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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Heterocycles 23: Synthesis, characterization and anticancer activity of new hydrazinoselenazole derivatives

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Abstract A series of novel functionalized 1,3-selenazole was synthesized by Hantzsch-type condensation reaction and evaluated for their in vitro cytotoxic activity against two human cancer cell lines. The structures of the synthesized selenosemicarbazones and functionalized 2-hydrazinyl-1,3-selenazole derivatives were assigned based on IR and NMR spectroscopic investigations, mass spectrometry, and elemental analysis data. Some of the synthesized compounds showed significant cytotoxic activities, the IC_{50} values below or around 10 μM were recorded with compounds **1d** and **4b** on both androgen-insensitive prostate cancer cells (DU-145) and hepatocarcinoma (Hep-G2) cell lines.

Keywords Selenazole · Hantzsch reaction · Cytotoxicity · Prostate cancer · Hepatocarcinoma

Introduction

Selenium used to be considered as an essential nutrient, constituent of selenoproteins involved in self-defense mechanism against oxidative stress (Wessjohann *et al*., 2007; Naithani, 2008; Lopez *et al*., 2009), in reducing certain inflammatory processes and in detoxification processes (Akbaraly *et al*., 2005), but on the other hand, selenium-accumulating plants grown in seleniferous soil (Pire *et al*., 2007) were identified as causing livestock poisoning. Based on the benefits associated to the presence of selenium, the synthesis of many organoselenium derivatives characterized by a large variety of biological activities was developed. The synthesis of heterocyclic systems is of potential importance in the field of medicinal chemistry because most of the compounds with biological properties are derived from heterocyclic structures.

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Similarly, heterocyclic compounds containing selenium have received a great deal of attention due to the wide variety of pharmacological activities reported for these compounds, including anticancer (Srivastava and Robins, 1983), antibacterial (Goldstein *et al*., 1990), and antiviral (Nguyen *et al*., 2009) properties. A number of recent studies have indicated that 1,3-selenazole derivatives inhibit the synthesis of nitric acid (Ueda *et al*., 2005) and they are antagonists for histamine H_2 receptors (van der Goot *et al*., 1994). In addition, it has also been shown that 2-dialkyl-amino-1,3-selenazole derivatives described by Kantlehner *et al*., 1996 were used as intermediates in the synthesis of some dyes (Keil and Hartmann, 2000). *N*-acyl-derivatives of 2-amino-4-(isothiocyanatomethyl)-1,3-selenazole have been reported to possess antitumor activity (Kumar *et al*., 1993). Recently, Nam *et al*., 2008 reported the inhibitory activity of 2-piperidin- and 4-phenyl-2-piperidin-1,3-selenazole upon anion-superoxidase. The literature survey regarding the biological effects of selenazole derivatives, the similarity between the chemical properties of selenium and sulfur equally balanced by our experience regarding the chemical synthesis and evaluation of antimicrobial and antitumoral effects of hydrazino-thiazole derivatives (Renson, 1956; Boik, 2001), encouraged us to synthesize a new series of hydrazino-1,3-selenazole derivatives and to investigate their biological properties. The 1,3-selenazole derivatives described below (Schemes 1, 2, 3, 4) were obtained by Hantzsch-type cyclization reaction between selenosemicarbazide precursors (selenosemicarbazones or aroyl-selenosemicarbazide derivatives respectively) and α-halogeno-carbonyl derivatives. The anticancer effects of selenazoles in prostate DU-145 and liver cancers have not been widely studied. Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to help bringing this disease under control. Prompted by the foregoing observations, coupled with our interest in the synthesis of heterocyclic compounds, we have reported below the synthesis of new hydrazinoselenazole derivatives and the results of our investigations regarding their cytotoxic activities.

Results and discussion

Chemical synthesis

The synthesis of the new selenazoles described in this work used two precursors. The first precursors are the selenosemicarbozones **1a–c**, obtained by the condensation of selenosemicarbazide with (hetero) aromatic carbaldehydes (Scheme 1).

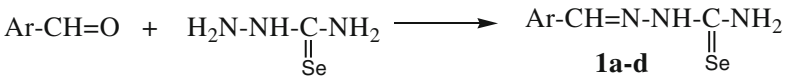
The second precursors are the aroyl-selenosemicarbazide derivatives **4a–b**, are prepared by acylation of selenosemicarbazide (Scheme 3). The Hantzsch-type condensation between the selenosemicarbazide derivatives with α-halogenocarbonyl derivatives resulted to a new aryliden-hydrazinoselenazoles **2a–h** (Scheme 2) and aroyl-hydrazinoselenazoles **5a–d** (Scheme 4).

For the synthesis of selenosemicarbazones **1a–d**, a series of aromatic carbaldehydes, such as benzaldehyde, *p*-chloro-, and *p*-methoxy-benzaldehyde, as well as a heteroaromatic carbaldehyde (2-phenyl-thiazole-4-carbaldehyde), was used (Scheme 1). The new thiazolyl-selenosemicarbazone **1d** was obtained in satisfactory yields (55 %) by a procedure previously applied for the preparation of **1a–c** (Renson, 1956).

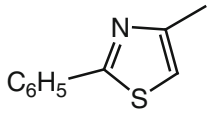
The selenosemicarbazones **1a–d** were further subjected to condensation using different α-halogenocarbonyl derivatives such as monochloroacetone, 1,3-dichloroacetone, α-bromoacetophenone, 3-chloro-acetylacetone, and α-bromoacetylacetic ester, in DMF/acetone solution. A series of new hydrazinoselenazoles **2a–h** were thus obtained in satisfactory yields (51–59 %) after 24-h reaction time at room temperature. The ^{1}H-NMR data indicate highly deshielded protons in the NH groups characterized by chemical shifts range 9.9–11.8 ppm for both semicarbazones **1a–d** and selenazoles **2a–h**. Acetylation of the selenazoles **2a–h** was readily accomplished using acetic anhydride in the presence of pyridine (Scheme 2), being obtained **3a–h** compounds, with yields between 46 and 68 %. The IR spectra of the stable crystalline acetyl derivatives **3a–h** display the typical absorption bands.

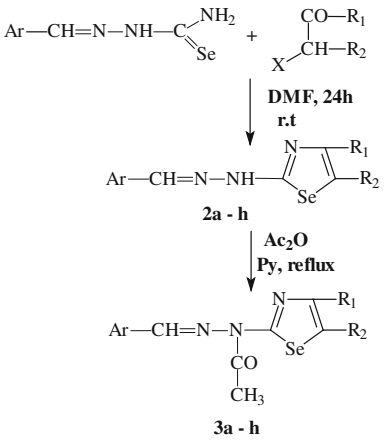
The aroyl-selenosemicarbazides **4a–b** were obtained in good yields (58 and 65 %, respectively) by the reaction of

Scheme 1 Synthetic route to selenosemicarbazones **1a–d**



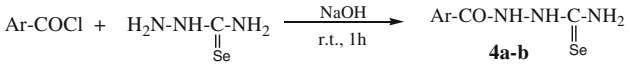
	1a	1b	1c	1d
Ar	C_6H_5-	*p*Cl-C_6H_4-	*p*CH_3O-C_6H_4-	C_6H_5-Th





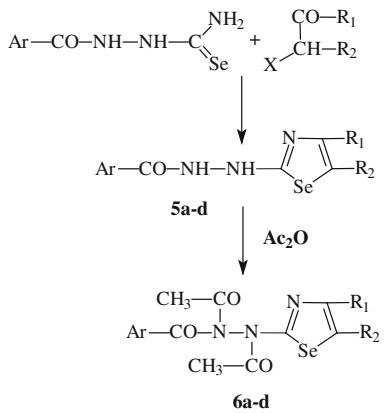
	2a, 3a	2b, 3b	2c, 3c	2d,3d	2e,3e	2f,3f	2g,3g	2h,3h
Ar	$C_6H_4Cl(p)$	$C_6H_4Cl(p)$	$C_6H_4Cl(p)$	$C_6H_4Cl(p)$	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	C_6H_5-Th	C_6H_5-Th
R_1	CH_3	CH_2Cl	C_6H_5	CH_3	CH_3	C_6H_5	CH_2Cl	CH_3
R_2	H	H	H	$COOC_2H_5$	H	H	H	$COCH_3$

Scheme 2 Hantzsch type condensation to hydrazinoselenazoles and their acetylation



	4a	4b
Ar	C_6H_5	C_6H_5Cl (*p*)

Scheme 3 Synthesis of aroyl-selenosemicarbazides



	5a,6a	5b,6b	5c,6c	5d,6d
Ar	C_6H_5	C_6H_5	C_6H_5Cl (*p*)	C_6H_5Cl (*p*)
R_1	CH_3	C_6H_5	CH_3	C_6H_5
R_2	H	H	H	H

Scheme 4 Hantzsch type condensation to aroyl-hydrazino-selenazoles **5a–d** and their acetylation

acid chlorides (benzoylchloride and *p*-chlorobenzoylchloride, respectively) with selenosemicarbazide (Scheme 3).

Aroyl-hydrazino-selenazoles **5a–d** were successfully obtained by the condensation of **4a–b** with monochloroacetone and α-bromoacetophenone, respectively, in the presence of aqueous NaOH (Scheme 4).

During the acetylation of **5a–d** with acetic anhydride, a double acylation of the hydrazine group occured, leading to diacetyl derivatives **6a–d** (Scheme 4) in satisfactory yields.

The selenohydrazone **1d**, the hydrazino-selenazoles **2a–h**, aroyl-selenosemicarbazide **4a–b** aroyl-hydrazino-selenazoles **5a–d**, and their acyl derivatives (**3a–h**, **6a–d**) were reported here for the first time as obtained via total synthesis based on the described protocols. The structures of the synthesized compounds were fully assigned based on spectroscopic data (IR, ^{1}H-, and ^{13}C-NMR), mass spectrometry, and elemental analysis (cf. "Experimental. Chemistry" section).

Biology

The synthetized compounds were tested for their cytotoxicity against two human cancer cell lines and the results are summarized in Table 1. In the US NCI plant screening program, a sample is generally considered to have in vitro cytotoxic activity if the IC_{50} value following incubation between 48 and 72 h is less than 4 μg/ml for pure compounds (Boik, 2001). This cutoff point for good cytotoxic compound has also been defined as 10 μM (Brahemi *et al.*, 2010). In the present work, some of the synthesized compounds showed significant cytotoxic activities, the IC_{50} values below or around 10 μM were recorded with compounds **1d** and **4b** on both androgen-insensitive prostate cancer cells (DU-145) and hepatocarcinoma (Hep-G2) cell lines. Compounds **1b** also exhibited significant cytotoxicity against DU-145. The activity of the most active compound, **1c** (IC_{50}: 6.14 and 9.18 μM respectively against DU-145 and Hep-G2) can be considered as important with regard to

Table 1 Activity of the studied compounds and doxorubicin on DU-145 and HepG-2 cancer cell lines

Compounds	Cell lines and IC_{50} values in μg/ml and in μM (in bracket)			
	DU-145		Hep-G2	
1a	9.22	(40.77)	8.84	(39.09)
1b	**2.25**	**(8.63)**	**3.38**	(12.97)
1c	7.76	(30.29)	**2.9**	(11.32)
1d	**1.9**	**(6.14)**	**2.84**	**(9.18)**
2a	14.74	(49.36)	16.86	(56.46)
2b	>25	(>75.06)	>25	(>75.06)
2c	19.54	(54.17)	15.33	(42.50)
2d	17.68	(47.69)	19.56	(52.77)
2e	>25	(>84.97)	24.49	(83.24)
2f	18.15	(50.94)	17.98	(50.47)
2g	6.25	(16.37)	7.16	(18.76)
2h	19.42	(49.88)	23.87	(61.31)
3a	20.63	(60.56)	20.18	(59.24)
3b	24.28	(64.73)	>25	(>66.65)
3c	>25	(>62.07)	>25	(>62.07)
3d	>25	(>60.57)	22.76	(55.15)
3e	>25	(>74.35)	>25	(>74.35)
3f	>25	(>62.76)	>25	(>62.76)
3g	8.31	(19.61)	8.17	(19.28)
3h	13.51	(31.32)	12.66	(29.35)
4a	8.5	(35.11)	8.43	(34.82)
4b	**2.22**	**(7.59)**	**3.12**	**(10.66)**
5a	>25	(>89.23)	>25	(>89.23)
5b	>25	(>73.05)	>25	(>73.05)
5c	>25	(>79.46)	>25	(>79.46)
5d	24.51	(65.07)	23.69	(62.89)
6a	>25	(>68.63)	>25	(>68.63)
6b	>25	(>58.64)	>25	(>58.64)
6c	>25	(>62.70)	>25	(>62.70)
6d	>25	(>54.26)	>25	(>54.26)
Doxorubicin	1.78	(3.27)	2.66	(4.89)

Bold values indicate best activities

that of the reference anticancer drug, doxorubicin (IC_{50}: 3.27 and 4.89 μM, respectively, against DU-145 and Hep-G2), as the compound was less than twofold less active. This compound can therefore be considered as potential cytotoxic drug and deserves further investigations on normals and other cancer cell lines as well as additional pharmacological studies, and complementary assays on normal cell lines.

When exploring the structure–activity relationship of the studied compounds, three main points can be highlighted. First, it appears that the presence of selenium is important for cytotoxic activity. However, it was found that when selenium atom is free in the structure, the activity was better than when it was included in an heterocycle. For example, only but all compounds of the series **1** (**1a–d**) and **4** (**4a** and **4b**) exhibited IC_{50} below 10 μg/ml. These compounds were generally the most active samples. Second, within the compounds of series **1** and **4**, those containing a chloride atom in position **4** of the benzene cycle **1b** (IC_{50}: 8.63 and 12.97 μM on DU-145 and Hep-G2, respectively) and **4b** (IC_{50}: 7.59 and 10.66 μM on DU-145 and Hep-G2, respectively) were among the most active. Finally, the presence of thiazolic and chloride substituents simultaneously in the structure of the compounds seemed to improve their activities as observed with compounds **2g** (IC_{50}: 16.37 and 18.76 μM on DU-145 and Hep-G2, respectively) and **3g** (IC_{50}: 19.61 and 19.28 μM on DU-145 and Hep-G2, respectively).

Conclusions

In conclusion, new hydrazinoselenazole derivatives were synthesized and their structure were determined and also assayed for their in vitro cytotoxic activity against two human cancer cell lines. This synthesis offers significant improvements over existing procedures and thus helps to facilitate the entry into a synthesis of a variety of selenazole compounds of potentially high synthetic and biological utilities. Also, this technique affords through one-pot reaction a various selenazoles derivatives with excellent yields and without formation of undesirable side products. The studies on the hydrazinoselenazoles and their derivatives as anticancer compounds are currently under investigation in our laboratory.

Experimental

Chemistry

Melting points were determined using an Electro thermal IA 9200 digital melting point apparatus and are uncorrected. Elemental analyses were performed using a Vario EL III instrument. The mass spectra were run on a Varian MAT-311A instrument and using a Bruker Esquire 3000 ESI-ion trap-mass spectrometer. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer fitted with a Golden Gate ATR accessory. NMR spectra were recorded on a WM-400 MHz and 300 MHz Bruker NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ, ppm).

*General procedure for the synthesis of aryliden-selenosemicarbazides (**1a–d**)*

2 mmol (0.27 g) selenosemicarbazide was dissolved in 5 ml glacial acetic acid and 2 ml ethanol. 2 mmol of aromatic carbaldehyde dissolved in 5 ml ethanol was added and the reaction mixture was refluxed for 4 h. The hot reaction mixture was filtered, the solution was cooled, and the precipitate thus obtained was collected by filtration. The purification of the product was performed by recrystallization from DMF/acetic acid mixture in volumetric ratio 1:2.

*Benzyliden-selenosemicarbazide (**1a**)* Yellow crystals, yield = 63 %, m.p. = 169–170 °C [m.p. = 167–168 °C lit. (Renson, 1956)], IR(cm^{-1}): 3372, 3263 (*v*NH); 3024, 2921 (*v*CH); 1643 (*v*C=N); 1600 (*v*C=$C_{aromatic}$). MS (*m/z*): 229/227/225 (M^+); 145; 119; 103; 104, 77(100 %); 51; 43; 28. ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.45 (t, 1H, C_6H_5-*para*, J = 7.3 Hz), 7.54 (t, 2H, C_6H_5-*meta*, J = 7.3 Hz), 7.77 (d, 2H, C_6H_5-*ortho*, J = 7.3 Hz), 7.85 (s, 1H, CH=N), 9.87 (s, 1H, NH), 10.16 (s, 2H, NH_2). ^{13}C-NMR (100.5 MHz, $CDCl_3$, δ ppm): 126.1, 126.4, 128.1, 129.4, 129.7, 135.7, 143.7, 163.2. Anal. Calcd. for $C_8H_9N_3Se$: C, 24.49 %; H, 4.01 %; N, 18.58 %. Found: C, 41.89 %; H, 4.03 %; N, 18.50 %.

*p-Chloro-benzyliden-selenosemicarbazide (**1b**)* Yellow crystals, yield = 61.23 %, m.p. = 220–221 °C [m.p. = 219–220 °C lit. (Renson, 1956)], IR(cm^{-1}): 3417, 3265 (*v*NH); 3154, 2981 (*v*CH); 1602 (*v*C=N); 1560, 1525 (*v*C=$C_{aromatic}$). MS (*m/z*): 263/261/259 (M^+); 220; 179; 153; 140; 138(100 %); 111; 89; 77; 75; 28. ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.39 (d, 2H, C_6H_5-*meta*, J = 8.5 Hz), 7.77 (d, 2H, C_6H_5-*ortho*, J = 8.5 Hz), 7.88 (s, 1H, CH=N), 9.92 (s, 1H, NH), 10.18 (s, 2H, NH_2). ^{13}C-NMR (100.5 MHz, $CDCl_3$, δ ppm): 127.7, 128.0, 128.9, 129.4, 130.4, 135.7, 145.7, 167.2. Anal. Calcd. for $C_8H_8ClN_3Se$: C, 36.87 %; H, 3.09 %; N, 16.13 %. Found: C, 36.45 %; H, 3.10 %; N,16.05 %.

*p-Methoxy-benzyliden-selenosemicarbazide (**1c**)* White crystals, yield = 62 %, m.p. = 183–184 °C [m.p. = 183–184 °C lit. (Renson, 1956)], IR (cm^{-1}): 3363, 3259 (*v*NH); 3123, 2973, (*v*CH); 1605 (*v*C=N); 1511 (*v*C=$C_{aromatic}$). MS (*m/z*): 255/257/259 (M^+); 175; 140; 134(100 %); 108; 92; 77; 64; 39. ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 3.82 (s, 3H, OCH_3), 7.02 (d, 2H, C_6H_5-*meta*, J = 8.6 Hz), 7.68 (d, 2H, C_6H_5-*ortho*, J = 8.6 Hz), 7.76 (s, 1H, CH=N), 9.95 (s, 1H, NH), 10.16 (s, 2H, NH_2). ^{13}C-NMR (100.5 MHz, $CDCl_3$, δ ppm): 55.4, 113.9, 114.0, 127.9, 128.4, 128.9, 146.1, 161.6, 167.2. Anal. Calcd. for $C_9H_{11}N_3OSe$: C, 24.20 %; H, 4.33 %; N, 16.40 %. Found: C, 23.99 %; H, 4.20 %; N, 16.35 %.

*2-Phenyl-1,3-thiazolo-5-methyliden-selenosemicarbazide (**1d**)* White crystals, yield = 55 %, m.p. = 189–190 °C, IR (cm^{-1}): 3359, 3265 (*v*NH); 3139 (*v*CH); 1620 (*v*C=N); 1529 (*v*C=$C_{aromatic}$). MS (*m/z*): 308/310/312 (M^+); 229; 189; 161; 121(100 %); 104; 83; 77; 71; 57; 43. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.49 (t, 2H,C_6H_5-*meta*, J = 8.6 Hz), 7.55 (t, 1H, C_6H_5-*para*, J = 8.6 Hz), 7.94 (d, 2H, C_6H_5-*ortho*, J = 8.6 Hz), 8.29 (s, 1H, Th–CH), 8.37 (s, 1H, CH=N), 11.84 (s, 1H, NH), 13.02 (s, 2H, NH_2). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 120.1, 126.3, 126.5, 128.7, 129.4, 129.5, 136.1, 143.1, 152.4, 159.2, 164.3. Anal. Calcd. for: $C_{11}H_{10}N_4SSe$: C, 42.72 %; H, 3.26 %; N, 18.12 %; S, 10.37 %. Found: C, 42.63 %; H, 3.20 %; N, 18.10 %; S, 10.30 %.

*General procedure for the preparation of aryliden-hydrazino-selenazole (**2a–h**)*

2 mmol selenosemicarbazone was dissolved in 5 ml DMF and 5 ml anhydrous acetone. 2 mmol of α-halogenocarbonyl derivative was added. The reaction mixture was stirred at room temperature for 24 h and then neutralized with $NaHCO_3$. The precipitate thus obtained was filtered and then recrystallized from ethanol.

*4-Methyl-2-[(4-chloro-benzyliden)-hydrazinyl]-1,3-selenazole (**2a**)* Pink crystals, yield = 52 %, m.p. = 193–195 °C, IR (cm^{-1}): 3179 (*v*NH); 2977, 2862 (*v*CH); 1630 (*v*C=N); 1575 (*v*C=$C_{aromatic}$). MS (*m/z*): 297/299/301 (M^+); 218; 188; 162(100 %); 160; 138; 120; 119; 111; 89; 75; 39; ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.41 (s, 3H, CH_3), 7.15 (s, 1H, CH–Se), 7.63 (d, 2H, C_6H_4-*meta*, J = 8.7 Hz), 7.89 (d, 2H, C_6H_4-*ortho*, J = 8.7 Hz), 8.02 (s, 1H, CH=N), 10.12 (s, 1H, NH). ^{13}C-NMR (75.5 MHz, $CDCl_3$, δ ppm): 17.5, 116.2, 127.2, 127.6, 128.3, 128.7, 133.0, 137.1, 139.8, 151.9, 173.5. Anal. Calcd. for $C_{11}H_{10}N_3ClSe$: C, 44.24 %; H, 3.38 %; N, 14.07 %. Found: C, 44.02 %; H, 3.33 %; N, 14.05 %.

*4-Chloromethyl-2-[(4-chloro-benzyliden)-hydrazinyl]-1,3-selenazole (**2b**)* White crystals, yield = 51 %, m.p. = 210–211 °C, IR (cm^{-1}): 3147 (*v*NH); 2960, 2862, 2786 (*v*CH); 1597 (*v*C=N); 1575 (*v*C=$C_{aromatic}$). MS (*m/z*):331/333/335 (M^+); 298; 222; 196(100 %); 161; 138; 111; 89; 75; 50; 39; 36; 28. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 4.68 (s, 2H, CH_2Cl), 7.72 (s, 1H, CH–Se), 7.45 (d, 2H, C_6H_4-*meta*, J = 8.7 Hz), 7.58 (d, 2H, C_6H_4-*ortho*, J = 8.7 Hz), 8.12 (s, 1H, CH=N) 10.16 (s, 1H, NH). ^{13}C-NMR (75.5 MHz, $CDCl_3$, δ ppm): 40.3, 118.2, 127.3, 127.6, 128.3, 128.7, 133.0, 137.1, 139.7, 151.1, 170.6. Anal. Calcd. for $C_{11}H_9N_3Cl_2Se$: C, 39.67 %; H, 2.72 %; N, 12.62 %. Found: C, 39.55 %; H, 2.69 %; N, 12.39 %.

*4-Phenyl-2-[(4-chloro-benzyliden)-hydrazinyl]-1,3-selenazole (**2c**)* White crystals, yield = 56 %, m.p. = 184–185 °C,

IR (cm^{-1}): 3140 (*v*NH); 3063, 2949 (*v*CH); 1595 (*v*C=N); 1570 (*v*C=$C_{aromatic}$). MS (*m/z*): 359/363/361(M^+); 359; 224; 182(100 %); 138; 111; 102; 89; 77; 51; 39; 28. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 7.35 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.48 (t, 2H, C_6H_5-*meta*, $J = 7.3$ Hz), 7.56 (d, 2H, C_6H_5-*ortho*, $J = 7.3$ Hz), 7.43 (d, 2H, C_6H_4-*meta*, $J = 8.4$ Hz), 7.91 (d, 2H, C_6H_5-*ortho*, $J = 8.4$ Hz), 7.85 (s, 1H, CH–Se), 8.08 (s, 1H, CH=N), 10.14 (s, 1H, *NH*). ^{13}C-NMR (75.5 MHz, $CDCl_3$, δ ppm): 113.6, 126.1, 126.2, 127.2, 127.3, 128.3, 128.5, 128.7, 128.8, 129.4, 133.0, 135.0, 137.1, 140.2, 149.7, 177.5. Anal. Calcd. For $C_{16}H_{12}N_3ClSe$: C, 53.28 %; H, 3.35 %; N, 11.65 %. Found: C, 53.19 %; H, 3.26 %; N, 11.49 %.

5-Ethoxycarbonyl-4-methyl-2-[(4-chloro-benzyliden)-hydrazinyl]-1,3-selenazole (***2d***) Brown crystals yield = 59 %, m.p. = 197–198 °C, IR (cm^{-1}): 3184 (*v*NH); 2980, 2862 (*v*CH); 1742 (*v*C=O ester); 1621 (*v*C=N); 1596 (*v*C=$C_{aromatic}$). MS (*m/z*): 369/371/373 (M^+); 326; 260; 234; 188; 160; 111; 89; 67; 42; 29(100 %). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 1.23 (t, 3H, CH_3, $J = 7.0$ Hz), 2.37 (s, 3H, CH_3), 4.14 (q, 2H, CH_2, $J = 7.0$ Hz), 7.47 (d, 2H, C_6H_4-*meta*, $J = 8.4$ Hz), 7.71 (d, 2H, C_6H_4-*ortho*, $J = 8.4$ Hz), 8.21 (s, 1H, CH=N), 10.52 (s, 1H, NH). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 14.7, 16.8, 60.7, 128.7, 129.0, 129.3, 129.5, 133.7, 134.8, 137.2, 138.7, 157.4, 163.4, 174.8. Anal. Calcd. for $C_{14}H_{14}ClN_3O_2Se$: C, 45.36 %; H, 3.81 %; N, 11.34 %. Found: C, 45.29 %; H, 3.36 %; N, 11.28 %.

4-Methyl-[2-(4-methoxy-benzyliden)-hydrazinyl]-1,3-selenazole (***2e***) Brown crystals, yield = 56 %, m.p. = 155–157 °C. IR (cm^{-1}): 3112 (*v*NH); 2924 (*v*CH); 1627 (*v*C=N) 1596 (*v*C=$C_{aromatic}$). MS (*m/z*): 293/295/297 (M^+); 162(100 %); 160; 134; 120; 92; 77; 64; 39. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.36 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.06 (d, 2H, C_6H_4-*meta*, $J = 8.8$ Hz), 7.12 (s, 1H, CH–Se), 7.82 (d, 2H, C_6H_4-*ortho*, $J = 8.8$ Hz), 8.37 (s, 1H, CH=N), 10.42 (s, 1H, *NH*). ^{13}C-NMR (75.5 MHz, DMSO-d_6, δ ppm): 18.9, 55.3, 114.6, 114.8, 115.2, 124.9, 128.1, 128.2, 138.4, 151.9, 161.8, 173.5. Anal. Calcd. for $C_{12}H_{13}N_3O_2Se$: C, 48.99 %; H, 4.45 %; N, 14.28 %. Found: C, 48.79 %; H, 4.41 %; N, 14.21 %.

4-Phenyl-[2-(4-metoxybenzyliden)-hydrazinyl]-1,3-selenazole (***2f***) White crystals, yield = 53 %, m.p. = 169–170 °C, IR (cm^{-1}): 3289 (*v*NH); 2965 (*v*CH); 1603 (*v*C=N); 1570 (*v*C=$C_{aromatic}$). MS (*m/z*):353/357/359 (M^+); 224; 182 (100 %); 179; 134; 109; 92; 77; 64; 51; 39. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 3.77 (s, 3H, OCH_3,), 6.98 (d, 2H, C_6H_4-*meta*, $J = 8.6$ Hz), 7.26 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.36 (t, 2H, C_6H_5-*meta*, $J = 7.3$ Hz), 7.58 (d, 2H, C_6H_5-*ortho*, $J = 8.6$ Hz), 7.81 (d, 2H, C_6H_4-*ortho*, $J = 7.3$ Hz), 7.65 (s, 1H, CH–Se), 8.01 (s, 1H, CH=N), 12.11 (s, 1H, NH). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 55.7, 114.4, 114.9, 115.3, 125.8, 126.6, 127.4, 127.9, 128.2, 128.5, 129.0, 129.1, 129.7, 160.8, 169.2, 171.5. Anal. Calcd. For $C_{17}H_{15}N_3OSe$ C, 57.31 %; H, 4.24 %; N, 11.79 %. Found: C, 57.29 %; H, 4.18 %; N, 11.46 %.

4-Chloromethyl-[2-(2-phenyl-1,3-thiazolo-4-methylidene)-hydrazinyl]-1,3-selenazole (***2g***) White crystals yield = 52 %, m.p. = 175–176 °C, IR (cm^{-1}): 3289 (*v*NH); 2965 (*v*CH); 1603 (*v*C=N); 1570 (*v*C=$C_{aromatic}$). MS (*m/z*): 381/383/384 (M^+); 224; 182(100 %); 179; 134; 109; 92; 77; 64; 51; 39. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 4.78 (s, 2H, CH_2Cl), 7.42 (s, 1H, CH–Se), 7.31 (s, 1H, CH–Th), 7.78 (t, 1H, C_6H_5-*para*, $J = 7.6$ Hz), 7.85 (t, 2H, C_6H_5-*meta*, $J = 7.6$ Hz), 7.98 (d, 2H, C_6H_4-*ortho*, $J = 7.6$ Hz), 8.07 (s, 1H, CH=N), 11.59 (s, 1H, NH). ^{13}C-NMR (75.5 MHz, DMSO-d_6, δ ppm): 40.3, 116.3, 118.9, 125.0, 126.6, 126.7, 128.7, 128.8, 129.9, 134.3, 149.6, 151.1, 167.0, 171.4. Anal. Calcd. for $C_{14}H_{11}ClN_4SSe$: C, 44.05 %; H, 2.90 %; N, 14.68 %; S, 8.40 %. Found: C, 44.02 %; H, 2.89 %; N, 14.63 %; S, 8.36 %.

4-Methyl-5-acetyl-[2-(2-phenyl-1,3-thiazolo-4-methylidene) -hydrazinyl]-1,3-selenazole (***2h***) White crystals, yield = 57 %, m.p. = 169–170 °C, IR (cm^{-1}): 3279 (*v*NH); 2935 (*v*CH); 1605 (*v*C=N); 1570 (*v*C=$C_{aromatic}$). MS (*m/z*): 388/390/391 (M^+); 224; 182(100 %); 179; 134; 109; 92; 77; 64; 51; 39. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.05 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.49–7.51 (m, 3H, Ar–H), 7.94 (d, 2H, Ar–H; $J = 7.6$ Hz), 7.99 (s, 1H, CH–Th), 8.22 (s, 1H, CH=N), 8.33 (s, 1H, NH). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 18.8, 24.5, 118.7, 126.4, 126.8, 127.0, 129.5, 129.9, 131.1, 133.2, 149.9, 152.8, 158.0, 160.0, 164.8, 167.7. Anal. Calcd. for $C_{16}H_{14}N_4O$ SSe: C, 49.36 %; H, 3.62 %; N, 14.39 %; S, 8.23 %. Found: C, 49.32 % H, 3.57 % N, 14.35 %; S, 8.20 %.

General procedure for the preparation of 2-(N_1-acetyl-N_2-aryliden-hydrazino)-1,3-selenazoles (***3a–h***)

2 mmol aryliden-hydrazinoselenazole (**2a**–**h**) was treated with 2 ml acetic anhydride and 2 drops of pyridine. The reaction mixture was refluxed for 5 min and then poured in water. The precipitate was filtered and purified by recrystallization from ethanol.

4-Methyl-2-[(4-chloro-benzyliden)-N-acetyl-hydrazinyl]-1, 3-selenazole (***3a***) White crystals, yield = 53 %, m.p. = 80–81 °C, IR (cm^{-1}): 2978 (*v*CH); 1685 (*v*C=O amide); 1629 (*v*C=N). MS (*m/z*): 339/341/343 (M^+); 299; 188; 162; 138; 111; 89; 75; 43(100 %); 39; 28. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.23 (s, 3H, CH_3), 2.63 (s, 3H, CH_3),

7.45 (d, 2H, C_6H_4-*meta*, $J = 8.7$ Hz), 7.75 (d, 2H, C_6H_4-*ortho*, $J = 8.7$ Hz), 8.23 (s, 1H, CH–Se), 9.61 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 17.2, 22.15, 110.3, 126.9, 127.2, 128.5, 128.6, 133.3, 135.1, 145.8, 152.1, 154.6, 171.2. Anal. Calcd. for $C_{13}H_{12}N_3ClOSe$: C, 45.83 %; H, 3.55 %; N, 12.33 %. Found: C, 45.76 %; H, 3.52 %; N, 12.23 %.

*4-Chloromethyl-2-[(4-chlorobenziliden)-N-acetyl-hydrazinyl]-1,3-selenazole (**3b**)* White crystals, yield = 52 %, m.p. = 132–133 °C, IR (cm^{-1}): 3041 (νCH); 1682 (νC=O amide); 1628 (νC=N). MS (*m/z*): 373/375 (M^+); 333; 196; 161; 124; 89; 43(100 %); 28. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.18 (s, 3H, CH_3), 4.63 (s, 2H, CH_2Cl), 7.45 (d, 2H, C_6H_4-*meta*, $J = 8.7$ Hz), 7.75 (d, 2H, C_6H_4-*ortho*, $J = 8.7$ Hz), 8.29 (s, 1H, CH–Se), 9.42 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 22.6, 40.3, 118.6, 127.3, 127.3, 128.4, 128.7, 133.1, 137.2, 144.3, 151.1, 154.3, 172.2. Anal. Calcd. for $C_{13}H_{11}N_3ClOSe$: C, 41.62 %; H, 2.96 %; N, 11.20 %;. Found: C, 41.60 %; H, 2.93 %; N, 11.05 %.

*4-Phenyl-2-[(4-chlorobenzyliden)-N-acetyl-hydrazinyl]-1,3-selenazole (**3c**)* White-yellowish crystals, yield = 68 %, m.p. = 162–163 °C, IR (cm^{-1}): 3072 (νCH); 1701 (νC=O amide); 1630 (νC=N). MS (*m/z*): 401/403/405 (M^+); 361; 266; 224; 182; 137; 102; 89; 77; 51; 43(100 %). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.06 (s, 1H, CH_3), 7.27 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.37 (t, 2H, C_6H_5-*meta*, $J = 7.6$ Hz), 7.61 (d, 2H, C_6H_5-*meta*, $J = 8.4$ Hz), 7.82 (d, 2H, C_6H_4-ortho, $J = 7.6$ Hz), 7.95 (d, 2H, C_6H_4-*ortho*, $J = 8.4$ Hz), 8.31 (s, 1H, CH–Se), 9.61 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 23.1, 117.3, 126.3, 126.8, 127.5, 128.1, 128.5, 129.2, 129.8, 130.3, 132.8, 135.2, 136.8, 149.3, 157.2, 159.7, 171.9. Anal. Calcd. for $C_{18}H_{14}ClN_3OSe$: C, 53.68 %; H, 3.50 %; N, 10.43 %. Found: C, 53.41 %; H, 3.39 %; N, 10.24 %.

*5-Ethoxycarbonyl-4-methyl-2-[(4-chlorobenzyliden)-N-acetyl-hydrazinyl]-1,3-selenazole (**3d**)* White-yellowish crystals, yield = 48 %, m.p. = 132–133 °C, IR (cm^{-1}): 3012 (νCH); 1712 (νC=O ester); 1672 (νC=O amide); 1621 (νC=N). MS (*m/z*): 411/413/416 (M^+); 371; 326; 298; 234; 188; 160; 111; 89; 67; 43(100 %); 28. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.42 (t, 3H, CH_3, $J = 6.9$ Hz), 2.38 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.51 (q, 2H, CH_2, $J = 6.9$ Hz), 7.64 (d, 2H, C_6H_4-*meta*, $J = 8.4$ Hz), 7.92 (d, 2H, C_6H_4-*ortho*, $J = 8.4$ Hz), 8.28 (s, 1H, CH–Se), 9.63 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 14.3, 16.5, 22.4, 61.7, 128.3, 129.1, 129.5, 129.7, 133.5, 134.8, 137.1, 138.3, 157.4, 159.33, 163.4, 174.8. Anal. Calcd. for $C_{16}H_{16}ClN_3O_3Se$: C, 46.56 %; H, 3.91 %; N, 10.18 %; Found: C, 46.12 %; H, 3.76 %; N, 10.02 %.

*4-Methyl-2-[(4-methoxybenzyliden)-N-acetyl-hydrazinyl]-1,3-selenazole (**3e**)* Brown-yellow, yield = 56 %, m.p. = 92–93 °C, IR (cm^{-1}): 2926 (νCH); 1693 (νC=O amide); 1627 (νC=N); 1591 (νC=$C_{aromatic}$). MS (*m/z*): 335/337/339 (M^+); 259; 204; 162; 134; 120; 92; 77; 51; 43(100 %). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.22 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3), 7.06 (d, 2H, C_6H_4-*meta*, $J = 8.8$ Hz), 7.36 (s, 1H, CH–Se), 7.82 (d, 2H, C_6H_4-*ortho*, $J = 8.8$ Hz), 9.07 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 19.0, 23.0, 55.9, 115.0, 115.7, 125.9, 126.1, 128.5, 130.7, 147.1, 157.1, 162.8, 163.7, 170.6. Anal. Calcd. for $C_{14}H_{15}N_3O_2Se$: C, 50.01 %; H, 4.50 %; N, 12.50 %. Found: C, 49.83 %; H, 4.46 %; N, 12.31 %.

*4-Phenyl-2-[(4-methoxybenzyliden)-N-acetyl-hydrazinyl]-1,3-selenazole (**3f**)* White-yellowish crystals, yield = 61 %, m.p. = 140–142 °C, IR (cm^{-1}): 2933 (νCH); 1678 (νC=O amide); 1624 (νC=N) 1593 (νC=$C_{aromatic}$). MS (*m/z*): 397/398/399 (M^+); 357/266/224; 182; 135; 102; 77; 43(100 %). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.47 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.10 (d, 2H, C_6H_4-*meta*, $J = 8.8$ Hz), 7.25 (t, 1H, C_6H_5-*para*, $J = 7.4$ Hz), 7.34 (t, 2H, C_6H_5-*meta*, $J = 7.4$ Hz), 7.80 (d, 2H, C_6H_5-*ortho*, $J = 7.3$ Hz), 7.89 (d, 2H, C_6H_4-*ortho*, $J = 8.8$ Hz), 8.24 (s, 1H, CH–Se), 9.33 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 23.0, 55.9, 115.1, 116.4, 126.0, 126.2, 128.0, 128.4. 129.0, 129.1, 130.6, 131.2, 134.8, 135.6, 149.1, 157.3, 162.8, 164.1, 171.1. Anal. Calcd. for $C_{19}H_{17}N_3O_2Se$: C, 57.29 %; H, 4.30 %; N, 10.55 %. Found: C, 57.29 %; H, 4.21 %; N, 10.43 %.

*4-Chloromethyl-2-[(2-phenyl-1,3-thiazolo-4-methyliden)-N-acetyl-hydrazinyl]-1,3–selenazole (**3g**)* White crystals, yield = 48 %, m.p. = 145–146 °C, IR (cm^{-1}): 2936 (νCH); 1693 (νC=O amide); 1625 (νC=N); 1570 (νC=$C_{aromatic}$). MS (*m/z*): 421/424/426 (M^+); 382; 253; 190; 161; 121; 59; 43(100 %). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.51 (s, 3H, CH_3), 4.83 (s, 2H, CH_2Cl), 7.58 (s, 1H, CH–Th), 7.82 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.86 (t, 2H, C_6H_5-*meta*, $J = 7.5$ Hz), 7.99 (d, 2H, C_6H_4-*ortho*, $J = 7.5$ Hz), 8.26 (s, 1H, CH–Se), 9.26 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 22.6, 40.3, 115.2, 118.5, 125.0, 126.3, 126.5, 128.8, 128.9, 130.0, 134.4, 149.5, 151.1, 155.3, 167.0, 171.4. Anal. Calcd. for $C_{16}H_{13}ClN_4OSSe$: C, 45.35 %; H, 3.09 %; N, 13.22 %; S, 7.57 %. Found: C, 45.32 %; H, 3.02 %; N, 13.21 %; S, 7.51 %.

*4-Methyl-5-acetyl-2-[(2-phenyl-1,3-thiazolo-4-methyliden-N-acetyl-hydrazinyl]-1,3-selenazole (**3h**)* White crystals, yield = 49 %, m.p. = 191–193 °C, IR (cm^{-1}): 3092 (νCH); 1691 (νC=O cetone); 1655 (νC=O amide). MS (*m/z*): 430/431/432 (M^+); 390; 342; 230; 188; 161; 121; 83; 57; 43(100 %). ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.05 (s, 3H, CH_3),

2.41 (s, 3H, *CH_3*), 2.52 (s, 3H, CH_3), 7.54 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.59 (t, 2H, C_6H_5-*meta*, $J = 8.8$ Hz), 7.97 (d, 2H, C_6H_5-*ortho*, $J = 8.8$ Hz), 7.98 (s, 1H, CH–Th), 9.14 (s, 1H, CH=N). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 18.6, 22.3, 24.2, 118.5, 126.6, 126.7, 127.0, 129.2, 129.3, 132.1, 133.4, 150.0, 152.6, 154.2, 158.1, 160.3, 164.5, 167.8. Anal. Calcd. for $C_{18}H_{16}N_4O_2SSe$: C, 50.12 %; H, 3.74 %; N, 12.99 %; S, 7.43 %. Found: C, 50.09 %; H, 3.68 %; N, 12.75 %; S, 7.39 %.

*General procedure for the synthesis of aroyl-selenosemicarbazide (**4a–b**)*

2 mmol (0.27 g) selenosemicarbazide was dissolved in 5 ml of NaOH 8 % aqueous solution and 2 mmol of acylchloride was added. The reaction mixture was stirred at room temperature for 1 h. Water was added until the volume became double and the solution was acidified with acetic acid. The precipitate thus obtained was collected by filtration. The purification was performed by recrystallization from water and ethanol in 1:2 volumetric ratios.

*Benzoyl-selenosemicarbazide (**4a**)* White crystals, yield = 58 %, m.p. = 182–183 °C, IR (cm^{-1}): 3343, 3271 (νNH); 3123, 2973, (νCH); 1679 (νC=O amide), 1521 (νC=$C_{aromatic}$). MS (*m/z*): 240/242/244 (M^+); 227; 166; 138(100 %); 105; 77; 50; 43; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.44 (t, 2H, C_6H_5-*meta*, $J = 7.2$ Hz); 7.53 (t, 1H, C_6H_5-*para,* $J = 7.2$ Hz), 7.87 (d, 2H, C_6H_5-*ortho,* $J = 7.2$ Hz), 8.12 (s, 1H, NH), 8.26 (s, 1H, NH), 9.72 (s, 2H, NH_2). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 125.5, 128.3, 128.6, 129.7, 130.9, 132.1, 133.0, 165.9. Anal. Calcd. for $C_8H_9N_3OSe$: C, 39.68 %; H, 3.75 %; N, 17.35 %. Found: C, 39.63 %; H, 3.68 %; N,17.31 %.

*p-Chloro-benzoyl-selenosemicarbazide (**4b**)* White crystals, yield = 65 %, m.p. = 199–200 °C, IR (cm^{-1}): 3336, 3262 (νNH); 3133, 2985 (νCH); 1682 (νC=O amide); 1592 (νC=$C_{aromatic}$). MS (*m/z*): 275/277/279, 280 (M^+); 196; 195; 152; 141; 139(100 %); 111; 75; 50; 43; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.53 (d, 2H, C_6H_5-*meta*, $J = 8.6$ Hz), 7.87 (d, 1H, C_6H_5-*ortho*, $J = 8.6$ Hz), 8.2 (s, 1H, NH), 8.32 (s, 1H, NH), 9.72 (s, 2H, NH_2). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 125.4, 128.1, 128.4, 129.7, 130.9, 132.1, 138.0, 165.9. Anal. Calcd. for $C_8H_8N_3ClOSe$: C, 34.74 %; H, 2.92 %; N, 15.19 %. Found: C, 34.56 %; H, 2.85 %; N, 15.10 %.

*General procedure for the preparation of aroyl-hydrazinyl-1,3-selenazole (**5a–d**)*

2 mmol (0.26 g) of aroyl-selenosemicarbazide was dissolved in 5 ml DMF and 5 ml anhydrous acetone and then 2 mmol of α-halogenocarbonyl derivative was added. The reaction mixture was stirred at room temperature for 24 h. The precipitate formed after neutralization with $NaHCO_3$ was collected by filtration. The product was purified by recrystallization from ethanol.

*4-Methyl-[2-(benzoyl-hydrazinyl)]-1,3-selenazole (**5a**)* White crystals, yield = 59 %, m.p. = 177–178 °C, IR (cm^{-1}): 3423, 3201 (νNH); 3099, 2983 (νCH); 1661 (νC=O amide); 1593 (νC=$C_{aromatic}$). MS (*m/z*): 279/281/283 (M^+); 204; 188; 162; 146; 105; 89; 77(100 %); 43; 39; 28. ^{1}H-NMR, (400 MHz, DMSO-d_6, δ ppm): 2.07 (s, 3H, CH_3), 6.72 (s, 1H, CH–Se), 7.49 (t, 2H, C_6H_5-*meta*, $J = 7.3$ Hz), 7.57 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.85 (d, 2H, C_6H_5-*ortho*, $J = 7.3$ Hz), 9.61 (s, 1H, NH), 10.79 (s, 1H, NH). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 18.8, 105.9, 127.8, 128.0, 129.0, 129.1, 132.5, 132.9, 148.9, 166.6, 175.2. Anal. Calcd. for $C_{11}H_{11}N_3OSe$: C, 47.15 %; H, 3.96 %; N, 15.00 %. Found: C, 47.03 %; H, 3.71 %; N, 14.96 %.

*4-Phenyl-2-(benzoyl-hydrazinyl)-1,3-selenazole (**5b**)* White crystals, yield = 63 %, m.p. = 172–173 °C, IR (cm^{-1}): 3426, 3204 (νNH); 3099, 2983 (νCH); 1663 (νC=O amide); 1593 (νC=$C_{aromatic}$). MS (*m/z*): 341/343/345 (M^+); 266; 238; 223; 162; 138; 120; 105; 77(100 %); 39; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.25 (t, 1H, C_6H_5-*para,* $J = 7.6$ Hz), 7.35 (t, 2H, C_6H_5-*meta*, $J = 7.6$ Hz), 7.52 (t, 2H, C_6H_5-*meta*, $J = 7.3$ Hz), 7.60 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.70 (s, 1H, CH–Se), 7.81 (d, 2H, C_6H_4-*ortho*, $J = 7.6$ Hz), 7.88 (d, 2H, C_6H_4-*ortho*, $J = 7.3$ Hz), 9.81 (s, 1H, NH), 10.93 (s, 1H, NH). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 108.2, 126.0, 126.3, 127.7, 127.9, 128.3, 128.3, 129.0, 129.1, 132.6, 132.7, 132.8, 136.0, 151.9, 168.0, 175.7. Anal. Calcd. for $C_{16}H_{13}N_3OSe$: C, 56.15 %; H, 3.83 %; N, 12.28 %. Found: C, 56.11 %; H, 3.79 %; N, 12.22 %.

*4-Methyl-2-[(4-chlorobenzoyl)-hydrazinyl]-1,3-selenazole (**5c**)* White crystals yield = 67 %, m.p. = 189–190 °C, IR (cm^{-1}): 3415, 3211 (νNH); 3097, 2846 (νCH); 1660 (νC=O amide); 1596 (νC=C aromatic). MS (*m/z*): 313/315/317 (M^+); 204; 162; 146; 138; 111;77(100 %); 39; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.07 (s, 3H, CH_3), 6.71 (s, 1H, CH–Se), 7.57 (d, 2H, C_6H_5-*meta*, $J = 8.5$ Hz), 7.86 (d, 2H, C_6H_4-*ortho*, $J = 8.5$ Hz), 9.65 (s, 1H, NH), 10.86 (s, 1H, NH). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 18.7, 105.7, 129.2, 129.8, 130.1, 131.6, 135.4, 137.3, 149.0, 165.5, 175.0. Anal. Calcd. for $C_{11}H_{10}ClN_3OSe$: C, 41.99 %; H, 3.20 %; N, 13.36 %. Found: C, 41.99 %; H, 3.16 %; N, 13.29 %.

*4-phenyl-2-[(4-chlorobenzoyl)-hydrazinyl]-1,3-selenazole (**5d**)* White-yellowish crystals, yield = 63 %, m.p. = 196–197 °C,

IR (cm^{-1}): 3410, 3212 (*v*NH); 3095, 2843 (*v*CH); 1665 (*v*C=O amide); 1596 (*v*C=C aromatic). MS (*m/z*): 375/377 (M^+); 299; 238; 223; 207; 169; 154; 146; 138; 77(100 %); 39; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 7.25 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.35 (t, 2H, C_6H_5-*meta*, $J = 7.3$ Hz), 7.60 (d, 2H, C_6H_5-*meta*, $J = 8.5$ Hz), 7.70 (s, 1H, CH–Se), 7.80 (d, 2H, C_6H_4-*ortho*, $J = 7.3$ Hz), 7.90 (d, 2H, C_6H_4-*ortho*, $J = 8.5$ Hz), 9.83 (s, 1H, *NH*), 11.00 (s, 1H, NH). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 108.6, 126.0, 126.3, 126.6, 127.0, 127.7, 129.0, 129.4, 129.6, 130.1, 131.5, 136.0, 137.5, 151.9, 165.9, 175.3. Anal. Calcd. for $C_{16}H_{12}ClN_3OSe$: C, 51.02 %; H, 3.21 %; N, 11.15 %. Found: C, 51.00 %; H, 3.19 %; N,11.12 %; .

*General procedure for the preparation of 2-(N,N-diacetyl-aroyl-hydrazinyl)-1,3-selenazole (**6a–d**)*

2 mmol aroyl-hydrazino-selenazole (**5a–d**) was treated with 2 ml acetic anhydride and 2 drops of pyridine. The mixture was stirred at room temperature for 24 h. The product was evaporated under vacuum to dryness and the residue was recrystallized from ethanol.

*4-Methyl-2-(benzoyl-N,N-diacetyl-hydrazinyl)-1,3-selenazole (**6a**)* White crystals, yield = 51 %, m.p. = 163–164 °C, IR (cm^{-1}): 3099, 2983, 2846 (*v*CH); 1634, 1661 (*v*C=O amide); 1593 (*v*C=$C_{aromatic}$). MS (*m/z*): 323/325 (M^+); 281; 203; 188; 162; 146; 116; 77; 43(100 %); 39; 28.

^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.30 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 7.35 (s, 1H, CH–Se), 7.46 (t, 2H, C_6H_5-*meta*, $J = 7.4$ Hz), 7.56 (t, 1H, C_6H_5-*para*, $J = 7.4$ Hz), 7.70 (d, 2H, C_6H_5-*ortho*, $J = 7.6$ Hz). ^{13}C-NMR 100.5 MHz, DMSO-d_6, δ ppm): 18.7, 31.1, 37.5, 116.7, 129,0, 129.0, 129.1, 130,3, 133.2, 137.5, 146.9, 158.0, 169.2, 171.7, 171.9. Anal. Calcd. for $C_{15}H_{15}N_3O_3Se$: C, 48.46 %; H, 4.07 %; N, 13.04 %. Found: C, 48.23 %; H, 4.02 %; N, 12.96 %.

*4-Phenyl-2-(benzoyl-N,N-diacetyl-hydrazinyl)-1,3-selenazole (**6b**)* White crystals, yield = 53 %, m.p. = 155–156 °C, IR (cm^{-1}): 3099, 2983 (*v*CH); 1632, 1663 (*v*C=O amide); 1593 (iC=$C_{aromatic}$). MS (*m/z*): 385/387 (M^+); 342; 266; 238; 223; 162; 138; 120; 105; 43(100 %); 39; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.41 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.24 (t, 1H, C_6H_5-*para*, $J = 7.2$ Hz), 7.33 (t, 2H, C_6H_5-*meta*, $J = 7.8$ Hz), 7.51 (t, 2H, C_6H_5-*meta*, $J = 7.2$ Hz), 7.60 (t, 1H, C_6H_5-*para*, $J = 7.8$ Hz), 7.80 (s, 1H, CH–Se), 7.81 (d, 2H, C_6H_4-*ortho*, $J = 7.2$ Hz), 7.82 (d, 2H, C_6H_4-*ortho*, $J = 7.8$ Hz). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 21.3, 24.4, 116.6, 126.0, 127.7, 127.9, 128.3, 129.0, 129.1, 132.6, 132.7, 132.8, 136.0, 151.9, 168.0, 166.9, 175.7, 175.9. Anal. Calcd. for $C_{20}H_{17}N_3O_3Se$: C, 56.26 %; H, 3.93 %; N, 10.93 %. Found: C, 56.21 %; H, 3.89 %; N, 10.85 %.

*4-Methyl-2-[(4-chloro-benzoyl)-N,N-diacetyl-hydrazinyl]-1,3-selenazole (**6c**)* White crystals, yield = 52 %, m.p. = 200–202 °C, IR (cm^{-1}): 3097, 2846 (*v*CH); 1660, 1631 (*v*C=O amide); 1596 (*v*C=C aromatic). MS (*m/z*): 357/359 (M^+); 204; 162; 146; 138; 111;77; 43(100 %); 39; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.32 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 7.35 (s, 1H, CH–Se), 7.55 (d, 2H, C_6H_5-*meta*, $J = 8.5$ Hz), 7.72 (d, 2H, C_6H_4-*ortho*, $J = 8.5$ Hz). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 18.7, 31.1, 32.4, 116.7, 129.0, 129.1, 130.1, 130.3, 133.2, 137.5, 146.9, 158.0, 169.2, 171.8, 172.0. Anal. Calcd. for $C_{15}H_{14}ClN_3O_3Se$: C, 43.78 %; H, 3.39 %; N, 11.78 %. Found: C, 43.65 %; H, 3.36 %; N, 11.67 %.

*4-Phenyl-2-[(4-chloro-benzoyl)-N,N-diacetyl-hydrazinyl]-1,3-selenazole (**6d**)* Yellow crystals, yield = 53 %, m.p = 140–141 °C, IR (cm^{-1}): 3095, 2843 (*v*CH); 1665, 1638 (*v*C=O amide); 1596 (*v*C=$C_{aromatic}$). MS (*m/z*): 419, 421 (M^+); 238/223/207; 169; 154; 146; 138; 43(100 %); 39; 28. ^{1}H-NMR (400 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.29 (t, 1H, C_6H_5-*para*, $J = 7.3$ Hz), 7.37 (t, 2H, C_6H_5-*meta*, $J = 7.3$ Hz), 7.55 (d, 2H, C_6H_5-*meta*, $J = 8.5$ Hz), 7.80 (d, 2H, C_6H_4-*ortho*, $J = 7.3$ Hz), 7.90 (d, 2H, C_6H_4-*ortho*, $J = 8.5$ Hz), 7.77 (s, 1H, CH–Se). ^{13}C-NMR (100.5 MHz, DMSO-d_6, δ ppm): 24.5, 25.2, 118.1, 125.9, 125.9, 126.6, 126.7, 128.3, 128.9, 129.0, 129.3, 130.5, 132.9, 135.2, 137.8, 148.9, 158.5, 171.87, 172.06. Anal. Calcd. for $C_{20}H_{16}ClN_3O_3Se$: C, 51.63 %; H, 3.37 %; N, 10.03 %. Found: C, 51.52 %; H, 3.27 %; N, 10.00 %.

Cytotoxicity assay

Antitumor assay on Human DU-145 prostate carcinoma and Hepatocarcinoma Hep-G2 cells

The cytotoxic activities of the samples were tested on human DU-145 (androgen-insensitive prostate cancer cells) and hepatocarcinoma Hep-G2 following the XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxyanilide inner salt) assay (Gerlier and Thomasset, 1986; Zee-Cheng, 1997; Itharat *et al*., 2004). Briefly, DU-145 and HepG-2 cells were cultured in Dulbecco's minimum essential medium (DMEM) supplemented with 5 % fetal calf serum (FCS), gentamicin sulfate (0.004 %), glucose (0.57 %), and $NaHCO_3$ (0.12 %) (Complete medium). Cells were seeded into 96-well flat-bottomed plates at a concentration of 3.0×10^5 cells/ml. After 24 h, cells were treated with samples, which were diluted with

culture medium at various concentrations to a final range of 1.57–25 μM (Gerlier and Thomasset, 1986; Itharat *et al*., 2004). XTT labeling reagent (50:1) was added and the absorbance (560 nm) read after 72 h (Itharat *et al*., 2004). Experiments were carried out three times in triplicate. The concentration of the sample that inhibited 50 % cell proliferation (IC_{50}) was determined graphically. Doxorubicin, a known antitumor agent, was used as positive control. The cells survival percentage was determined by the formula: % Survival Cell = (ODT/ODC) × 100; OD_T and OD_C being the absorbance of the test sample-treated group and the control group (0.1 % DMSO), respectively. IC_{50} value was the concentration of sample required to inhibit 50 % of the cell proliferation and was calculated from a calibration curve by a linear regression (Joshi *et al*., 2010) using Microsoft Excel.

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