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Research article

Highly bioactive novel aryl-, benzyl-, and piperazine-selenoureas: synthesis, structural characterization and in vitro biological evaluation

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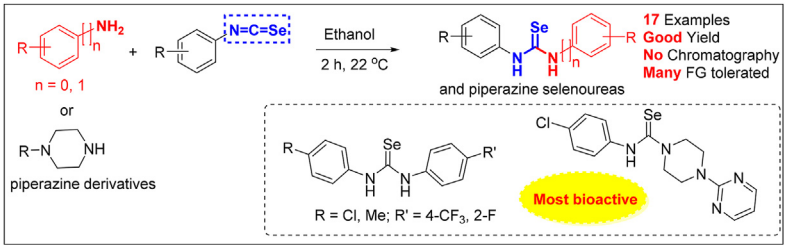
GRAPHICAL ABSTRACT



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ABSTRACT

Selenoureas are widespread as useful elements for constructing important species and biologically active molecules. Finding an efficient and straightforward method to prepare this motif and biologically screen derivatives thereof is crucial. Herein, we demonstrate the effectiveness of using ethanol as a solvent in the preparation of various substituted aryl-, benzyl-, and piperazine-selenoureas from isoselenocyanates and amines. The synthetic method includes mild reaction conditions, large substrate scope, and good isolated yields. Biological evaluation of the prepared products on MDA-MB-231 and MCF-7 cancer cell lines revealed several remarkably active compounds (IC_{50} < 10 μM) with the best one exhibiting IC_{50} values of 1.8 μM and 1.2 μM observed against the challenging former triple-negative breast cancer cell line and the latter one, respectively. The chemical structures of all new compounds were fully characterized by multinuclear nuclear magnetic resonance (NMR) spectroscopy and high accuracy mass measurements.

1. Introduction

Selenoureas are organoselenium compounds which contain two amine (–NH) groups flanking the sides of a selenocarbonyl (C=Se) function. In recent years, the synthesis, and applications of selenourea derivatives have received growing attention and have been the subject of several reviews [1, 2]. Such species are close structural analogs of thiourea and urea compounds, although the presence of larger and more polarizable selenium atom introduces significant changes to their chemical and biological properties [2, 3]. As such, they have been widely

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used as key precursors to make biologically important heterocycles [4, 5, 6, 7] and have been utilized in many applications in the fields of organic and analytical chemistry, agriculture, and medicine.

To highlight the versatile utility of selenoureas, several compounds (**1**–**14**) performing various biological and chemical functions have been summarized in Figure 1. Thus, in organic chemistry, chiral selenoureas containing Cinchona alkaloid scaffolds such as **1** have been utilized as bifunctional organocatalysts in the asymmetric Michael addition of nitromethane to *trans*-chalcone [8] and dithiomalonate to nitrostyrene [9], promoting the reactions with product enantioselectivities reaching 96% enantiomeric excess. Another chiral selenourea Brønsted acid catalyst **2** was found to facilitate the asymmetric conjugate additions of cyclic amines to unactivated α,β-unsaturated esters to produce β-amino esters with high levels of enantioselectivity [10]. 1,3-disubstituted selenoureas have been deselenized by iodine to carbodiimides **3** [11], which have extensive synthetic utility in peptide coupling chemistry, oxidation of alcohols with DMSO, and dehydration reactions, just to list a few. They also serve as precursors like **4** to access bicyclic sugar isoureas [12].

In analytical chemistry, selenoureas have proven useful receptors for the binding, recognition, and transport of various anions [13] and can generate mono- and bi-coordinated adducts [14]. Recently, molecular logic gate founded on selenoureas/anions host-guest interaction using substrate **13** was reported to perform a ternary (conditional) logic operation employing proton NMR to measure response output [15]. Another innovative application exploiting the binding properties of selenoureas involves utilizing them as suitable phasing agents for macromolecular X-ray crystal structures in parallel to the traditional practice of heavy-atom derivatization. In this strategy, selenourea ($SeC(NH_2)_2$) (**5**) is introduced into protein crystals and binds to reactive moieties at the surface of macromolecules via H-bonding, where the amide groups may act as donors and selenium atom as acceptor [16]. Bis-selenoureas in the form of bis(imidazoline selone) (**9**) have been employed as ligands for iridium photocatalyst complexes to promote oxidative coupling reaction of benzylamines to imines with visible light [17]. Such bidentate organochalcogens have also found success as ligands for iridium(III) and rhodium(III) complexes for application as catalysts for norbornene polymerization [18]. Other cyclic selenoureas like *N*′-dimethylimidazole selone have been used as starting materials to zinc selenides [19] and copper complexes of biological importance [20, 21, 22]. The related NHC-derived cyclic selenourea **7** and its related derivatives, beneficial for the quantification of NHC electronic properties, have also been explored in the coordination to gold(I) [23]. *N,N,N*′-trisubstituted selenourea **10** and other derivatives serve as precursors to lead selenide (PbSe) nanocrystals, useful for optoelectronics and photovoltaic devices [24].

Biologically, selenourea derivatives containing diselenide linkage **14** were found cytotoxic against *Leishmania infantum* axenic intracellular amastigotes and demonstrated improved antileishmanial activity in infected macrophages over the reference drug miltefosine (EC50 < 2.84 μM). *N*-Aliphatic substituents such as hexyl in selenourea was necessary for

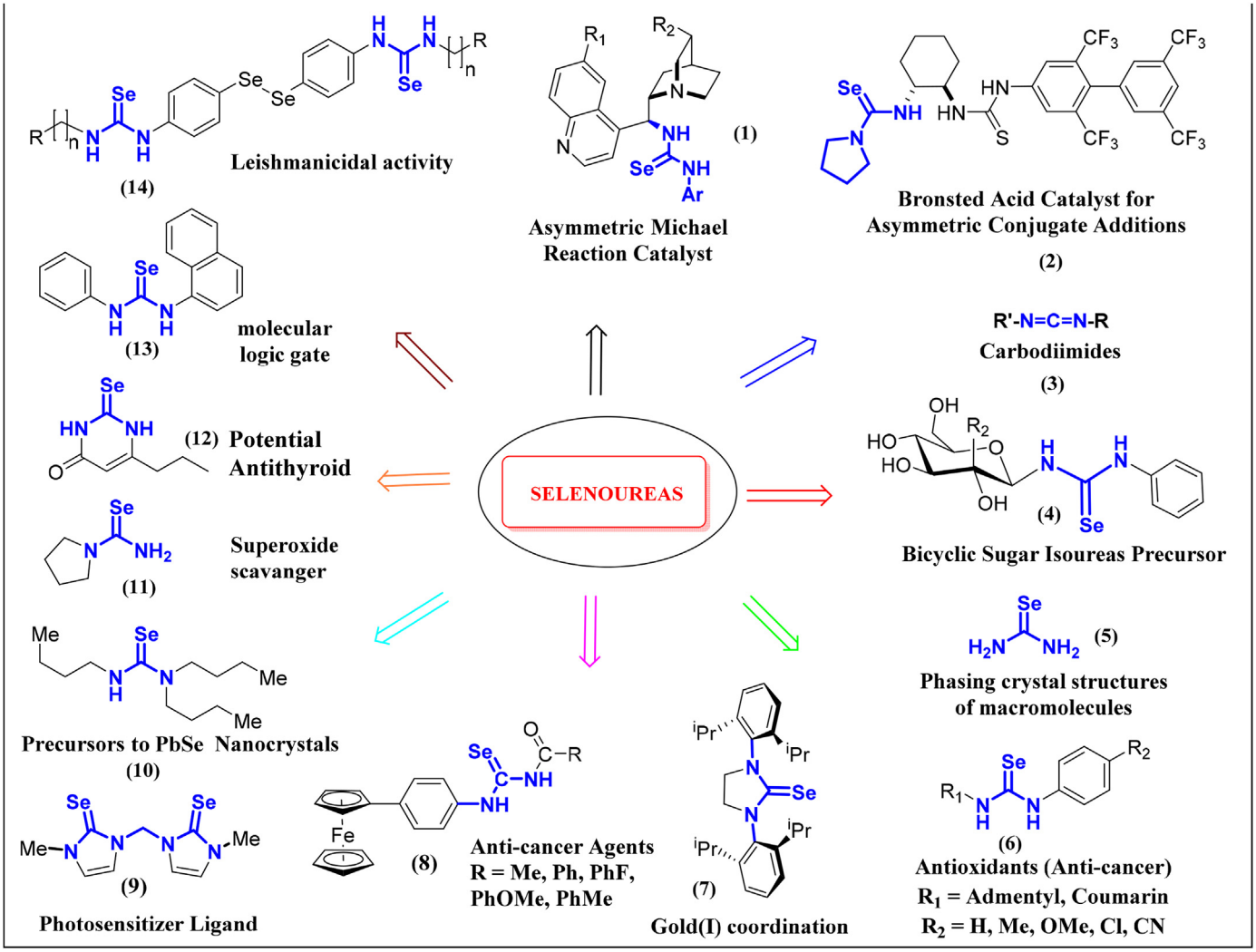


Figure 1. Examples of prevalence of the selenourea motif in several products and its usefulness in chemical and bioactive agents.

optimum leishmanicidal activity [25]. In biological systems, hydroxyl radical produced from the reaction of H_2O_2 with Cu^+ damages DNA oxidatively, causing cancer, ageing, and other diseases. Selenium-containing compounds like *N, N′*-dimethylimidazole selone can act as antioxidants by coordinating to copper as one justification for their observed antioxidant activity [19]. The related pyrrolidine-1-carboselenoamide (**11**) exhibits superoxide scavenging activity (SOSA) and scavenges superoxide radical species from 4-β-phorbol-12-myristate-13-actate (PMA) (71.1% inhibition) [26]. Generally, selenoureas were several folds more active than their corresponding thioureas. The existence of selenoureas as zwitterions in biological systems is a crucial property that reverses their interactions with biomolecules, restoring their original redox state. This distinctive advantage enhances their redox efficiency in comparison to sulfur analogs. The sulfur analogues of cyclic selenourea **12** are in use as drugs for the therapy of hyperthyroidism, rendering the latter as potential antithyroid agent as it significantly reduces the tyrosine nitration of both cytochrome c and BSA [27]. Presently, various forms of selenium species are being utilized as a preventative agent for cancer. Recently, some of the ferrocene incorporated selenoureas **8** [28, 29] were reported to exhibit electrostatic DNA binding capabilities through the positively charged Fe(II) with the negatively charged phosphate backbone of DNA, confirming their efficiency against cancer initiation, propagation, and termination. In some cases, derivatives of **8** interacted with DNA through partial intercalation, indicating that **8** can be useful as antitumor agent. Studies exploring the antibacterial potential of selenoureas have been scarce. In a recent study, selenourea-dihydropyrrol-2-one derivatives showed promising antibacterial activity, notably exceeding the activity of the sulfur series of analogs, highlighting the importance of selenoureas as antibacterial agents [30].

Cancer is one of the principal reasons of human death [31] where seventy percent of cancer-related mortality happens in under-developed and developing nations [31, 32, 33]. There is an increasing trend of deaths resulting from various types of cancers worldwide, with a projected twelve million deaths in the year 2030 according to WHO [34]. Despite the progress in cancer therapy, the success of cancer treatment is a significant challenge due to the varied responses of tumor cells and toxicity associated with existing chemotherapeutic drugs. Henceforth, developing new chemotherapeutic agents for cancer therapy continues to represent an inspiring endeavor for chemists.

As of the end of 2020, the number of women who were diagnosed with breast cancer (BC) in the previous five years had reached 7.8 million, rendering it the world's most frequently diagnosed type of cancer [35]. Despite showing similar pathological characteristics, patients react differently to treatments and show considerably different outcomes, highlighting the variety of BC subtypes. Thus, BC is classified into molecular subtypes according to their gene expression profile. Indeed, proteomic, genomic, transcriptional, and epigenetic results have revealed that BC cells may differ in progression, pathway activity [36], proliferative potential, apoptosis, vascularization, and ability to metastasize and invade tissues [37, 38]. Consequently, this provided us the impetus to contribute to cancer research to address some of the above shortcomings. Thus, the intention of this investigation was to assess the cytotoxic effect of selenoureas **21**–**37** against MDA-MB-231$^{ER(-)/PR(-)/HER2(-)}$ and MCF-7$^{ER(+)/PR(+)/HER2(-)}$ breast cancer cell lines as models. These were selected because they show key differences regarding the presence of receptors for estrogen (ER), progesterone (PR), and epidermal growth factor (HER2) [39]. Additionally, these cancer cell lines vary in the level of malignancy [40]. Tumors that are negative for hormone receptors are more aggressive, causing limited treatment options [41] than positive ones [40].

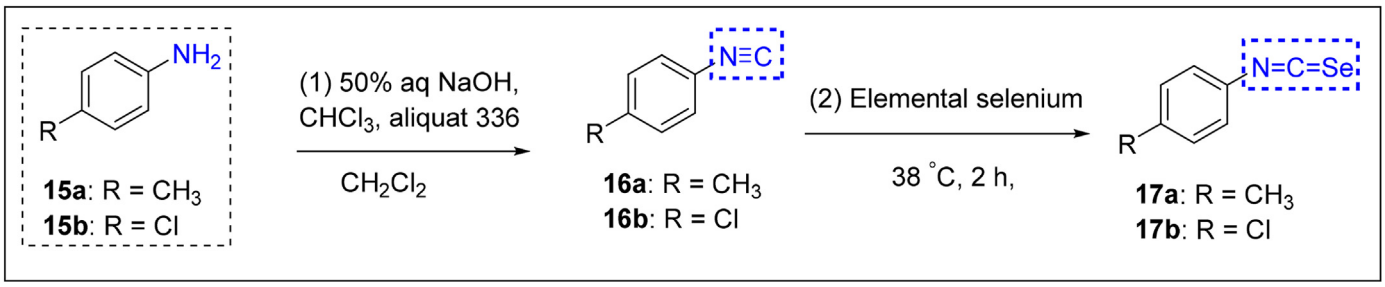
2. Results and discussion

2.1. Chemical synthesis of 21–35

Herein, we report the efficient synthesis and characterization of a new set of novel aryl, benzyl, and piperazine selenoureas **21–35**, and demonstrate their *in vitro* biological activities. Although several methods in the literature describe the synthesis of selenoureas [42, 43, 44], the best-known protocol remains the reaction of isoselenocyanates with primary and secondary amines. The critical step in the synthetic process is the preparation of isoselenocyanates, which, unlike their isothiocyanates equivalents, are commercially unavailable. There is several synthetic strategies that have been reported in the literature towards their preparation [45, 46, 47]. Among these, using elemental black selenium to oxidize isocyanides appears the most reliable synthetic method [48, 49, 50, 51]. In the current work, the first coupling partner for the preparation of the desired selenoureas, 4-methylphenyl isoselenocyanate **17a** and its 4-chloro analogue **17b** [25], were prepared in 55 and 85 % yields, respectively, from toluidine and 4-chloroaniline (Scheme 1). These isoselenocyanates were prepared in one pot according to the published procedure by Zakrzewski [40] which, incidentally, is one of the most useful and good yielding protocols. The preparation of **17a,b** initially involves *in situ* preparation of isocyanides **16a**,**b** by a Hofmann isonitrile synthesis utilizing anilines **15a**,**b** as reactants together in $CHCl_3$ and 50% aqueous NaOH solution and the ammonium salt Aliquat 336 as phase transfer reagent. Both isoselenocyanates **17a and 17b** were fully characterized by mp, Infrared, nuclear magnetic resonance, and high accuracy mass measurement techniques and the acquired data is consistent with what has been reported in the literature [25].

At the outset of our current work, we were aiming to prepare selenoureas in high purity without the need of resorting to purification by column chromatography since they could not be separated and purified by this method due to their instability and similar R_f values to the corresponding requisite amines. Pleasingly, the choice of chemical solvent and duration of reaction was key to obtaining pure products. Thus, using the less electrophilic isoselenocyanate **17a** as a model substrate and 2-nitroaniline as the least nucleophilic reactant, we explored several reaction conditions to determine the optimal conditions for best yields and purity of the target products. Initially, the reaction between **17a** and the anilines was stirred overnight at ambient temperature using a series of solvents including DMF, DMSO, and acetonitrile. Equimolar amount of triethylamine was added to foster mild basic conditions which are more suitable for the formation of the acid-sensitive selenourea. Unfortunately, the desired product was not observed as the *ortho* nitro group was detrimental to the nucleophilicity and reactivity of the aniline due to its very strong negative mesomeric effect. As an alternative aniline derivative, the more electron-rich *p*-toluidine was used instead, and the reaction was re-run under the same conditions outlined earlier. Progress of chemical reaction was followed carefully by 1H NMR spectroscopy. While extensive decomposition of the selenourea product was observed in DMSO and DMF solvents overnight as indicated by the observed copious quantities of black selenium deposited in the reaction vessel, acetonitrile proved a more suitable solvent, although affording only 36% yield of selenourea **21**. However, with ethanol as solvent and shorter reaction time to circumvent decomposition of the selenourea product, 1,3-di-p-tolylselenourea (**21**) crashed out of the reaction and was successfully isolated by filtration using Whatman filter paper in 76% yield.

Having established the optimized reaction conditions (1:1:1 equimolar amounts of the aniline, isoselenocyanate, and TEA, rt, 2h), the applicability of this synthetic route was then verified using 4-methylphenyl isoselenocyanate (**17a**) and 4-chlorophenyl isoselenocyanate (**17b**) on a variety of substituted aniline, benzyl and piperazine derivatives. Aniline and derivatives **18** carrying substituents able of exhibiting positive and negative mesomeric (+M, −M) and inductive (+I and –I) effects furnished the desired selenoureas **21**–**37** in 59–94% yield. While 2-nitroaniline was completely unreactive because of the strong negative mesomeric (−M) effect of the nitro group, the corresponding aniline analogs with negative inductive effect substituents (−I) like 4-Cl, produced the desired selenoureas **22** and **30**, although in low yields, in 63% and 59% yields, respectively. Interestingly, **22** could be prepared in 82% yield from selenourea **17b**, highlighting the crucial impact of the aromatic substituents on the reactivity of the aniline derivative and



Scheme 1. Preparation of isoselenocyanates **17a** and **17b**.

formation of selenourea product. Thus, aniline analogs with positive inductive effect (+I), positive mesomeric (+M) effect, and no substituents are expected to be more suitable reactants. Indeed, the yields of the resulting selenoureas were generally higher and reactions were faster with *p*-toluidine (Table 1, compound **21**, 76%), aniline (Table 1, compound **23**, 81%; compound **32**, 77%), and *p*-anisidine (Table 1, compound **31**, 80%). As expected, the reactivity of the benzylamine substrates was not impacted by the nature of aromatic substituent and these species were more reactive nucleophiles, as anticipated, than the aniline analogs. For instance, having strongly electron withdrawing substituents such as 4-F (−I) afforded the product **27** in 97% yield, meanwhile using an amine with a very strong electron donating group like 4-OMe (+M) afforded the selenourea **35** in a relatively lower 78% yield (Table 1). In many cases, higher yields of products ranging from 63 to 97% were obtained with benzylamines. Finally, the substituted piperazine, which presents a more sterically challenging scenario, afforded the selenourea products in high yields (Table 1, compound **36**, 94%; compound **37**, 88%). The above synthetic approach could be executed on gram scale as proven by the synthesis of selenoureas **22** and **24** on a larger quantities from *p*-touidine and benzylamine (10 mmol scale) to afford the products in 79% and 75% yields, respectively.

The resulting light peach/off-white/brown/grey products could be separated cleanly as solids without the need for purification by chromatography and are reasonably stable. All products were amenable to storage at ambient temperature for months. High-resolution mass spectrometry (HRMS) and NMR measurements validated the proposed chemical structures of selenoureas **21**–**37** shown in Table 1.

The chemical structures of all new selenoureas (**21–36**) (Table 1) and formation of the key R-**N-C**=Se(NH)-Ar bonds were confirmed and initially verified by routine spectroscopic and analytical techniques including melting point, IR, 1-Dimension NMR, and high resolution mass spectrometry. In addition, 2-dimension homonuclear and heteronuclear NMR spectroscopic measurements (^{1}H–^{1}H-gDQFCOSY, ^{1}H–^{13}C-gHSQC, ^{1}H–^{13}C-gHMBC, and ^{1}H–^{1}H-ROESYAD) were performed on one member of each group of derivatives (aryl, benzyl and piperazine) as representative examples to trace the ^{1}H–^{1}H and ^{1}H–^{13}C connectivity and identify the chemical shifts of each carbon and proton (see supporting information section S9-S84). The physical and IR/NMR spectral data of known selenoureas agreed with those stated in the literature (see experimental and supporting information sections). The most distinguishing and informative IR signals of the selenoureas are those of the N–H and C=Se groups which appear around 3100-3400 cm^{-1} and 1500–1600 in the IR region, respectively.

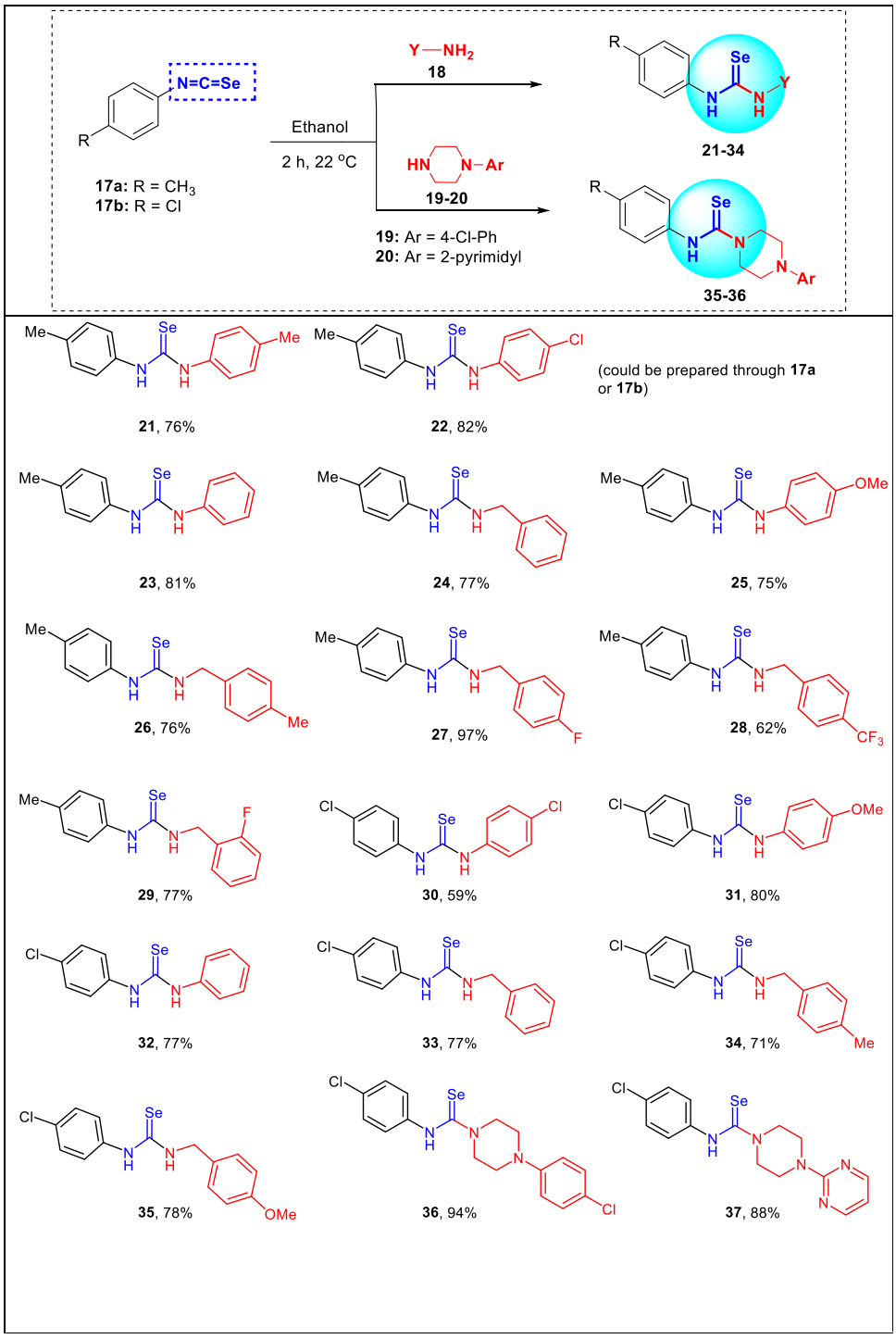
Next, using *N*-(4-chlorophenyl)-4-(pyrimidin-2-yl)piperazine-1-carboselenoamide (**37**) as a representative model example for NMR analysis and studies, the relevant 1-D and 2-D NMR spectra that were employed for structural elucidation and chemical shift assignments are shown in Figure 2. The ^{13}C-CRAPT NMR spectrum (Figure 2, spectrum b) clearly confirms the occurrence of the expected 10 signals (4 aromatic CH's, 3 aromatic quaternary carbons, 1 C=Se carbon, and 2 piperazine methylenes which is agreeable with 5 carbon atoms being magnetically equivalent. The most prominent aspect of the ^{13}C-CRAPT NMR of **37** is the existence of 4 aromatic CH's (δ 158.3, 128.4, 128.3 & 110.9 ppm) as suggested by their negative phase. The two chemical shifts at δ 158.3 and 110.9 ppm were traced to the pyrimidine $C_{5'}$H and $C_{6'}$H, respectively, based on strong correlation cross peaks observed in the ^{1}H–^{13}C-gHSQC spectrum (Figure 2d). Clearly, $C_{5'}$H and $C_{6'}$H are part of same the spin system as additionally supported by ^{1}H–^{1}H-gDQFCOSY which verified them as a totally correlated system (4-contour green square in the aromatic region (Figure 2c). The scalar coupling between the vicinal $C_{5'}$H doublet and $C_{6'}$H triplet is 4.0 Hz. The N_5–H (δ 9.68 ppm/^{1}H NMR) group of the selenourea group (Figure 2a) offered the only starting point to provide definite pairing of the observed chemical shifts in the ^{1}H and ^{13}C NMR to the appropriate structural positions. In this regard, the ^{1}H–^{1}H-ROESYAD NMR spectrum (Figure 2f) shows two strong spatial proximity correlation contours between the N_1–H proton (δ 9.68 ppm) and C_2H (d, J = 8.0 Hz, δ 7.33 ppm) as well as $C_{2'}$-H (m, 4H). Thus, identification of C_2–H and $C_{2'}$-H sparked the full assignment of the chlorophenyl and piperazine ring protons. Notably, C_2–H and $C_{2'}$-H showed strong correlation cross peaks in the ^{1}H–^{1}H-gDQFCOSY spectrum with C_3–H (d, J = 8.0 Hz, δ 7.36 ppm, red correlation square) and $C_{3'}$-H (m, 4H, δ 3.87 ppm, blue correlation square), respectively (Figure 2c).

Having totally matched the chemical shift values to the protons of the three independent spin systems of the pyrimidine, piperazine, and chlorophenyl, the respective ^{13}C chemical shifts were traced and paired with the proton chemical shifts through the ^{1}H–^{13}C-gHSQC spectrum (Figure 2d and Table 2). Finally, the quaternary carbons were identified through the ^{1}H–^{13}C-gHMBC spectrum (Figure 2e). While the most deshielded C=Se carbon (δ 181.2 ppm) was identified through long-range HMBC correlation with the $C_{2'}$-H protons (δ 4.10/181.2 ppm), the pyrimidine $C_{4'}$ chemical shift was established by a strong cross peak with the $C_{3'}$-H protons (δ 3.87/161.2 ppm). Interestingly, the latter correlation proves the creation of the new selenourea N–C=Se quaternary center and is an indication of a successful coupling between the piperazine and isoselenocyanatestarting material. The remaining chlorophenyl quaternary carbons C_1 and C_4 were identified through strong correlation contours with C_3H (^{3}J, δ 7.36/141 ppm) and C_2H (^{3}J, δ 7.33/129.5 ppm), respectively. The chemical shifts of the assigned ^{1}H and ^{13}C NMR of selenourea **37** have been summarized in Table 2 along with the structure showing the most relevant NOE and HMBC correlations.

2.2. Biological evaluation

The MTT assay was employed to assess the cytotoxic potentials of **21**-**37** on MDA-MB-23v1$^{ER(-)/PR(-)/HER2(-)}$ and MCF-7$^{ER(+)/PR(+)/HER2(-)}$ cells. The selenoureas were tested at concentrations ranging from 0.006 to 123.4 μg/mL to examine the impact of different concentrations on cancer cell survival. The correlation between surviving fraction and concentration of each compound was plotted to determine the survival curve of each cancer cell line against each selenourea. Generally, both cell lines were very responsive to increase in drug dose between 10 and 40 μg/mL as shown by the subsequent reduction in cell surviving fraction, while higher dose–response was less evident between 40 and 125 μg/mL (Figures 3a-f). Notably, the cell lines showed variation in cytotoxic responses, as would be anticipated. The observed variation in cytotoxic responses suggest different cytotoxicity on tumor cell lines,

Table 1. Preparation of variously substituted aryl, benzyl and piperazine selenoureas



[a]Compound **22** was prepared in 63% yield from **17a** and 82% yield from **17b**.

which could be due to differential expression of receptors in these cells, involving numerous intracellular signaling paths [52]. Because of the overlapping nature of the dose–response curves in Figures 3a-f and the high number of data points collected, we elected to utilize the half-maximal inhibitory concentration (IC_{50}) values extracted from the plotted dose–response curves and converted to μM since this comprises a more convenient method to compare the cytotoxic activities of **21**-**37** as antitumor agents (see Table 3).

As indicated in Table 3, all synthesized selenoureas showed mild to strong cytotoxic activity with IC_{50} values varying between 1.2 and 252.4 μM against tested breast cancer cells. Compound **31** was the most potent selenoureas with IC_{50} value of 1.8 μM against MDA-MB-231 followed by 34 (7.7 μM) and 28 (7.8 μM) respectively. Similarly, Compound **31** was the most potent selenoureas in the series with IC_{50} values of 1.2 μM against MCF7 followed by **37** (5.1 μM), **29** (6.5 μM), and **28** (6.5 μM) respectively. The IC_{50} values calculated for Carbonyl cyanide 3-

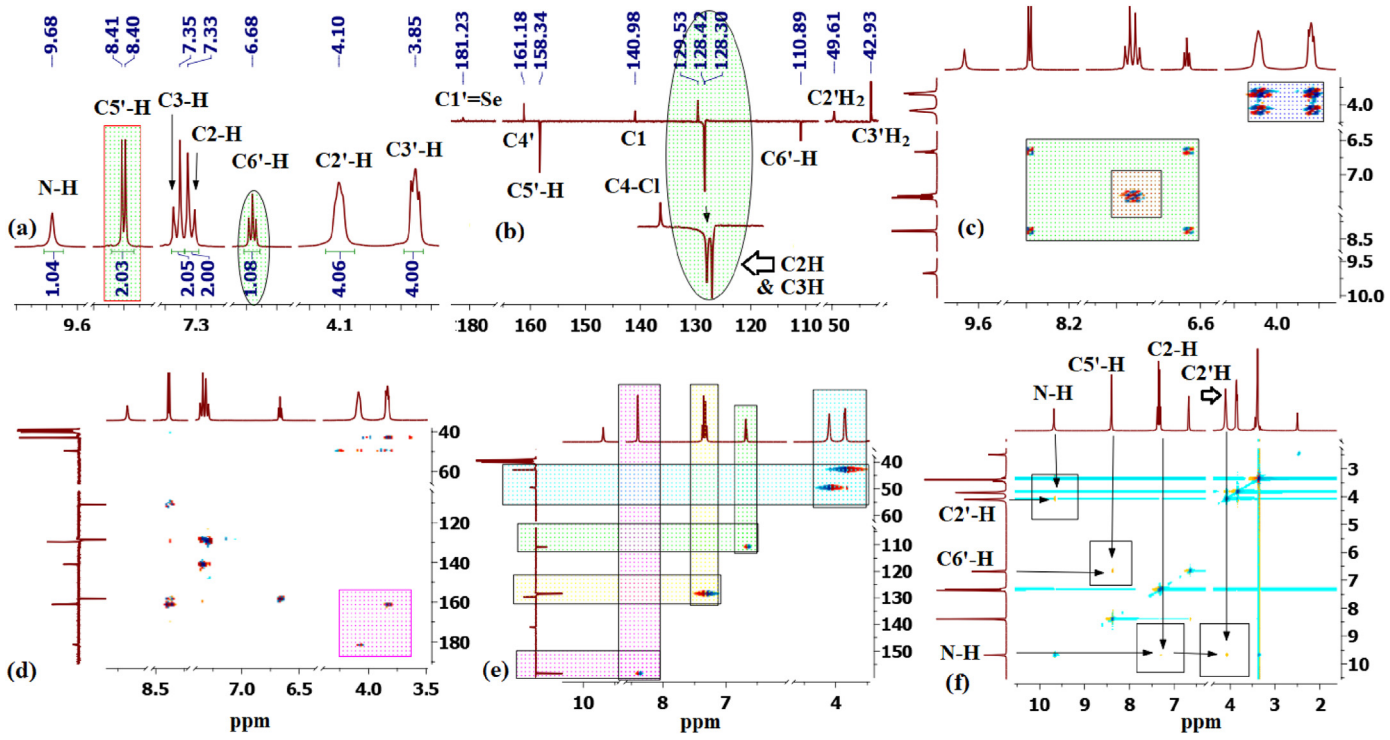
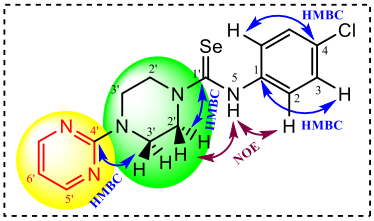


Figure 2. Truncated 1D and 2D NMR spectra of selenourea **37**: (a) ^{1}H-NMR; (b) ^{13}C-CRAPT NMR; (c) ^{1}H–^{1}H -gDQFCOSY NMR; (d) ^{1}H–^{13}C-gHSQC NMR; (e) ^{1}H–^{13}C-gHMBC NMR; (f) ^{1}H–^{1}H -ROESYAD NMR.

Table 2. Assigned ^{1}H- and ^{13}C-NMR chemical shifts of selenourea **37**.

^{1}H-NMR	Proton Number	Selenourea 37 Protons (ppm)	Selenourea 37
	5	9.68 (s, 1H, NH)	
	5'	8.40 (d, J = 4.0 Hz, 2H)	
	3	7.36 (d, J = 8.0 Hz, 2H)	
	2	7.33 (d, J = 8.0 Hz, 2H)	
	6'	6.68 (t, J = 4.0 Hz, 1H)	
	2'	4.10 (m, 4H)	
	3'	3.87 (m, 4H)	

^{13}C-NMR	Carbon Number	Selenourea 17 Carbons (ppm)	Carbon Number	Selenourea 17 Carbons (ppm)
	1	141.0	2'	42.9
	2	128.3	3'	49.6
	3	128.4	4'	161.2
	4	129.5	5'	158.5
	1'	181.2	6'	110.9

chlorophenylhydrazone (positive control) were 2.26, and 18.39 μg/mL, against MDA-MB-231 and MCF7 cells, respectively.

The much higher rates of cell growth inhibition in the more aggressive cell line MDA-MB 231, highlight the promising potential of selenoureas **21** (IC_{50} = 9.6 μM, Entry 1, Table 3), **28** (IC_{50} = 7.8 μM, Entry 8, Table 3), **29** (IC_{50} = 9.6 μM, Entry 9, Table 3), **31** (IC_{50} = 1.8 μM, Entry 11, Table 3), **34** (IC_{50} = 7.7 μM, Entry 14, Table 3), and **35** (IC_{50} = 21.7 μM, Entry 15, Table 3) tested in this study. Triple-negative breast cancer constitutes about 15% of all BC incidents and exemplifies a therapeutic challenge because of its poor prognosis and unavailability of standard treatment [53]. Likewise, the efficacy of these species in the MDA-MB-231 cell line emphasizes their capacity to conquer shortcomings in treating triple-negative breast cancer.

The sharp variation in cytotoxic activity among selenoureas in the same series is a tribute to the impact wielded by manipulating the *N*-(1) and *N*-(3) substituent and how subtle differences in aryl substitution pattern may have a prominent influence on activity. Hence, while the most active compound **31** [(*N*-(1): 4-chlorophenyl; *N*-(3): 4-methoxyphenyl)], having the strongest electron donating methoxy group on the *N*-(3) aryl substituent, exhibited the highest cytotoxic activity against

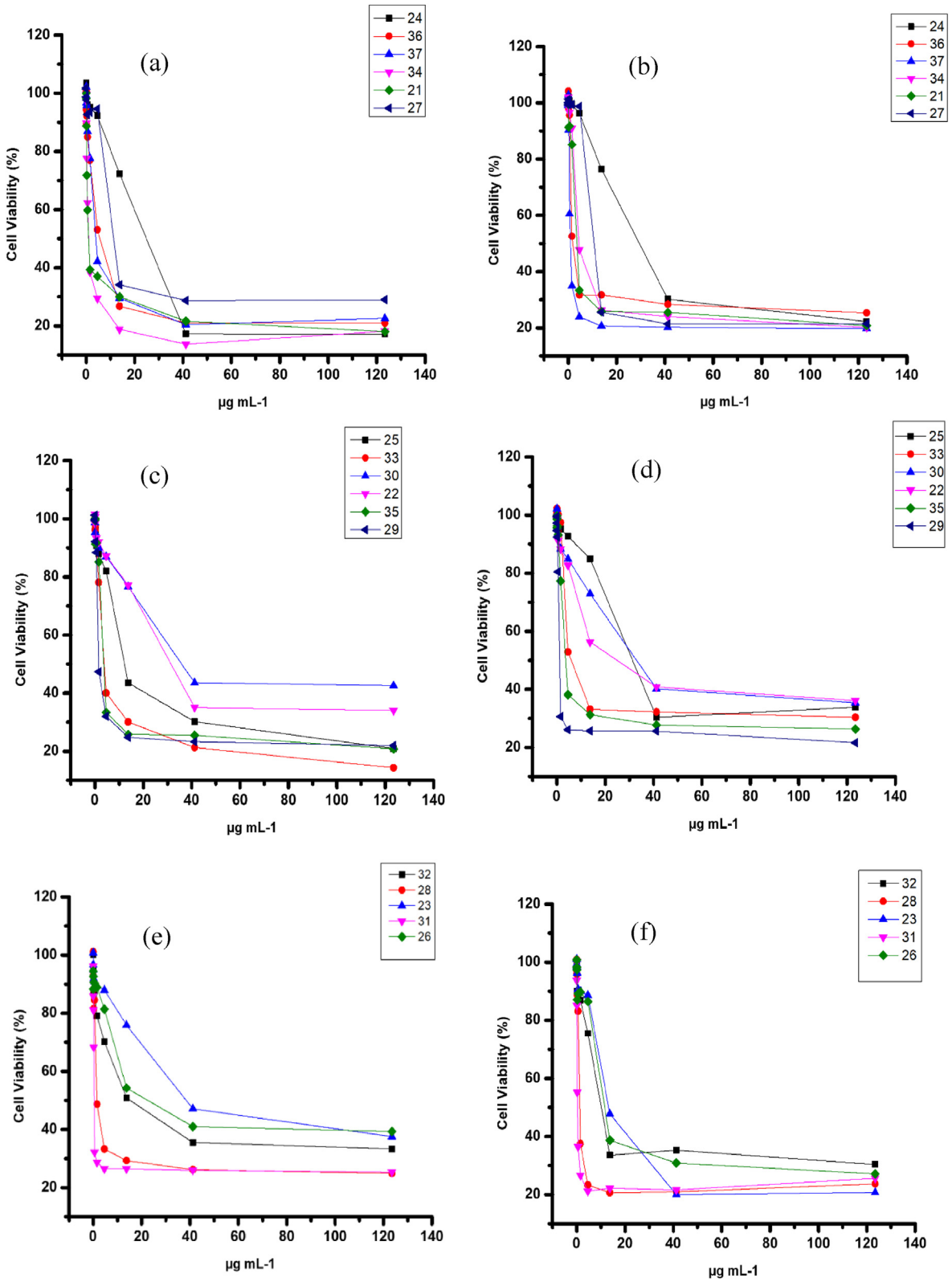


Figure 3. Survival curves of breast cancer cell lines MDA-MB-231 and MCF7 against various concentrations of selenoureas **21–37**: (a) survival curves of MDA-MB-231 against **21**, **24**, **27**, **34**, **36**, **37**. (b) survival curves of MCF7 against **21**, **24**, **27**, **34**, **36**, **37**. (c) survival curves of MDA-MB-231 against **22**, **25**, **29**, **30**, **33**, **35**. (d) survival curves of MCF7 against **22**, **25**, **29**, **30**, **33**, **35**. (e) survival curves of MDA-MB-231 against **23**, **26**, **28**, **31**, **32**. (f) survival curves of MCF7 against **23**, **26**, **28**, **31**, **32**.

Table 3. The half-maximal inhibitory concentration (IC_{50} in μM) of derivatives **21–37** against breast cancer cell lines, MCF7, and MDA-MB231.

Entry	Compound	MDA-MB-231 (IC_{50} in μM)	MCF-7 (IC_{50} in μM)
1	**21**	9.6	10.6
2	**22**	159.4	91.4
3	**23**	252.4	131.7
4	**24**	193.6	181.7
5	**25**	106.8	185.1
6	**26**	86.4	82.6
7	**27**	94.2	68.5
8	**28**	7.8	6.5
9	**29**	9.6	6.5
10	**30**	140.9	163.0
11	**31**	1.8	1.2
12	**32**	80.4	59.7
13	**33**	35.4	54.8
14	**34**	7.7	37.7
15	**35**	21.7	18.9
16	**36**	30.0	10.1
17	**37**	28.2	5.1
18	**Carbonyl cyanide 3-chlorophenylhydrazone**	11.1	90.1

both, MDA-MB 231 (IC_{50} = 1.8 μM) and MCF-7 (IC_{50} = 1.2 μM), its analog **30** [(*N*-(1): 4-chlorophenyl; *N*-(3): 4-chlorophenyl)], where the *N*-(3) aryl *para* position bears another chloro group, exhibited one of the lowest cytotoxic activity against both, MDA-MB 231 (IC_{50} = 140.9 μM) and MCF-7 (IC_{50} = 163.0 μM). Clearly, the 4-methoxy group on the *N*-(3) phenyl substituent is a crucial feature necessary for optimum activity. Likewise, the presence of the 4-chloro group on the *N*-(1) phenyl substituent is a critical as analog **25** [(*N*-(1): 4-methylphenyl; *N*-(3): 4-methoxyphenyl)] exhibited one of the lowest cytotoxic activity against both, MDA-MB 231 (IC_{50} = 106.8 μM) and MCF-7 (IC_{50} = 185.1.0 μM). Clearly, the *N*-(1) 4-chlorophenyl and *N*-(3): 4-methoxyphenyl are synergetic groups and required for high activity. Interestingly, disrupting conjugation in the benzyl analog of **31**, namely compound **35** [(*N*-(1): 4-chlorophenyl; *N*-(3): 4-methoxybenzyl)], attenuated the cytotoxic activity against both, MDA-MB 231 (IC_{50} = 21.7 μM) and MCF-7 (IC_{50} = 18.9 μM). It appears that electronic factors also play a role in controlling activity of the aforementioned analogs through positive mesomeric (+M) effect which donates electron density into the *N*-(3) phenyl ring. The involvement of the *N*-(3) atom of the selenourea function in resonance seems necessary for optimum activity since the benzyl analog **35** was significantly less active (comparing **31** → **35**). 1,3-di-p-tolylselenourea (**21**) also proved highly active conceivably due to the synergetic effect of the *N*-(1) and *N*-(3) hydrophobic substituents [MDA-MB 231 (IC_{50} = 9.6 μM) and MCF-7 (IC_{50} = 10.6 μM)]. Removal of the *N*-(3) 4-methyl group (**23**; IC_{50} = 252.4 μM against MDA-MB 231 and IC_{50} = 131.7 μM against MCF-7), substitution with chlorine atom (**22**; IC_{50} = 159.4 μM against MDA-MB 231 and IC_{50} = 91.4 μM against MCF-7), or disrupting conjugation by introducing a benzyl group (**26**; IC_{50} = 86.4 μM against MDA-MB 231 and IC_{50} = 82.6 μM against MCF-7) renders analogs **22**, **23**, **26** with debilitated cytotoxic activities. Interestingly, for the 1,3-di-p-tolylselenourea system, both methyl as required for high activity as removal of one of the methyls reduced activity. For instance, the monomethylated analog **24** (IC_{50} = 193.6 μM against MDA-MB 231 and IC_{50} = 181.7 μM against MCF-7) (comparing **21** → **24**) displayed multiple fold reduction in activity compared to **24**. Notably, while replacing the *N*-(3) methyl group in **26** with a 4-F group (**27**) did not impact activity to any significant extent, however, replacing it with a 2-F (**29**) or 4-CF_3 group (**28**) enhanced activity against both cell line drastically (**29**; IC_{50} = 9.6 μM against MDA-MB 231 and IC_{50} = 6.5 μM against MCF-7; **28**; IC_{50} = 7.8 μM against MDA-MB 231 and IC_{50} = 6.5 μM against MCF-7), supporting the necessary presence of a 4-methyl group or, in this case, a more polar and electronegative isostere (4-CF_3) or group (2-F). Finally, replacing the entire *N*-(3) group of the most active analog **31** with a piperazine group produced very active species **36** (IC_{50} = 10.1 μM against MCF-7) and **37** (IC_{50} = 5.1 μM against MCF-7), although more so towards MCF7 cells. Though this may suggest that the presence of both selenourea *N*-(1)-H and *N*-(3)-H is not a requirement for high activity, it seems that both may be needed for optimum dual potency.

Selenium-based molecules have shown efficacy as chemotherapeutic compounds [54]. Noteworthy, different Se compounds have been reported for their anti-cancer effects based on different mechanisms of action. Selenium compounds may trigger cell death through apoptosis, and non-apoptotic events such as cell cycle arrest [55, 56], oxidative stress [57], necrosis [58], autophagy [56], ferroptosis [59], necroptosis [60], entosis [61] and anoikis [62].

The antitumor, antiviral, antibacterial and antifungal properties of imidazolidineiminothiones and their derivatives have been systematically established over the past recent years [64, 65, 66, 67, 68, 69, 70]. While the 1-position contains a carbonyl (C=O) group flanked by two nitrogen atoms (N^1 and N^3), the heterocyclic ring contains adjoining imino and thione functional groups in the 4 and 5 locations, respectively. This type of arrangement serves as a reactive template for subsequent cyclizations in which one or both exocyclic nitrogen and oxygen can be integrated into further fused heterocyclic rings. Imidazolidineiminothiones and the many heterocycles based on them have been demonstrated to exhibit broad scale of pharmacological impacts against tumor cells, and viral, microbial, and fungal strains. In this context, we were further interested in transforming the selenoureas, as part of planned future extension to this work, to *N*1-, *N*3-disubstituted selenoxo-imidazolidine-4,5-diones. There are no known or reported general literature methods to prepare such species [71], which will not only serve as potentially bioactive Se analogs to imidazolidineiminothiones and their derivatives but will be crucial in finding the impact of masking the *N*-(1)-H and *N*-(3)-H of selenoureas and determining their role in obtaining optimum potency. Thus, after solvent, temperature, base and reagent optimization, we found that treatment of selenourea **22** with oxalyl chloride (2 equivalents) in DCM with triethylamine as base (2 equivalents) were the best conditions to furnish the desired selenoxo-imidazolidine-4,5-dione **38** in 77% yield (Scheme 2). In continuation to the work reported herein, the newly developed methodology will be employed to prepare a series of selenoxo-imidazolidine-4,5-dione derivatives for biological testing and comparative analysis.

3. Materials and methods

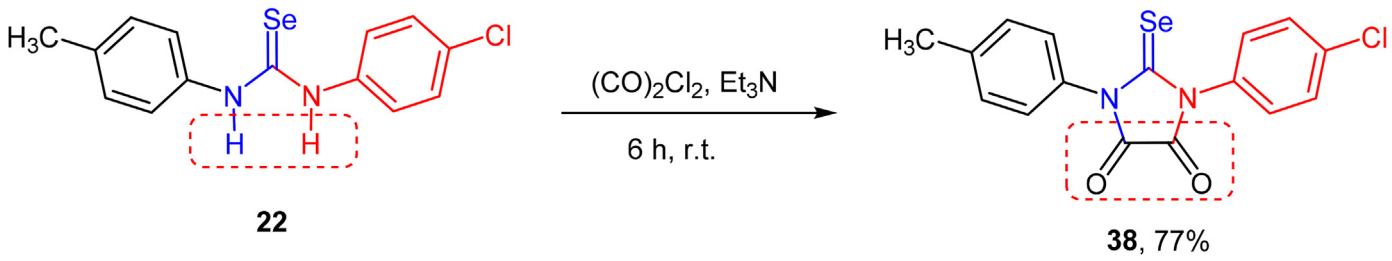
3.1. Material

3.1.1. Reagents

All chemical transformations were performed with magnetic stirring in oven-dried glassware. All chemical reagents and reaction solvents were used as received from MilliporeSigma. Analytical thin-layer chromatography (TLC) used to track the progress of reactions or test purity of isolated products was performed on precoated silica gel plates (HSGF 254) and visualized under UV irradiation at λ = 254 nm.

3.1.2. Equipment

^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Varian 400 MHz NMR spectrometer. The NMR chemical shifts (δ) are reported in parts per million (ppm) relative to the residual solvent peak (^{1}H-NMR δ 2.50 for DMSO-d_6; δ 39.52 for DMSO-d_6). The following abbreviations were used to explain NMR peak multiplicities: br s = broad signal, s = singlet, d = doublet, t = triplet, and m = multiplet. IR spectra were recorded using a Thermo Nicolet Nexus 470 FT-IR. High-resolution mass analyses (HRMS) were obtained using a Waters Q-TOF Premier mass spectrometer [electrospray ionization (ESI)]. Melting points were



Scheme 2. First time successful preparation of a *N*1-, *N*3-disubstituted selenoxo-imidazolidine-4,5-dione from a selenourea

measured using a capillary melting point apparatus (MEL-TEMP) in degrees Celsius (°C) and are uncorrected.

3.2. Methods

3.2.1. General synthesis of 4-methylphenyl isoselenocyanate (17a) and 4-chlorophenyl isoselenocyanate (17b)

3.2.1.14-Methylphenyl isoselenocyanate (17a). A mixture of the amine (0.015 mol), chloroform (2.4 g, 1.62 mL, 0.02 mol), dichloromethane (10 mL), Aliquat 336 (0.2 g, 0.0005 mol), and NaOH (6.84 g, 4.5 mL, 0.086 mol) was vigorously stirred and heated at 45 °C for 12 h. The reaction was cooled to room temperature, chloroform (10 mL) and water (mL) were added, and the mixture was further heated at 45 °C for another 6 h. Progress of the reaction and consumption of the amine was monitored by ^{1}HNMR. The mixture was cooled to room temperature, and finely divided black elemental selenium powder (1.5 g, 0.019 mol) was added. The heterogeneous mixture was vigorously stirred at 45 °C for 1 h. The isoselenocyanate formation was monitored by ^{1}HNMR. The reaction mixture was cooled to room temperature, then water (15 mL) and dichloromethane (15 mL) were added, and the remaining unreacted selenium was filtered off under reduced pressure using a sintered glass Büchner funnel (250 mL capacity). The organic layer was separated and dried using anhydrous $MgSO_4$. The drying agent was then removed by filtration and the solvent was evaporated under reduced pressure in a well-ventilated fume hood due to a strong stench. The residue was purified by column chromatography on silica gel using a gradient of petroleum ether and ethyl acetate as the mobile phase (gradient elution starting with 100% pet. Ether, followed by 1–3% EtOAc-pet.ether) to afford 15 g (50%) of the isoselenocyanate **4** as a light pink solid; R_f = 0.33 (hexane), 0.56 (hexane–EtOAc, 9:1). Mp 67–69; IR (KBr) 2159, 1500, 817 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05 (merged dd, J = 9.0 Hz, 4H, Ar–H), 2.25 (s, 3H, CH_3) ppm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.41 (d, J = 9.0 Hz, 2H, Ar–H), 7.28 (d, J = 9.0 Hz, 2H, Ar–H), 2.33 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.3 ($\mathbf{C}_q$-CH_3), 130.0 (2xCH), 126.7 (C=Se), 125.7 (2xCH), 123.7 (C_q-N), 21.2 (CH_3) ppm; HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for C_8H_8NSe: 197.9822; found: 197.9836.

3.2.1.24-Chlorophenyl isoselenocyanate (17b). Light pink solid; yield: 15 g (85%; starting 4-chloroaniline: 0.015 mol); Mp 68–69; IR (KBr) 2149, 1481, 1085, 823 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34 (d, J = 8.0 Hz, 2H, Ar–H), 7.22 (d, J = 8.0 Hz, 2H, Ar–H) ppm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.34 (d, J = 8.0 Hz, 2H, ArC$_3$-H), 7.22 (d, J = 8.0 Hz, 2H, Ar$\mathbf{C}_2$-H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 133.8 (**C**–Cl), 131.3 (C=Se), 129.9 (2xAr$\mathbf{C}_3$-H), 128.4.7 (C_q-N), 127.3 (2xAr$\mathbf{C}_2$-H) ppm; HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for C_7H_4NSe: 182.9587; found: 182.9598.

3.2.2. General synthesis of selenoureas 21-37

To a stirred solution of isoselenocyanate **17** (0.5 mmol) in ethanol (3 mL), triethylamine (70 μL, 0.5 mmol) and the corresponding aniline or benzylamine derivatives **18** (1 eq) or piperazine **19**–**20** (1 eq) was added. The reaction mixture was stirred in dark under nitrogen conditions at room temperature for maximum 2 h. The formed precipitate was filtered using Whatman filter paper, washed with minimum amount of ethanol and hexane, and air-dried to afford the desired selenourea product.

3.2.3. Experimental data

The yields, m.p. and spectral data of compounds (**21–37**) are shown below, and the related spectra have been included in the supporting information section:

3.2.3.11,3-Di-p-tolylselenourea (21). Grey solid (76% yield); Mp 146–148 °C; IR (KBr) 3171 (N–H), 1555 (C=Se), 1337, 820 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.92 (s, 2H, NH), 7.22 (d, J = 8.0 Hz, 4H, Ar–H), 7.13 (d, J = 8.0 Hz, 4H, Ar–H), 2.26 (s, 6H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.4 (C=Se), 137.1 (N-$\mathbf{C}_q$), 134.4 (CH_3-$\mathbf{C}_q$), 128.9 (2xCH_{tolyl}), 124.8 (2xCH_{tolyl}), 20.5 (CH_3) ppm; HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{15}H_{17}N_2Se$: 305.0557; found: 305.0552.

3.2.3.21-(4-Chlorophenyl)-3-(p-tolyl)selenourea (22). Grey solid (82% yield); Mp 164–166 °C; IR (KBr) 3173 (N–H), 1553 (C=Se), 1333, 1091, 825 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.19 (s, 1H, NH), 10.06 (s, 1H, NH), 7.42 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-}chlorophenyl}$), 7.37 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-}chlorophenyl}$), 7.25 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 7.15 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 2.28 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.7 (C=Se), 138.9 (N_3-$\mathbf{C}_q$), 136.9 (N_1-$\mathbf{C}_q$), 134.7 (CH_3-$\mathbf{C}_q$), 130.3 (C–Cl), 129.2 (2x$CH_{p\text{-}chlorophenyl}$), 128.3 (2x$CH_{p\text{-}chlorophenyl}$), 126.6 (2x$CH_{tolyl}$), 124.8 (2x$CH_{tolyl}$), 20.6 ($CH_3$) ppm; HRMS (ESI$^+$): m/z [M + H]$^+$ calcd for $C_{14}H_{14}ClN_2Se$: 325.0011; found: 325.0019.

3.2.3.31-Phenyl-3-(p-tolyl)selenourea (23). Grey solid (81% yield); Mp 142–144 °C; IR (KBr) 3170 (N–H), 1557 (C=Se), 1336 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.04 (s, 1H, NH), 10.01 (s, 1H, NH), 7.38–7.29 (m, 4H, Ar-H_{phenyl}), 7.23 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 7.19–7.15 (m, 1H, Ar-H_{phenyl}), 7.13 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 2.26 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.8 (C=Se), 139.9 (N_1-$\mathbf{C}_q$), 137.2 (N_3-$\mathbf{C}_q$), 134.9 (CH_3-$\mathbf{C}_q$), 129.3 (2xCH_{tolyl}), 128.8 (2xCH_{tolyl}), 125.5 (CH_{phenyl}), 125.1 (2xCH_{phenyl}), 125.0 (2xCH_{phenyl}), 20.8 (CH_3) ppm; HRMS (ESI$^+$): m/z [M + Na]$^+$ calcd for $C_{14}H_{14}N_2SeNa$: 313.0220; found: 313.0214.

3.2.3.41-Benzyl-3-(p-tolyl)selenourea (24). Brown solid (77% yield); Mp 102–103 °C; IR (KBr) 3290 (N–H), 3200 (N–H), 1556 (C=Se), 1237 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.93 (s, 1H, NH), 8.34 (s, 1H, NH), 7.33–7.29 (m, 3H, Ar–H), 7.26–7.21 (m, 2H, Ar–H), 7.18–7.2111 (m, 4H, Ar–H), 4.81 (s, 2H, $BnCH_2$), 2.26 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.5 (C=Se), 139.1 (N_3-$\mathbf{C}_q$), 135.8 (CH_3-$\mathbf{C}_q$), 135.4 (N_1-$\mathbf{C}_q$), 129.9 (2xCH), 128.5 (2xCH), 127.5 (2xCH), 127.1 (CH), 125.1 (2xCH), 50.3 (CH_2), 20.8 (CH_3) ppm; HRMS (ESI$^+$): m/z [M + Na]$^+$ calcd for $C_{15}H_{16}N_2SeNa$: 327.0376; found: 327.0371.

3.2.3.51-(4-Methoxyphenyl)-3-(p-tolyl)selenourea (25). Light yellow solid (75% yield); Mp 156–158 °C; IR (KBr) 3175 (N–H), 1556 (C=Se), 1510, 1248, 1035 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.87 (s, 1H, NH), 7.25 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-}methoxyphenyl}$), 7.24 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-}tolyl}$), 7.13 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-}tolyl}$), 6.89 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-}methoxyphenyl}$), 3.74 (s, 3H, OCH_3) 2.27 (s, 3H, CH_3) ppm; ^{13}C NMR

(DMSO-d_6, 100 MHz) δ 178.5 (C=Se), 157.0 (C–O), 137.1 (N_3-**C_q**), 134.5 (CH_3-**C_q**), 132.5 (N_1-**C_q**), 129.0 (2x$CH_{p\text{-methoxyphenyl}}$), 126.9 (2x$CH_{tolyl}$), 125.0 (2x$CH_{tolyl}$), 113.7 (2x$CH_{p\text{-methoxyphenyl}}$), 55.3 ($OCH_3$), 20.6 ($CH_3$) ppm; HRMS ($ESI^+$): m/z $[M + H]^+$ calcd for $C_{15}H_{17}N_2OSe$: 321.0506; found: 321.0501.

3.2.3.61-(4-Methylbenzyl)-3-(p-tolyl)selenourea ***(26)***. Off -white solid (76% yield); Mp 128–130 °C; IR (KBr) 3300 (N–H), 3215 (N–H), 1550 (C=Se), 1514 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.87 (s, 1H, NH), 8.26 (s, 1H, NH), 7.20–7.08 (m, 8H, Ar-$H_{p\text{-tolyl}}$), 4.74 (s, 2H, $BnCH_2$), 2.24 (s, 6H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.3 (C=Se), 136.3 (CH_3-**C_q**), 136.0 (N_3-**C_q**), 135.9 (N_1-**C_q**), 135.3 (CH_3-**C_q**), 129.9 (2xCH_{tolyl}), 129.0 (2xCH_{tolyl}), 127.6 (2xCH_{tolyl}), 125.0 (2xCH_{tolyl}), 50.1 ($BnCH_2$), 20.9 (CH_3), 20.8 (CH_3) ppm; HRMS (ESI^+): m/z $[M + H]^+$ calcd for $C_{16}H_{19}N_2Se$: 319.0713; found: 319.0708.

3.2.3.71-(4-Fluorobenzyl)-3-(p-tolyl)selenourea ***(27)***. Off -white solid (97% yield); Mp 128–130 °C; IR (KBr) 3384 (N–H), 3140 (N–H), 1544 (C=Se), 1508, 1220 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.94 (s, 1H, NH), 8.34 (s, 1H, NH), 7.38–7.33 (m, 2H, Ar–H), 7.20–7.10 (m, 6H, Ar–H), 4.78 (s, 2H, $BnCH_2$), 2.26 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.6 (C=Se), 162.4 (d, J = 241.0 Hz, C–F), 137.4 (CH_3-**C_q**), 135.8 (), 135.6 (N_1-**C_q**), 131.1 (2xCH_{tolyl}), 130.2 (d, J = 8.0 Hz, 2x$CH_{p\text{-fluorobenzyl}}$), 126.3 (2x$CH_{tolyl}$), 116.1 (d, J = 21.0 Hz, 2x$CH_{p\text{-fluorobenzyl}}$), 50.5 ($BnCH_2$), 21.5 ($CH_3$) ppm; HRMS ($ESI^+$): m/z $[M + Na]^+$ calcd for $C_{15}H_{15}FN_2SeNa$: 345.0282; found: 345.0277.

3.2.41-(p-Tolyl)-3-(4-(trifluoromethyl)benzyl)selenourea ***(28)***

Off-white solid (62% yield); Mp 138–139 °C; IR (KBr) 3265 (N–H), 3161 (N–H), 1563 (C=Se), 1327, 1113 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.04 (s, 1H, NH), 8.43 (s, 1H, NH), 7.68 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-(trifluoromethyl)phenyl}}$), 7.50 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-(trifluoromethyl)phenyl}}$), 7.18 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 7.14 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 4.89 (s, 2H, $BnCH_2$), 2.27 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.9 (C=Se), 144.2 (N_1-**C_q**), 135.7 (Bn**C_q**), 135.5 (CH_3-**C_q**), 129.9 (2xCH_{tolyl}), 128.1 (2xCH), 127.6 (q, J = 31.0 Hz, **C**-CF_3), 125.2 (q, J = 3.0 Hz, 2xCH), 125.1 (2xCH_{tolyl}), 124.5 (q, J = 270.0 Hz, CF_3), 49.8 ($BnCH_2$), 20.8 (CH_3) ppm; HRMS (ESI^+): m/z $[M + Na]^+$ calcd for $C_{16}H_{15}F_3N_2SeNa$: 395.0250; found: 395. 0245.

3.2.4.11-(2-Fluorobenzyl)-3-(p-tolyl)selenourea ***(29)***. Brown solid (77% yield); Mp 126–127 °C; IR (KBr) 3347 (N–H), 3178 (N–H), 1552 (C=Se), 1347, 1223, 764 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.00 (s, 1H, NH), 8.31 (s, 1H, NH), 7.50–6.98 (8H, Ar–H), 4.84 (s, 2H, $BnCH_2$), 2.26 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 180.1 (C=Se), 160.1 (d, J = 243.0 Hz, C–F), 135.9 (N_3-**C_q**), 135.5 (CH_3-**C_q**), 129.9 (2xCH_{tolyl}), 129.4 ($CH_{2\text{-fluorophenyl}}$), 129.1 (d, J = 8.0 Hz, $CH_{2\text{-fluorophenyl}}$), 125.8 (d, J = 15.0 Hz, NCH_2-**C_q**), 125.1 (2xCH_{tolyl}), 124.5 (d, J = 3.0 Hz, $CH_{2\text{-fluorophenyl}}$), 115.3 (d, J = 21.0 Hz, $CH_{2\text{-fluorophenyl}}$), 44.3 ($BnCH_2$), 20.8 ($CH_3$) ppm; HRMS ($ESI^+$): m/z $[M + Na]^+$ calcd for $C_{15}H_{15}FN_2SeNa$: 345.0282; found: 345.0277.

3.2.4.21,3-Bis(4-chlorophenyl)selenourea ***(30)***. Light peach solid (59% yield); Mp 185–187 °C; IR (KBr) 3186 (N–H), 1555 (C=Se), 1336, 1088, 825 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.24 (s, 2H, NH), 7.42–7.34 (m, 8H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.4 (C=Se), 138.7 (N-**C_q**), 129.7 (C–Cl), 128.7 (2xCH), 126.9 (2xCH) ppm; HRMS (ESI^+): m/z $[M + Na]^+$ calcd for $C_{13}H_{10}Cl_2N_2SeNa$: 366.9284; found: 366.9279.

3.2.51-(4-Chlorophenyl)-3-(4-methoxyphenyl)selenourea ***(31)***

Grey solid (80% yield); Mp 178–180 °C; IR (KBr) 3183 (N–H), 1555 (C=Se), 1342, 1249, 1091, 825 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.09 (s, 1H, NH), 9.96 (s, 1H, NH), 7.40 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.36 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.24 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-methoxyphenyl}}$), 6.90 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-methoxyphenyl}}$), 3.73 (s, 3H, OCH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.1 (C=Se), 157.4 (C–O), 139.0 (N_1-**C_q**), 132.3 (N_3-**C_q**), 129.5 (C–Cl), 128.6 (2x$CH_{p\text{-chlorophenyl}}$), 127.1 (2x$CH_{p\text{-chlorophenyl}}$ and 2x$CH_{p\text{-methoxyphenyl}}$), 114.1 (2x$CH_{p\text{-methoxyphenyl}}$), 55.5 ($OCH_3$) ppm; HRMS ($ESI^+$): m/z $[M + Na]^+$ calcd for $C_{14}H_{13}ClN_2OSeNa$: 362.9779; found: 362.9774.

3.2.5.11-(4-Chlorophenyl)-3-phenylselenourea ***(32)***. Light brown solid (77% yield); Mp 159–160 °C; IR (KBr) 3175 (N–H), 1553 (C=Se), 1338, 1089, 843 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.25 (s, 1H, NH), 10.15 (s, 1H, NH), 7.50–7.11 (m, 9H, Ar–H) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.1 (C=Se), 139.6 (N_1-**C_q**), 138.9 (N_3-**C_q**), 129.5 (C–Cl), 128.9 (2x$CH_{p\text{-chlorophenyl}}$), 128.6 (2x$CH_{p\text{-chlorophenyl}}$), 126.9 (2x$CH_{phenyl}$), 125.9 ($CH_{phenyl}$), 125.0 (2x$CH_{phenyl}$) ppm; HRMS ($ESI^+$): m/z $[M + Na]^+$ calcd for $C_{13}H_{11}ClN_2SeNa$: 332.9674; found: 332.9668.

3.2.5.21-Benzyl-3-(4-chlorophenyl)selenourea ***(33)***. Off-white solid (77% yield); Mp 157–158 °C; IR (KBr) 3380 (N–H), 3133 (N–H), 1541 (C=Se), 1348, 1096, 817 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.04 (s, 1H, NH), 8.61 (s, 1H, NH), 7.60–7.06 (m, 9H, Ar–H), 4.82 (s, 2H, CH_2) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 180.2 (C=Se), 138.8 (Ph**C_q**), 137.8 (N_3-**C_q**), 129.7 (C–Cl), 129.1 (2x$CH_{p\text{-chlorophenyl}}$), 128.5 (2x$CH_{p\text{-chlorophenyl}}$), 127.6 (2x$CH_{phenyl}$), 127.2 ($CH_{phenyl}$), 126.5 (2x$CH_{phenyl}$), 50.3 ($CH_2$) ppm; HRMS ($ESI^+$): m/z $[M + Na]^+$ calcd for $C_{14}H_{13}ClN_2SeNa$: 346.9830; found: 346.9825.

3.2.5.31-(4-Chlorophenyl)-3-(4-methylbenzyl)selenourea ***(34)***. Light brown solid (71% yield); Mp 166–168 °C; IR (KBr) 3262 (N–H), 3171 (N–H), 1562 (C=Se), 1338, 1090, 801 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.01 (s, 1H, $NH_{p\text{-chlorophenyl}}$), 8.56 (s, 1H, NH_{Bn}), 7.39 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.33 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.21 (d, J = 8.0 Hz, 2H, ArC_2-$H_{p\text{-tolyl}}$), 7.13 (d, J = 8.0 Hz, 2H, ArC_3-$H_{p\text{-tolyl}}$), 4.77 (s, 2H, CH_2), 2.27 (CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 180.0 (C=Se), 137.8 (N_1-**C_q**), 136.3 (CH_3-**C_q**), 135.7 (CH_2-**C_q**), 129.6 (C–Cl), 129.0 (2xC_3H_{tolyl} & 2x$CH_{p\text{-chlorophenyl}}$), 127.6 (2x$C_2H_{tolyl}$), 126.5 ($CH_{p\text{-chlorophenyl}}$), 50.1 ($CH_2$), 20.9 ($CH_3$) ppm; HRMS ($ESI^+$): m/z $[M + Na]^+$ calcd for $C_{15}H_{15}ClN_2SeNa$: 360.9987. found: 360.9977.

3.2.5.41-(4-Chlorophenyl)-3-(4-methoxybenzyl)selenourea ***(35)***. Off-white solid (78% yield); Mp 158–159 °C; IR (KBr) 3207 (N–H), 3166 (N–H), 1547 (C=Se), 1489, 1340, 1245, 1091, 1027, 828 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.98 (s, 1H, NH), 8.54 (s, 1H, NH), 7.39 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.33 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.25 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-methoxyphenyl}}$), 6.89 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-methoxyphenyl}}$), 4.74 (s, 2H, CH_2), 3.72 (OCH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.8 (C=Se), 158.6 (O-**C_q**), 137.8 (N_1-**C_q**), 130.6 (CH_2-**C_q**), 129.6 (C–Cl), 129.1 (2x$CH_{p\text{-chlorophenyl}}$), 129.0 (2x$CH_{p\text{-chlorophenyl}}$), 126.4 (2x$CH_{p\text{-methoxyphenyl}}$), 113.9 (2x$CH_{p\text{-methoxyphenyl}}$), 55.3 ($OCH_3$), 49.8 ($CH_2$) ppm; HRMS ($ESI^+$): m/z $[M + Na]^+$ calcd for $C_{15}H_{15}ClN_2O$-SeNa: 376.9936; found: 376.9930.

3.2.5.5N,4-bis(4-chlorophenyl)piperazine-1-carboselenoamide ***(36)***. Off-white solid (94% yield); Mp 203–204 °C; IR (KBr) 3192 (N–H), 1535 (C=Se), 1320, 1218, 1089, 1017, 806 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.73 (s, 1H, NH), 7.35 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.28 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.25 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 6.96 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 4.11 (br s, 4H, 2x$NCH_2$), 3.26 (br s, 4H, 2x$NCH_2$) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.2 (C=Se), 149.3 (*N*-**C_q**), 140.9 (*N*-**C_q**), 129.5 (C–Cl), 129.0 (2x$CH_{p\text{-chlorophenyl}}$), 128.3 (2x$CH_{p\text{-chlorophenyl}}$), 128.2 (2x$CH_{p\text{-chlorophenyl}}$), 122.9 (C–Cl), 117.1 (2x$CH_{p\text{-chlorophenyl}}$), 49.7 (2x$NCH_2$), 47.7 (2x$NCH_2$) ppm; HRMS ($ESI^+$): m/z $[M + H]^+$ calcd for $C_{17}H_{18}Cl_2N_3SeNa$: 414.0043; found: 414.0038.

*3.2.5.6N-(4-chlorophenyl)-4-(pyrimidin-2-yl)piperazine-1-carboselenoamide **(37)**.* Off-white solid (88% yield); Mp 173–174 °C; IR (KBr) 3141 (N–H), 1585 (C=Se), 1484, 1309, 1091, 1020, 802 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.70 (s, 1H, NH), 8.39 (d, J = 4.0 Hz, 2H, Ar–H), 7.36 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.31 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 6.68 (t, J = 4.0 Hz, 1H, Ar–H), 4.10 (m, 4H, 2xNCH_2), 3.87 (m, 4H, 2xNCH_2) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.2 (C=Se), 161.2 (pyrimidine *N*-$\mathbf{C_q}$), 158.3 (pyrimidine 2xCH), 141.0 (*N*-$\mathbf{C_q}$), 129.5 (C–Cl), 128.4 (2x$CH_{p\text{-chlorophenyl}}$), 128.3 (2x$CH_{p\text{-chlorophenyl}}$), 110.9 (pyrimidine CH), 49.6 (2xNCH_2), 42.9 (2xNCH_2) ppm; HRMS (ESI^+): m/z $[M + Na]^+$ calcd for $C_{15}H_{16}ClN_5SeNa$: 404.0157; found: 404.0152.

*3.2.6. Synthesis of 1-(4-chlorophenyl)-2-selenoxo-3-(p-tolyl)imidazolidine-4,5-dione **(38)***

1-(4-Chlorophenyl)-3-(p-tolyl)selenourea (**22**) (70.3 mg, 0.22 mmol) and Et_3N (2 equiv.) was suspended in anhydrous DCM (2 mL) and the reaction mixture placed in an ice bath and oxalyl chloride (1 equiv.) was added dropwise, producing a milky green solution. Then reaction mixture was stirred at room temperature overnight under nitrogen atmosphere. The reaction was subsequently extracted with DCM (3 × 10 mL), then the organic layer was washed once with water (10 mL) and once with 5% $NaHCO_3$ (10 mL), then again with water (10 mL). The organic layer was collected and dried over sodium sulfate and evaporated under *vacuum* to furnish an oily residue which was dissolved in ethanol (with sonication/no heat). The formed precipitation was filtered off to afford the product as red fluffy solid.

*3.2.6.11-(4-chlorophenyl)-2-selenoxo-3-(p-tolyl)imidazolidine-4,5-dione **(38)**.* Red fluffy solid (77% yield); Mp 246–247 °C; IR (KBr) 1774 (C=O), 1514 (C=Se), 1494, 1390, 1283, 816 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.44 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.29 (d, J = 8.0 Hz, 2H, Ar-$H_{p\text{-chlorophenyl}}$), 7.27 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 7.19 (d, J = 8.0 Hz, 2H, Ar-H_{tolyl}), 2.37 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 183.7 (C=Se), 154.0 (C=O), 153.9 (C=O), 140.5 (CH_3-$\mathbf{C_q}$), 136.2 (C–Cl), 131.1 (N_3-$\mathbf{C_q}$), 130.2 (2x$CH_{p\text{-chlorophenyl}}$), 129.9 ($N_1$-$\mathbf{C_q}$), 129.8 (2x$CH_{p\text{-chlorophenyl}}$), 129.6 (2x$CH_{tolyl}$), 127.9 (2x$CH_{tolyl}$), 21.4 ($CH_3$) ppm.

3.2.7. Biological activity methods

3.2.7.1. 7Cell culture. Human breast adenocarcinoma MDA-MB-$231^{ER(-)/PR(-)/HER2(-)}$ and MCF-$7^{ER(+)/PR(+)/HER2(-)}$ cells were obtained from DSMZ (GmbH, Germany). The cells were seeded in DMEM supplemented with FBS 10%, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C and 5% CO_2 in a humidified atmosphere.

3.2.7.2. 7Cytotoxicity assay. The cells viability was investigated by MTT assay [63]. Cells were trypsinized (0.25% trypsin) and aliquots of 120 μL of the suspended cells (5 × 10^4 cells/mL) were added to 96-well plates to yield 6000 cells/well. After 24h, 60 μL of serially diluted compounds in DMSO were added to the prepared cells. After 48 h, 10 μL of a 5% MTT solution in PBS was added for 2 h. The medium was aspirated, and the formazan crystals were dissolved in 200 μL of DMSO for 10 min on a shaker. The optical density was read in a microplate reader (ChroMate, USA) at 570 nm.

3.2.7.3. 7Biological activity tests. Biological evaluation the newly synthesized compounds **21**–**37** were assessed for their in vitro anti-cancer activity against two cancer cell lines, MDA-MB-$231^{ER(-)/PR(-)/HER2(-)}$ and MCF-$7^{ER(+)/PR(+)/HER2(-)}$, using MTT assay. Carbonyl cyanide 3-chlorophenylhydrazone (USA, Sigma) was used as a positive control. The results are reported as growth inhibitory concentration (IC_{50}) values after 48 h of treatment, compared with the untreated controls (Table 3). OriginPro 8.5 software was used to calculate the IC_{50} values.

4. Conclusion

The synthesis of 17 novel selenoureas was successfully accomplished through the reaction of 4-methylphenyl isoselenocyanate (**17a**) and 4-chlorophenyl isoselenocyanate (**17b**) with various aryl, benzyl, and piperazine amines in ethanol at room temperature. The selenoureas were attained in good yields, isolated by filtration, and required no further purification by chromatography. The bioactivity results revealed that the synthesized selenoureas promote in vitro cytotoxicity in the breast cancer cell lines tested. These selenium containing compounds are potential anticancer agents for breast cancer and may act as valuable precursors to for further structural optimization for optimum bioactivity.

Declarations

Author contribution statement

Ziad Moussa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools and data; Wrote the paper.

Ranem Kaddoura, Saleh A. Ahmed: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Haythem A. Saadeh, Nael Abutaha: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools and data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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References

[1] R.A. Hussain, A. Badshah, A. Shah, Synthesis and biological applications of selenoureas, Appl. Organomet. Chem. 28 (2014) 61–73.
[2] M. Koketsu, H. Ishihara, Thiourea and selenourea and their applications, Curr. Org. Synth. 3 (2006) 439–455.
[3] M. Ninomiya, D.R. Garud, M. Koketsu, Biologically significant selenium-containing heterocycles, Coord. Chem. Rev. 255 (2011) 2968–2990.
[4] I.V. Sekov, T.M. Serova, A.N. Proshin, S.O. Bachurin, Synthesis of selenoureas and heterocycles based thereon, Russ. J. Org. Chem. 51 (2015) 471–490.

[5] M. Ninomiya, D.R. Garud, M. Koketsu, Selenium-containing heterocycles using selenoamides, selenoureas, selenazadienes, and isoselenocyanates, Heterocycles 81 (2010) 2027–2055.

[6] G. Mugesh, W.W. du Mont, H. Sies, Chemistry of biologically important synthetic organoselenium compounds, Chem. Rev. 101 (2001) 2125–2179.

[7] M. Koketsu, H. Ishihara, Synthesis of 1,3-selenazine and 1,3-selenazole and their biological activities, Curr. Org. Chem. 7 (2003) 175–185.

[8] M. Zielińska-Błajet, J. Najdek, Novel selenoureas based on Cinchona alkaloid skeleton: synthesis and catalytic investigations, Materials 14 (2021) 600.

[9] M. Zielińska-Błajet, Ż.A. Mała, R. Kowalczyk, Efficacy of selenourea organocatalysts in asymmetric Michael reactions under standard and solvent-free conditions, Molecules 26 (2021) 7303.

[10] Y. Lin, W.J. Hirschi, A. Kunadia, A. Paul, I. Ghiviriga, K.A. Abboud, R.W. Karugu, M.J. Vetticatt, J.S. Hirschi, D. Seidel, A selenourea-thiourea Brønsted acid catalyst facilitates asymmetric conjugate additions of amines to α,β-unsaturated esters, J. Am. Chem. Soc. 142 (2020) 5627–5635.

[11] S.L. Shekharappa, L.R. Kumar, V.V. Sureshbabu, Synthesis of carbodiimides from 1,3-disubstituted selenoureas by iodine-mediated deselenization, Indian J. Chem., Sect. B 57B (2018) 1291–1294.

[12] J.G. Fernández-Bolaños, Ó. López, V.C. Ulgar, I. Maya, J. Fuentes, Synthesis of O-unprotected glycosyl selenoureas. A new access to bicyclic sugar isoureas, Tetrahedron Lett. 45 (2004) 4081–4084.

[13] (a) A. Molter, J. Rust, C.W. Lehmann, F. Mohr, Synthesis and structural studies of some selenoureas and their metal complexes, ARKIVOC (Gainesville, FL, U. S.) (vi) (2011) 10–17;
(b) M.J. Spooner, P.A. Gale, A tripodal tris-selenourea anion transporter matches the activity of its thio- analogue but shows distinct selectivity, Supramol. Chem. 30 (2018) 514–519.

[14] A. Casula, A. Llopis-Lorente, A. Garau, F. Isaia, M. Kubicki, V. Lippolis, F. Sancenón, R. Martínez-Máñez, A. Owczarzak, C. Santi, M. Andrea Scorciapino, C. Caltagirone, A new class of silica-supported chromo-fluorogenic chemosensors for anion recognition based on a selenourea scaffold, Chem. Commun. 53 (2017) 3729–3732.

[15] A. Casula, P. Begines, A. Bettoschi, J.G. Fernandez-Bolaños, F. Isaia, V. Lippolis, Ó. López, G. Picci, M.A. Scorciapino, C. Caltagirone, Selenoureas for anion binding as molecular logic gates, Chem. Commun. 53 (2017) 11869–11872.

[16] Z. Luo, Selenourea: a convenient phasing vehicle for macromolecular X-ray crystal structures, Sci. Rep. 6 (2016), 37123.

[17] J. Jin, H.-W. Shin, J.H. Park, J.H. Park, E. Kim, T.K. Ahn, D.H. Ryu, S.U. Son, Iridium complexes containing bis(imidazoline thione) and bis(imidazoline selone) ligands for visible-light-induced oxidative coupling of benzylamines to imines, Organometallics 32 (2013) 3954–3959.

[18] W.-G. Jia, Y.-B. Huang, Y.-J. Lin, G.-X. Jin, Syntheses and structures of half-sandwich iridium(III) and rhodium(III) complexes with organochalcogen (S, Se) ligands bearing N-methylimidazole and their use as catalysts for norbornene polymerization, Dalton Trans. (2008) 5612–5620.

[19] D.J. Williams, K.M. White, D. VanDerveer, A.P. Wilkinson, Dichlorobis[1,3-dimethyl-2(3H)-imidazoleselone]zinc(II): a potential zinc selenide synthon, Inorg. Chem. Commun. 5 (2002) 124–126.

[20] M.M. Kimani, J.L. Brumaghim, D. VanDerveer, Probing the antioxidant action of selenium and sulfur using Cu(I)-chalcogenone tris(pyrazolyl)methane and -borate complexes, Inorg. Chem. 49 (2010) 9200–9211.

[21] M.M. Kimani, C.A. Bayse, J.L. Brumaghim, Synthesis, characterization, and DFT studies of thione and selone Cu(I) complexes with variable coordination geometries, Dalton Trans. 40 (2011) 3711–3723.

[22] M.M. Kimani, H.C. Wang, J.L. Brumaghim, Investigating the copper coordination, electrochemistry, and Cu(II) reduction kinetics of biologically relevant selone and thione compounds, Dalton Trans. 41 (2012) 5248.

[23] D.J. Nelson, F. Nahra, S.R. Patrick, D.B. Cordes, A.M.Z. Slawin, S.P. Nolan, Exploring the coordination of cyclic selenoureas to gold(I), Organometallics 33 (2014) 3640–3645.

[24] M.P. Campos, M.P. Hendricks, A.N. Beecher, W. Walravens, R.A. Swain, G.T. Cleveland, Z. Hens, M.Y. Sfeir, J.S. Owen, A library of selenourea precursors to PbSe nanocrystals with size distributions near the homogeneous limit, J. Am. Chem. Soc. 139 (2017) 2296–2305.

[25] M. Díaz, H. de Lucio, E. Moreno, S. Espuelas, C. Aydillo, A. Jiménez-Ruiz, M.Á. Toro, K.J. Gutiérrez, V. Martínez-Merino, A. Cornejo, J.A. Palop, C. Sanmartín, D. Plano, Synthesis and leishmanicidal activity of novel urea, thiourea, and selenourea derivatives of diselenides, Antimicrob. Agents Chemother. 63 (2019) e02200–e02218.

[26] (a) H. Tsukagoshi, M. Koketsu, M. Kato, M. Kurabayashi, A. Nishina, H. Kimura, Superoxide radical-scavenging effects from polymorphonuclear leukocytes and toxicity in human cell lines of newly synthesized organic selenium compounds, FEBS J. 274 (2007) 6046–6054;
(b) H. Takahashi, A. Nishina, R.H. Fukumoto, H. Kimura, M. Koketsu, H. Ishihara, Selenoureas and thioureas are effective superoxide radical scavengers in vitro, Life Sci. 76 (2005) 2185–2192.

[27] (a) K.P. Bhabak, G. Mugesh, Antithyroid drugs and their analogues protect against peroxynitrite-mediated protein tyrosine nitration—a mechanistic study, Chem. Eur J. 16 (2010) 1175–1185;
(b) K.P. Bhabak, K. Satheeshkumar, S. Jayavelu, G. Mugesh, Inhibition of peroxynitrite- and peroxidase-mediated protein tyrosine nitration by imidazole-based thiourea and selenourea derivatives, Org. Biomol. Chem. 9 (2011) 7343–7350.

[28] R.A. Hussain, A. Badshah, M.N. Tahir, B. Lal, I.A. Khan, Synthesis, chemical characterisation, and DNA binding studies of ferrocene-incorporated selenoureas, Aust. J. Chem. 66 (2013) 626–634.

[29] (a) R.A. Hussain, A. Badshah, M. Sohail, B. Lal, K. Akbar, Synthesis, chemical characterization, DNA binding and antioxidant studies of ferrocene incorporated selenourea, J. Mol. Struct. 1048 (2013) 367–374;
(b) R.A. Hussain, A. Badshash, M. Sohail, B. Lal, A.A. Altaf, Synthesis, chemical characterization, DNA interaction and antioxidant studies of ortho, meta and para fluoro substituted ferrocene incorporated selenoureas, Inorg. Chim. Acta 402 (2013) 133–139;
(c) R.A. Hussain, A. Badshah, K. Akbar, Comparative DNA binding and antioxidant studies of acetyl and benzoyl substituted ferrocene incorporated selenoureas, Russ. J. Electrochem. 51 (2015) 198–208.

[30] S. Sabir, T.T. Yu, R. Kuppusamy, B. Almohaywi, G. Iskander, T. Das, M.D.P. Willcox, D.S. Black, N. Kumar, Novel seleno- and thio-urea containing dihydropyrrol-2-one analogues as antibacterial agents, Antibiotics 10 (2021) 321.

[31] O.O. Fadeyi, S.T. Adamson, E.L. Myles, C.O. Okoro, Novel fluorinated acridone derivatives. Part 1: synthesis and evaluation as potential anticancer agents, Bioorg. Med. Chem. Lett 18 (2008) 4172–4176. .

[32] N.S.H.N. Moorthy, C. Karthikeyan, P. Trivedi, Synthesis, cytotoxic evaluation and in silico pharmacokinetic prediction of some benzo [a] phenazine-5-sulfonic acid derivatives, Med. Chem. 5 (2009) 549–557. .

[33] A. Sherif, F. Rostom, Synthesis and in vitro antitumor evaluation of some indeno [1,2-c]pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems, Bioorg. Med. Chem. 14 (2006) 6475–6485.

[34] D. Chowrasia, C. Karthikeyan, L. Choure, M. Gupta, M. Arshad, P. Trivedi, Synthesis, characterization and anti cancer activity of some fluorinated 3, 6-diaryl-[1, 2, 4] triazolo [3, 4-b][1, 3, 4] thiadiazoles, Arab. J. Chem. 10 (2017) S2424–S2428. .

[35] WHO website, Breast cancer (who.int). https://www.who.int/news-room/fact-sheets/detail/breast-cancer, 2022.

[36] L.M. Heiser, A. Sadanandam, W.L. Kuo, S.C. Benz, T.C. Goldstein, S. Ng, W.J. Gibb, N.J. Wang, S. Ziyad, F. Tong, N. Bayani, Z. Hu, J.I. Billig, A. Dueregger, S. Lewis, L. Jakkula, J.E. Korkola, S. Durinck, F. Pepin, Y. Guan, E. Purdom, P. Neuvial, H. Bengtsson, K.W. Wood, P.G. Smith, L.T. Vassilev, B.T. Hennessy, J. Greshock, K.E. Bachman, M.A. Hardwicke, Subtype and pathway specific responses to anticancer compounds in breast cancer, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 2724–2729.

[37] L.G. Martelotto, C.K. Ng, S. Piscuoglio, B. Weigelt, J.S. Reis-Filho, Breast cancer intra-tumor heterogeneity, Breast Cancer Res. 16 (2014) 210.

[38] Y.A. Fouad, C. Aanei, Revisiting the hallmarks of cancer, Am. J. Cancer Res. 7 (2017) 1016–1036.

[39] D.L. Holliday, V. Speirs, Choosing the right cell line for breast cancer research, Breast Cancr Res. 13 (2011) 215.

[40] G.M. Nagaraja, M. Othman, B.P. Fox, R. Alsaber, C.M. Pellegrino, Y. Zeng, R. Khanna, P. Tamburini, A. Swaroop, R.P. Kandpal, Gene expression signatures and biomarkers of noninvasive and invasive breast cancer cells: comprehensive profiles by representational difference analysis, microarrays and proteomics, Oncogene 25 (2006) 2328–2338.

[41] U. Kronenwett, A. Ploner, A. Zetterberg, J. Bergh, P. Hall, G. Auer, Y. Pawitan, Genomic instability and prognosis in breast carcinomas, Cancer Epidemiol. Biomarkers Prev. 15 (2006) 1630–1635.

[42] P. Merino-Montiel, S. Maza, S. Martos, Ó. López, I. Maya, J.G. Fernández-Bolaños, Synthesis and antioxidant activity of *O*-alkylselenocarbamates, selenoureas and selenohydantoins, Eur. J. Pharmaceut. Sci. 48 (2013) 582–592.

[43] R.A. Hussain, A. Badshah, A. Shah, Synthesis, and biological applications of selenoureas, Appl. Organomet. Chem. 28 (2014) 61–73.

[44] H. Ishihara, M. Koketsu, Y. Fukuta, F. Nada, Reaction of lithium aluminum hydride with elemental selenium: its application as a selenating reagent into organic molecules, J. Am. Chem. Soc. 123 (2001) 8408–8409.

[45] Y. Takikawa, H. Watanabe, R. Sasaki, K. Shimada, Conversion of amides, N,N,N0,N0-tetramethylurea, and esters to the corresponding selenoxo compounds by treatment with bis(trimethylsilyl) selenide and $BF_3.OEt_2$, Bull. Chem. Soc. Jpn. 67 (1994) 876–878.

[46] Y. Zhou, M.K. Denk, Synthesis and reactivity of subvalent compounds. Part 13: reaction of triethyl orthoformate with amines and selenium - a convenient one-step three-component synthesis for selenoureas, Tetrahedron Lett. 44 (2003) 1295–1299.

[47] M. Koketsu, N. Takakura, H. Ishihara, Efficient synthesis of selenoureas from the corresponding carbodiimides, Synth. Commun. 32 (2002) 3075–3079.

[48] M. Ninomiya, D.R. Garud, M. Koketsu, Selenium-containing heterocycles using selenoamides, selenoureas, sulfadiazines, and isoselenocyanates, Heterocycles 81 (2010) 2027–2055.

[49] Ó. López, S. Maza, V. Ulgar, I. Maya, J.G. Fernández-Bolaños, Synthesis of sugar-derived isoselenocyanates, selenoureas, and selenazoles, Tetrahedron 65 (2009) 2556–2566.

[50] J.G. Fernández-Bolaños, Ó. López, V. Ulgar, I. Maya, J. Fuentes, Synthesis of O-unprotected glycosyl selenoureas. A new access to bicyclic sugar isoureas, Tetrahedron Lett. 45 (2004) 4081–4084.

[51] J. Zakrzewski, B. Huras, A. Kiełczewska, Synthesis of isoselenocyanates, Synthesis 48 (2016) 85–96.

[52] P. Gupta, S.K. Srivastava, Antitumor activity of phenethyl isothiocyanate in HER2-positive breast cancer models, BMC Med. 10 (2012) 80.

[53] A. Bramati, S. Girelli, V. Torri, G. Farina, E. Galfrascoli, S. Piva, A. Moretti, M.C. Dazzani, P. Sburlati, N.M. La Verde, Efficacy of biological agents in metastatic triple-negative breast cancer, Cancer Treat Rev. 40 (2014) 605–613.

[54] A.P. Fernandes, V. Gandin, Selenium compounds as therapeutic agents in cancer, Biochim. Biophys. Acta, Gen. Subj. 1850 (2015) 1642–1660.

[55] R. Sinha, K. El-Bayoumy, Apoptosis is a critical cellular event in cancer chemoprevention and chemotherapy by selenium compounds, Curr. Cancer Drug Targets 4 (2004) 13–28.

[56] Y. Chen, S. Liu, J. Li, Z. Li, J. Quan, X. Liu, Y. Tang, B. Liu, The latest view on the mechanism of ferroptosis and its research progress in spinal cord injury, Oxid. Med. Cell. Longev. 2020 (2020), 6375938.

[57] T.G. Back, Z. Moussa, M. Parvez, The exceptional glutathione peroxidase-like activity of di (3-hydroxypropyl) selenide and the unexpected role of a novel spirodioxaselenanonane intermediate in the catalytic cycle, Angew. Chem., Int. Ed. Engl. 43 (2004) 1268–1270.

[58] C. Sanmartín, D. Plano, A.K. Sharma, J.A. Palop, Selenium compounds, apoptosis and other types of cell death: an overview for cancer therapy, Int. J. Mol. Sci. 13 (2012) 9649–9672.

[59] K. Subburayan, F. Thayyullathil, S. Pallichankandy, A.R. Cheratta, S. Galadari, Superoxide-mediated ferroptosis in human cancer cells induced by sodium selenite, Transl. Oncol. 13 (2020), 100843.

[60] S. Misra, M. Boylan, A. Selvam, J.E. Spallholz, M. Björnstedt, Redox-active selenium compounds-from toxicity and cell death to cancer treatment, Nutrients 7 (2015) 3536–3556.

[61] P. Khalkar, N. Díaz-Argelich, J. Antonio Palop, C. Sanmartín, A.P. Fernandes, Novel methylselenoesters induce programed cell death via entosis in pancreatic cancer cells, Int. J. Mol. Sci. 19 (2018) 2849.

[62] C. Jiang, Z. Wang, H. Ganther, J. Lu, Caspases as key executors of methyl selenium-induced apoptosis (Anoikis) of DU-145 prostate cancer cells, Cancer Res. 61 (2001) 3062–3070.

[63] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.

[64] A.M. Sh. El-Sharief, Z. Moussa, Synthesis, characterization and derivatization of some novel types of mono- and bis-imidazolidineiminothiones and imidazolidineiminodithiones with antitumor, antiviral, antibacterial and antifungal activities - part I, Eur. J. Med. Chem. 44 (2009) 4315–4334.

[65] Z. Moussa, A.M. Sh. El-Sharief, A.M. Sh. El-Sharief, Synthesis and characterization of new types of halogenated and alkylated imidazolidineiminothiones and a comparative study of their antitumor, antibacterial, and antifungal activities, Eur. J. Med. Chem. 46 (2011) 2280–2289.

[66] A.M. Sh. El-Sharief, Z. Moussa, A.M. Sh. El-Sharief, Synthesis, characterization, and derivatization of some novel types of fluorinated mono- and bis-imidazolidineiminothiones with antitumor, antiviral, antibacterial, and antifungal activities, J. Fluor. Chem. 132 (2011) 596–611.

[67] M.A. M. Sh. El-Sharief, Z. Moussa, A.M. Sh. El-Sharief, Synthesis and biological evaluation of bis-imidazolidineiminothiones: a comparative study, Arch. Pharm. Chem. Life Sci. 346 (2013) 542–555.

[68] Z. Moussa, M.A. M. Sh. El-Sharief, S.Y. Abbas, New imidazolidineiminothione derivatives: synthesis, spectral characterization and evaluation of antitumor, antiviral, antibacterial, and antifungal activities, Eur. J. Med. Chem. 122 (2016) 419–428.

[69] M.A. M. Sh. El-Sharief, S.Y. Abbas, A.M.Sh. El-Sharief, N.M. Sabry, Z. Moussa, S.M. El-Messery, A.R. Elsheakh, G.S. Hassan, M.T. El Sayed, 5-Thioxoimidazolidine-2-one derivatives: synthesis, anti-inflammatory activity, analgesic activity, COX inhibition assay and molecular modelling study, Bioorg. Chem. 87 (2019) 679–687.

[70] Z. Moussa, Z.M.A. Judeh, M.A. M. Sh. El-Sharief, A.M.Sh. El-Sharief, N-arylcyanothioformanilides: preparation methods and application in the synthesis of bioactive molecules, ChemistrySelect 5 (2020) 764–798.

[71] L. Annika, V. Kathrin, B. Hannes, S. Klaus, G. Christian, Determining the π-acceptor properties of N-heterocyclic carbenes by mmeasuring the ^{77}Se NMR chemical shifts of their selenium adducts, Organometallics 32 (2013) 5269–5272.