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Enhancing the chemosensitivity of HepG2 cells towards cisplatin by organoselenium pseudopeptides

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ABSTRACT

Despite all recent advances in the treatment of hepatocellular carcinoma (HCC), chemotherapy resistance still represents a major challenge in its successful clinical management. Chemo-sensitization offers an attractive strategy to counter drug resistance. Herein we report the identification of novel organoselenium-based pseudopeptides as promising highly effective chemo-sensitizers in treating HCC with cisplatin. A series of functionalized pseudopeptide- (**5**–**9** and **17**–**19**), peptidomimetic- (**10**–**12** and **20**–**23**), and tetrazole-based (**13**–**16** and **24**–**27**) organoselenium compounds were synthesized via isonitrile-based multicomponent reactions from two novel selenium-containing isocyanides. All compounds were evaluated for their cytotoxicity against HepG2 and the non-cytotoxic doses were used to restor the sensitivity of the cells to cisplatin. New organoselenium compounds (**7**, **9**, **15,** or **23**) led to an effective chemo-sensitization of HepG2 cells towards cisplatin (up-to 27-fold). Cell cycle studies indicate that the most potent peptidomimetic diselenide **23** arrested cells at the S phase and induced apoptosis via ROS modulation.

1. Introduction

Primary liver cancer is among the most frequent cancer types and one of the leading causes of cancer-related deaths globally [1,2]. Hepatocellular carcinoma (HCC) is the most frequent kind of primary liver cancer with a high incidence in Africa and Asia [3]. Despite its spread, the successful treatment of HCC still represents a tremendous challenge, leading to a poor long-term prognosis, particularly for patients with advanced stages of HCC. Whereas radical therapeutic approaches, such as surgical tumor removal or liver transplantation, are indicated only for early stages, therapies for advanced HCC are still lacking. Currently, sorafenib is the only approved drug for systemic chemotherapy of HCC, unfortunately with only a modest efficacy [4]. Treatment of HCC with standard chemotherapeutic agents [5-7], such as cisplatin or 5-fluorouracil, fails in the majority of patients [8]. Due to its genomic instability and complex morphology, HCC cancer cells are either inherently resistant to common chemotherapeutics or develop resistance within a short timeframe after an initial response [9]. Due to its low inherent chemosensitivity and its acquired drug resistance, there is an urgent need to identify novel compounds, sensitizing HCC towards therapy with common therapeutic agents [10].

Chemo-sensitization represents an attractive and very effective approach to counter the development of chemoresistance in tumors [11,12]. Tumor cells can become more susceptible to the initial chemotherapeutic drug using an additional agent, a so-called chemosensitizer. This is where organic selenides come into play. In recent years, organic selenides have evolved as an attractive compound class in medicinal chemistry and drug development [13]. They are being used as chemopreventive, chemoprotective, and antioxidant agents, as confirmed in several epidemiological studies and pre-clinical trials (e.g., SELEBLAT, SELECT, and NPC) [14,15]. For example, the two synthetic organoselenium compounds, methylseleninic acid (**I**) and diphenylmethyl selenocyanate (**II**) exhibited good chemoprotective and chemopreventive activities against different cancer types in combination

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with cisplatin, cyclophosphamide or paclitaxel therapy (Fig. 1) [16,17]. Furthermore, ebselen (**III**) has shown excellent antioxidant and anti-inflammatory activities and currently being explored as a neuro-protective agent in clinical trials [18-20].

Moreover, the selenocyanate-containing compounds BSC (**IV**) and p-XSC (**V**) have shown excellent cancer chemopreventive activity [14,21]. Indeed, we have reported several organoselenium compounds (**VI-VIII**) with exciting and, at the same time, highly selective anti-HCC activities [18,22-28]. Interestingly, all these compounds exhibited superior cytotoxicity to the traditional anticancer drug fluorouracil, up-regulated the expression levels of CD40 protein and interleukin 2 and 6, and down-regulated the expression of Bcl-2 in HepG2 cells. Recently, we also reported cytoprotective selenorganic compounds with potential glutathione peroxidase (GPx)-like activity (**IX**) and antiapoptotic properties (**X**) for the 158 N myelin-forming cells (Fig. 1) [29]. Despite the interesting chemopreventive and anticancer activities of organoselenium compounds, their chemo-sensitization effect with chemotherapeutic drugs has just begun.

Diversity-oriented synthesis (DOS) is a robust chemical tool that allows the construction of skeletally diverse small molecules efficiently and deliberately [30-33]. Furthermore, it enables the understanding of the biological mechanism of sophisticated diseases (e.g., cancer and cardiovascular diseases) and frequently employed for drug discovery. In this context, lead candidates are identified via small-molecule libraries' high-throughput screening, which can be optimized to be used as probes or modulators for specific biological targets [30-33]. Multicomponent reactions (MCRs) are among the most explored DOS pathways in terms of combinatorial synthesis and the context of molecular diversity and complexity [34,35]. Fortunately, isocyanide-based Multicomponent reactions (IMCRs) are among the most well-known and explored MCR subclass [36-41]. The isonitrile (a.k.a. isocyanide) group's extraordinary nature accounts for the great potential of IMCRs. They are stable organic compounds with a formally divalent carbon that readily reacts with both electrophiles and nucleophiles to give α-adducts [38,41,42]. Amongst all known IMCRs, Groebke–Blackburn–Bienayme (GBB), Ugi, and Passerini reactions are by far the most popular [34,38,43,44]. These one-pot reactions give fused imidazole, peptidomimetic, and pseudopeptide scaffolds in a high diversity and combinatorial-controlled fashion from readily available components such as amines, acids, and oxo compounds (e.g., aldehydes or ketones). While these reactions are a convenient tool for constructing skeletally diverse small molecules, they suffer from the lack of commercially available isonitriles. Unlike the other utilized building blocks, isonitriles have an unpleasant odor and are of limited commercial availability, i.e., fewer than three-dozen can be bought[1]. In 2009, we reported the synthesis of sulfur-, selenium- and tellurium-based isonitriles and their applications in IMCRs [22].

In continuation of our previous projects, we herein report an expansion of the chemical space of isocyanides via designing and synthesizing two novel selenium-based isocyanides. The newly synthesized isonitriles' reactivity was explored in different IMCRs, leading to a structurally diverse library of highly functionalized organoselenium compounds. The latter was tested for cytotoxicity against HepG2 cells. Furthermore, the chemo-sensitizing and chemoprotective efficacy of the synthesized novel organoselenium compounds was investigated, and their potential mode of action was evaluated by cell cycle analysis, apoptosis analysis, and evaluation of reactive oxygen species (ROS) level. Our studies revealed a so far unprecedented sensitization of HCC cells against the standard anticancer drug cisplatin with organoselenium agents.

[1] Twenty-six isocyanides are commercially available at sigmaaldrich.com.

2. Results and discussion

2.1. Synthesis

IMCRs enable the expeditious generation of molecular diversity and structural complexity from simple starting building blocks in an atom-convergent and economical fashion. They are a versatile tool in medicinal chemistry, and plenty of biologically active small molecules have been synthesized via the Passerini, Ugi, or Groebke–-Blackburn–Bienayme reactions over the years [41,42,45,46]. Although IMCRs provide straightforward and practical access to drug-like scaffolds, their utility is significantly hampered by the low structural diversity of readily available isocyanide building blocks, which restricts the accessible structural space. Isonitriles bearing an additional functional group handle, such as the chalcogens (e.g., sulfur, selenium, tellurium) functionality, are not commercially available and are limited to few examples the literature. Since our initial report on the first three phenyl-sulfide, -selenide, and -telluride containing isocyanides, no further chalcogen-based isonitriles were reported in the literature [23,26]. In continuation of our previous work, we set out to expand the chemical space accessible via isocyanide-based multicomponent reactions (ICMR) by establishing novel chalcogen-containing isonitriles.

Therefore we studied the conversion of the two selenium-based amines, 4-selenocyanatoaniline (**1**) and 4,4′-diselanediyldianiline (**2**), [22,25,47] into the corresponding isonitriles **5** and **6** via a two-step formylation/dehydration procedure [48,49].

4-Selenocyanatoaniline (**1**) was prepared directly from aniline according to an established procedure and further converted into the diselenide 2. Formylation was initially performed using formic acid under neat conditions; however, the corresponding formamides **3** and **5** were obtained in low yields (<40%). Next, formylation was performed employing formic acid/acetic anhydride mixture. Unfortunately, a mixture of the corresponding acetanilides and formamides was obtained. Further formylation of **1** and **2** was achieved with acetic formic anhydride, prepared *in situ* from acetyl chloride and sodium formate in dry ether, delivering the desired formamides **3** and **4** in quantitative yields [50]. We then directed our attention to the dehydration of both formamides. Reaction with various dehydrating agents, such as phosphorus oxychloride ($POCl_3$), cyanuric chloride, triphenylphosphine, 4-toluenesulfonyl chloride, Burgess reagents, or phosgene were performed [41,42,51-54].

Interestingly, the formation of nonpolar products (using TLC) with the characteristic isonitriles odor was only observed in the case of $POCl_3$. Initial attempts to isolate the observed nonpolar products failed. Due to the reported instability of isonitriles, we suspected that isonitriles **5** and **6** might be unstable at room temperature. Indeed, both the desired selenocyanate isocyanide **5** and diselenide diisonitrile **6** were obtained in 92 and 94% yields after performing purification and isolation at low temperatures (≤0 °C) (Scheme 1). Both compounds show a characteristic isocyanide carbon triplet peak at 165 ppm in the ^{13}C NMR spectra (see supporting information). It is worth noting that isocyano-4-selenocyanatobenzene (**5**) is not stable and must *be* prepared directly before usage.

With established access to the two novel isocyanides **5** and **6**, we set out to incorporate the selenocyanate and diselenides functionality into a small set of structurally diverse molecules using different IMCRs. In accordance with our aim, the reactivity of the selenocyanate isocyanide **5** and diselenide diisonitrile **6** was explored in three typical IMCRs, the Passerini, the Ugi, and the azido-Ugi reaction (Scheme 2 and Scheme 3).

By variation of the other building blocks, we prepared a small library of 21 different organoselenium compounds (Scheme 4).

We selected four different aldehyde (paraformaldehyde, isobutyraldehyde, isovaleraldehyde, and 4-nitrobenzaldehyde) for our initial study and three different amines (benzyl amine, aniline, and 4-methoxyaniline) components. As an acid component, only acetic acid (Passerini/Ugi) or trimethylsilyl azide ($TMSN_3$) (azido-Ugi reaction)

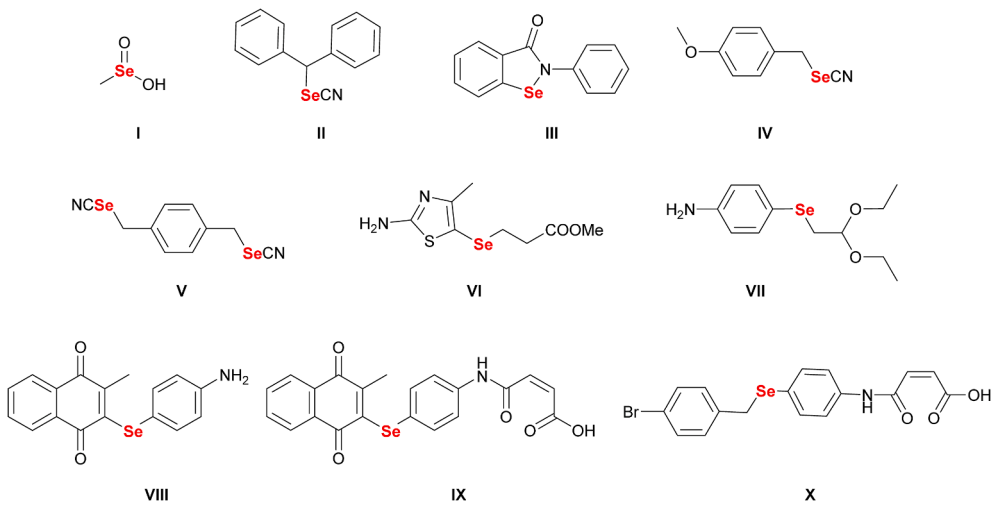
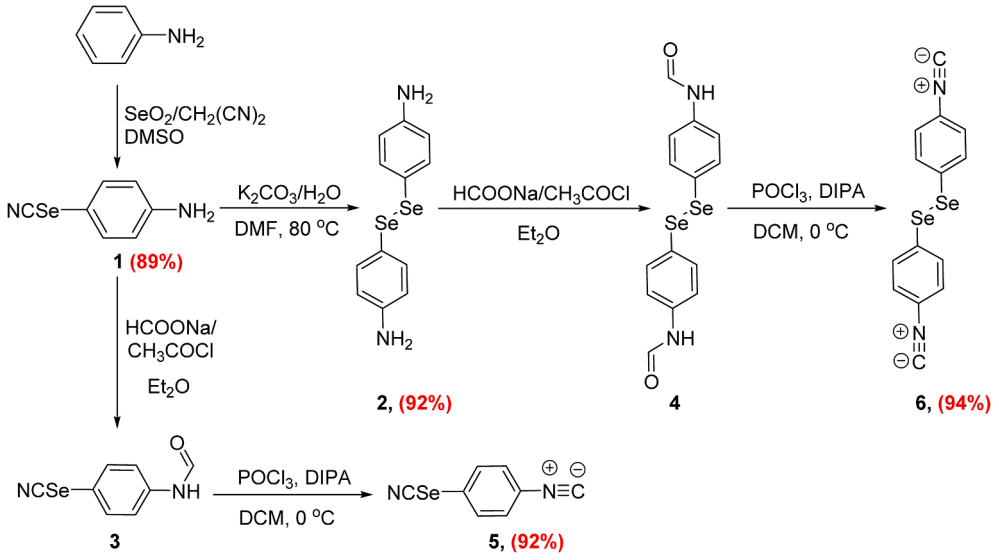
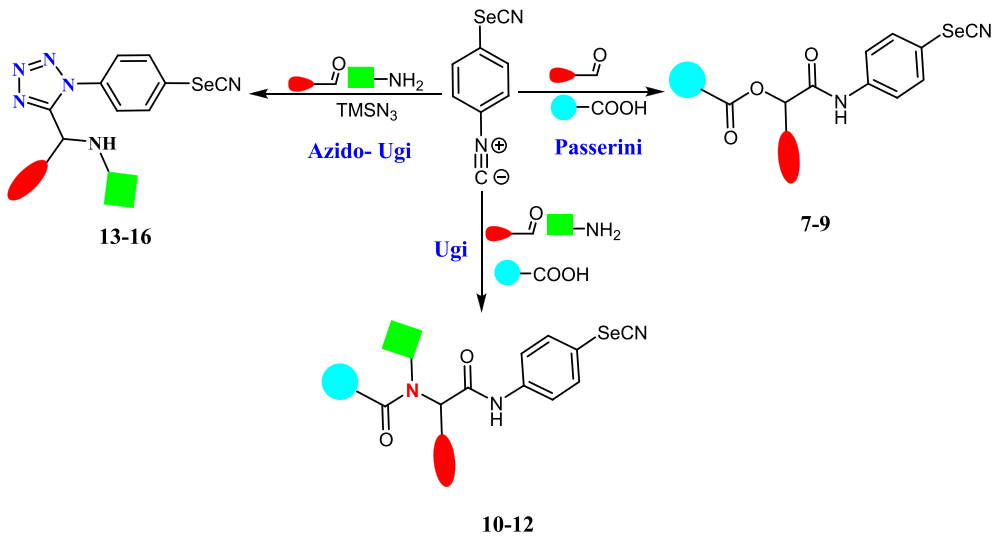


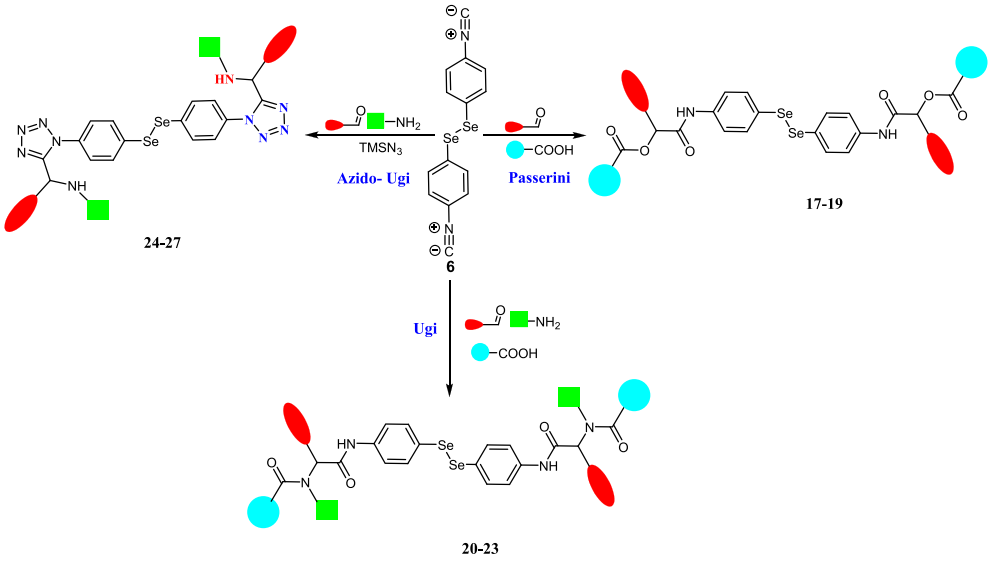
Fig. 1. Structures of selected organoselenium compounds (**I-X**) with anticancer, chemopreventive, and antioxidant properties.



Scheme 1. Synthesis of selenocyanate and diselenide isocyanides **5** and **6**.



Scheme 2. Schematic overview of the participation of the selenocyanate isocyanide **5** in Passerini, Ugi, and azido-Ugi multicomponent reactions.



Scheme 3. Schematic overview of the participation of the diselenide diisonitrile **6** in Passerini, Ugi, and azido-Ugi multicomponent reactions.

were used. The Passerini reaction was performed in dichloromethane, whereas the Ugi and azido-Ugi reactions were performed in methanol. Peptidomimetic selenocyanates **7**–**16** and diselenides **17**–**26** were synthesized in moderate-excellent yields (up to 94%).

2.2. Biological evaluation

2.2.1. Organoselenium anti-HepG2 activity

After preparing a small set of structurally diverse, novel organoselenium compounds, we started to assay their biological activities. Based on our previously established broad-spectrum cytotoxicity screening, we evaluated the cytotoxic activity of compounds **7**–**27** against HepG2 cells, a simple model system for hepato-carcinogenesis *in vitro* drug targeting studies [3,19,29,47,55]. Cell viability was evaluated using MTT assay, and IC_{50} values were calculated after 48 h using GraphPad prism (Table 1).

Most of the tested organoselenium agents did not exhibit any cytotoxic effect ($IC_{50} \geq 50$ μM) against HepG2 cells in the tested concentration range used, except for pseudopeptides **10**, **17**, and **19**. The latter two exhibited pronounced cytotoxic activities ($IC_{50} = 7 \pm 0.1$ for compound **17** and 9 ± 1.5 μM for compound **19**), which are approx. two-fold higher than cisplatin ($IC_{50} = 16.3 \pm 0.5$ μM). Furthermore, pseudopeptide **10** showed moderate activity with an $IC_{50} = 28 \pm 0.4$ μM. Observed morphological alterations in cells treated with compounds **17** or **19**, as depicted in Fig. 2, S1 and S2 (Supplementary Materials Figure S1 and S2), indicate that both organoselenium compounds induce apoptosis as observed in the formation of black round apoptotic bodies. In our previous work, we could already show that peptidomimetic organoselenides can induce apoptosis via arresting cells at the G0/G1 phase and disruption of endoplasmic reticulum and cytoskeleton organization (stress fibers) [23,26].

From a chemistry point of view, compounds **10**, **17**, and **19** share some common structural features other than being organoselenium derivatives. Compounds **17** and **19** are both Ugi adducts and diselenides at the same time. Furthermore, compounds **10** and **17** have a common isopropyl fragment. Collectively, the herein reported novel selenium-containing pseudopeptides **17** and **19** warrant further investigation as potential anticancer agents.

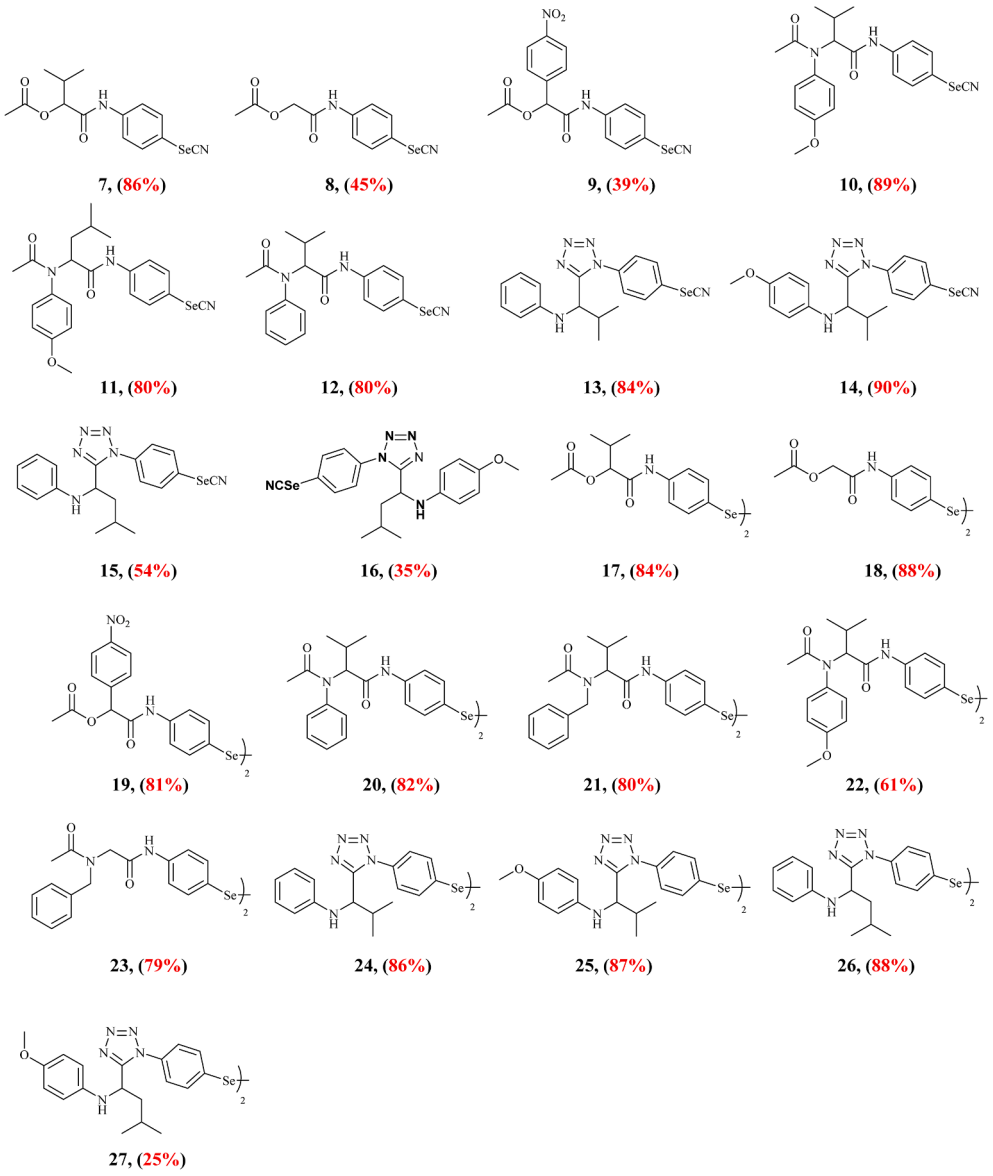
2.2.2. Chemo-sensitization/chemoprevention of HepG2 cells to cisplatin cytotoxicity

After the initial evaluation of the organoselenium compounds' cytotoxicity, we turned our attention towards potential chemo-sensitizing/chemoprotective effects of the non-cytotoxic compounds.

Cisplatin is one of the main chemotherapeutic drugs used for the treatment of different cancer types. Its cytotoxic mechanism is the formation of platinum-DNA adducts and ROS induction, thereby triggering DNA damage response, subsequent cell cycle arrest, and finally apoptosis [56]. Moreover, cisplatin is effective in combination with radiotherapy and immunotherapy [57]. Unfortunately, in particular HCC cells, cancer cells can develop multiple mechanisms of resistance towards cisplatin [58]. It has been reported that a selenium-containing diet can restore prostate cancer sensitivity to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis [16]. Besides, diphenylmethyl selenocyanate **II** has been used to sensitize a murine tumor model to cisplatin via the overproduction of reactive oxygen species, p53 activation, downregulation of the antiapoptotic protein Bcl-2, and overexpression of apoptotic protein Bax [17]. Based on this literature precedence, we decided to investigate further the use of our novel organoselenium compounds as chemo-sensitizers to improve the treatment of HCC with cisplatin using HepG2 cells as a model system.

All fourteen organoselenium compounds displaying no cytotoxic effect in the initial MTT assay were evaluated for their chemo-sensitizing activities combined with cisplatin. Therefore, HepG2 cells were initially treated with 20 μM of the selected organoselenium compounds for 4 h, and then serial dilutions of cisplatin were applied (50, 25, 12.5, 6.25, 3.125, 1.56 μM) (Table 2). IC_{50} values of the respective combinations were determined by MTT assay after 48 h overall treatment (Fig. 3).

To our delight, cells pretreated with the organoselenium compounds **7**, **9**, **15**, and **23** were more susceptible towards treatment with cisplatin ($IC_{50} = 3 \pm 1.8$, 3 ± 0.2, 6 ± 0.5, 0.6 ± 0.3 μM, respectively), leading to a significantly increased response compared to treatment with cisplatin alone ($IC_{50} = 16.3 \pm 0.5$ μM) (Table 2). The peptidomimetic diselenide **23** displayed the most promising chemo-sensitizing effect (27-fold increase; Table 2). Pretreatment experiments were carried out following the literature reported procedures [59-63] to find out the lowest doses with the best chemo-sensitization effect. Our experiments started by treating HepG2 cells with compound **23** (40, 20, 10, 5 or 2.5 μM) for four h followed by incubation with cisplatin (50, 25, 12.5, 6.25, 3.125, 1.56 μM) for an additional 44 h. A drastic cell growth inhibition was observed after 20 μM of compound **23** where the IC_{50} of cisplatin was declined from 16.3 to 0.6 μM (Fig. 4). Next, the optimum time of pre-incubation was investigated at different time points (1, 2, 3, 4, 5, or 6 h). Interestingly, only one-hour pretreatment of HepG2 cells with compound **23** (20 μM) was sufficient to sensitize the cells towards cisplatin ($IC_{50} = 0.6$



Scheme 4. Organoselenium pseudopeptides, **7**–**27**, synthesized employing the Passerini, Ugi, and azido-Ugi multicomponent reactions.

μM) toxicity (Fig. 4 and Supplementary Material Figure S3). The short time sensitizing activity indicates the modulation of antiapoptotic components in HepG2 cells, leading to increased sensitivity towards cisplatin, according to Chakraborty *et al.*, [17]. It worth mentioning that the study presented here is only an entry point for considerably broader and more in-depth investigations, which ultimately will consider the concomitant and posttreatment group as well as mechanistic aspects underlying the chemosensitization of compound **23**.

Most anticancer drugs act by perturbating the cell cycle, either by overexpressing cell cycle inhibitors (p15, p16, p21, or p27), down-regulating cyclin-dependent kinases, damaging DNA, or affecting cyto-skeleton organization [64,65]. Therefore, we further investigated the sensitizing mechanism of compound **23** by cell cycle analysis employing flow cytometry. Cisplatin was reported to induce HL-60 cells to be initially arrested at S-phase, and then over time, gradually accumulated and arrested at the sub-G1 phase [66]. Our results also showed that cisplatin induces cell arrest at G1-phase. Interestingly, HepG2 cells sensitized with the peptidomimetic diselenide **23** were arrested at S-phase (Fig. 5), which indicates the induction of early apoptosis. Therefore, the level of apoptotic cells was evaluated after treatment with cisplatin alone and with cells pretreated with compound **23**. The staining for the early apoptotic marker phosphatidylserine with Annexin V-PE by flow cytometry revealed that cisplatin alone was less efficient in inducing apoptosis (2% early apoptotic cells *vs.* 36.6% with combined treatment), but preincubation for just four h increased the sensitivity of HepG2 cells to cytotoxicity of cisplatin (11.8% apoptotic cells *vs.* 3.7% with single-drug treatment).

The results showed that the apoptosis index in the sensitized cells was higher than the cells treated with cisplatin alone or the DMSO-treated cells (36.3%±0.8 *vs.* 2.3%±0.3 and 0.4%±0.2, respectively (Fig. 6).

In our former studies, we could confirm possible mechanism(s) for some organoselenium agent's cytotoxicity against different types of cancer, such as modulation of the ROS and GSH levels, activation of caspase 3/7, cell cycle arrest, and subsequent induction of apoptosis [3,19,22,23,29,38]. The peptidomimetic diselenide **23** could therefore chemo-sensitize HepG2 cells via an analogous perturbation of the cell's redox system. To evaluate this possible mode of action, the intracellular ROS levels were estimated using H2-DCFDA assay by flow cytometry in sensitized HepG2 cells and compared to treatment with cisplatin alone. H2-DCFDA is a lipophilic probe used to detect several types of ROS, such as peroxides, peroxynitrites, and lipid hydroperoxides. As expected,

Table 1
Selenorganic compounds effect on the viability of HepG2 cells.[a]

Compounds	IC_{50} (μM)[b]
Cisplatin[c]	16.3 ± 0.5
7	-d
8	-d
9	-d
10	28 ± 0.4
11	-d
12	-d
13	-d
14	-d
15	-d
17	7 ± 0.1
18	-d
19	9 ± 1.5
20	-d
21	-d
22	-d
23	-d
24	-d
25	-d
26	-d
27	-d

[a] The cytotoxicity was assessed after 48 h treatment of HepG2 cells with various concentrations of the organoselenium agents using the MTT assay. [b] IC_{50} values in μM were estimated, and the standard deviation (±) is for three parallel experiments. [c]The positive control used in this experiment is cisplatin; [d] no apparent cytotoxicity was observed at the employed concentration range.

cisplatin-induced significant ROS elevation (24.9%± 0.26). On the other hand, the peptidomimetic diselenide **23** alone could attenuate the ROS level (1.1%± 0.21). Notably, the organoselenium compound **23** displayed a similar attenuating effect in combination with cisplatin (5.4%± 0.17) (Fig. 7).

Organoselenium compounds were reported to use pre-existing ROS to oxidize redox-sensitive cellular compartments leading to cellular malfunction and subsequently cell death [67-72]. It is also reported that the cisplatin cytotoxic mechanism is correlated to ROS's overproduction [73-75].

In the case of the HepG2, compound **23** did not exhibit any cytotoxicity (IC_{50} > 50 μM), whereas cisplatin cytotoxicity was in the micromolar scale, i.e., 16.3 μM (Table 1). Surprisingly,

a drastic inhibition of HepG2 cell growth was observed upon treatment with 20 μM of compound **23** and 0.6 μM cisplatin (Figs. 4 and 5). It is likely that the ROS levels (HepG2 pre-existing and cisplatin-induced) are now enough to activate compound **23**. The latter facilitates ROS's

Table 2
Effect of the selenorganic compounds on the modulation of the cytotoxicity of cisplatin against HepG2 cells.[a]

Compounds	Chemo-sensitization IC_{50} (μM)[b]	Chemo-sensitization fold[c]
Cisplatin	16.3 ± 0.5	–
7	3 ± 0.2	5
9	3 ± 0.2	5
11	11.6 ± 0.2	1
12	17.8 ± 0.2	1
13	8 ± 0.5	2
14	10 ± 0.4	2
15	6 ± 0.5	3
20	20 ± 0.4	1
21	17.8 ± 0.2	1
22	13.8 ± 0.2	1
23	0.6 ± 0.1	27
25	29.2 ± 1.5	1
26	29.2 ± 0.2	1
27	8 ± 0.7	2

[a] HepG2 cells were pretreated for four h with 20 μM of organoselenium compounds, and then serial dilutions of cisplatin (50, 25, 12.5, 6.25, 3.125, 1.56 μM) was added, and the viability was determined after 48 h by MTT assay. [b] IC_{50} values of cisplatin in μM were estimated, and the standard deviation (±) is for three parallel experiments. [c]Chemo-sensitization fold was calculated as follows: IC_{50} value of cisplatin (16.3 μM)/cytotoxicity resulted from a combination of selenium compounds with cisplatin.

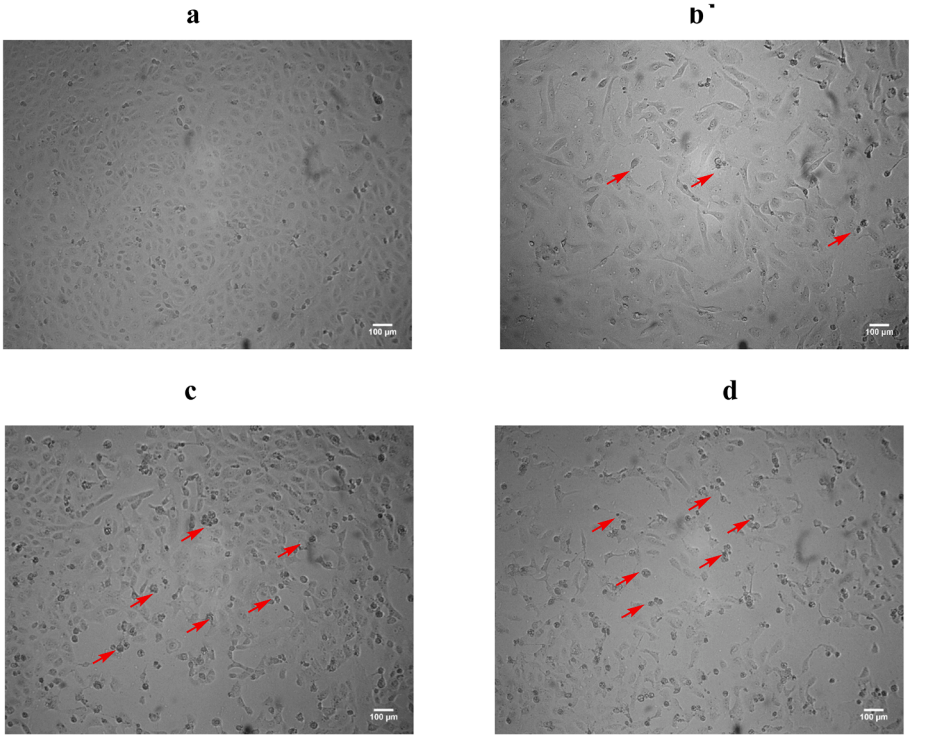


Fig. 2. Morphological changes of HepG2 cells after treatment with compound DMSO vehicle (panel a), cisplatin (panel b), compound **17** (panel c), and compound **19** (panel d). Cells were treated with different concentrations, and morphology was observed after 48 h of incubation. The presented figures show the morphological alterations (red arrow) induced after treatment with 6.25 μM of compound **17**, **19**, or cisplatin. The cells were round and show apoptotic debris (black round cells indicated by a red arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

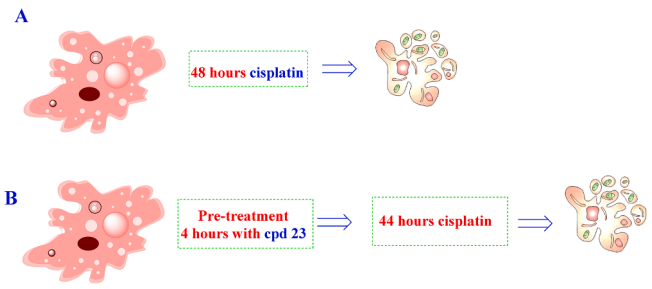


Fig. 3. Chemo-sensitization experiment design. A) HepG2 cells were treated for forty-eight h with cisplatin; B) HepG2 cells were treated for four h with 20 μM of the organoselenium compounds, and then serial dilutions of cisplatin (50, 25, 12.5, 6.25, 3.125, 1.56 μM) were applied.

reaction- pre-existing in HepG2 and induced by cisplatin-with redox-sensitive cellular compartments and subsequently triggers cellular injury and apoptosis [67-72]. However, the exact organoselenium compounds' potential has not been thoroughly studied in the context of cancer chemosensitization research.

These results reveal compound **23** as a new chemo-sensitizer to cisplatin to overcome the emergence of resistance and improve the clinical outcomes for patients. Notably, the exact underlying mechanism is still not understood and merit further studies.

3. Conclusion

HCC is the most common and aggressive kind of liver cancer with an overall poor long-term prognosis. Treatment of HCC with classical cytotoxic drugs is limited due to its inherent or readily acquired chemoresistance. Chemo-sensitization is a desirable strategy to overcome this limitation and provide a still lacking general systemic therapy for HCC. Herein, we disclose the synthesis of small set of novel pseudo-peptide- (**5**–**9** and **17**–**19**), peptidomimetic- (**10**–**12** and **20**–**23**), and tetrazole-based (**13**–**16** and **24**–**27**) organoselenium compounds from two novel isocyanide building blocks (**5** and **6**) via common IMCRs.

Among the twenty-one novel organoselenium compounds, **17** and **19** exhibited promising cytotoxic activity against HepG2 cells, presumably by inducing apoptosis. Interestingly, most organoselenium compounds (**7**–**9**, **11**–**15**, **18**, and **20**–**27**) did not display any cytotoxic effect. Further studies revealed that four compounds **7**, **9**, **15**, or **23** exhibited an enhanced chemo-sensitizing effect on HepG2 cells to cisplatin (up-to 27-fold increase in cisplatin activity). The peptidomimetic diselenide **23** lead to an effective chemo-sensitization of HepG2 cells in 20 μM concentration within only one hour.

Cell cycle analysis revealed a cell cycle delay at S-phase in HepG2 cells treated with cisplatin combined with the peptidomimetic diselenide **23**, which indicates a sensitizing effect via enhancing the cisplatin-induced apoptosis. Additionally, peptidomimetic diselenide **23** enhanced the chemo-sensitizing effect by exerted its cytotoxicity via amplification of the ROS severity. In summary, the present study entails a novel efficient synthetic avenue towards structurally diverse organoselenium compounds, which significantly increases the chemical space accessible via ICMRs. The herein synthesized compounds can act as lead structures for the development of novel chemotherapeutics and chemosensitizers for the effective treatment of HCC. Furthermore, our work provides numerous chances for future studies at the biology/chemistry interface. This research is worth further investigations and probably follow-up studies in different scientific areas such as medicinal chemistry, pharmacology, and drug development.

4. Experimental protocols

4.1. Material and methods

All chemicals (e.g., solvents, reagents) were purchased from Sigma-Aldrich and used directly. TLC was performed on aluminum sheets, SiO_2 coated, (60 F_{254}) and ultraviolet light was employed to visualize spots were by. Column chromatography was performed silica 60.

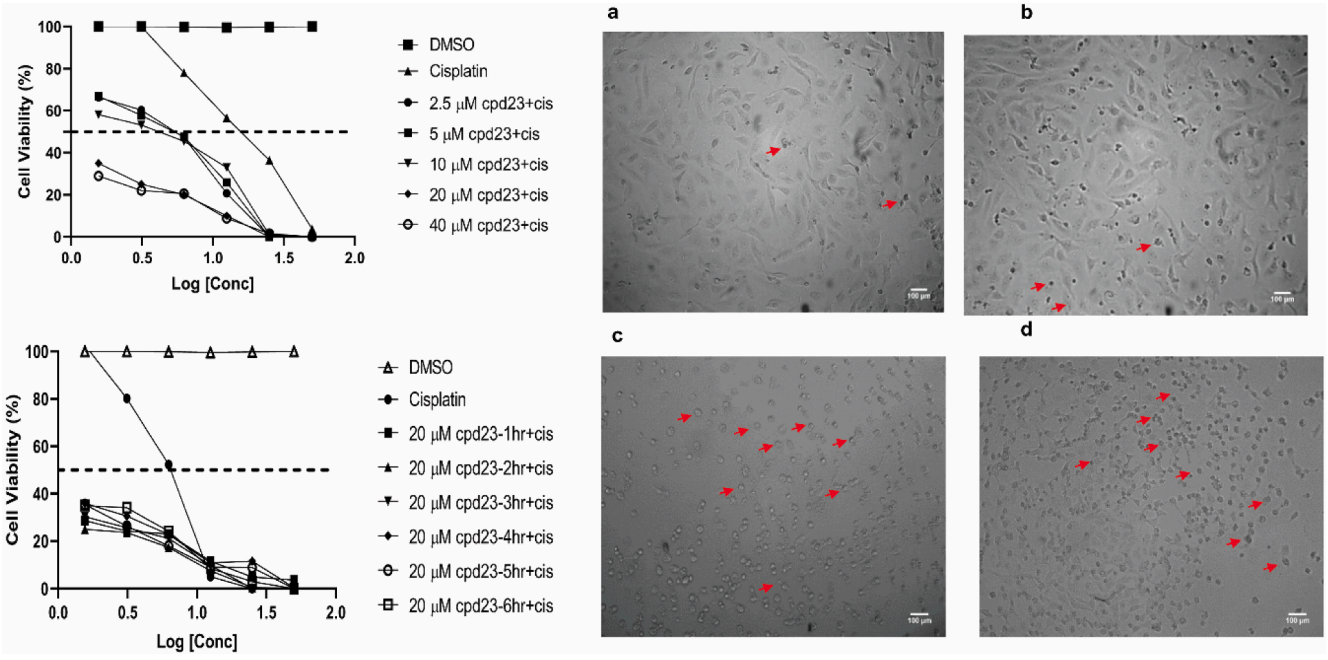


Fig. 4. Pretreatment with compound **23** restored the sensitivity of HepG2 to cisplatin. Top left panel: cells were pre-treated with different concentrations (40, 20, 10, 5, 2.5 μM) of compound **23** for 4 h then treated with serial dilutions of cisplatin (50, 25, 12.5, 6.25, 3.125, 1.56 μM) for total 48 h. MTT evaluated the cell viability. Lower left panel: cells were pretreated with 20 μM of compound **23** for the indicated time points then treated with a serial dilution of cisplatin (50, 25, 12.5, 6.25, 3.125, 1.56 μM). Right panel: morphological changes of cells treated either with 6.25 μM (panel a) or 3.125 μM (panel b) of cisplatin alone shows fewer apoptotic cells (red arrow) while cells pretreated with 20 μM for four h increased the apoptosis in cells treated with 6.25 μM (panel c) or 3.125 μM (panel d) of cisplatin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

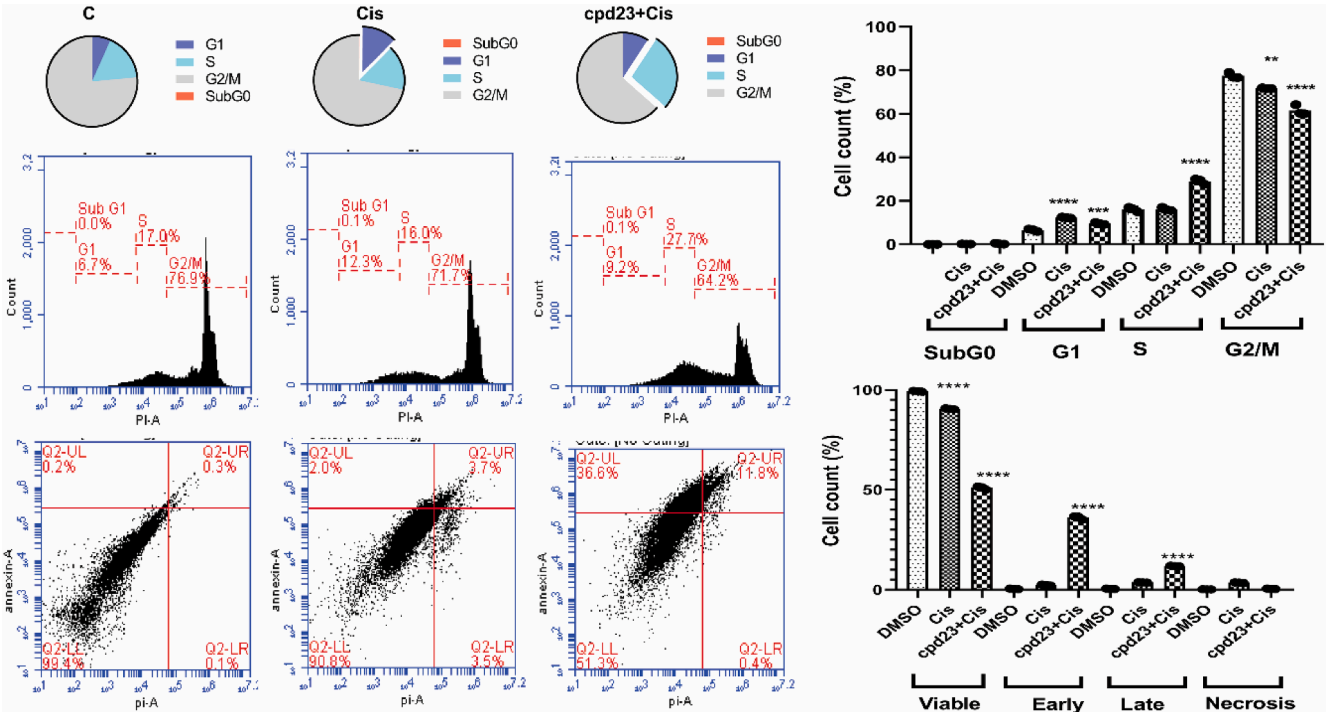


Fig. 5. Cell cycle arrest at S phase after combination treatment with both cisplatin and diselenide **23**. The cells were treated either with 0.6 μM cisplatin alone or treated with 20 μM compound **23** for four h then treated with 0.6 μM cisplatin for a total of 48 h. The DNA content was evaluated by flow cytometry. The induction of apoptosis was analyzed by staining cells with Annexin-V. Statistical analysis was performed using GraphPad prism 8.0 by one-way ANOVA test. **** $p < 0.0001$ and ** $p < 0.0069$.

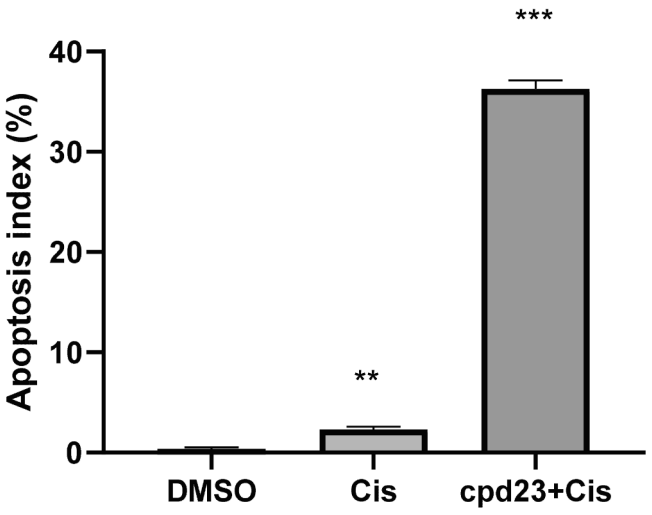


Fig. 6. Apoptosis index increased in the sensitized cells with compound **23**. The apoptotic index was calculated as the percentage of Annexin V-positive and PI-negative cells (early apoptosis) divided by the total number of cells in the gated region (viable cells).

Melting points are reported uncorrected. Electrospray ionization (ESI) techniques were used to measure the mass spectra (MS), while, matrix-assisted laser desorption/ionization (MALDI) techniques were used to measure the high-resolution mass spectra (HRMS).^{1}H NMR and ^{13}C NMR spectra were recorded at 300.03 M*Hz*, 500.18 M*Hz*, 600.31 M*Hz* (^{1}H), 75.44 M*Hz*, 125.77 M*Hz*, 150.95 M*Hz* (^{13}C), respectively. Chemical shifts (δ) are relative to the residual $CDCl_3$ ($\delta = 77.16$ ppm for ^{13}C and $\delta = 7.26$ ppm for ^{1}H). Coupling constants (J) are given in *Hz* and multiplicities of the signals are abbreviated as follows: dt = doublet of triplets, dd = doublet of doublets, m = multiplet, q = quartett, t = triplet, d = doublet and s = singlet. The structure and purity (≥97%) of the compounds were established using HRMS spectrometry and NMR spectroscopy. 4-Selenocyanatoaniline (1) and 4,4′-diselanediyldianiline (2) were synthesized according to literature reported methods [3,19,29].

4.2. Biological assays

4.2.1. Cell lines and reagents

HepG2 cells were obtained from VACSERA company, Egypt. Cells were grown in a modified medium (Eagle's Dulbecco) (BioWhittaker™) and kept at 5% CO_2 and 37 °C. Bovine fetal serum (10%) was supplemented to the medium (Life Science Group L UK, Cat No: S-001B-BR) and (100 IU/mL) penicillin/ streptomycin (100 μg/mL) (Lonza, 17-602E).

4.2.2. Dose selection and sample preparation

Cisplatin was obtained from Sigma-Aldrich, solubilized in saline (0.9%), and then kept at –20 °C as 1666 μM stock solution. Ten mM stock solutions of organoselenium compounds were prepared in Dimethyl sulfoxide (DMSO Cat. No. 20385.02, Serva, Heidelberg, Germany) and stored at –20 °C. The compounds were diluted to the desired concentration using a modified medium so that the DMSO final concentration is less than 0.5%.

In the case of chemosensitization experiment: Five different concentrations of compound **23** (40, 20, 10, 5, 2.5 μM) were tested together with six different concentrations of cisplatin (50, 25, 12.5, 6.25, 3.125, 1.56 μM). The best chemosensitization effect was observed using 20 μM of compound **23** for 4 h and applied in cell cycle, apoptosis, and ROS assays.

4.2.3. Cytotoxicity assay

Liver cancer cells (HepG2) were treated with the new organoselenium pseudo peptides to screen for the most active compound. Cells

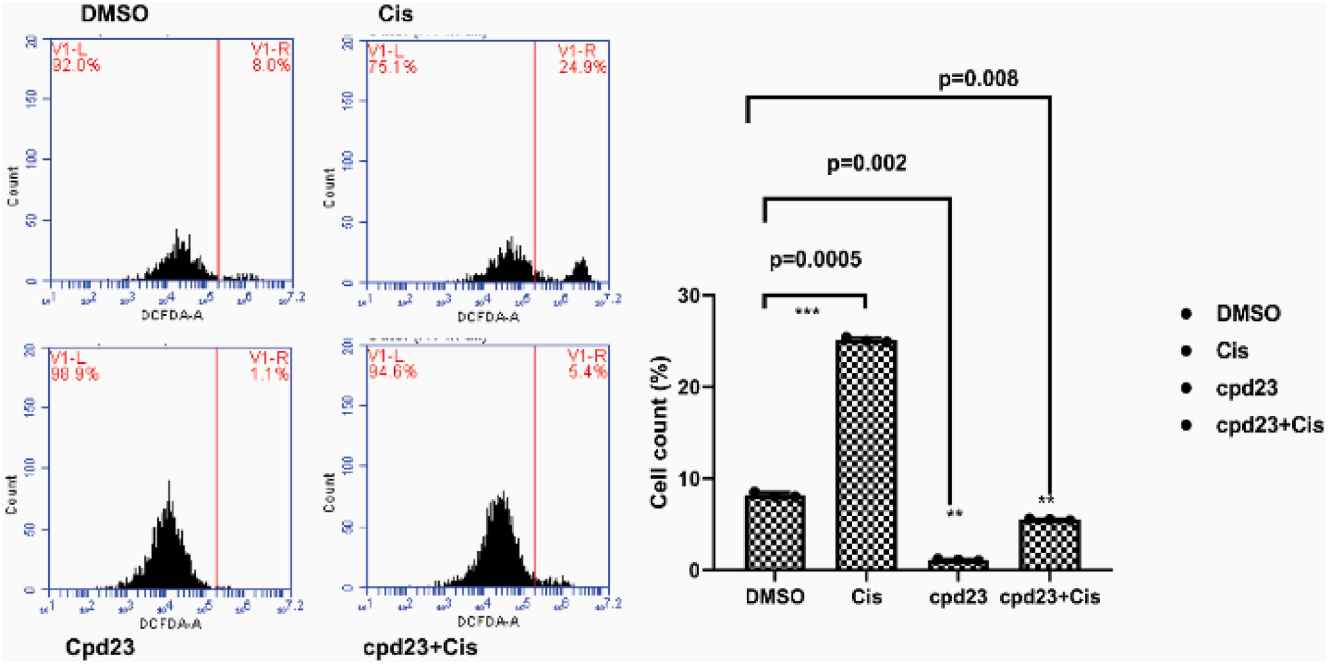


Fig. 7. Estimation of the intracellular ROS levels was estimated using H_2-DCFDA by flow cytometry. Pretreatment of HepG2 cells with compound **23** decreased the ROS levels. The cells were treated either with 0.6 μM cisplatin alone, 20 μM of compound **23** alone or treated with 20 μM of compound **23** for four h then treated with 0.6 μM cisplatin for a total of 48 h. ROS level was evaluated using DCFDA as an indicator for oxidative stress by flow cytometry, the experiment was repeated 3X, and statistical significance was calculated by One-way ANOVA test by Prism software.

were seeded in a 96-well plate ($3*10^4$ cells/mL, 100 μL/well). After overnight incubation at 37 °C and 5% CO_2, the cells were incubated with 50 μM (final concentration in the assay) of the tested compounds. DMSO (0.5% V/V) was used as a negative control while cisplatin (50 μM) was used as a positive control. After 48 h of 37 °C and 5% CO_2 incubation, MTT (5 mg/mL PBS) was added and the plate was incubated for 4 h [76]. Afterward, acidified sodium dodecyl sulfate solution (10% SDS in 1x PBS/0.01 N HCl) was used to solubilize the produced formazan crystals. The absorbance was measured at $\lambda_{570\text{-}630}$ nm by Biotek plate reader (Gen5™) after fourteen h of incubation at 37 °C and 5% CO_2

Based on the initial screening assay results, a serial dilution of the most cytotoxic organoselenium pseudo peptides was used for the evaluation of its IC_{50}. The HepG2 cells were seeded as above then were treated with different concentrations (50, 25, 12.5, 6.25, 3.125, and 1.56 μM) of the most active compound. After 48 h of incubation, the viability of the cells was determined by using MTT assay as described above. IC50 of compounds were calculated by GraphPad Prism 8 software.

4.2.4. Chemo-sensitization activity

The cells were seeded as mentioned above, and on the next day, the cells were initially pretreated with 20 or 40 μM of the non-toxic compounds (the concentration that showed 100% cell viability, data not shown) for 4 h then treated with a serial dilution of cisplatin (50, 25, 12.5, 6.25, 3.125, and 1.56 μM) for another 44 h. After incubation at 37 °C and 5% CO_2, the viability was determined by MTT reagent. The viability was calculated by GraphPad Prism 8 software. The compound that should chemo-sensitizing activity was further used in the study where different concentrations and time points of preincubation were tested [77,80,81].

4.2.5. Cell cycle analysis

Cells were treated either with 0.6 μM cisplatin alone or treated with 20 μM compound **23** for 4 h then treated with 0.6 μM cisplatin for a total of 48 h. Then the cells were fixed by using ethanol (ice-cold) for two h at −20 °C. Cells were then washed with PBS and stained with PI (20 μg/mL, 500 μL) containing RNase (1 mg/mL) for 30 min [78]. The cells were analyzed by Accuri C6 Flow cytometer within one hour.

4.2.6. Apoptosis analysis

Cells were treated either with 0.6 μM cisplatin alone or treated with 20 μM compound **23** for 4 h then treated with 0.6 μM cisplatin for total 48 h. The cells were combined after trypsinization and washed two times with 1x PBS. The induction of apoptosis was analyzed by staining cells with PE-Annexin-V [79] in presence of propidium iodide (BD Pharmingen™, Cat. No. 556547) and analyzed by Accuri C6 Flow cytometer. The apoptotic index was calculated as the percentage of Annexin V-positive and PI-negative cells (early apoptosis) divided by the total number of cells in the gated region (viable cells).

4.2.7. Reactive oxygen species determination

Cells were treated either with 0.6 μM cisplatin alone or treated with 20 μM compound **23** for 4 h then treated with 0.6 μM cisplatin for total 48 h. After that, the cells were collected, washed then stained with 20 μM DCFDA (Abcam®, ab113851) and incubated for 30 min at 37 °C. The stained cells were analyzed by Accuri C6 Flow cytometer.

4.3. Synthesis and characterization

4.3.1. Preparation of acetic formic anhydride [50]

In a dry, three-necked flask, 1.88 mol (133 mL) acetyl chloride is added to 2.2 mol (150 g) sodium formate in 125 mL of diethyl ether (anhydrous) while the temperature is maintained at 0 °C. The mixture is then allowed to stir for 6 h at 26 °C and filtered. The residue solid is washed twice with ether (2 X 50 mL). The ether organic solvent is removed by under low pressure, and the resulted acetic formic anhydride residue is further used without any purification.

General procedure I: Preparation of isocyanide derivatives 5 & 6 (Scheme 1)

Freshly prepared acetic formic anhydride (1.2 mmol) was added dropwise at room temperature to a solution of selenoamine (1 mmol) in THF solution (10 mL). The reaction progress was monitored with TLC,

and after the reaction completion, the corresponding *N*-formyl derivatives obtained quantitatively was used without any purification.

$POCl_3$ (1.2 mmol) in THF (5 mL) was added carefully, drop-by-drop, to a solution of *N*-formyl derivatives (1 mmol) and Et_3N (3 mmol) in THF (5 mL) at 00°C. The reaction progress was monitored with TLC, and after the reaction completion, a saturated Na_2CO_3 aqueous cold solution was added, and the THF layer was washed with brine (2 X 50 mL). At 0°C, the THF layer was removed under low pressure and purified by flash "column" chromatography (petroleum ether/ ethyl acetate = 8/1).

Isocyano-4-selenocyanatobenzene ***(5)***[2]

Compound 5 synthesized following procedure I from 4-selenocyanatoaniline 1 (198 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 10:1, $R_f = 0.35$, purified employing "flash" chromatography using petrol ether: ethyl acetate = 8:1. White solid; Yield: 191 mg (92%). ^{1}H NMR (300 MHz, DMSO) δ 7.89–7.82 (dd, J = 7.8, 1.3 Hz, 2H, Ar-H), 7.69–7.62 (dd, J = 7.8, 1.3 Hz, 2H, Ar-H); ^{13}C NMR (75 MHz, DMSO) δ 166.15, 134.43, 128.22, 127.06, 105.37. MS (ESI): m/z = found 182.23 [M^+−CN], 324.12 [M^++3K]; calcd. 207.98 [M^+].

1,2-bis(4-isocyanophenyl)diselane ***(6)***

Compound 6 synthesized following procedure I from 4,4′-diselanediyldianiline 2 (342 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 10:1, R_f = 0.33, purified employing "flash" chromatography using petrol ether: ethyl acetate = 8:1. Yellow solid; Yield: 342 mg (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56–7.46 (dd, J = 7.7, 1.4 Hz, 4H, Ar-H), 7.23–7.14 (dd, J = 7.7, 1.4 Hz, 4H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.68, 132.02, 132.19, 131.84, 127.14. MS (ESI): m/z = found 365.90 [M^++2H]; calcd 363.90 [M^+]; HRMS calcd. for $C_{14}H_8N_2Se_2$ [M^+]: 363.90179, found 366.16024 [M^++2H]. Anal. Calcd for $C_{14}H_8N_2Se_2$ (363.90): C, 46.43; H, 2.23; N, 7.74. Found: C, 46.40; H, 2.20; N, 7.71.

General procedure II: Synthesis of functionalized pseudopeptide organoselenium derivatives (7–9 & 17–19) via Passerini reaction (Scheme 2 and 3)

Isonitrile (1.1 mmol) was added to a mixture of acid component (1 mmol), and aldehyde (1 mmol) in CH_2Cl_2 (1 mL), and the mixture was allowed to stir for 12 h at room temperature. Water was added and the organic layer was separated, dried with Sodium sulfate, and evaporated under low pressure. The residue obtained was purified by column "flash" chromatography using petroleum ether/ ethyl acetate = 5/3.

3-Methyl-1-oxo-1-((4-selenocyanatophenyl)amino)butan-2-yl acetate ***(7)***

Compound 7 synthesized following procedure II from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), acetic acid (63 μL, 1 mmol), and isopropyl aldehyde (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.30, purified employing "flash" chromatography using petrol ether: ethyl acetate = 4:3. White solid; Yield: 342 mg (86%). IR (KBr) ν = 506 (Se-C), 1590 (C═O), 1695 (C═O), 2222 (CN), 3565 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (s, 1H, N—H), 7.54–7.36 (m, 4H, Ar-H), 4.91 (d, J = 5.2 Hz, 1H, CH-O), 2.27–2.15 (m, 1H, CH), 2.12 (s, 3H, H_3CC═O), 0.98–0.87 (m, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.53, 168.33, 139.15, 134.38, 121.75, 115.69, 102.30, 78.55, 30.76, 20.88, 18.64, 17.34. MS (ESI): m/z = found 340.87 [M^+]; calcd 340.03 [M^+]; HRMS calcd. for $C_{14}H_{16}N_2O_3Se$ [M^+]: 340.03261, found 340.03156 [M^+]. Anal. Calcd for $C_{14}H_{16}N_2O_3Se$ (calcd 340.03): C, 49.57; H, 4.75; N, 8.26. Found: C, 49.54; H, 4.74; N, 8.28.

2-oxo-2-((4-selenocyanatophenyl)amino)ethyl acetate ***(8)***

Compound 8 synthesized following procedure II from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), acetic acid (63 μL, 1 mmol), and paraformaldehyde (30 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.31, purified employing "flash" chromatography using petrol ether: ethyl acetate = 4:3. White solid; Yield: 133 mg (45%). IR (KBr) ν = 490 (Se-C), 1587 (C═O), 1684 (C═O), 2238 (CN), 3665 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H, N—H), 7.52 (m, 4H, Ar-H), 4.61 (s, 2H, CH_2), 2.15 (s, 3H, H_3CC═O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.72, 165.68, 159.43, 138.85, 134.48, 119.76, 116.17, 102.03, 63.14, 20.76. MS (ESI): m/z = found 297.02 [M^+−H]; calcd 297.98 [M^+]; HRMS calcd. for $C_{11}H_{10}N_2O_3Se$ [M^+]: 297.98566, found 297.98491 [M^+]. Anal. Calcd for $C_{11}H_{10}N_2O_3Se$ (calcd 297.98): C, 44.46; H, 3.39; N, 9.43. Found: C, 44.48; H, 3.41; N, 9.46.

1-(4-nitrophenyl)-2-oxo-2-((4-selenocyanatophenyl)amino)ethyl acetate ***(9)***

Compound 9 synthesized following procedure II from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), acetic acid (63 μL, 1 mmol), and *p*-nitrobenzaldehyde (151 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.29, purified employing "flash" chromatography using petrol ether: ethyl acetate = 4:3. White solid; Yield: 163 mg (39%). IR (KBr) ν = 504 (Se-C), 1610 (C═O), 1730 (C═O), 2245 (CN), 3681 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 1H, N—H), 8.19–8.12 (m, 2H, Ar-H), 7.65–7.60 (m, 2H, Ar-H), 7.53 (m, 2H, Ar-H), 7.51 (m, 2H, Ar-H), 6.17 (s, 1H), 2.23 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.05, 165.60, 159.15, 141.64, 138.64, 134.39, 128.23, 124.01, 121.71, 101.85, 74.66, 20.97. MS (ESI): m/z = found 419.79 [M^+−H]; calcd 419.00 [M^+]; HRMS calcd. for $C_{17}H_{13}N_3O_5Se$ [M^+]: 419.00204, found 419.00149 [M^+]. Anal. Calcd for $C_{17}H_{13}N_3O_5Se$ (calcd 419.00): C, 48.82; H, 3.13; N, 10.05. Found: C, 48.80; H, 3.10; N, 10.03.

((diselanediylbis(4,1-phenylene))bis(azanediyl))bis(3-methyl-1-oxobutane-1,2-diyl) diacetate ***(17)***

Compound 17 synthesized following procedure II from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), acetic acid (63 μL, 1 mmol), and isopropyl aldehyde (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.27, purified employing "flash" chromatography using petrol ether: ethyl acetate = 4:3. Yellow solid; Yield: 528 mg (84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 2H, 2 N—H), 7.41–7.25 (m, 8H, Ar-H), 4.96 (d, J = 5.2 Hz, 2H, 2CH), 2.21 (ddd, J = 14.2, 7.1, 5.8 Hz, 2H, 2CH), 2.10 (s, 6H, $2H_3CC$═O), 0.91 (d, J = 6.8 Hz, 12H, $4CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.30, 167.95, 137.13, 133.47, 126.39, 120.92, 78.56, 60.47, 30.77, 20.98, 18.67, 17.33, 14.20. MS (ESI): m/z = found 628.97 [M^+]; calcd 628.06 [M^+]; HRMS calcd. for $C_{26}H_{32}N_2O_6Se_2$ [M^+]: 628.05908, found 628.05876 [M^+]. Anal. Calcd for $C_{26}H_{32}N_2O_6Se_2$ (calcd 628.06): C, 49.85; H, 5.15; N, 4.47. Found: C, 49.83; H, 5.12; N, 4.45.

((diselanediylbis(4,1-phenylene))bis(azanediyl))bis(2-oxoethane-2,1-diyl) diacetate ***(18)***

Compound 18 synthesized following procedure II from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), acetic acid (63 μL, 1 mmol), and paraformaldehyde (60 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.27, purified employing "flash" chromatography using petrol ether: ethyl acetate = 4:3. Yellow solid; Yield: 479 mg (88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (s, 2H, 2 N—H), 7.54–7.44 (m, 4H, Ar-H), 7.42–7.33 (m, 4H, Ar-H), 4.61 (s, 4H, $2CH_2$), 2.15 (s, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.32, 165.08, 136.82, 133.65, 126.68, 120.75, 63.30, 20.78. MS (ESI): m/z = found 544.87 [M^+]; calcd 543.96 [M^+]; HRMS calcd. for $C_{20}H_{20}N_2O_6Se_2$ [M^+]: 543.96518, found 544.96363 [M^++H]. Anal. Calcd for $C_{20}H_{20}N_2O_6Se_2$ (calcd 543.96): C, 44.30; H, 3.72; N, 5.17. Found: C, 44.32; H, 3.75; N, 5.20.

((diselanediylbis(4,1-phenylene))bis(azanediyl))bis(1-(4-nitrophenyl)-2-oxoethane-2,1-diyl) diacetate ***(19)***

Compound 19 synthesized following procedure II from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), acetic acid (63 μL, 1 mmol), and para nitrobenzaldehyde (151 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.25, purified employing "flash" chromatography using petrol ether: ethyl acetate = 4:3. Yellow solid; Yield: 637 mg (81%). ^{1}H NMR (300 MHz,

[2] Isocyano-4-selenocyanatobenzene (5) is not stable and must be prepared directly before usage and therefore our trials to obtain HRMS was not possible.

$CDCl_3$) δ 8.60 (s, 2H, 2 N—H), 7.97 (d, J = 8.8 Hz, 4H, Ar-H), 7.53 (d, J = 8.9 Hz, 4H, Ar-H), 7.24 (d, J = 8.6 Hz, 4H, Ar-H), 7.15 (d, J = 8.8 Hz, 4H, Ar-H), 6.08 (s, 2H, 2CH), 2.13 (s, 6H, $2H_3CC$═O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.54, 165.97, 148.07, 141.84, 136.59, 132.95, 128.24, 126.98, 123.88, 121.15, 74.71, 20.88. MS (ESI): m/z = found 803.61 [M^++H_2O]; calcd 785.99 [M^+]; HRMS calcd. for $C_{32}H_{26}N_4O_{10}Se_2$ [M^+]: 785.99794, found 787.09301 [M^++H]. Anal. Calcd for $C_{32}H_{26}N_4O_{10}Se_2$ (calcd 785.99): C, 48.99; H, 3.34; N, 7.14. Found: C, 48.97; H, 3.32; N, 7.11.

General procedure III: Synthesis of functionalized peptidomimetic organoselenium derivatives (10–12 & 20–23) via Ugi reaction (Scheme 2 and 3)

Isonitrile (1.1 mmol) was added to a mixture of acid components (1 mmol), aldehyde (1 mmol), and amine (1 mmol) in CH_3OH (1 mL), and the mixture was allowed to stir for 12 h at room temperature. Water was added and extracted with 3x10 mL dichloromethane. The organic layer was separated, dried with anhydrous Sodium sulfate, and evaporated under low pressure. The residue obtained was purified by column "flash" chromatography using petroleum ether/ ethyl acetate = 5/3.

2-(N-(4-methoxyphenyl)acetamido)-3-methyl-N-(4-selenocyanatophenyl)butanamide ***(10)***

Compound 10 synthesized following procedure III from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), acetic acid (63 μL, 1 mmol), *p*-anisidine (123 mg, 1 mmol), and isopropyl aldehyde (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.36, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 396 mg (89%). IR (KBr) ν = 494 (Se-C), 1592 (C═O), 1744 (C═O), 2256 (CN), 3623 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.46 (s, 1H, N—H), 7.62–7.51 (m, 4H, Ar-H), 7.08–7.00 (m, 2H, Ar-H), 6.86–6.78 (m, 2H, Ar-H), 4.26–4.12 (m, 1H, CH), 3.74 (s, 3H, H_3CO), 2.50–2.26 (m, 1H, CH), 1.86 (s, 3H, H_3CC═O), 1.01 (d, J = 6.5 Hz, 3H, H_3C), 0.93 (d, J = 6.6 Hz, 3H, H_3C); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.77, 169.31, 159.46, 140.24, 134.74, 129.29, 121.32, 114.94, 101.65, 55.50, 26.71, 23.74, 20.13, 19.71. MS (ESI): m/z = found 445.91 [M^+]; calcd 445.09 [M^+]; HRMS calcd. for $C_{21}H_{23}N_3O_3Se$ [M^+]: 445.09046, found 446.09742 [M^+]. Anal. Calcd for $C_{21}H_{23}N_3O_3Se$ (calcd 445.09): C, 56.76; H, 5.22; N, 9.46. Found: C, 56.78; H, 5.25; N, 9.49.

2-(N-(4-methoxyphenyl)acetamido)-4-methyl-N-(4-selenocyanatophenyl)pentanamide ***(11)***

Compound 11 synthesized following procedure III from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), acetic acid (63 μLl, 1 mmol), *p*-anisidine (123 mg, 1 mmol), and isovaleraldehyde (110 μl, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.29, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 367 mg (80%). IR (KBr) ν = 504 (Se-C), 1654 (C═O), 1739 (C═O), 2243 (CN), 3601 (NH) cm^{-1}; ^{1}H NMR (300 MHz, DMSO) δ 10.37 (s, 1H, N—H), 7.80–7.64 (m, 4H, Ar-H), 7.33 (d, J = 8.5 Hz, 2H, Ar-H), 6.98 (dd, J = 7.8, 1.3 Hz, 2H, Ar-H), 5.21 (t, J = 7.2 Hz, 1H, CH), 3.79 (s, 3H, H_3CO), 1.75 (s, 3H, H_3CC═O), 1.59–1.43 (m, 2H, CH_2), 1.41–1.25 (m, 1H, CH), 0.87 (dd, J = 12.2, 6.2 Hz, 6H, $2CH_3$); ^{13}C NMR (75 MHz, DMSO) δ 170.84, 159.17, 140.79, 135.12, 132.60, 131.55, 121.45, 116.96, 114.57, 105.57, 57.95, 55.72, 24.89, 23.52, 23.00. MS (ESI): m/z = found 481.87 [M^++Na]; calcd 459.10 [M^+]; HRMS calcd. for $C_{22}H_{25}N_3O_3Se$ [M^+]: 459.10611, found 482.09533 [M^++H + Na]. Anal. Calcd for $C_{22}H_{25}N_3O_3Se$ (calcd 459.10): C, 57.64; H, 5.50; N, 9.17. Found: C, 57.62; H, 5.53; N, 9.20.

3-methyl-2-(N-phenylacetamido)-N-(4-selenocyanatophenyl)butanamide ***(12)***

Compound 12 synthesized following procedure III from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), acetic acid (63 μL, 1 mmol), aniline (91 μL, 1 mmol), and isopropyl aldehyde (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.29, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 367 mg (80%). IR (KBr) ν = 502 (Se-C), 1644 (C═O), 1733 (C═O), 2239 (CN), 3591 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.68 (s, 1H, N—H), 7.62–7.48 (m, 4H, Ar-H), 7.38–7.28 (m, 3H, Ar-H), 7.17 (ddd, J = 5.5, 2.8, 1.2 Hz, 2H, Ar-H), 4.26 (d, J = 11.2 Hz, 1H, CH), 2.41 (ddq, J = 19.5, 13.0, 6.6 Hz, 1H, CH), 1.83 (s, 3H, H_3CC═O), 1.01 (d, J = 6.5 Hz, 3H, CH_3), 0.92 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.21, 169.33, 141.63, 140.27, 134.67, 129.77, 128.73, 128.38, 121.36, 115.06, 101.76, 72.11, 27.00, 23.87, 20.23, 19.74. MS (ESI): m/z = found 415.88 [M^+]; calcd 415.07 [M^+]; HRMS calcd. for $C_{20}H_{21}N_3O_2Se$ [M^+]: 415.08, found 438.06710 [M^++Na]. Anal. Calcd for $C_{20}H_{21}N_3O_2Se$ (calcd 415.08): C, 57.97; H, 5.11; N, 10.14. Found: C, 57.99; H, 5.13; N, 10.16.

N,N′-(diselanediylbis(4,1-phenylene))bis(3-methyl-2-(N-phenylacetamido)butanamide) ***(20)***

Compound 20 synthesized following procedure III from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), acetic acid (63 μL, 1 mmol), aniline (46 μL, 1 mmol), and isopropyl aldehyde (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.33, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:2. Yellow solid; Yield: 638 mg (82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.40 (s, 2H, 2 N—H), 7.53–7.41 (m, 8H, Ar-H), 7.37–7.27 (m, 6H, Ar-H), 7.20–7.12 (m, 4H, Ar-H), 4.21 (d, J = 11.2 Hz, 2H, 2CH), 2.44 (tt, J = 12.8, 6.6 Hz, 2H, 2CH), 1.82 (s, 6H, $2H_3CC$═O), 1.00 (d, J = 6.5 Hz, 6H, $2CH_3$), 0.93 (d, J = 6.6 Hz, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.09, 168.98, 141.76, 138.48, 133.79, 129.72, 128.59, 128.33, 125.75, 120.41, 72.34, 26.92, 23.87, 20.19, 19.80. MS (ESI): m/z = found 779.17 [M^++H]; calcd 778.15 [M^+]; HRMS calcd. for $C_{38}H_{42}N_4O_4Se_2$ [M^+]: 778.15365, found 801.13910 [M^++Na]. Anal. Calcd for $C_{38}H_{42}N_4O_4Se_2$ (calcd 778.15): C, 58.76; H, 5.45; N, 7.21. Found: C, 58.74; H, 5.43; N, 7.22.

N,N′-(diselanediylbis(4,1-phenylene))bis(2-(N-benzylacetamido)-3-methylbutanamide) ***(21)***

Compound 21 synthesized following procedure III from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), acetic acid (63 μL, 1 mmol), benzylamine (108 μL, 1 mmol), and isopropyl aldehyde (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.34, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:2. Yellow solid; Yield: 645 mg (80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.29 (s, 2H, 2 N—H), 7.42–7.38 (m, 3H, Ar-H), 7.29–7.24 (m, 5H, Ar-H), 7.13–7.02 (m, 10*H*, Ar-H), 4.74–4.61 (d, J = 17.1 Hz, 2H, 2CH), 4.52 (d, J = 17.1 Hz, 4H, $2CH_2$), 2.48 (tt, J = 12.9, 6.3 Hz, 2H, 2CH), 2.03 (s, 6H, $2H_3CC$═O), 0.91 (d, J = 6.5 Hz, 6H, $2CH_3$), 0.81 (d, J = 6.6 Hz, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.56, 168.53, 138.29, 137.51, 136.76, 133.57, 128.81, 127.46, 126.25, 125.82, 120.91, 120.60, 66.80, 50.40, 26.95, 22.71, 19.82, 19.08. MS (ESI): m/z = found 807.24 [M^++H]; calcd 806.18 [M^+]; HRMS calcd. for $C_{40}H_{46}N_4O_4Se_2$ [M^+]: 806.18495, found 829.17511 [M^++Na]. Anal. Calcd for $C_{40}H_{46}N_4O_4Se_2$ (calcd 806.18): C, 59.70; H, 5.76; N, 6.96. Found: C, 59.68; H, 5.74; N, 6.93.

N,N′-(diselanediylbis(4,1-phenylene))bis(2-(N-(4-methoxyphenyl)acetamido)-3-methylbutanamide) ***(22)***

Compound 22 synthesized following procedure III from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), acetic acid (63 μL, 1 mmol), *p*-anisidine (123 mg, 1 mmol), and isopropyl aldehyde (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.30, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:2. Yellow solid; Yield: 511 mg (61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54–7.32 (m, 8H, Ar-H), 7.09–7.02 (m, 4H, Ar-H), 6.87–6.76 (m, 4H, Ar-H), 4.22 (d, J = 11.7 Hz, 2H, 2CH), 3.74 (s, 6H, $2H_3CO$), 2.36 (dd, J = 11.3, 5.7 Hz, 2H, 2CH), 1.81 (s, 6H, 2 H_3CC═O), 0.99 (d, J = 6.5 Hz, 6H, $2CH_3$), 0.93 (d, J = 6.6 Hz, 6H, $2CH_3$).^{13}C NMR (75 MHz, $CDCl_3$) δ 173.54, 168.96, 159.38, 138.45, 137.70, 133.86, 129.38, 125.85, 120.49, 114.76, 55.49, 26.69, 23.73, 20.13, 19.75. MS (ESI): m/z = found 837.25 [M^+−H]; calcd 838.17 [M^+]; HRMS calcd. for $C_{40}H_{46}N_4O_6Se_2$ [M^+]: 838.17478, found 838.17561 [M^+]. Anal. Calcd for $C_{40}H_{46}N_4O_6Se_2$ (calcd 838.17): C, 57.42; H, 5.54; N, 6.70. Found: C, 57.39; H, 5.51; N, 6.68.

N,N′-(diselanediylbis(4,1-phenylene))bis(2-(N-benzylacetamido)acetamide) ***(23)***

Compound 23 synthesized following procedure III from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), acetic acid (63 μL, 1 mmol), benzylamine (108 μL, 1 mmol), and paraformaldehyde (30 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 2:1, R_f = 0.20, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:2. Yellow solid; Yield: 570 mg (79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (s, 2H, 2 N—H), 7.36–7.29 (m, 4H, Ar-H), 7.26–7.18 (m, 10*H*, Ar-H), 7.09 (d, J = 6.6 Hz, 4H, Ar-H), 4.56 (d, J = 16.5 Hz, 4H, $2CH_2$), 3.95 (d, J = 23.6 Hz, 4H, $2CH_2$), 2.11 (s, 6H, $2H_3CC$═O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.63, 167.28, 138.06, 135.58, 133.53, 129.03, 128.24, 127.92, 126.74, 125.72, 120.65, 120.33, 53.74, 50.96, 21.47. MS (ESI): m/z = found 745.08 [M^++Na]; calcd 722.09 [M^+]; HRMS calcd. for $C_{34}H_{34}N_4O_4Se_2$ [M^+]: 722.09105, found 723.09540 [M^++H]. Anal. Calcd for $C_{34}H_{34}N_4O_4Se_2$ (calcd 722.09): C, 56.67; H, 4.76; N, 7.78. Found: C, 56.65; H, 4.74; N, 7.76.

General procedure IV: Synthesis of functionalized organoselenium-based tetrazoles (13–16 & 24–27) via azido-Ugi reaction (Scheme 2 and 3)

Isonitrile (1.1 mmol) was added to a mixture of $TMSN_3$ (1.1 mmol), aldehyde (1 mmol), and amine (1 mmol) in CH_3OH (1 mL), and the mixture was allowed to stir for 12 h at room temperature. Water was added and extracted with 3x10 mL dichloromethane. The organic layer was separated, dried with anhydrous Sodium sulfate, and evaporated under low pressure. The residue obtained was purified by column "flash" chromatography using petroleum ether/ ethyl acetate = 5/3.

N-(2-methyl-1-(1-(4-selenocyanatophenyl)-1H-tetrazol-5-yl)propyl)aniline ***(13)***

Compound 13 synthesized following procedure IV from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isopropyl aldehyde (91 μL, 1 mmol), and aniline (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.25, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 334 mg (84%). IR (KBr) ν = 489 (Se-C), 1660 (C═C), 1633 (C═N), 2239 (CN), 3586 (NH) cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.72–7.60 (m, 2H, Ar-H), 7.25–7.12 (m, 2H, Ar-H), 6.99 (dd, J = 8.3, 7.5 Hz, 2H, Ar-H), 6.63 (t, J = 7.3 Hz, 1H, Ar-H), 6.36 (d, J = 7.7 Hz, 2H, Ar-H), 4.52 – (s, 1H, N—H), 4.01 (q, J = 7.1 Hz, 1H, CH), 2.26–2.04 (m, 1H, CH), 0.98 (d, J = 6.6 Hz, 3H, CH_3), 0.83 (d, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.81, 145.99, 134.72, 133.18, 129.50, 127.66, 125.66, 119.25, 114.06, 100.61, 55.32, 33.28, 19.40. MS (ESI): m/z = found 398.94 [M^+]; calcd 398.07 [M^+]; HRMS calcd. for $C_{18}H_{18}N_6Se$ [M^+]: 398.07582, found 398.08613 [M^+]. Anal. Calcd for $C_{18}H_{18}N_6Se$ (calcd 398.07): C, 54.41; H, 4.57; N, 21.15. Found: C, 54.43; H, 4.60; N, 21.17.

4-methoxy-N-(2-methyl-1-(1-(4-selenocyanatophenyl)-1H-tetrazol-5-yl)propyl)aniline ***(14)***

Compound 14 synthesized following procedure IV from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isopropyl aldehyde (91 μL, 1 mmol), and *p*-anisidine (123 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.20, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 385 mg (90%). IR (KBr) ν = 505 (Se-C), 1656 (C═C), 1623 (C═N), 2221 (CN), 3614 (NH) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.65 (m, 2H, Ar-H), 7.18–7.09 (m, 2H, Ar-H), 6.66–6.58 (m, 2H, Ar-H), 6.42–6.32 (m, 2H, Ar-H), 4.28–4.18 (d, J = 6.7 Hz, 1H, N—H), 3.68–3.63 (s, 3H, H_3CO), 2.27–2.07 (m, 1H, CH), 1.24–1.15 (m, 1H, CH), 1.08–0.99 (d, J = 6.4 Hz, 3H, CH_3), 0.83 (d, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.03, 153.83, 139.78, 134.80, 133.08, 127.67, 125.43, 117.01, 114.92, 100.16, 57.51, 55.68, 33.35, 19.42. MS (ESI): m/z = found 429.29 [M^++H]; calcd 428.08 [M^+]; HRMS calcd. for $C_{19}H_{20}N_6OSe$ [M^+]: 428.08638, found 429.08873 [M^++H]. Anal. Calcd for $C_{19}H_{20}N_6OSe$ (calcd 428.08): C, 53.40; H, 4.72; N, 19.66. Found: C, 53.38; H, 4.69; N, 19.64.

N-(3-methyl-1-(1-(4-selenocyanatophenyl)-1H-tetrazol-5-yl)butyl)aniline ***(15)***

Compound 15 synthesized following procedure IV from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isovaleraldehyde (110 μL, 1 mmol), and aniline (46 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.20, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 222 mg (54%). IR (KBr) ν = 506 (Se-C), 1651 (C═C), 1633 (C═N), 2251 (CN), 3650 (NH) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72–7.62 (m, 2H, Ar-H), 7.30–7.24 (m, 1H, Ar-H), 7.19–7.15 (m, 1H, Ar-H), 7.09–6.94 (m, 2H, Ar-H), 6.71–6.59 (m, 1H, Ar-H), 6.40–6.29 (m, 2H, Ar-H), 4.90 (s, 1H, N—H), 3.90–3.73 (m, 1H, CH), 1.88–1.69 (m, 2H, CH_2), 1.64–1.50 (m, 1H, CH), 0.80 (ddd, J = 29.1, 15.1, 7.1 Hz, 6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.11, 145.45, 136.88, 134.90, 134.30, 133.63, 133.20, 131.91, 129.55, 127.36, 126.37, 125.49, 119.32, 113.79, 47.42, 43.78, 24.92, 22.32. MS (ESI): m/z = found 457.12 [M^++2Na]; calcd 412.09 [M^+]; HRMS calcd. for $C_{19}H_{20}N_6Se$ [M^+]: 412.09147, found 412.10317 [M^++H]. Anal. Calcd for $C_{19}H_{20}N_6Se$ (calcd 412.09): C, 55.48; H, 4.90; N, 20.43. Found: C, 55.50; H, 4.92; N, 20.45.

4-methoxy-N-(3-methyl-1-(1-(4-selenocyanatophenyl)-1H-tetrazol-5-yl)butyl)aniline ***(16)***

Compound 16 synthesized following procedure IV from isocyano-4-selenocyanatobenzene (5) (229 mg, 1.1 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isovaleraldehyde (110 μL, 1 mmol), and *p*-anisidine (123 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.20, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 155 mg (35%). IR (KBr) ν = 497 (Se—C), 1643 (C═C), 1596 (C═N), 2249 (CN), 3648 (NH) cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (t, J = 10.3 Hz, 2H, Ar-H), 7.27 (d, J = 8.6 Hz, 2H, Ar-H), 6.68–6.58 (m, 2H, Ar-H), 6.38–6.29 (m, 2H, Ar-H), 4.68 (d, J = 7.3 Hz, 1H, N—H), 4.05 (s, CH_3O), 1.87–1.72 (m, 2H, CH_2), 1.63–1.54 (m, 1H, CH), 1.25–1.13 (m, 1H, CH), 0.85–0.74 (dd, J = 6.6, 3.9 Hz, 6H, $2CH_3$).^{13}C NMR (151 MHz, $CDCl_3$) δ 157.29, 153.70, 139.23, 134.94, 133.05, 127.32, 125.34, 116.19, 115.03, 100.10, 55.66, 49.04, 43.76, 24.95, 22.32. MS (ESI): m/z = found 443.02 [M^++H]; calcd 442.10 [M^+]; HRMS calcd. for $C_{20}H_{22}N_6OSe$ [M^+]: 442.10203, found 442.100982 [M^+]. Anal. Calcd for $C_{20}H_{22}N_6OSe$ (calcd 442.10): C, 54.42; H, 5.02; N, 19.04. Found: C, 54.39; H, 5.00; N, 19.01.

N,N′-(((diselanediylbis(4,1-phenylene))bis(1H-tetrazole-1,5-diyl))bis(2-methylpropane-1,1-diyl))dianiline ***(24)***

Compound 24 synthesized following procedure IV from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isopropyl aldehyde (91 μL, 1 mmol), and aniline (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.23, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 640 mg (86%).^{1}H NMR (250 MHz, $CDCl_3$) δ 7.72–7.60 (m, 4H, Ar-H), 7.25–7.12 (m, 4H, Ar-H), 6.99 (dd, J = 8.3, 7.5 Hz, 4H, Ar-H), 6.63 (t, J = 7.3 Hz, 2H, Ar-H), 6.36 (d, J = 7.7 Hz, 4H, Ar-H), 4.52 (s, 2H, 2 N—H), 4.01 (q, J = 7.1 Hz, 2H, 2CH), 2.26–2.04 (m, 2H, 2CH), 0.98 (t, J = 6.6 Hz, 6H, $2CH_3$), 0.83 (d, J = 6.7 Hz, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.67, 145.99, 133.66, 133.22, 131.79, 129.43, 126.71, 119.26, 114.19, 55.18, 33.42, 19.38. MS (ESI): m/z = found 745.16 [M^++H]; calcd 744.14 [M^+]; HRMS calcd. for $C_{34}H_{36}N_{10}Se_2$ [M^+]: 744.14548, found 783.10836 [M^++K]. Anal. Calcd for $C_{34}H_{36}N_{10}Se_2$ (calcd 744.14): C, 54.99; H, 4.89; N, 18.86. Found: C, 54.97; H, 4.86; N, 18.83.

N,N′-(((diselanediylbis(4,1-phenylene))bis(1H-tetrazole-1,5-diyl))bis(2-methylpropane-1,1-diyl))bis(4-methoxyaniline) ***(25)***

Compound 25 synthesized following procedure IV from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isopropyl aldehyde (91 μL, 1 mmol), and *p*-anisidine (123 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.20, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 699 mg (87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, J =

8.5 Hz, 4H, Ar-H), 6.57–6.46 (m, 4H, Ar-H), 6.39–6.24 (m, 4H, Ar-H), 4.24 (d, J = 7.9 Hz, 2H), 3.82 (s, 2H, 2 N—H), 3.56 (s, 6H, H_3CO), 2.22–2.03 (m, 2H, 2CH), 0.96 (d, J = 6.7 Hz, 6H, $2CH_3$), 0.78 (d, J = 6.7 Hz, 6H, $2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.97, 153.41, 140.01, 133.54, 133.10, 131.70, 126.66, 116.59, 114.82, 56.92, 55.63, 33.24, 19.43. MS (ESI): m/z = found 805.24 [M^++H]; calcd 804.16661 [M^+]; HRMS calcd. for $C_{36}H_{40}N_{10}O_2Se_2$ [M^+]: 804.16661, found 843.12783 [M^++K]. Anal. Calcd for $C_{36}H_{40}N_{10}O_2Se_2$ (calcd 804.16): C, 53.87; H, 5.02; N, 17.45. Found: C, 53.85; H, 5.00; N, 17.42.

N,N′-(((diselanediylbis(4,1-phenylene))bis(1H-tetrazole-1,5-diyl))bis (3-methylbutane-1,1-diyl))dianiline ***(26)***

Compound 26 synthesized following procedure IV from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isovaleraldehyde (110 μL, 1 mmol)., and aniline (91 μL, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.20, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. White solid; Yield: 679 mg (88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.58 (m, 4H, Ar-H), 7.20–7.10 (m, 4H, Ar-H), 6.93 (dd, J = 8.4, 7.5 Hz, 4H, Ar-H), 6.57 (t, J = 7.4 Hz, 2H, Ar-H), 6.36–6.26 (m, 4H, Ar-H), 4.78 (d, J = 6.9 Hz, 2H, 2 N—H), 4.00 (dt, J = 14.3, 6.3 Hz, 2H, 2CH), 1.87–1.63 (m, 4H, $2CH_2$), 1.61–1.45 (m, 2H, 2CH), 0.74 (dd, J = 8.7, 6.6 Hz, 12H, $4CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.16, 145.68, 133.62, 133.25, 131.90, 129.46, 126.39, 119.02, 113.71, 60.41, 47.26, 43.72, 24.89, 22.48, 22.19, 21.11, 14.27. MS (ESI): m/z = found 773.22 [M^++H]; calcd 772.17 [M^+]; HRMS calcd. for $C_{36}H_{40}N_{10}Se_2$ [M^+]: 772.17678, found 774.04152 [M^++2H]. Anal. Calcd for $C_{36}H_{40}N_{10}Se_2$ (calcd 772.17): C, 56.10; H, 5.23; N, 18.17. Found: C, 56.13; H, 5.25; N, 18.19.

N,N′-(((diselanediylbis(4,1-phenylene))bis(1H-tetrazole-1,5-diyl))bis (3-methylbutane-1,1-diyl))bis(4-methoxyaniline) ***(27)***

Compound 27 synthesized following procedure IV from 1,2-bis(4-isocyanophenyl)diselane (6) (200 mg, 0.55 mmol), $TMSN_3$ (145 μL, 1.1 mmol), isovaleraldehyde (110 μL, 1 mmol), and *p*-anisidine (123 mg, 1 mmol). TLC was used to monitor its formation petrol ether: ethyl acetate = 3:1, R_f = 0.20, purified employing "flash" chromatography using petrol ether: ethyl acetate = 5:3. Yellow solid; Yield: 723 mg (25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.61 (m, 4H, Ar-H), 7.18–7.08 (m, 4H, Ar-H), 6.61–6.52 (m, 4H, Ar-H), 6.37–6.25 (m, 4H, Ar-H), 4.66 (t, J = 7.3 Hz, 2H, 2CH), 3.61 (s, 6H, $2H_3CO$), 1.88–1.65 (m, 4H, $2CH_2$), 1.55 (td, J = 13.2, 6.6 Hz, 2H, 2CH), 0.77 (dd, J = 6.6, 3.9 Hz, 12H, $4CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.28, 153.43, 139.47, 133.40, 131.86, 126.34, 116.05, 114.93, 55.64, 48.77, 43.82, 24.90, 22.33. MS (ESI): m/z = found 833.29 [M^++H]; calcd 832.19 [M^+]; HRMS calcd. for $C_{38}H_{44}N_{10}O_2Se_2$ [M^+]: 832.19791, found 833.05157 [M^++H]. Anal. Calcd for $C_{38}H_{44}N_{10}O_2Se_2$ (calcd 832.19): C, 54.94; H, 5.34; N, 16.86. Found: C, 54.96; H, 5.37; N, 16.88.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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