1634 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 2.45 (s, 3H, SeCH_3); 2.52 (s, 3H, SCH_3); 5.46 (br s, 2H, CH_2–NH); 7.24 (s, 4H, $H_{2'} + H_{3'} + H_{5'} + H_{6'}$); 7.6 (t, 1H, H_6, $J_{6-5} = J_{6-7} = 7.8$ Hz); 7.62 (d, 1H, H_5); 7.86 (t, 1H, H_7, $J_{7-8} = 8$ Hz); 8.37 (d, 1H, H_8); 9.69 (s, 1H, –NH–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 11.5 (SeCH_3); 15.7 (SCH_3); 53.4 (CH_2); 116.5 (C_9); 126.3 (C_8); 126.9 (C_5); 127.4 (C_7); 127.9 ($C_{3'} + C_{5'}$); 129.2 ($C_{2'} + C_{6'}$); 133.6 (C_6); 134.7 ($C_{1'}$); 137.1 ($C_{4'}$); 151.2 (C_{10}); 155.8 (C_4); 161.0 (C_2). MS (*m*/*z* % abundance): 375 (M^+, 5); 281 (13); 159 (18); 137 (100); 121 (46); 91 (39); 77 (23). Elemental Analysis for $C_{17}H_{17}N_3SSe \cdot 0.8$ HI Calcd/Found (%): C: 42.84/42.62; H: 3.68/4.03; N: 8.82/9.19.

*4.1.29.4. 4-Benzylamino-2-pentylselenoquinazoline (**7h**).* From **7a** and pentyl iodide. Yield: 64%; mp 177–179 °C. IR (KBr) cm^{-1}: 3334–3306 (m, N–H); 3131 (m, C–H); 2938 (m, C–H); 1645 (s, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.86 (t, 3H, $(CH_2)_4$–CH_3); 1.33 (m, 4H, $(CH_2)_2$–(CH_2)$_2$–CH_3); 1.75 (q, 2H, CH_2–CH_2–$(CH_2)_2$–CH_3); 3.32 (t, 2H, CH_2–$(CH_2)_3$–CH_3); 5.71 (s, 2H, CH_2–Ph); 7.35 (m, 5H, CH_2–*Ph*); 7.77 (m, 2H, $H_6 + H_5$); 8.06 (t, 1H, H_7); 8.53 (d, 1H, H_8); 10.12 (br s, 1H, NH–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.9 (Se–$(CH_2)_4$–CH_3); 22.4 (Se–$(CH_2)_3$–CH_2–CH_3); 29.4 (Se–CH_2–CH_2–$(CH_2)_2$–CH_3); 31.3 (Se–CH_2–$(CH_2)_3$–CH_3); 32.2 (Se–$(CH_2)_2$–CH_2–CH_2–CH_3); 53.2 (CH_2); 114.0 (C_9); 126.3 (C_8); 126.9 (C_5); 127.5 (C_7); 128.3 ($C_{4'}$); 128.7 ($C_{2'} + C_{6'}$); 129.1 ($C_{3'} + C_{5'}$); 135.1 (C_6); 138.0 ($C_{1'}$); 146.2 (C_{10}); 155.0 (C_4); 158.2 (C_2). MS (*m*/*z* % abundance): 385 (M^+, 10); 314 (100); 234 (64); 207 (55); 145 (10); 129 (24); 106 (22); 91 (68). Elemental Analysis for $C_{20}H_{23}N_3Se \cdot 1$ HI Calcd/Found (%): C: 46.87/47.25; H: 4.49/4.49; N: 8.20/8.63.

*4.1.29.5. 4-(4′-Methylthiobenzyl)amino-2-pentylselenoquinazoline (**7i**).* From **7c** and pentyl iodide. Yield: 37%; mp 169–170 °C. IR (KBr) cm^{-1}: 3321 (m, N–H); 3131 (m, C–H); 2958–2892 (m, C–H); 1589 (f, C=N). ^1H NMR (400 MHz, DMSO-d_6) δ: 0.86 (t, 3H, $(CH_2)_4$–CH_3); 1.34 (m, 4H, $(CH_2)_2$–(CH_2)$_2$–CH_3); 1.78 (q, 2H, CH_2–CH_2–$(CH_2)_2$–CH_3); 2.50 (s, 3H, SCH_3); 3.31 (t, 2H, CH_2–$(CH_2)_3$–CH_3); 5.53 (s, 2H, CH_2–Ph); 7.25 (m, 4H, Ph); 7.76 (m, 2H, $H_6 + H_5$); 8.07 (t, 1H, H_7); 8.53 (d, 1H, H_8); 10.32 (s, 1H, NH–CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.6 (Se–$(CH_2)_4$–CH_3); 15.5 (SCH_3); 22.4 (Se–$(CH_2)_3$–CH_2–CH_3); 29.2 (Se–CH_2–CH_2–$(CH_2)_2$–CH_3); 31.3 (Se–CH_2–$(CH_2)_3$–CH_3); 31.8 (Se–$(CH_2)_2$–CH_2–CH_2–CH_3); 53.1 (CH_2); 113.8 (C_9); 126.2 (C_8); 126.6 (C_5); 127.3 (C_7); 127.9 ($C_{3'} + C_{5'}$); 128.7 ($C_{2'} + C_{6'}$); 134.8 (C_6); 136.1 ($C_{1'}$); 139.0 ($C_{4'}$); 146.2 (C_{10}); 155.0 (C_4); 157.9 (C_2). MS (*m*/*z* % abundance): 431 (M^+, 9); 360 (43); 280 (7); 253 (28); 233 (9); 137 (100); 121 (11); 91 (9). Elemental Analysis for $C_{21}H_{25}N_3SSe \cdot 0.9$ HI Calcd/Found (%): C: 46.22/45.91; H: 4.58/4.60; N: 7.70/7.74.

4.2. Cytotoxic and antiproliferative activities

The cytotoxic effect of each substance was tested at five different concentrations between 0.01 and 100 μM. Each substance was initially dissolved in DMSO at a concentration of 0.1 M and serial dilutions were prepared using culture medium. The plates with cells from the different lines, to which medium containing the substance under test was added, were incubated for 72 h at 37 oC in a humidified atmosphere containing 5% CO_2. Human tumor cell lines were provided by the European Collection of Cell Cultures (ECACC) or the American Type Culture Collection (ATCC). Four cell lines were used: one human lymphocytic leukemia (CCRF-CEM) and three human solid tumors, one colon carcinoma (HT-29), one lung carcinoma (HTB-54) and one breast adenocarcinoma (MCF-7). CCRF-CEM, HT-29 and HTB-54 cells were grown in RPMI 1640

medium (Invitrogen) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin and 10 mM HEPES buffer (pH = 7.4). MCF-7 cells were grown in EMEM medium (Clonetics) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin. 184B5 cells were grown in Hams F-12/DMEM (50:50) supplemented as described by Li et al. [61]. BEAS-2B were grown in 25 mL fetal bovine serum (FBS), 5 mL insulin–transferrin–sodium selenite (ITS), 1 mL hydrocortisone, 10 mL sodium pyruvate, 5 mL glutamine, 5 mL penicillin/gentamicin, 10 μL epidermal growth factor (EGF), and 150 μL retinoic acid (1 μM). Cytotoxicity was then determined by the MTT method. Results are expressed as GI_{50}, the concentration that reduces by 50% the growth of treated cells with respect to untreated controls, TGI, the concentration that completely inhibits cell growth, and LC_{50}, the concentration that kills 50% of the cells. Data were obtained from at least 3 independent experiments performed in quadruplicate.

4.3. Evaluation of cell cycle progression and cell death

For breast adenocarcinoma MCF-7 cells, the cell death status and cell cycle analysis of the cells were determined using the Apo-Direct kit (BD Pharmingen), based on the TUNEL technique, under the conditions described by the manufacturer. Briefly, for fixation step cells were suspended in 1% paraformaldehyde in PBS (pH = 7.4) at a concentration of 1×10^6 cells/mL, incubated on ice for 1 h, collected by centrifugation, washed, adjusted to 1×10^6 cells/mL in 70% ice-cold ethanol and incubated at −20 °C for 30 min. After fixation, cells were recovered by centrifugation, washed, resuspended in FITC dUTP-DNA labeling solution and incubated for 1 h at 37 °C. Cells were then rinsed, resuspended in PI/RNase staining buffer, incubated in the dark for 30 min at RT and analyzed using a Coulter Epics XL flow cytometer.

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