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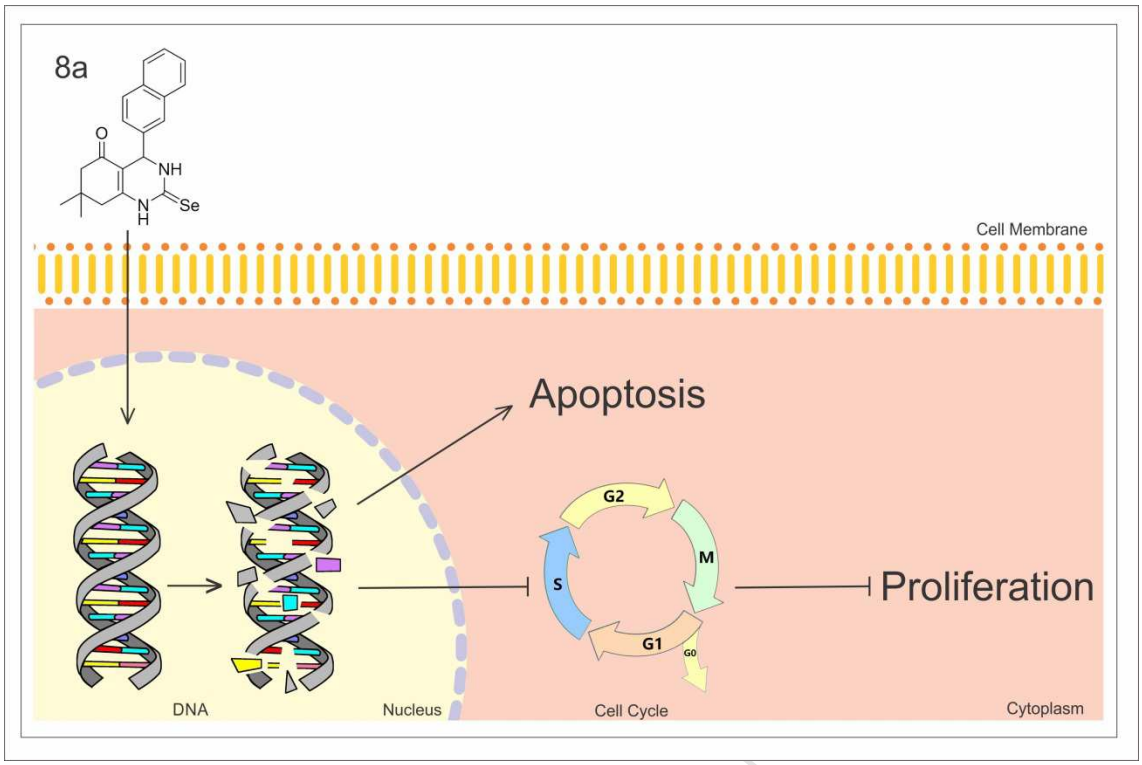

Novel pyrimidinic selenourea induces DNA damage, cell cycle arrest, and apoptosis in human breast carcinoma

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ABSTRACT

Novel pyrimidinic selenoureas were synthesized and evaluated against tumour and normal cell lines. Among these, the compound named **3j** initially showed relevant cytotoxicity and selectivity for tumour cells. Three analogues of **3j** were designed and synthesized keeping in view the structural requirements of this compound. Almost all

the tested compounds displayed considerable cytotoxicity. However, **8a**, one of the **3j** analogues, was shown to be highly selective and cytotoxic, especially for breast carcinoma cells (MCF-7) (IC_{50} = 3.9 µM). Furthermore, **8a** caused DNA damage, inhibited cell proliferation, was able to arrest cell cycle in S phase, and induced cell death by apoptosis in human breast carcinoma cells. Moreover, predictions of pharmacokinetic properties showed that **8a** may present good absorption and permeation characteristics for oral administration. Overall, the current study established **8a** as a potential drug prototype to be employed as a DNA interactive cytotoxic agent for the treatment of breast cancer.

Keywords: dihydropyrimidinones, organoselenium, cytotoxicity, DNA damage, cell cycle arrest, apoptosis, selenoureas

1.Introduction

Dihydropyrimidinones (DHPMs) are a very important class of six-membered heterocycles that are obtained by the Biginelli multicomponent reaction [1]. In the field of medicinal chemistry, DHPMs have been much explored due to the modular nature of the multicomponent reaction that enables the use of several aldehydes, dicarbonylic compounds, and ureas, which readily leads to the preparation of a diverse library of compounds. In addition, these compounds are known for presenting multiple biological activities, such as antioxidant action [2–4], acetylcholinesterase inhibition [5,6], and mPGES-1 inhibition [7] among other activities [8]. However, the anticancer activities of DHPMs are more pronounced, since various reports have described compounds such as Monastrol and its derivatives as good cytotoxic and antiproliferative agents [9–15]. Molecular hybrids containing the DHPM core are also reported as potential anticancer

agents, such as DHPM-Coumarin hybrids for breast tumours [16] and DHPM-semicarbazone hybrids with anticancer activity due to their ability to inhibit human DNA ligase 1 [17]. More recently, DHPMs containing a fatty acid moiety have been found to possess antiproliferative activity against C6 glioma cell line [18] and cytotoxic effects in the melanoma B16F10 cell line [19].

Organoselenium compounds are well known for their antioxidant activity with the ability to mimic selenoenzyme Glutathione Peroxidase (GPx-like activity) [20,21]; there is also emerging evidence indicating the potential of selenium (Se) compounds in cancer chemotherapy; these compounds are well known to inhibit cell proliferation and induce cell death in human cancer cells by apoptosis [22,23]. Se-compounds induce inhibition of proliferation and promote cellular apoptosis, presumably by decreased phosphorylation of Akt [24,25] which, through the phosphatidylinositol 3-kinase PI3K/Akt/mTOR pathway, has been shown to inhibit apoptosis in cancer cells [26] and promote proliferation and angiogenesis [27]. Furthermore, Se-compounds are related to apoptotic processes, including cytochrome *c*-independent caspase-3 activation, down-regulation of IAP family proteins, and Bax cleavage [28]. Moreover, Suzuki et al. [24], demonstrated that Se-induced apoptosis in some carcinoma cells is a caspase-dependent process involving p53 activation. In addition, Se-compounds are involved in the generation of reactive oxygen species (ROS), DNA damage, mitochondrial dysfunction, and imbalance of Bcl-2 family expression, which leads to cell death in several human cancer cells [29].

The pro-oxidant effects of Se-containing compounds have been used as new tools for developing novel anticancer molecules [30–32]. The insertion of selenium atom into biologically relevant nuclei such as as indoles [33], naphtoquinones [34,35], flavonoids [36], and acetylsalicylic acid [37] has been described in the recent years and

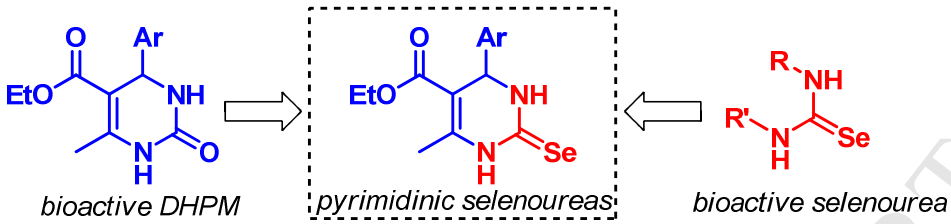
such modification leads to more cytotoxic compounds. The synthesis of molecular hybrids between ebselen and resveratrol also leads to very potent cytotoxic compounds with possible mechanisms of action related to inhibition of Thioredoxin Reductase (TrxR), another selenoenzyme involved in the redox balance, which leads to cell death by oxidative stress [38].

Selenoureas, isoselenoureas, and their derivatives have been reported as antioxidant and anticancer agents used mainly in the design of highly potent molecular hybrids [39–42]. To the best of our knowledge, the study of pyrimidinic selenoureas has only two reports in the literature. Klein and co-workers reported the synthesis of a single seleno-analogue of Monastrol and evaluated its ability to inhibit the Kinesin Eg5 enzyme, however, it caused a two-fold decrease in the activity when compared to Monastrol [43]. In the second report, Kolb and colleagues evaluated a pyrimidinic selenourea and its selenazolopyrimidinic derivative as CDC25B phosphatase inhibitors [44].

DNA is also an important target for the development of new antitumor drugs, because interactions between drugs and DNA can result in blockage of cell division and triggering of cell death [45]. DHPMs and their derivatives have potential to overlap with DNA base pairs or to bind to the grooves; therefore, they are regarded as potential new DNA-targeted drugs [46–48]. Organoselenium compounds are also able to cause DNA damage leading to cell death [49,50].

As part of our research program focused on the synthesis and biological evaluation of new seleno-DHPMs [51,52], we here report the design, synthesis, and anticancer evaluation of a series of pyrimidinic selenoureas. These compounds were designed through molecular hybridization [53] by merging the dihydropyrimidinone nucleus and the selenourea moiety (Scheme 1) to form one molecule that has the

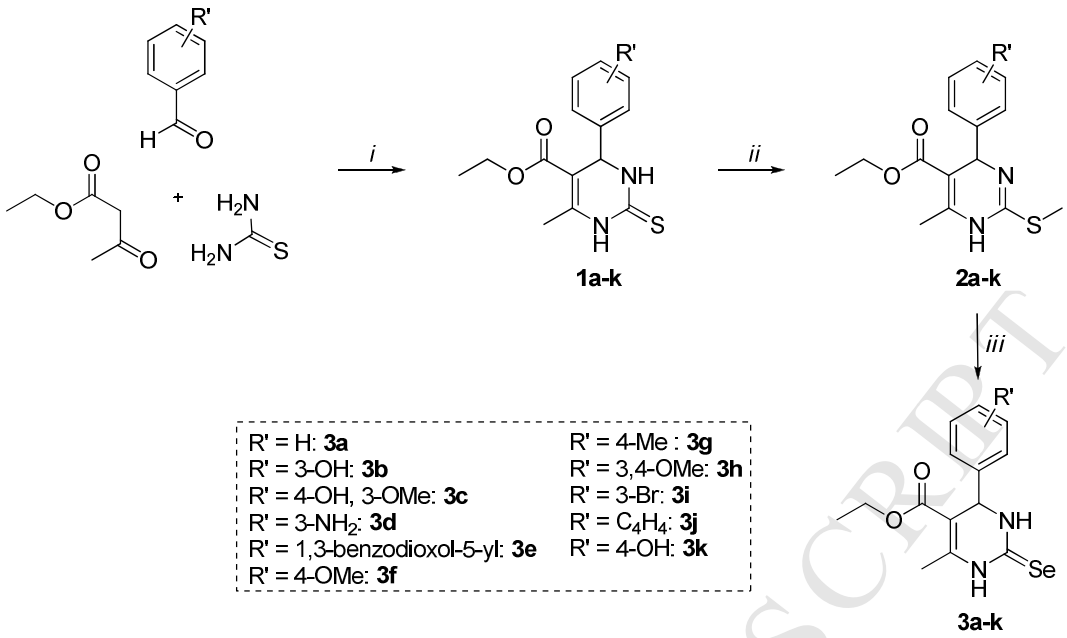
pharmacological potential of both classes of compounds.



Scheme 1- Molecular hybridization towards pyrimidinic selenoureas

2.Results and Discussion

Pyrimidinic selenoureas **3a-k** were synthesized via a three-step route (Scheme 2), following the previous literature [54], which is based on transforming thioureas into selenoureas using an iso-thiourea intermediate. Thioureas **1a-k** were prepared through the classical Biginelli multicomponent reaction, using different substituted aromatic aldehydes, ethyl acetoacetate, thiourea and HCl as catalyst [51]. Methylation of thioureas **1a-k** with methyl iodide followed by basic treatment leads to the formation of iso-thioureas **2a-k** with excellent yields. Finally, pyrimidinic selenoureas **3a-k** were obtained by the reaction of iso-thioureas **2a-k** with the nucleophilic selenium species NaSeH in refluxing ethanol for 15 hours. By this strategy, the Se anion adds to the carbon atom of iso-thiourea, followed by the elimination of methanethiolate as a leaving group. The compounds were obtained in good to excellent yields (65-99%). Compound **2d** bearing a nitro group was obtained as an aryl amine **3d** after reaction with NaSeH, this reduction was expected since sulphur species such as sodium sulphide have been used as reducing agents for aromatic nitro compounds [55].



Scheme 2 - *i*) HCl, 100 °C, neat, 55-99%; *ii*) MeI, EtOH, reflux, 1 h, then $NaHCO_3$, 72-99%; *iii*) NaSeH, EtOH, reflux, 15h, 65-99%.

Initially, pyrimidinic selenoureas **3a-k** were evaluated against two cancer cell lines, MCF-7 and HeLa, and the normal cell line McCoy. The cells were treated for different periods of time with different concentrations of the pyrimidinic selenoureas **3a-k** followed by screening for cell cytotoxicity by MTT assay. Table 1 shows values obtained from IC_{50} (half-maximal inhibitory concentration) for compounds **3a-k** and two standards, Monastrol and the anticancer drug Fluorouracil (5-FU), 72 h post-treatment. As previously demonstrated, both DHPMs and Se-compounds induced time-dependent cytotoxicity and growth inhibition in different cancer cell lines [14,24].

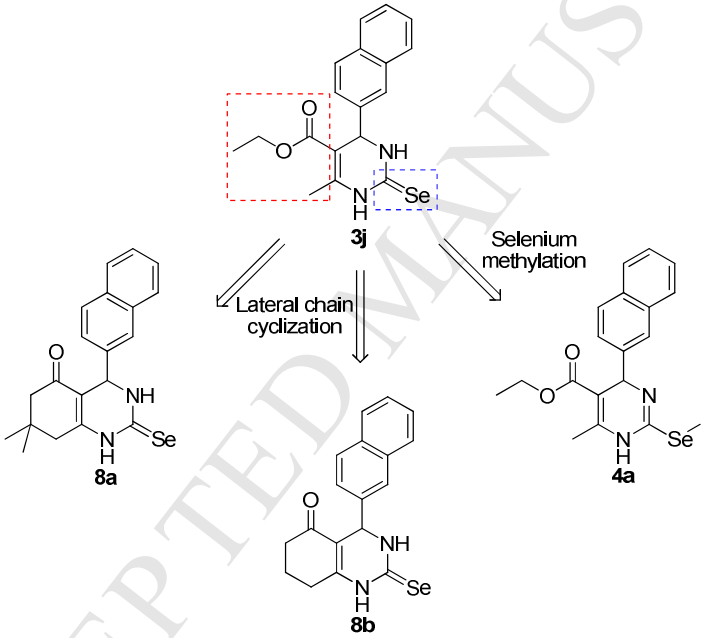
Table 1 - IC_{50} values of the pyrimidinic selenoureas **3a-k** in different cancer cell lines and in normal cells.

	IC_{50} (µM)		
Compound	**MCF-7**	**HeLa**	**McCoy**
3a	452.2	909.1	251.8
3b	92.9	205.1	175.9
3c	175.8	390.2	252.7
3d	207.1	512.3	289.1
3e	200.6	283.8	212.9
3f	301	254.3	199.1
3g	169.4	258.3	107.9
3h	247.8	149.1	135.6
3i	251.7	152.6	143.9
3j	25.2	87.6	57.7
3k	181.1	224.3	194
Monastrol	59.1	176.9	26.8
5-FU	63.0	7.00	15.7

Cells were treated for 72 h with compounds (0.1 – 1000 µM) and the cytotoxicity data were obtained by MTT assay. Data from three independent experiments.

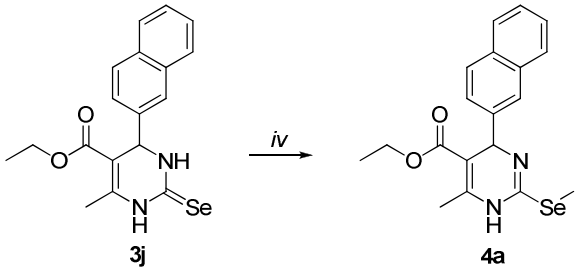
As seen in Table 1, most of the compounds exhibited high IC_{50} values for both cancer cell lines, however, compound **3j** presented the lowest IC_{50} value among other pyrimidinic selenoureas, with IC_{50} of 25.2 µM for MCF-7 cells and 87.6 µM for HeLa, more than two times the activity of the standard Monastrol. Also, compound **3j** was 2,5 times more efficient than the 5-FU for MCF-7 cells.

Based on these results, we designed three new analogues of compound **3j**, keeping the important naphthyl moiety in the structure, aiming for an enhancement in antitumor activity. Two main modifications were planned for its structure: modification of the selenourea moiety in **3j** by methylation of the selenium atom, leading to iso-selenourea **4a**, and lateral chain cyclization of the heterocycle, leading to compounds **8a-b** (Scheme 3). The latter modifications are well known for the structure-activity relationships in DHPM cores as antitumor agents, and these modifications usually lead to more active compounds [56].



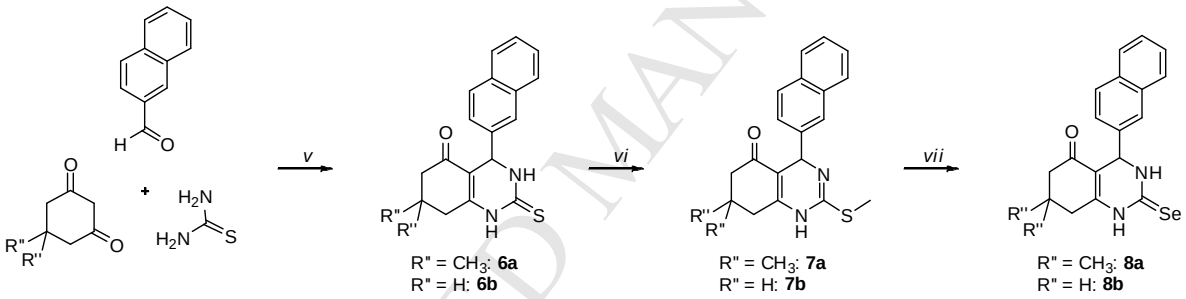
Scheme 3 -Design of **3j** analogues by methylation of selenium and lateral chain cyclization

Methylation of the selenium of **3j** occurred successfully applying the same methodology usually used for sulphur analogues, affording the product **4a** in 98% yield (Scheme 4).



Scheme 4 - *iv*) MeI, EtOH, reflux, 1 h, then $NaHCO_3$, 98%

Two other analogues, **8a-b**, were synthesized using the same strategy applied for selenoureas **3a-k**, replacing the ethyl acetoacetate by cyclohexanedione and dimedone. The desired compounds were obtained in good to excellent yields, except for **6b,** which was obtained in a moderate yield (Scheme 5).



Scheme 5 - *v*) HCl, 100°C, neat, 66-35%; *vi*) MeI, EtOH, reflux, 1 h, then $NaHCO_3$, 90-99%; *vii*) NaSeH, EtOH, reflux, 15 h, 92-86%.

With the three new analogues **4a** and **8a-b** in hand, it was possible to evaluate their activity against MCF-7 and HeLa cells; the results for their IC_{50} values are presented in Table 2. In general, the three modifications resulted in more cytotoxic compounds when compared to **3j**. Compound **4a** containing an isoselenourea showed an IC_{50} value lower than **3j**. However, **4a** also showed a decrease in selectivity, since it was very toxic to normal cells. According Badisa et al. [57], selectivity index (SI) demonstrates the differential activity of a compound for a tumour cell, and a SI value

greater than two indicate low toxicity for normal cells and high toxicity against tumour cell lines.

The compounds presenting the cyclized lateral chain also lead to more cytotoxic compounds (**8a** and **8b**). Compound **8a** was particularly interesting because the presence of a dimethyl group in the lateral chain caused a significant increase in cytotoxicity, leading to a decrease in viability of tumour cells, with IC_{50} values showing activities approximately 15 times more pronounced than Monastrol and 16 times more citotoxic when compared to the drug 5-FU for MCF-7 cells. In addition, these compounds showed substantial activity without further damage to normal cells (Table 2). To our delight, **8a** presented higher selectivity than the standards monastrol and 5-FU, showing its great potential for further studies. This structural alteration and potentiation of the antitumor effect for **8a** is in accord with that proposed by Müller et al. [58], who reported that enastron and dimethylenastron, both cyclized analogues of Monastrol, increased cytotoxic effects against human glioblastoma cells.

Table 2 - IC_{50} and SI values of the pyrimidinicselenoureas**4a**, **8a-b** and **3j** in different cancer cell lines and in normal cells.

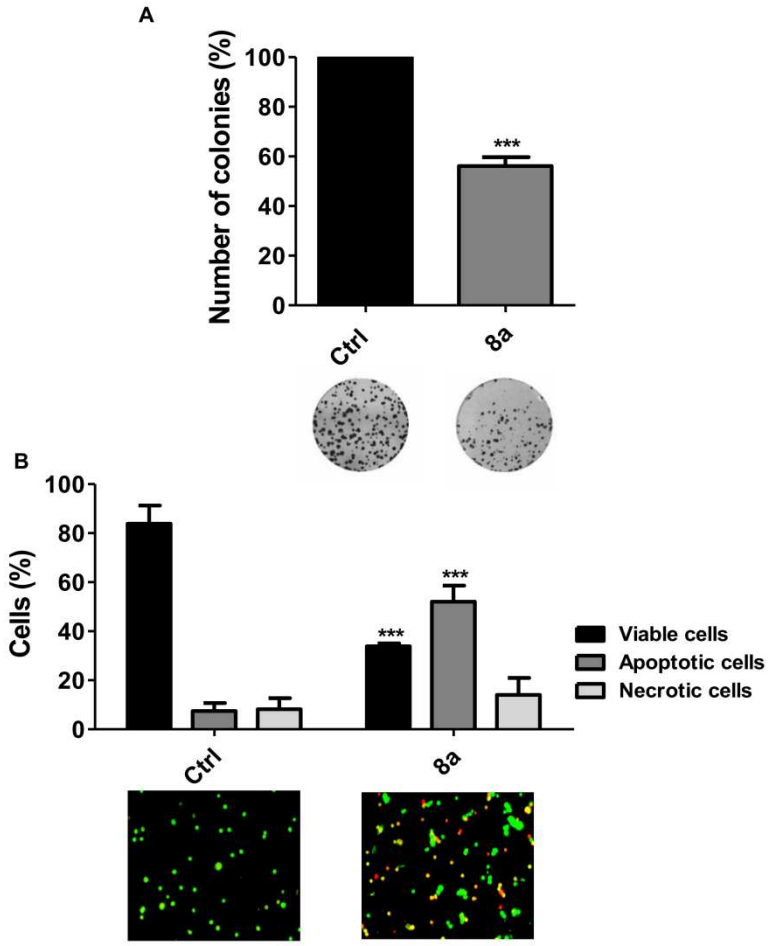
	IC_{50} (µM)			**Selectivity Index (SI)**	
Compound	**MCF-7**	**HeLa**	**McCoy**	**MCF-7**	**HeLa**
4a	*8.8*	*11.2*	*0.01*	0.001	0.0009
8a	*3.9*	*12.2*	*425.8*	109.1	34.9
8b	*13.1*	*26.2*	*22.4*	1.7	0.8
3j	*25.2*	*87.6*	*57.7*	2.2	0.6
Monastrol	59.1	176.9	26.8	0.4	0.2
5-FU	63.0	7.00	15.7	0.2	2.2

SI = IC_{50} of compound in normal cell line/IC_{50} of the compound in cancer cell line. Data from the IC_{50} mean of three independent experiments.

As seen that **8a** presented the most relevant results in terms of viability and selectivity, other biological assays were conducted in human breast carcinoma in order to elucidate the mechanisms of such effects.

The antiproliferative effect of **8a** (2.4 μM) against MCF-7 cells was evaluated over 72 h of treatment and the results are presented in Fig. 2A. The results indicated that **8a** inhibited cell proliferation, as shown by the reduction in the number of colonies (40%) in comparison to non-treated cells. Guido et al. [59] reported that DHPMs have been associated with inhibition of proliferation and angiogenesis. These effects may be related to kinesin Eg5 [60], a protein involved in the formation and function of the mitotic spindle, and to DNA damage [61]. Compound **8a** was also able to induce MCF-7 cell death (Fig. 2B) by apoptosis and necrosis. Apoptosis, the most pursued goal in anticancer drug development [62], was significantly increased after treatment with compound **8a** ($p < 0.001$). This result is very interesting since MCF-7 cells may be resistant to apoptosis due to the possible absence of caspase-3 [63].

Figure 2. Antiproliferative effect (A) and induction of cell death (B) after treatment of MCF-7 with non-toxic concentrations of compound **8a** (2.4 µM) for 72 h. Data were obtained from three independent experiments. (***) denotes statistical differences at $p < 0.001$ compared to control cells (non-treated).

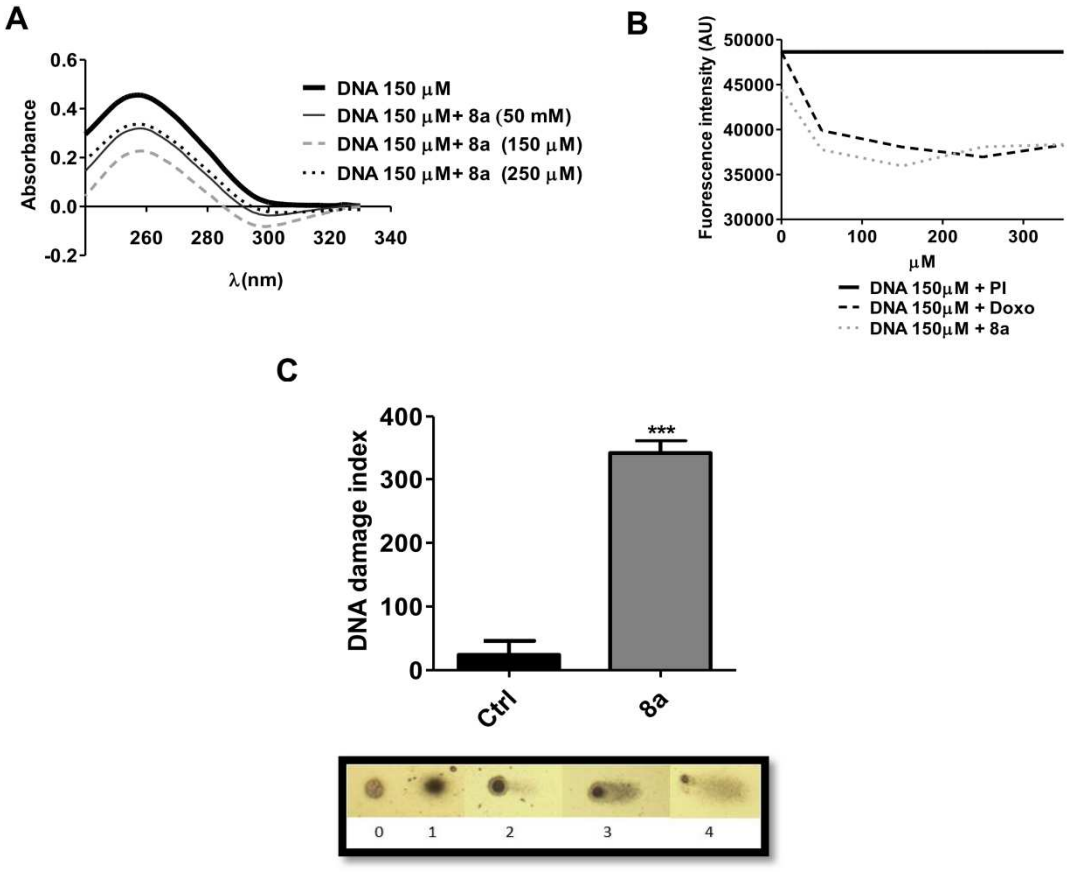


DNA is also a target for developing new potential drugs [64], and we investigated whether **8a** is able to interact with DNA. Figure 3A and 3B show data concerning the absorption scanning of CT-DNA alone and after treatment with compound **8a**. Electronic absorption spectroscopy is an effective method to examine the binding modes of drug with DNA. Hypochromism was observed when **8a**, in increasing concentrations, was placed in the presence of CT-DNA (Fig. 3A). This effect results

from contraction and changes in the conformation of DNA [65]. The possible DNA intercalation effect of **8a** was investigated (Fig. 3B). For this, propidium iodide was used because it displays an enhancement of DNA fluorescence efficiency due to its strong intercalation between adjacent base pairs. **8a** was able to remove propidium iodide and insert between the DNA strands, decreasing CT-DNA fluorescence, which is indicative of intercalation. Our results are in agreement with those obtained by Wang et al. [46] who showed that the potential antitumor effects of dihydropyrimidinone derivatives are related to its DNA binding properties.

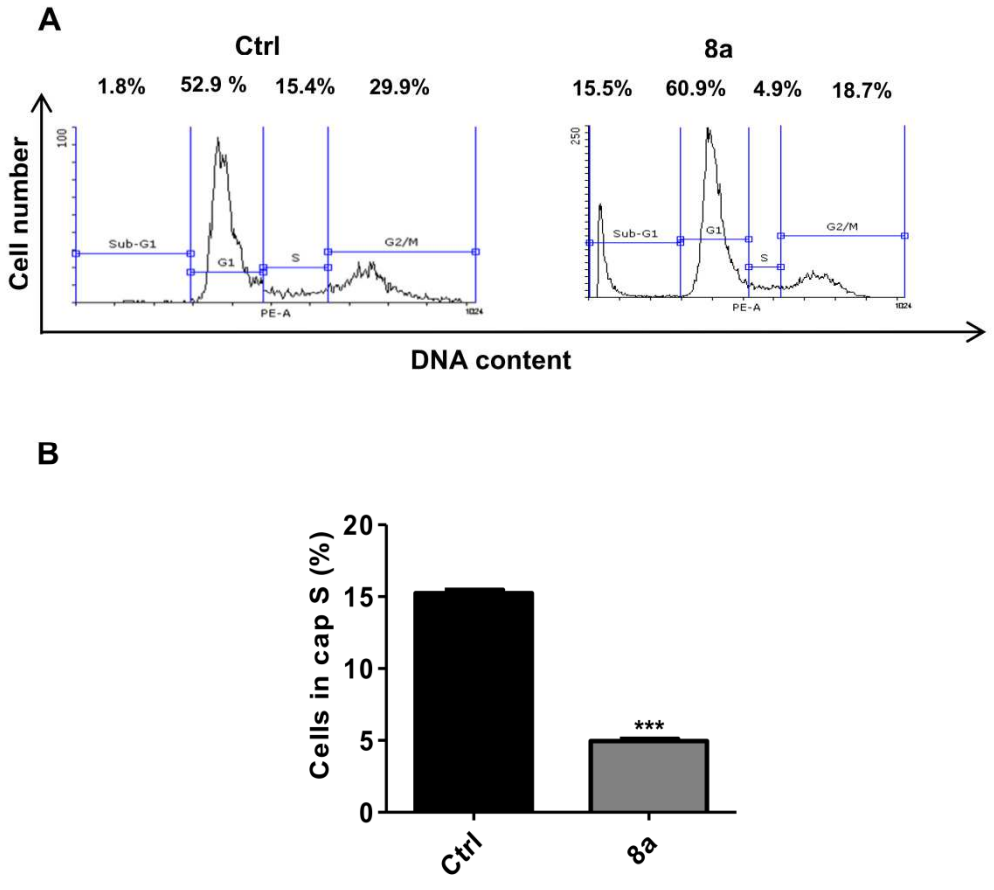
Next, the ability of compound **8a** to cause DNA fragmentation in MCF-7 cells was evaluated by Comet assay and the results are shown in Figure 3C. After 72 h of treatment we observed a significant increase in the total DNA damage caused by **8a** ($p < 0.001$) in MCF-7 cells treated with non-toxic concentrations. Double-stranded DNA breaks have been shown to correlate with DNA-targeting chemotherapeutics leading to inhibition of proliferation and cell death [64]. Planar and non-planar, aromatic and heterocyclic compounds, such as dihydropyrimidinone derivatives have potential for overlap with DNA base pairs or binding to the grooves [46]. Also, Se-compounds may be involved in induction of DNA breakdown caused by generation of intracellular ROS [66]. Compounds that cause DNA damage and consequent cell cycle arrest have been considered promising agents for chemotherapy [67].

Figure 3. The interaction (A) and intercalating ability via fluorescence intensity of propidium iodide (300 µM) and doxorubicin as a positive control for intercalation (B) of compound **8a** (0-350 µM) with CT-DNA (150 µM) were evaluated. MCF-7 cells were treated with **8a** (2.4 µM) for 72 h. DNA damage index was determined by Comet assay (C) and DNA fragmentation scores are shown in the box below the graph. (***) denotes statistically significant differences at $p < 0.001$ compared to control cells (non-treated).



The assessment of the cell cycle of cells treated with **8a** was realized and Figure 4A shows distribution of MCF-7 cells in different phases of the cell cycle. The treatment with **8a** leads to an increase in the sub-G1 area of 15.5% (indicative of apoptosis). Additionally, the number of cells was significantly reduced in S phase by up to 4.9%, as shown in Figure 4B. Therefore, **8a** showed a large safety to normal cells, and the cytotoxic effects on breast carcinoma cells are related to DNA damage and cell cycle arrest, which leads to cell death by apoptosis.

Figure 4. Changes in MCF-7 cell cycle induced by treatment were measured (A) and percentage of cell distribution in cap S was evaluated (B). Data were obtained from three independent experiments. (***) denotes statistically significant differences at $p < 0.001$ compared to control cells (non-treated).



Moreover, the pharmacokinetic properties for compound **8a** were predicted using the open-source software Molinspiration [68] and ChemBioDraw Ultra 11.0. According to Lipinski's rule of 5 [69] poor absorption or permeation will be more likely if a drug candidate presents molecular weight > 500, number of hydrogen bond donors > 5, number of hydrogen bond acceptors > 10 and Clog P > 5.0. Another two relevant properties in this type of analysis were introduced by Veber and co-workers [70], and these state that a drug prototype should not have more than 10 rotatable bonds and its topological polar surface area should be ≤ 140 Å^2.

As seen in Table 3, compound **8a** satisfied such criteria for presenting potentially- good absorption and permeation after oral administration, which enhance its potential as a drug prototype.

Table 3. Predictions of pharmacokinetic properties for compound **8a**.

Property	Value
CLogP	3.58
Molecular Weight	383.35
tPSA[a](Å^2)	41.12
HBA[b]	3
HBD[c]	2
NRB[d]	1

[a] tPSA, Topological polar surface area; [b] HBA, H-bond acceptors; [c] HBD, H-bond donors; [d] NRB, Number of rotatable bonds.

3. Conclusion

DNA damage, cell cycle arrest, and induction of cell death by apoptosis are attractive strategies to selectively kill cancer cells. In this work, we demonstrated the synthesis of novel pyrimidinic selenoureas and their cytotoxicity against tumour cell lines. Among the compounds evaluated, **8a** appears to be promising for the development of an antitumor agent. This compound caused DNA damage, cell cycle arrest, antiproliferative effects and cell death by apoptosis. Compound **8a** also showed remarkable selective cytotoxicity for tumour cells, being more selective than the drug 5-FU, mainly for human breast carcinoma. The prediction of pharmacokinetic properties indicated that **8a** may present good absorption and permeation characteristics. Hence, compound **8a** is a potential drug prototype for the treatment of breast cancer. More in-depth studies are currently being conducted in our laboratories to clarify and offer more molecular details concerning the mechanisms of action of compound **8a**.

4.Materials and Methods

4.1. Chemicals

NMR spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Varian AS-400 or Bruker Avance 200 spectrometer. Chemical shifts (δ) are reported (in ppm) relative to the TMS (^{1}H NMR) and the solvent (^{13}C NMR). APPI or APCI-micrOTOF-Q II measurements were performed with a micrOTOF Q-II (Bruker Daltonics) mass spectrometer equipped with an automatic syringe pump (KD Scientific) for sample injection. The mass spectrometer was operated in the positive ion mode. The sample was injected using a constant flow (3 µL/min). The solvent was a chloroform/methanol mixture. The APPI-micrOTOF-Q II instrument was calibrated in the mass range of 50–3000 m/z using an internal calibration standard (low concentration tuning mix solution)

supplied by Agilent Technologies. Data were processed employing Bruker Compass Data Analysis software (version 4.0). Thin layer chromatography (TLC) was conducted using Merck Silica Gel GF254 (0.25 mm thickness). For visualization, the TLC plates were either placed under ultraviolet light or stained with iodine vapour or acidic vanillin. Melting points were determined using a microscope coverslip on a Micro Chemical MQA PF digital apparatus and are uncorrected. Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS) and antibiotics were purchased from Gibco (USA). The following material was purchased from Sigma-Aldrich: calf thymus DNA (CT-DNA; Cat. D4522), agarose (Cat. A6013), dimethyl sulphoxide (DMSO; Cat. D8418), bovine serum albumin (BSA; Cat. A2153), propidium iodide (Cat. P4170), acridine orange (Cat. A6014), nocodazole (Cat. M1404) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Cat. M5655). All common reagents and solvents were used as purchased unless otherwise noted.

4.2.Synthetic procedures

*4.2.1. General procedure for the synthesis of compounds **1a-k** and **6a-b**:*

The procedure described in the literature [51] was followed. To a two-necked round-bottom flask, the appropriated aldehyde (10 mmol), ethyl acetoacetate or dimedone or cyclohexanedione (10 mmol), thiourea (20 mmol) and 5 drops of concentrated HCl were added. The reaction mixture was stirred at 100°C for the time required for consumption of the starting materials, which was verified by TLC. After this time, the reaction mixture was poured into crushed ice and water. The precipitate was filtered and dried. The compounds were used in the next step without further purification.

*4.2.1.1. Ethyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1a**)*

White solid, m.p. 204-207 ºC [Lit. 210ºC][44], 72% yield; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 9.67 (s, 1H), 7.34 (t, *J* = 7.3 Hz, 2H), 7.28 – 7.22 (m, 3H), 5.19 (d, *J* = 3.6 Hz, 1H), 4.00 (q, *J* = 6.9 Hz, 2H), 2.30 (s, 3H), 1.09 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.30, 165.19, 145.10, 143.57, 128.62, 127.75, 126.47, 100.79, 59.67, 54.14, 17.25, 14.07.

*4.2.1.2. Ethyl 4-(3-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1b**)*

White solid, m.p. 150-153 ºC [Lit. 152-154ºC][44], 70% yield; **^{1}H NMR** (200 MHz, DMSO- d_6) δ 10.30 (s, 1H), 9.61 (s, 1H), 9.46 (bs, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 6.66 (d, *J* = 6.5 Hz, 3H), 5.10 (d, *J* = 2.6 Hz, 1H), 4.01 (q, *J* = 6.8 Hz, 2H), 2.28 (s, 3H), 1.11 (t, *J* = 6.9 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.25, 165.28, 157.54, 144.89, 129.59, 117.11, 114.72, 113.34, 100.89, 59.72, 54.05, 17.27, 14.13.

*4.2.1.3. Ethyl 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1c**)*

White solid, m.p. 231-233 ºC [Lit. 238-239ºC][12], 55% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 10.26 (s, 1H), 9.57 (s, 1H), 9.03 (bs, 1H), 6.79 (d, *J* = 1.9 Hz, 1H), 6.73 (d, *J* = 8.1 Hz, 1H), 6.59 (dd, *J* = 8.1, 1.9 Hz, 1H), 5.09 (d, *J* = 3.5 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.73 (s, 3H), 2.28 (s, 3H), 1.11 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.09, 165.34, 147.41, 146.22, 144.70, 134.65, 118.62, 115.48, 110.98, 101.07, 59.64, 55.64, 53.76, 17.22, 14.17.

*4.2.1.4. Ethyl 6-methyl-4-(3-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1d**)*

White solid, m.p. 200-205 ºC [Lit. 206-209ºC][71], 65% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 10.52 (s, 1H), 9.78 (s, 1H), 8.17 – 8.12 (m, 1H), 8.08 (s, 1H), 7.67 (d, *J* = 5.3 Hz, 2H), 5.34 (d, *J* = 3.6 Hz, 1H), 4.07 – 3.95 (m, 2H), 2.31 (s, 3H), 1.09 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.56, 164.91, 147.86, 146.09, 145.56, 133.10, 130.47, 122.78, 121.25, 99.89, 59.86, 53.58, 17.31, 14.00.

*4.2.1.5. Ethyl 4-(benzo[d][1,3]dioxol-5-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1e**)*

Yellowish solid, m.p. 156-159 ºC [Lit. 175ºC][44], 70% yield; **^{1}H NMR** (200 MHz, DMSO- d_6) δ 10.32 (s, 1H), 9.61 (s, 1H), 6.87 (d, *J* = 7.9 Hz, 1H), 6.72 – 6.65 (m, 2H), 5.99 (s, 2H), 5.09 (d, *J* = 3.5 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 2.29 (s, 3H), 1.11 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.05, 165.14, 147.41, 146.73, 145.04, 137.48, 119.67, 108.18, 106.75, 101.11, 100.73, 59.62, 53.72, 17.20, 14.07.

*4.2.1.6. Ethyl 4-(4-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1f**)*

Beige solid, m.p. 151-154 ºC [Lit. 150-151ºC][72], 99% yield; **^{1}H NMR** (200 MHz, DMSO- d_6) δ 10.29 (s, 1H), 9.60 (s, 1H), 7.13 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 5.12 (d, *J* = 3.5 Hz, 1H), 4.00 (q, *J* = 7.0 Hz, 2H), 3.72 (s, 3H), 2.29 (s, 3H), 1.10 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.04, 165.20, 158.77, 144.79, 135.74, 127.66, 113.91, 101.00, 59.60, 55.13, 53.49, 17.19, 14.08.

*4.2.1.7. Ethyl 6-methyl-2-thioxo-4-p-tolyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1g**)*

Beige solid, m.p. 180-182 ºC [Lit. 185-188ºC][73], 85% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 10.31 (s, 1H), 9.63 (s, 1H), 7.14 (d, *J* = 8.2 Hz, 2H), 7.11 (d, *J* = 8.3 Hz, 2H), 5.15 (d, *J* = 3.6 Hz, 1H), 4.00 (q, *J* = 7.1 Hz, 2H), 2.29 (s, 3H), 2.25 (s, 3H), 1.10 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.21, 165.20, 144.93, 140.66, 136.96, 129.11, 126.36, 100.88, 59.62, 53.81, 20.72, 17.21, 14.08.

*4.2.1.8. Ethyl 4-(3,4-dimethoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1h**)*

Orange solid, m.p. 171-174 ºC [Lit. 178ºC][44], 72% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 10.32 (s, 1H), 9.62 (s, 1H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.84 (d, *J* = 1.8 Hz, 1H), 6.71 (dd, *J* = 8.3, 1.9 Hz, 1H), 5.13 (d, *J* = 3.6 Hz, 1H), 4.02 (q, *J* = 7.1 Hz, 3H), 3.72 (s, 3H), 3.71 (s, 3H), 2.29 (s, 3H), 1.12 (t, *J* = 7.1 Hz, 3H).**^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.22, 165.28, 148.57, 148.38, 144.92, 136.02, 118.21, 111.83, 110.48, 100.88, 59.66, 55.56, 55.46, 53.65, 17.21, 14.15.

*4.2.1.9. Ethyl 4-(3-bromophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1i**)*

White solid, m.p. 165-168 ºC [Lit. 171ºC][74], 71% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 10.43 (s, 1H), 9.69 (s, 1H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.38 (s, 1H), 7.32 (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 5.17 (s, 1H), 4.08 – 3.95 (m, 2H), 2.30 (s, 3H), 1.10 (t, *J* = 7.1 Hz, 4H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 174.38, 164.99, 146.07, 145.63, 131.01, 130.62, 129.33, 125.46, 121.74, 100.15, 59.77, 53.64, 17.28, 14.04.

*4.2.1.10. Ethyl 6-methyl-4-(naphthalen-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1j**)*

White solid, m.p. 170-174 ºC [Lit. 177ºC][44], 76% yield; **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.41 (s, 1H), 9.77 (s, 1H), 7.93 – 7.89 (m, 3H), 7.69 (s, 1H), 7.52 – 7.46 (m, 3H), 5.37 (s, 1H), 4.00 (q, *J* = 7.0 Hz, 2H), 2.35 (s, 3H), 1.08 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO- d_6) δ 174.24, 165.15, 145.27, 140.81, 132.65, 132.46, 128.53, 127.92, 127.52, 126.43, 126.16, 124.97, 124.82, 100.51, 59.60, 54.39, 17.27, 14.04.

*4.2.1.11. Ethyl 4-(4-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1k**)*

Yellowish solid, m.p. 202-206 ºC [Lit. 202ºC][44], 73% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 10.25 (s, 1H), 9.56 (s, 1H), 9.46 (s, 1H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.71 (d, *J* = 8.4 Hz, 2H), 5.07 (d, *J* = 3.3 Hz, 1H), 3.99 (q, *J* = 6.9 Hz, 2H), 2.28 (s, 3H), 1.09 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 173.95, 165.32, 157.01, 144.63, 134.23, 127.79, 115.26, 101.22, 59.64, 53.69, 17.25, 14.14.

*4.2.1.12. 7,7-dimethyl-4-(naphthalen-2-yl)-2-thioxo-1,2,3,4,7,8-hexahydroquinazolin-5(6H)-one (**6a**)*

White solid, m.p. above 250 ºC, 66% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.65 (s, 1H), 9.80 (s, 1H), 7.92 – 7.88 (m, 3H), 7.70 (s, 1H), 7.53 – 7.48 (m, 2H), 7.40 (d, *J* = 8.3 Hz, 1H), 5.37 (d, *J* = 2.7 Hz, 1H), 2.45 (s, 2H), 2.25 (d, J = 16.2 Hz, 1H), 2.06 (d, *J* = 16.1 Hz, 1H), 1.03 (s, 3H), 0.89 (s, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 193.66, 174.63, 148.81, 140.67, 132.61, 132.35, 128.38, 127.84, 127.48, 126.38, 126.08, 125.01, 124.68, 107.93, 52.54, 49.85, 38.55, 32.23, 28.77, 26.72. **IR** (KBr), (ν, cm^{-1}): 3530, 3451, 3249, 3204, 3008, 2957, 2927, 2888, 1668, 1646, 1623, 1601, 1575, 1466,

1373. **HRMS** (APPI) m/z calculated for $C_{20}H_{20}N_2OS$ [M+H] 337.1369; found 337.1368.

*4.2.1.13. 4-(naphthalen-2-yl)-2-thioxo-1,2,3,4,7,8-hexahydroquinazolin-5(6H)-one (**6b**)*

Off white solid, m.p. above 250 ºC, 35% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.66 (s, 1H), 9.78 (s, 1H), 7.92 – 7.87 (m, 3H), 7.68 (s, 1H), 7.53 – 7.48 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 1H), 5.37 (d, *J* = 3.0 Hz, 1H), 2.51 – 2.50 (m, 2H), 2.32 – 2.25 (m, 2H), 2.03 – 1.77 (m, 2H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 193.93, 174.48, 150.85, 140.57, 132.60, 132.34, 128.35, 127.85, 127.42, 126.29, 126.03, 124.79, 108.82, 52.18, 36.32, 25.34, 20.49. **IR** (KBr), (ν, cm^{-1}): 3261, 3212, 3182, 3106, 3055, 3021, 2958, 2886, 2860, 1622, 1568, 1507, 1458. **HRMS** (APPI) m/z calculated for $C_{18}H_{16}N_2OS$ [M+H] 309.1056; found 309.1052.

*4.2.2. General procedure for the synthesis of isothioureas **2a-k**, **7a-b** and isoselenourea **4a***

The procedures described previously in the literature [75] were followed. In a two-necked round-bottom flask, the appropriate thioureas **1a-k**, **6a-b** or selenourea **3j** (5 mmol) were solubilized in ethanol, followed by the addition of methyl iodide (6 mmol). The reaction mixture was stirred at reflux temperature for the time required for consumption of the starting material, which was verified by TLC. After this time, the reaction mixture was treated with 20 ml of a $NaHCO_3$ (Sat.) solution. The reaction mixture was extracted with ethyl acetate/water and the organic phase dried over $MgSO_4$

and concentrated under vacuum. The resulting products presented high purity and were used in the next step without further purification.

*4.2.2.1. Ethyl 6-methyl-2-(methylthio)-4-phenyl-1,4-dihydropyrimidine-5-carboxylate (**2a**)*

White solid, m.p. 172-174 ºC [Lit. 171-172ºC][76], 99% yield; **^{1}H NMR** (200 MHz, DMSO- d_6) δ 9.59 (s, 1H), 7.32 – 7.20 (m, 5H), 5.49 (s, 1H), 4.00 (q, *J* = 7.0 Hz, 2H), 2.30 (s, 3H), 2.24 (s, 3H), 1.11 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO- d_6) δ 166.21, 145.46, 128.15, 126.76, 126.43, 58.98, 14.07, 12.52.

*4.2.2.2. Ethyl 4-(3-hydroxyphenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**2b**)*

Beige solid, m.p. 201-204ºC, 91% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 9.59 (s, 1H), 9.28 (s, 1H), 7.05 (t, *J* = 7.1 Hz, 1H), 6.66 (s, 1H), 6.63 (d, *J* = 1.4 Hz, 1H), 6.58 (d, *J* = 6.2 Hz, 1H), 5.43 (s, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 2.29 (s, 3H), 2.22 (s, 3H), 1.13 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO- d_6) δ 166.37, 157.28, 145.77, 129.09, 117.22, 113.48, 59.09, 14.20, 12.63. **IR** (KBr) (ν, cm^{-1}): 3290, 3192, 3070, 2978, 2931, 2811, 2713, 2592, 2468, 1664, 1591, 1485, 1452. **HRMS** (APPI) m/z calculated for $C_{15}H_{18}N_2O_3S$ 307.1111 [M+H]; found 307.1116.

*4.2.2.3. Ethyl 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**2c**)*

Beige solid, m.p. 163-164ºC, 87% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 9.54 (s, 1H), 8.83 (s, 1H), 6.78 (s, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 7.3 Hz, 1H), 5.41

(s, 1H), 4.04 – 3.98 (m, 3H), 3.71 (s, 3H), 2.30 (s, 3H), 2.22 (s, 3H), 1.13 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO- d_6) δ 166.40, 147.17, 118.73, 115.25, 111.07, 58.96, 55.60, 14.17, 12.58. **IR** (KBr) (ν, cm^{-1}): 3304, 2994, 2930, 2831, 2737, 2592, 1658, 1611, 1595, 1519. **HRMS** (APPI) m/z calculated for $C_{16}H_{20}N_2O_4S$ 337.1217 [M+H]; found 337.1217.

*4.2.2.4. Ethyl 6-methyl-2-(methylthio)-4-(3-nitrophenyl)-1,4-dihydropyrimidine-5-carboxylate (**2d**)*

White solid, m.p. 215-218 ºC [Lit. 220-221ºC][77], 99% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.08 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.69 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.49 – 7.45 (m, 1H), 5.78 (s, 1H), 4.18 – 4.10 (m, 2H), 2.40 (s, 3H), 2.34 (s, 3H), 1.23 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.60, 148.28, 147.05, 133.42, 129.26, 122.12, 122.00, 60.15, 14.24, 13.48.

*4.2.2.5. Ethyl 4-(benzo[d][1,3]dioxol-5-yl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**2e**)*

Yellow solid, m.p. 118-121 ºC, 95% yield; **^{1}H NMR** (200 MHz, $CDCl_3$) δ 6.82 (s, 1H), 6.72 (t, *J* = 8.5 Hz, 2H), 5.90 (s, 2H), 5.53 (s, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 2.40 (s, 3H), 2.31 (s, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 190.59, 166.98, 147.61, 146.63, 139.19, 120.13, 107.93, 107.60, 100.91, 59.87, 14.29, 13.47. **IR** (KBr) (ν, cm^{-1}): 3325, 3278, 3223, 3078, 2978, 2931, 2906, 2782, 1854, 1668, 1617, 1501, 1485. **HRMS** (APCI) m/z calculated for $C_{16}H_{18}N_2O_4S$ [M+H] 335.1060; found 335.1059.

*4.2.2.6. Ethyl 4-(4-methoxyphenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**2f**)*

Yellowish solid, m.p. 126-129 ºC [Lit. 135-136ºC][77], 91% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.6 Hz, 2H), 5.55 (s, 1H), 4.09 (q, *J* = 7.1 Hz, 2H), 3.77 (s, 3H), 2.41 (s, 3H), 2.32 (s, 3H), 1.20 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.00, 158.96, 137.47, 128.14, 113.86, 59.85, 55.34, 14.36, 13.59.

*4.2.2.7. Ethyl 6-methyl-2-(methylthio)-4-p-tolyl-1,4-dihydropyrimidine-5-carboxylate (**2g**)*

Beige solid, m.p. 169-171 ºC [Lit. 175-176ºC][77], 99% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.1 Hz, 2H), 7.10 (d, *J* = 7.9 Hz, 2H), 5.61 (s, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 2.44 (s, 3H), 2.34 (s, 3H), 2.32 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.88, 141.90, 137.05, 129.47, 129.18, 128.46, 126.92, 126.61, 59.90, 21.20, 14.34, 13.80, 13.65.

*4.2.2.8. Ethyl 4-(3,4-dimethoxyphenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**2h**)*

Orange solid, m.p. 58-61 ºC, 99% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.84 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.77 (d, *J* = 8.3 Hz, 1H), 5.58 (s, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.84 (s, 6H), 2.42 (s, 3H), 2.33 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.02, 148.88, 148.33, 137.87, 118.94, 111.21, 110.69, 59.83, 55.93, 14.36, 13.55. **IR** (KBr) (ν, cm^{-1}): 3594, 3427, 3217, 3078, 2986, 2962, 2929, 2904, 2833, 2042, 1850, 1648, 1593, 1513, 1477. **HRMS** (APCI) m/z calculated for $C_{17}H_{22}N_2O_4S$ [M+H] 351.1373; found 351.1375.

*4.2.2.9. Ethyl 4-(3-bromophenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**2i**)*

Yellow solid, m.p. 101-104 ºC, 98% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.25 (d, *J* = 7.7 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 6.63 (s, 1H), 5.67 (s, 1H), 4.17 – 4.05 (m, 2H), 2.41 (s, 3H), 2.31 (s, 3H), 1.21 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.71, 147.29, 130.21, 130.03, 125.78, 122.49, 60.03, 14.35, 13.62. **IR** (KBr) (ν, cm^{-1}): 3308, 3231, 3092, 3064, 2988, 2927, 1658, 1619, 1589, 1566, 1489. **HRMS** (APCI) m/z calculated for $C_{15}H_{17}BrN_2O_2S$ [M+H] 369.0267; found 369.0245.

*4.2.2.10. Ethyl 6-methyl-2-(methylthio)-4-(naphthalen-2-yl)-1,4-dihydropyrimidine-5-carboxylate (**2j**)*

Yellowish solid, m.p. 127-130 ºC, 98% yield; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.82 – 7.78 (m, 3H), 7.71 (s, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.46 – 7.44 (m, 2H), 5.82 (s, 1H), 4.09 (q, *J* = 7.1 Hz, 2H), 2.42 (s, 3H), 2.36 (s, 3H), 1.19 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 166.99, 142.16, 133.38, 132.90, 128.32, 128.17, 127.63, 125.97, 125.73, 125.35, 77.48, 77.16, 76.84, 59.91, 14.32, 13.60. **IR** (KBr) (ν, cm^{-1}): 3326, 3218, 3051, 2994, 2965, 2953, 2933, 1652, 1474; **HRMS** (APPI) m/z calculated for $C_{19}H_{20}N_2O_2S$ 341.1318 [M+H]; found 341.1319.

*4.2.2.11. Ethyl 4-(4-hydroxyphenyl)-6-methyl-2-(methylthio)-1,4-dihydropyrimidine-5-carboxylate (**2k**)*

Beige solid, m.p. 193-195ºC, 90% yield; **^{1}H NMR** (400 MHz, DMSO- d_6) δ 9.49 (s, 1H), 9.21 (s, 1H), 7.00 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 7.7 Hz, 2H), 5.40 (s, 1H), 3.99 (q, *J* = 7.0 Hz, 2H), 2.27 (s, 3H), 2.21 (s, 3H), 1.12 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101

MHz, DMSO- d_6) δ 166.29, 127.49, 114.82, 58.85, 14.08, 12.47. **IR** (KBr) (ν, cm^{-1}): 3310, 3068, 2992, 2935, 2798, 2725, 2672, 2590, 2513, 2468, 1658, 1609, 1589, 1511. **HRMS** (APPI) m/z calculated for $C_{15}H_{18}N_2O_3S$ 307.1111 [M+H]; found 307.1111.

*4.2.2.12. 7,7-dimethyl-2-(methylthio)-4-(naphthalen-2-yl)-4,6,7,8-tetrahydroquinazolin-5(1H)-one (**7a**)*

Beige solid, m.p. 125-128 ºC, 90% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 7.88 – 7.84 (m, 3H), 7.65 (s, 1H), 7.53 – 7.41 (m, 3H), 2.44 – 2.35 (m, 5H), 2.22 (d, *J* = 16.2 Hz, 1H), 2.05 (d, *J* = 15.2 Hz, 1H), 1.02 (s, 3H), 0.92 (s, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 132.67, 132.13, 127.77, 127.35, 126.03, 125.57, 124.57, 50.44, 32.16, 28.92, 12.58. **IR** (KBr), (ν, cm^{-1}): 3249, 3182, 3110, 3076, 2951, 2925, 2888, 2867, 2817, 2798, 2731, 1740, 1709, 1656, 1630, 1605, 1487. **HRMS** (APPI) m/z calculated for $C_{21}H_{22}N_2OS$ [M+H] 351.1526; found 351.1525.

*4.2.2.13. 2-(methylthio)-4-(naphthalen-2-yl)-4,6,7,8-tetrahydroquinazolin-5(1H)-one (**7b**)*

Yellowish solid, m.p. 232-234 ºC, 99% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 7.90 – 7.83 (m, 3H), 7.65 (s, 1H), 7.52 – 7.45 (m, 3H), 5.64 (s, 1H), 2.51 – 2.45 (m, 2H), 2.38 (s, 3H), 2.26 – 2.23 (m, 2H), 1.93 – 1.85 (m, 2H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 194.99, 142.24, 132.73, 132.20, 128.07, 127.84, 127.36, 126.05, 125.70, 125.38, 124.56, 36.86, 20.89, 12.65. **IR** (KBr), (ν, cm^{-1}): 3245, 3168, 3055, 2933, 2872, 1709, 1646, 1626, 1601, 1481. **HRMS** (APPI) m/z calculated for $C_{19}H_{18}N_2OS$ [M+H] 323.1213; found 323.1215.

*4.2.2.14. Ethyl 6-methyl-2-(methylselanyl)-4-(naphthalen-2-yl)-1,4-dihydropyrimidine-5-carboxylate (**4a**)*

Yellowish oil, 98% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 9.72 (s, 1H), 7.87 – 7.83 (m, 3H), 7.66 (s, 1H), 7.50 – 7.44 (m, 3H), 5.70 (s, 1H), 4.00 (q, *J* = 7.0 Hz, 2H), 2.29 (s, 3H), 2.21 (s, 3H), 1.10 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 166.28, 145.83, 145.27, 142.77, 132.77, 132.18, 128.00, 127.78, 127.36, 126.00, 125.59, 124.53, 97.82, 59.72, 59.01, 17.55, 14.10, 5.89. **IR** (KBr), (ν, cm^{-1}): 3241, 3053, 2955, 2925, 2870, 2853, 1697, 1685, 1648, 1464, 1369. **HRMS** (APPI) m/z calculated for $C_{19}H_{20}N_2O_2Se$ [M+H] 389.0764; found 389.0762.

*4.2.3. General procedure for the synthesis of pyrimidinicselenoureas**3a-k** and **8a-b***

The procedure previously reported [54] was followed with modifications. In a two-necked round-bottom flask at room temperature and under argon atmosphere, Se^0 (5.0 mmol) and ethanol (20 mL) were added followed by addition of $NaBH_4$ in a portionwise manner (10 mmol). The mixture was stirred until the solution became colourless. After this time, the appropriate isothioureas**2a-k** were added (1mmol). The reaction temperature was allowed to reach reflux temperature and left stirring for 15 h. The reaction mixture was extracted with ethyl acetate/water and the organic phase was dried over $MgSO_4$ and concentrated under vacuum. The resulting products presented high purity and were used without further purification.

*4.2.3.1. Ethyl 6-methyl-4-phenyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3a**)*

White solid, m.p. 191 – 193ºC, 92% yield. **^{1}HNMR** (200 MHz, DMSO-d_6) δ 10.66 (s, 1H); 10.19 (s, 1H); 7.20 – 7.40 (m, 5H); 5.19 (d, *J* = 3.6 Hz, 1H); 4.01 (q, *J* = 7.0 Hz, 2H); 2.30 (s, 1H); 1.10 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.39; 165.21; 144.12; 143.05; 128.66; 127.85; 126.48; 101.25; 59.76; 54.22; 17.03; 14.01. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 279.35. **IR** (KBr), (ν, cm^{-1}): 3326; 3153; 3094; 2977; 2871; 1670; 1574; 1466. **HRMS** (APPI) m/z calculated for $C_{14}H_{16}N_2O_2Se$ [M+H] 325.0450; found 325.0455.

*4.2.3.2. Ethyl 4-(3-hydroxyphenyl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3b**)*

Yellowish solid, m.p. 193-195 ºC, 80% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.62 (s, 1H), 10.14 (d, *J* = 2.2 Hz, 1H), 9.48 (s, 1H), 7.13 (t, *J* = 8.0 Hz, 1H), 6.69 – 6.64 (m, 3H), 5.10 (d, *J* = 3.7 Hz, 1H), 4.02 (q, *J* = 7.1 Hz, 2H), 2.29 (s, 3H), 1.12 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.29, 165.27, 157.52, 144.37, 143.90, 129.59, 117.08, 114.78, 113.31, 101.31, 59.76, 54.13, 17.02, 14.13, 14.04. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 277.41. **IR** (KBr), (ν, cm^{-1}): 3308, 3163, 3110, 3012, 2982, 2951, 2935, 2870, 2472, 2366, 2219, 1925, 1847, 1666, 1617, 1589, 1573, 1475. **HRMS** (APPI) m/z calculated for $C_{14}H_{16}N_2O_3Se$ [M+H] 341.0399; found 341.0400.

*4.2.3.3. Ethyl 4-(4-hydroxy-3-methoxyphenyl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3c**)*

Yellowish solid, m.p. 180-183 ºC, 95% yield. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 10.09 (s, 1H), 9.03 (s, 1H), 6.79 (s, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.59 (d, *J* = 7.6 Hz, 1H), 5.10 (s, 1H), 4.02 (q, *J* = 6.9 Hz, 2H), 3.73 (s, 3H), 2.29 (s, 3H), 1.11 (t, *J* = 6.8 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.10, 165.33, 147.40, 146.29,

143.63, 134.13, 118.68, 115.47, 111.03, 101.58, 59.66, 55.62, 53.86, 16.95, 14.05. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 274.68. **IR** (KBr), (ν, cm^{-1}): 3480, 3321, 3151, 3094, 3002, 2972, 2937, 2906, 2847, 2362, 2217, 2076, 1887, 1668, 1648, 1603, 1572, 1517. **HRMS** (APPI) m/z calculated for $C_{15}H_{18}N_2O_4Se$ [M+H] 371.0505; found 371.0503.

*4.2.3.4. Ethyl 4-(3-aminophenyl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3d**)*

Yellowish solid, m.p. 189-192 ºC, 80% yield. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 10.07 (s, 1H), 6.96 (t, *J* = 7.7 Hz, 1H), 6.47 – 6.44 (m, 1H), 6.41 (dd, *J* = 1.8 Hz, 1H), 6.37 (d, *J* = 7.7 Hz, 1H), 5.10 (s, 2H), 5.03 (d, *J* = 3.7 Hz, 1H), 4.01 (q, *J* = 7.1 Hz, 2H), 2.29 (s, 3H), 1.11 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.02, 165.25, 148.67, 143.51, 143.40, 128.88, 113.98, 113.33, 111.78, 101.38, 59.53, 54.42, 16.88, 13.93. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 274.04. **IR** (KBr), (ν, cm^{-1}): 3390, 3363, 3294, 3165, 2974, 2900, 2874, 1929, 1717, 1672, 1654, 1607, 1572. **HRMS** (APPI) m/z calculated for $C_{14}H_{17}N_3O_2Se$ [M+H] 340.0559; found 340.0560.

*4.2.3.5. Ethyl 4-(benzo[d][1,3]dioxol-5-yl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3e**)*

Yellowish solid, m.p. 173-175 ºC, 99% yield. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 10.13 (s, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.73 (d, *J* = 1.7 Hz, 1H), 6.68 (dd, *J* = 8.0, 1.7 Hz, 1H), 6.00 (s, 2H), 5.11 (d, *J* = 3.6 Hz, 1H), 4.05 – 3.98 (m, 2H), 2.30 (s, 3H), 1.11 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.21, 165.18, 147.44, 146.81, 144.03, 136.99, 119.77, 108.17, 106.76, 101.28, 101.12, 59.70, 53.85, 16.98, 13.99. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 278.71. **IR** (KBr), (ν, cm^{-1}): 3316,

3159, 3102, 3011, 2976, 2935, 2894, 2782, 2613, 2360, 1850, 1666, 1573, 1499. **HRMS** (APPI) m/z calculated for $C_{15}H_{16}N_2O_4Se$ [M+H] 369.0349; found 369.0344.

*4.2.3.6. Ethyl 4-(4-methoxyphenyl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3f**)*

Yellowish solid, m.p. 125-127 ºC, 95% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.62 (s, 1H), 10.15 (s, 1H), 7.15 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 5.14 (d, *J* = 1.4 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.73 (s, 3H), 2.31 (s, 3H), 1.10 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 170.09, 165.26, 158.87, 143.85, 135.29, 127.75, 113.95, 101.52, 59.70, 55.13, 53.65, 17.00, 14.02. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 276.26. **IR** (KBr), (ν, cm^{-1}): 3308, 3155, 3104, 2978, 2933, 2902, 2835, 2360, 2046, 1889, 1707, 1689, 1664, 1609, 1573, 1509. **HRMS** (APPI) m/z calculated for $C_{15}H_{18}N_2O3Se$ [M+H] 355.0556; found 355.0551.

*4.2.3.7. Ethyl 6-methyl-2-selenoxo-4-p-tolyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3g**)*

Yellowish solid, m.p. 155-158 ºC, 65% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.63 (s, 1H), 10.16 (s, 1H), 7.16 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 7.9 Hz, 2H), 5.16 (d, J = 3.0 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 2.30 (s, 3H), 2.27 (s, 3H), 1.10 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 170.28, 165.24, 143.96, 140.17, 137.11, 129.15, 126.40, 101.37, 59.72, 53.93, 20.70, 17.00, 14.02. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 277.74. **IR** (KBr), (ν, cm^{-1}): 3325, 3153, 3096, 3021, 2978, 2931, 2872, 2729, 2486, 2364, 2225, 1899, 1675, 1575, 1509, 1466. **HRMS** (APPI) m/z calculated for $C_{15}H_{18}N_2O_2Se$ [M+H] 339.0607; found 339.0603.

*4.2.3.8. Ethyl 4-(3,4-dimethoxyphenyl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3h**)*

Orange solid, m.p. 108-110 ºC, 91% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.63 (s, 1H), 10.15 (s, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 6.85 (d, *J* = 1.5 Hz, 1H), 6.72 (dd, *J* = 8.3, 1.6 Hz, 1H), 5.16 (d, *J* = 3.4 Hz, 1H), 4.03 (q, *J* = 7.1 Hz, 2H), 3.73 (s, 6H), 2.31 (s, 3H), 1.13 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 170.29, 165.32, 148.59, 148.46, 143.93, 135.54, 118.30, 111.87, 110.51, 101.41, 59.74, 55.55, 55.47, 53.78, 17.00, 14.08. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 276.38. **IR** (KBr), (ν, cm^{-1}): 3531, 3306, 3155, 2976, 2935, 2906, 2835, 2600, 2370, 2278, 2043, 1882, 1705, 1662, 1573, 1515, 1462. **HRMS** (APPI) m/z calculated for $C_{16}H_{20}N_2O_4Se$ [M+H] 385.0662; found 385.0665.

*4.2.3.9. Ethyl 4-(3-bromophenyl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3i**)*

Yellowish solid, m.p. 201-203 ºC, 95% yield. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.72 (s, 1H), 10.20 (s, 1H), 7.50 (ddd, *J* = 7.9, 2.0, 1.0 Hz, 1H), 7.38 (dd, *J* = 1.8 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.8 Hz, 1H), 5.19 (d, *J* = 3.6 Hz, 1H), 4.08 – 3.95 (m, 2H), 2.31 (s, 3H), 1.10 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.81, 164.92, 145.44, 144.43, 130.87, 130.57, 129.25, 125.34, 121.62, 100.67, 59.70, 53.66, 16.93, 13.84. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 286.76. **IR** (KBr), (ν, cm^{-1}): 3300, 3164, 3098, 2980, 2923, 2866, 1978, 1940, 1872, 1666, 1576, 1562, 1464. **HRMS** (APPI) m/z calculated for $C_{14}H_{15}BrN_2O_2Se$ [M+H] 402.9552; found 402.9549.

*4.2.3.10. Ethyl 6-methyl-4-(naphthalen-2-yl)-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3j**)*

Yellow solid, m.p. 176-179 ºC, 95% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.74 (s, 1H), 10.32 (s, 1H), 7.96 – 7.86 (m, 3H), 7.71 (s, 1H), 7.54 – 7.42 (m, 3H), 5.40 (s, 1H), 4.01 (q, *J* = 6.9 Hz, 2H), 2.37 (s, 3H), 1.08 (t, *J* = 7.0 Hz, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 170.47, 165.21, 144.33, 140.35, 132.64, 132.50, 128.61, 127.95, 127.55, 126.48, 126.25, 125.11, 124.80, 101.03, 59.72, 54.54, 17.10, 13.99. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 280.93. **IR** (KBr), (ν, cm^{-1}): 3310, 3157, 3098, 3055, 2974, 2939, 2866, 1950, 1921, 1905, 1821, 1664, 1601, 1568, 1456. **HRMS** (APPI) m/z calculated for $C_{18}H_{18}N_2O_2Se$ [M+H] 375.0607; found 375.0605.

*4.2.3.11. Ethyl 4-(4-hydroxyphenyl)-6-methyl-2-selenoxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**3k**)*

Yellowish solid, m.p. 159-161 ºC, 86% yield. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 10.08 (s, 1H), 9.45 (s, 1H), 7.01 (d, *J* = 8.6 Hz, 2H), 6.72 (d, *J* = 8.6 Hz, 2H), 5.07 (d, *J* = 3.7 Hz, 1H), 4.03 – 3.96 (m, 2H), 2.28 (s, 3H), 1.09 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 169.93, 165.22, 156.96, 143.42, 133.62, 127.66, 115.15, 101.68, 59.53, 53.71, 16.87, 13.92. **^{77}Se NMR** (76 MHz, DMSO-d_6) δ 274.33. **IR** (KBr), (ν, cm^{-1}): 3323, 3178, 3000, 2980, 2935, 2694, 2554, 2466, 2417, 1923, 1899, 1715, 1664, 1611, 1573. **HRMS** (APPI) m/z calculated for $C_{14}H_{16}N_2O_3Se$ [M+H] 341.0399; found 341.0404.

*4.2.3.12. 7,7-dimethyl-4-(naphthalen-2-yl)-2-selenoxo-1,2,3,4,7,8-hexahydroquinazolin-5(6H)-one (**8a**)*

Off white solid, m.p. 236-238 ºC, 92% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 10.95 (s, 1H), 10.33 (s, 1H), 7.93 – 7.88 (m, 3H), 7.69 (s, 1H), 7.54 – 7.49 (m, 2H), 7.39 (dd,

J = 8.7, 1.2 Hz, 1H), 5.36 (d, *J* = 3.0 Hz, 1H), 2.46 (s, 2H), 2.26 (d, *J* = 16.2 Hz, 1H), 2.06 (d, *J* = 16.3 Hz, 1H), 1.03 (s, 3H), 0.89 (s, 3H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 194.05, 171.21, 147.75, 140.18, 132.59, 132.37, 128.45, 127.85, 127.49, 126.43, 126.16, 125.17, 124.64, 108.24, 52.81, 49.85, 38.37, 32.23, 28.78, 26.72. **IR** (KBr), (ν, cm^{-1}): 3233, 3159, 3061, 2990, 2962, 2925, 2890, 2868, 2849, 1966, 1911, 1846, 1811, 1738, 1707, 1658, 1626, 1568, 1509, 1460. **HRMS** (APPI) m/z calculated for $C_{20}H_{20}N_2OSe$ [M+H] 385.0814; found 385.0817.

*4.2.3.13. 4-(naphthalen-2-yl)-2-selenoxo-1,2,3,4,7,8-hexahydroquinazolin-5(6H)-one (**8b**)*

Off white solid, m.p. 230-233 ºC, 86% yield. **^{1}H NMR** (200 MHz, DMSO-d_6) δ 11.00 (s, 1H), 10.36 (s, 1H), 7.93 – 7.86 (m, 3H), 7.70 (s, 1H), 7.53 – 7.49 (m, 2H), 7.43 (d, *J* = 8.6 Hz, 1H), 5.40 (d, *J* = 3.0 Hz, 1H), 2.54 – 2.47 (m, 2H), 2.32 – 2.25 (m, 2H), 2.02 – 1.80 (m, 2H). **^{13}C NMR** (50 MHz, DMSO-d_6) δ 194.33, 171.05, 149.85, 140.10, 132.61, 132.38, 128.44, 127.89, 127.46, 126.36, 126.12, 125.00, 124.78, 109.11, 52.47, 36.35, 25.21, 20.43. **IR** (KBr), (ν, cm^{-1}): 3245, 3159, 3055, 2953, 2923, 2870, 1626, 1564, 1507, 1456. **HRMS** (APPI) m/z calculated for $C_{18}H_{16}N_2OSe$ [M+H] 357.0501; found 357.0501.

4.3. Biological and molecular assays

4.3.1. Cell culture

MCF-7 cells (human breast carcinoma), HeLa (human cervical adenocarcinoma) and McCoy (normal fibroblasts) were obtained from the Rio de Janeiro cell bank, Brazil, and were cultured at 37ºC under 5% CO_2 atmosphere with 95% air humidity and

allowed to reach confluence. Dulbecco's modified Eagle's medium used in cell culture was supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 µg/mL).

4.3.2. Citotoxicity assay

The cytotoxic effects of pyrimidinic selenoureas on tumour and normal cells were measured using the tetrazolium salt method (MTT) as described previously by Mosmann [78].Briefly, MCF-7, HeLa and McCoy cells (10^4/well) were plated into 96-well plates. At confluence, the cells were exposed to pyrimidinic selenoureas and to a positive control agents (Monastrol and 5-FU) at increasing concentrations (0.1, 1, 10, 100 and 1000 µM) for 72 h. In control wells, the cells were incubated in a medium containing 1%DMSO. The cells were then washed twice with PBS and incubated for 2 h with MTT (0.5 mg/mL). The formazan crystals were solubilized by adding DMSO (100 µL/well), and the colored solutions were spectrophotometrically measured at 550 nm. Three independent experiments were conducted, and the results are presented as IC_{50} values.

The degree of selectivity of pyrimidinic selenoureas for tumour cells was calculated according to Koch et al [39]: SI = IC_{50} of the compound in normal cell line/IC_{50} of the compound in cancer cell line.

4.3.3. Antiproliferative effects

The effects on cell proliferation were examined by the colony formation assay, according to Franken et al [79]. MCF-7 cells (500/well) were allowed to set in six-well plates for 24 h. The medium was then replaced by one containing pyrimidinic selenoureas at non-cytotoxic concentrations and incubated for 72 h. In control wells,

cells were incubated in medium containing 1% DMSO. After treatment, cells were washed twice with warm PBS, and fresh medium was added. The cells were incubated for 15 days and then stained with crystal violet and colonies were counted.

4.3.4. Cell death induced by pyrimidinic selenoureas

MCF-7 cells (2×10^5/well) were plated into 6-well plates and, after confluence, received treatments with the pyrimidinic selenoureas at non-toxic concentrations for 72 h. After washing, cells were stained with a solution (6 µL, 1:1) of acridine orange (100 µg/mL) plus propidium iodide (100 µg/mL). Subsequently, cells (300/glass slide) from triplicates of each treatment were categorized through microscopy as being viable, apoptotic or necrotic [80].

4.3.5. DNA binding and intercalation

DNA-binding experiments were performed by UV-Vis spectrometry [81]. Absorption titration experiments were done using different concentrations of pyrimidinic selenoureas (50-350 µM), while keeping constant the concentration of CT-DNA (150 µM). Spectra were obtained by reading the absorption from 230 to 800 nm in a Hitachi U-2910 spectrophotometer. General changes in terms of absorption as well as the displacement of maximum absorption were evaluated.

DNA intercalation was evaluated by fluorescence measurements using a TECAN Infinity M200 microplate reader, according to a protocol adapted from Silveiraet al [82]. CT-DNA (150 µM) was saturated with the DNA-intercalating agent propidium iodide (300 µM) in a 50 mM phosphate buffer containing 0.1 M NaCl (pH 7.4). Doxorubicin, an antitumor drug known for its ability to intercalate DNA base pairs, was used as a positive control. Fluorescence titrations were conducted by keeping

constant the concentration of CT-DNA and propidium iodide and varying the concentration of pyrimidinic selenoureas (0-350 µM). The excitation and emission wavelengths were 535 nm and 617 nm, respectively.

4.3.6. Evaluation of DNA fragmentation by Comet Assay

Cells treated with non-toxic concentrations of pyrimidinic selenoureas for 72 h were suspended in low-melting temperature agarose (0.75%) and then deposited on slides containing a thin layer of agarose (1%) and allowed to set for 10 min at room temperature. After this, the slides were submerged for 5-7 days in a lysis solution (2.5 mMNaCl, 100 mM EDTA, 1% Triton X-100, 10% DMSO, 10 mMTris, pH 10.0) at 4ºC. Slides were then subjected to horizontal electrophoresis at 300 mA, 8ºC, for 20 min in a tank with buffer (NaOH 300 mM, EDTA 1 mM, pH 13) and subsequently washed with a neutralizing solution (Tris-HCl 0.4 M, pH 7.5). A fixing solution (15% trichloroacetic acid, 5% $ZnSO_4$, 5% glycerol) was added for 10 min, followed by washing and drying. The slides were stained with $AgNO_3$ (0.001 g/mL) and analysed under an optical microscope. The results are expressed as damage index (score 0-4) [83].

4.3.7. Cell cycle arrest

The distribution of cells in phases of the cell cycle was assessed according to the cellular DNA content as measured by flow cytometry using a PI/RNAse solution kit from Immunostep® (Salamanca, Spain) and followed the procedure provided by the manufacturer. MCF-7 cells (2×10^5) were plated in 6-well plates. After adhesion, cells were synchronized using nocodazole (30 ng/mL) for 12 h. Then, the medium was replaced by one containing pyrimidinic selenoureas at non-toxic concentrations and the

cells were incubated for 72 h. The cells were washed and fixed carefully in cold ethanol (70%) at -20 ºC overnight. Again, the cells were washed with PBS, resuspended and incubated with PI/RNAse solution for 15 min at room temperature. Finally, cells were evaluated by FACSCanto II (BD Biosciences) flow cytometer. Data were processed using Flowing Software 2.5.

4.3.8. Data analysis

Assays were performed in triplicate. Data were analysed with the ANOVA test followed by Bonferroni's and Tukey's test. Comparisons and IC_{50} values were processed using GraphPad Prism 5.0 software (San Diego, USA). Values of $p < 0.05$ were considered to be statistically significant.

Conflict of interest

The authors declare no conflicts of interest associated with this paper.

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- 14 Pyrimidinic selenoureas synthesized and evaluated against cancer cell lines
- Pyrimidinic selenourea **8a** caused DNA damage, inhibited cell proliferation and cell cycle arrest
- The pharmacokinetic parameters were favorable for compound **8a**