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SYNTHESIS AND CYTOTOXICITY PROPERTIES OF SOME NOVEL FUNCTIONALIZED 2-{2-[(4-OXO-4*H*-CHROMEN-3-YL)METHYLENE]-HYDRAZINYL}-1,3-SELENAZOLES

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Abstract – A series of novel functionalized 1,3-selenazoles bearing 4-oxo-4*H*-chromen-3-yl moiety was constructed by Hantzsch-type condensation reaction. The methodology depended on the cyclization of N-[(4-oxo-4*H*-chromen-3-yl)methylene]selenosemicarbazide with bis-halogen and α -halocarbonyl compounds in DMF. The structures of the synthesized compounds were assigned based on the spectral tools. The cytotoxicity properties of the products were evaluated. Both compounds **9** and **11** showed significant cytotoxic properties against HCT-116, Hep-G2, A-549 and MCF-7 cancer cell lines.

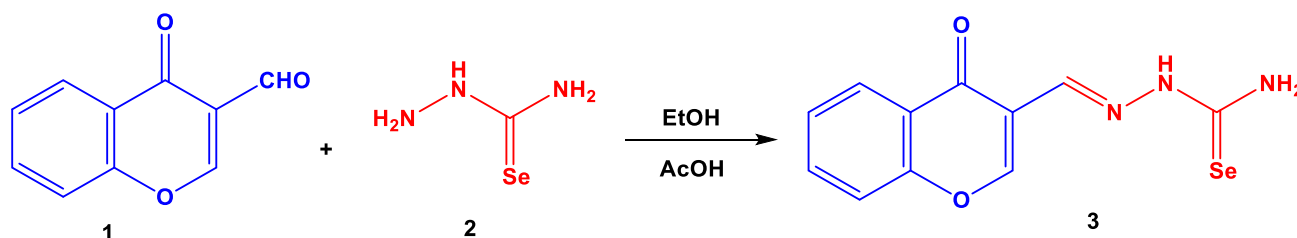
INTRODUCTION

Selenium element is considered an essential nutrient and constituent of selenoproteins involved in self-defense mechanisms against oxidative stress.^{1,2} Also, selenoproteins are used in reducing certain inflammatory processes and in detoxification processes.³ The heterocyclic compounds containing selenium atom have a great attention due to the wide variety of pharmacological activities such as anticancer,⁴ antibacterial⁵ and antiviral properties.⁶ Several of recent studies have confirmed that 1,3-selenazole compounds are antagonists for histamine H₂ receptors⁷ and effective antitumor agents⁸ as well as anion-superoxidase.⁹ On the other hand, chromone compounds are a group of bioactive molecules found substantially in nature with a wide range of structural modifications.¹⁰ Moreover, chromones are privileged oxygen heterocycles widely distributed in various plants and animals and microbial

metabolites that play an essential role in the agricultural and pharmaceutical industries.¹¹ They exhibited antitumor,¹² anti-inflammatory,¹³ antioxidant,¹⁴ anti-HIV properties.¹⁵ Depending on the above facts and our program research on developing new biologically active chromone compounds containing sulfur, selenium or phosphorus atom,¹⁶⁻²⁰ we decide to synthesize a novel series of 1,3-selenazole compounds bearing a chromone ring. The target products were designed by Hantzsch-type cyclization reaction of the novel precursor, N-[(4-oxo-4*H*-chromen-3-yl)methylene]selenosemicarbazide with bis-halogen or α -halocarbonyl reagents under mild reaction conditions. The cytotoxic activities of the products were investigated against human colon carcinoma HCT-116, hepatocellular carcinoma Hep-G2, lung carcinoma A-549 and breast adenocarcinoma MCF-7 cancer cell lines.

RESULTS AND DISCUSSION

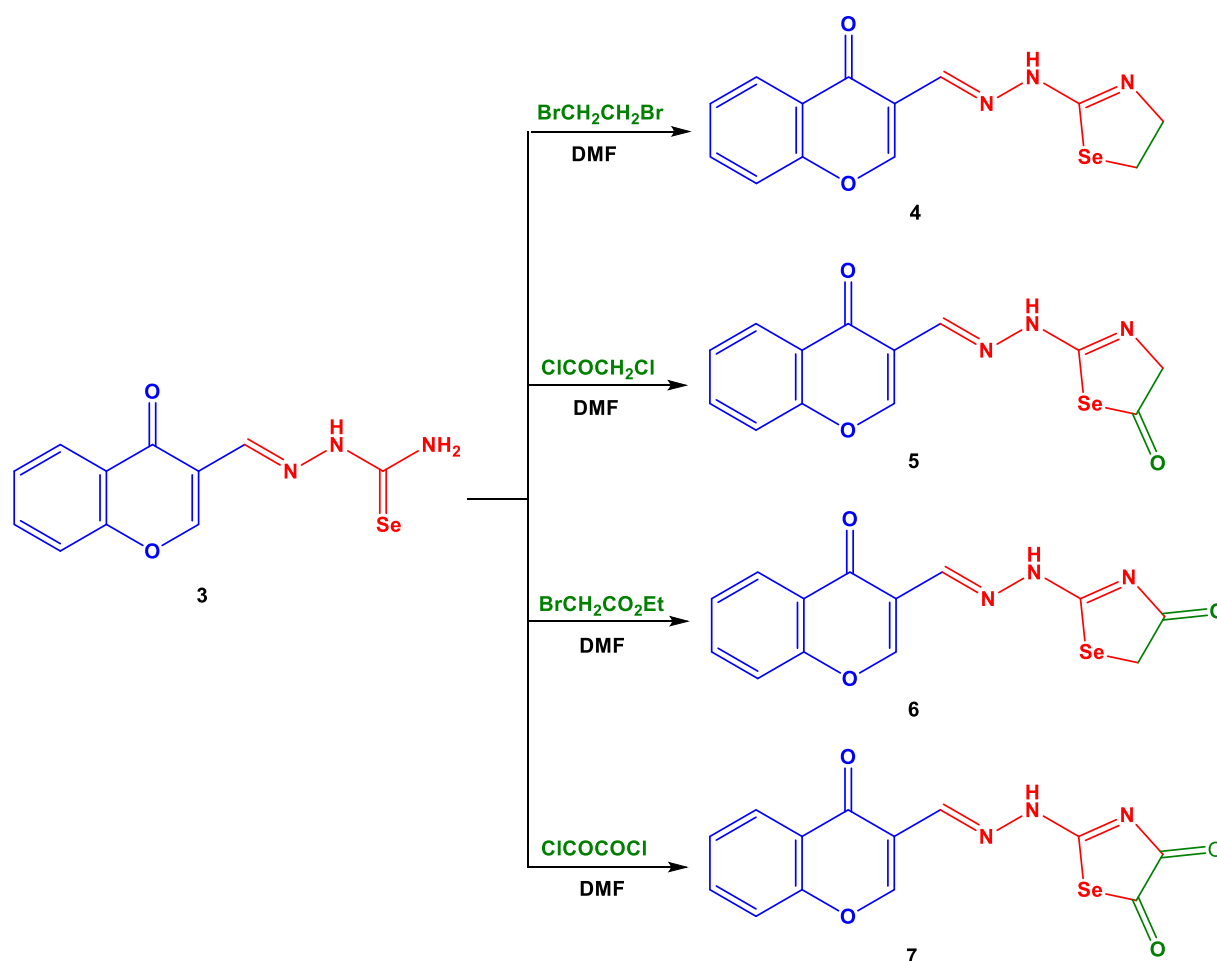
The synthesis of the new chromonyl selenazoles **4-11** described in this work was performed through two steps. The first step was the preparation of the novel precursor, N-[(4-oxo-4*H*-chromen-3-yl)methylene]selenosemicarbazide (**3**) by the condensation of 4-oxo-4*H*-chromene-3-carboxaldehyde (**1**)²¹ and selenosemicarbazide (**2**)^{22,23} in absolute ethanol containing a few drops of glacial acetic acid (Scheme 1). The IR spectrum of the selenosemicarbazone **3** showed intense bands at 1654 and 1609 cm^{-1} due to $\text{C}=\text{O}_{\text{pyrone}}$ and $\text{CH}=\text{N}$ groups, respectively. Whereas the NH_2 and NH stretching bands were observed at 3332, 3219, and 3121 cm^{-1} . It was further confirmed by $^1\text{H-NMR}$ spectrum, wherein the NH_2 and NH protons appeared as singlets at δ 9.27 and 10.80 ppm, respectively. Moreover, its $^{13}\text{C-NMR}$ spectrum recorded the specific carbon atoms of $\text{CH}=\text{N}$, $\text{C}=\text{Se}$, and $\text{C}=\text{O}$ at δ 159.0, 167.2 and 175.2 ppm, respectively. The mass spectrum of compound **3** displayed its molecular ion peaks at m/z 295, 293 and 291 due to the isotopes of selenium atom.²⁴



Scheme 1

The second step was performing of the Hantzsch-type condensation²⁵ between the selenosemicarbazide **3** with bis-halogen and α -halocarbonyl compounds such as 1,2-dibromoethane, chloroacetyl chloride, ethyl bromoacetate, oxalyl chloride, chloroacetone, phenacyl bromide, 1,3-dichloroacetone and ethyl α -chloroacetoacetate in dry DMF containing a few drops of pyridine (Schemes 2 and 3).^{26,27} A series of

the novel chromonyl selenazoles **4–11** were obtained in satisfactory yields (54–71%) after refluxing for 6–10 hours. The IR spectra for the synthesized compounds exhibited one NH group that confirmed the cyclization process. However, they displayed the vibrational absorption bands for the functions NH, C=O_{pyrone}, and CH=N at 3196–3111, 1646–1636 and 1613–1606 cm⁻¹, respectively. The ¹H-NMR spectra indicated highly downfield protons for the NH groups at chemical shifts in range δ 10.42–10.03 ppm for all products. The methylene protons in the products **4–6** were shown at regions δ 3.12–1.68 ppm. The specific protons at position 5 of selenazole compounds **8–10** appeared as singlets at δ 7.21–6.98 ppm. The ¹³C-NMR spectra of the target products displayed the specific carbon atoms at δ 175.4–173.6 (C=O_{pyrone}), 159.6–158.4 (CH=N), 169.1–164.4 (C-2_{selenazole}), while C=O_{selenazole} in products **5–7** were shown at regions δ 189.3–171.8 ppm.²⁸ The mass spectrometry and elemental analysis supported the proposed structures.

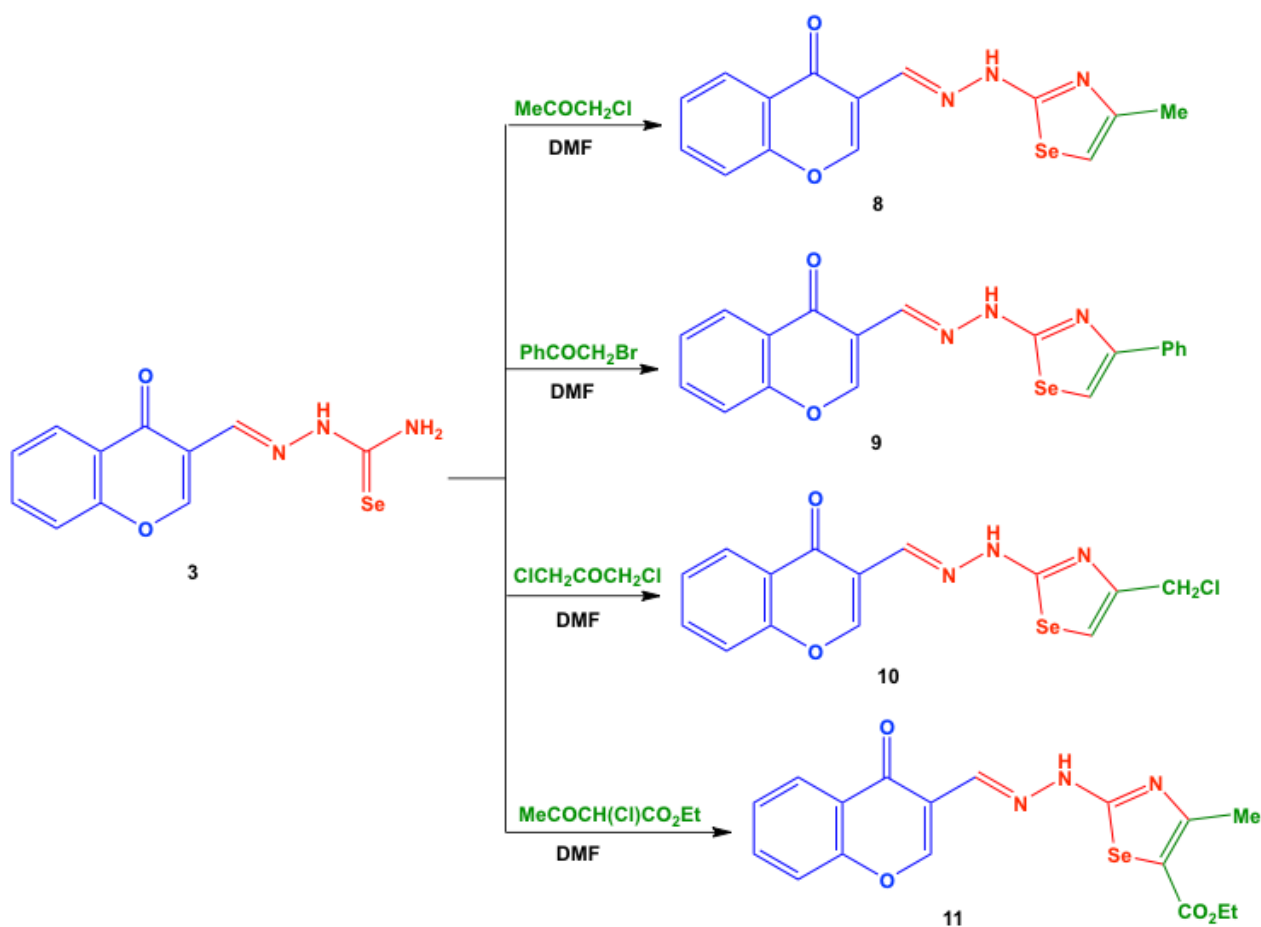


Scheme 2

CYTOTOXICITY EVALUATIONS

The *in vitro* cytotoxicity of the synthesized compounds **3–11** was evaluated against human colon carcinoma HCT-116, hepatocellular carcinoma Hep-G2, lung carcinoma A-549 and breast

adenocarcinoma MCF-7, using the crystal violet viability assay.^{29–31} The IC_{50} values of the synthesized compounds ($IC_{50} = 4.9 \rightarrow 500 \mu\text{g/mL}$) were comparable to that of Doxorubicin and summarized in Table 1. Compounds **3**, **4**, **5**, **7** and **10** were found to be less active while compounds **6** and **8** showed moderate cytotoxicity against all the investigated cancer cell lines. Compound **9** had the acceptable IC_{50} values against all cancer cells, while compound **11** exhibited the best cytotoxicity properties in comparison with all synthesized compounds and doxorubicin. Based on the results in Table 1, it was cleared that merge of the chromonyl hydrazinyl with 1,3-selenazole moieties improved the cytotoxicity properties in comparison with starting material **3**. Furthermore, it can be concluded that the type of substituent attached to selenazole ring is a major determinant of the pharmacological properties of the parent structures **4–11**. Therefore, the presence of *endo* N-C=O, Me, Ph and CO₂Et substituents attached to selenazole ring could be considered to be the factor responsible for the high cytotoxicity against cancer cells. These results provide evidence that the characteristic chemical features of Me and CO₂Et groups attached to selenazole ring are key factors for their cytotoxicity and play a useful role in elucidating the mechanisms of action in relation to the synthesized compounds in future research programs.



Scheme 3

Table 1. Cytotoxicity (IC₅₀, µg/mL) of the synthesized compounds **3-11** against human cancer cell lines in comparison with Doxorubicin

Compound	IC ₅₀ (µg/mL)			
	HCT-116	Hep-G2	A-549	MCF-7
3	>500	423	>500	>500
4	111.6	213.6	209.6	300.5
5	250.3	238.7	292.3	210.2
6	28.3	35.6	45.1	36.8
7	243.0	310.7	224.3	290.8
8	24.6	46.9	39.4	72.1
9	15.8	11.1	14.7	21.6
10	190.6	230.6	246.7	218.1
11	4.9	5.4	4.9	7.2
Doxorubicin	0.493	0.467	0.426	0.469

EXPERIMENTAL

The melting points were measured on a digital Stuart SMP-3 apparatus in an open capillary tube. Infrared spectra were measured on FT-IR spectrophotometer (Nicolet iS10) using KBr disks. ¹H- and ¹³C-NMR spectra were determined on Gemini-300BB (400 and 100 MHz) spectrometer, using DMSO-*d*₆ as a solvent and TMS (δ) as an internal standard. Mass spectra were recorded on direct probe controller inlet part to single quadropole mass analyzer in (Thermo Scientific GCMS). Elemental microanalysis was performed on Perkin-Elmer 2400II at the Chemical War department, Ministry of Defense. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental microanalysis.

N-[(4-Oxo-4*H*-chromen-3-yl)methylene]selenosemicarbazide (**3**)

A mixture of 4-oxo-4*H*-chromene-3-carboxaldehyde (2.5 mmol, 0.43 g) and selenosemicarbazide (2.5 mmol, 0.33 g) in absolute EtOH (25 mL) in the presence of glacial acetic acid (1 mL) was heated under reflux for 3 h. The obtained solid was filtered off, washed with water and crystallized from diluted DMF to give pale yellow solid in 83% yield; mp 238–240 °C. IR (KBr), (ν max, cm⁻¹): 3332, 3219 (NH₂), 3121 (NH), 1654 (C=O_{pyrone}), 1609 (C=N), 1583 (C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 7.48 (t, 1H, *J*=7.2 Hz, H-6_{chromone}), 7.63 (d, 1H, *J*=7.6 Hz, H-8_{chromone}), 7.79–7.81 (m, 1H, H-7_{chromone}), 8.09 (d, 1H, *J*=8.0 Hz, H-5_{chromone}), 8.55 (s, 1H, H-2_{chromone}), 8.82 (s, 1H, CH=N), 9.27 (brs, 2H, NH₂), 10.80 (s, 1H, NH). ¹³C-NMR (100 MHz, DMSO-*d*₆): 112.2 (C-3_{chromone}), 117.1 (C-8_{chromone}), 121.3 (C-4_achromone), 123.2 (C-6_{chromone}), 125.8 (C-5_{chromone}), 134.5 (C-7_{chromone}), 154.6 (C-2_{chromone}), 157.6 (C-8_achromone), 159.0 (CH=N), 167.2 (C=Se), 175.2 (C=O_{pyrone}). MS (*m/z*, I%): 295, 293, 291 (M⁺, 15%). Anal. Calcd for C₁₁H₉N₃O₂Se (294.17): C, 44.91%; H, 3.08%; N, 14.28%. Found: C, 44.72%; H, 2.99%; N, 14.03%.

General procedure for synthesis of the chromonyl selenazoles 4-11.

A mixture of selenosemicarbazide **3** (2.5 mmol, 0.73 g) and bis-halogen or α -halocarbonyl compounds (2.5 mmol) in dry DMF (30 mL) in the presence of pyridine (0.2 mL) as a catalyst, was heated under reflux for 6-10 h. The reaction mixture was poured onto cold water. The obtained solid was filtered off, washed with water, and crystallized from EtOH.

2-{2-[(4-Oxo-4H-chromen-3-yl)methylene]hydrazinyl}-4,5-dihydro-1,3-selenazole (4): Beige solid in 66% yield; mp 172–174 °C. IR (KBr), (ν max, cm^{-1}): 3172 (NH), 1644 ($\text{C}=\text{O}_{\text{pyrone}}$), 1611 ($\text{C}=\text{N}$), 1581 ($\text{C}=\text{C}$). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 1.68–1.73 (m, 2H, CH_2), 2.92–3.09 (m, 2H, CH_2), 7.41 (t, 1H, $J=6.8$ Hz, H-6_{chromone}), 7.59 (d, 1H, $J=7.2$ Hz, H-8_{chromone}), 7.74–7.79 (m, 1H, H-7_{chromone}), 8.15 (d, 1H, $J=6.8$ Hz, H-5_{chromone}), 8.48 (s, 1H, H-2_{chromone}), 8.79 (s, 1H, $\text{CH}=\text{N}$), 10.23 (s, 1H, NH). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): 17.3 (CH_2), 37.8 (CH_2), 113.1 (C-3_{chromone}), 118.2 (C-8_{chromone}), 120.8 (C-4_achromone), 123.6 (C-6_{chromone}), 126.0 (C-5_{chromone}), 134.9 (C-7_{chromone}), 155.1 (C-2_{chromone}), 157.3 (C-8_achromone), 159.1 ($\text{CH}=\text{N}$), 164.4 (C-2_{selenazole}), 175.4 ($\text{C}=\text{O}_{\text{pyrone}}$). MS (m/z , I%): 321, 319, 317 (M^+ , 12%). Anal. Calcd for $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}_2\text{Se}$ (320.21): C, 48.76%; H, 3.46%; N, 13.12%. Found: C, 48.57%; H, 3.29%; N, 13.03%.

2-{2-[(4-Oxo-4H-chromen-3-yl)methylene]hydrazinyl}-1,3-selenazol-5(4H)-one (5): Yellow solid in 62% yield; mp 181–183 °C. IR (KBr), (ν max, cm^{-1}): 3196 (NH), 1663 ($\text{C}=\text{O}_{\text{ketone}}$), 1646 ($\text{C}=\text{O}_{\text{pyrone}}$), 1612 ($\text{C}=\text{N}$), 1585 ($\text{C}=\text{C}$). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 3.12 (s, 2H, CH_2), 7.46 (t, 1H, $J=6.8$ Hz, H-6_{chromone}), 7.63 (d, 1H, $J=7.6$ Hz, H-8_{chromone}), 7.77–7.85 (m, 1H, H-7_{chromone}), 8.12 (d, 1H, $J=7.6$ Hz, H-5_{chromone}), 8.49 (s, 1H, H-2_{chromone}), 8.83 (s, 1H, $\text{CH}=\text{N}$), 10.16 (s, 1H, NH). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): 42.6 (CH_2), 113.6 (C-3_{chromone}), 117.7 (C-8_{chromone}), 120.6 (C-4_achromone), 124.1 (C-6_{chromone}), 126.6 (C-5_{chromone}), 135.4 (C-7_{chromone}), 155.4 (C-2_{chromone}), 156.9 (C-8_achromone), 158.7 ($\text{CH}=\text{N}$), 166.7 (C-2_{selenazole}), 174.6 ($\text{C}=\text{O}_{\text{pyrone}}$), 189.3 ($\text{C}=\text{O}_{\text{selenazole}}$). MS (m/z , I%): 335, 333, 331 (M^+ , 10%). Anal. Calcd for $\text{C}_{13}\text{H}_9\text{N}_3\text{O}_3\text{Se}$ (334.19): C, 46.72%; H, 2.71%; N, 12.57%. Found: C, 46.53%; H, 2.53%; N, 12.39%.

2-{2-[(4-Oxo-4H-chromen-3-yl)methylene]hydrazinyl}-1,3-selenazol-4(5H)-one (6): Yellow solid in 71% yield; mp 198–199 °C. IR (KBr), (ν max, cm^{-1}): 3152 (NH), 1659 ($\text{C}=\text{O}_{\text{amide}}$), 1639 ($\text{C}=\text{O}_{\text{pyrone}}$), 1607 ($\text{C}=\text{N}$), 1579 ($\text{C}=\text{C}$). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 2.68 (s, 2H, CH_2), 7.51 (t, 1H, $J=7.6$ Hz, H-6_{chromone}), 7.68 (d, 1H, $J=7.2$ Hz, H-8_{chromone}), 7.79–7.81 (m, 1H, H-7_{chromone}), 8.19 (d, 1H, $J=7.6$ Hz, H-5_{chromone}), 8.43 (s, 1H, H-2_{chromone}), 8.69 (s, 1H, $\text{CH}=\text{N}$), 10.03 (s, 1H, NH). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): 35.6 (CH_2), 113.8 (C-3_{chromone}), 117.6 (C-8_{chromone}), 121.6 (C-4_achromone), 124.3 (C-6_{chromone}), 126.8 (C-5_{chromone}), 135.6 (C-7_{chromone}), 155.6 (C-2_{chromone}), 156.8 (C-8_achromone), 158.5 ($\text{CH}=\text{N}$), 168.3 (C-2_{selenazole}), 171.8 ($\text{C}=\text{O}_{\text{selenazole}}$), 175.3 ($\text{C}=\text{O}_{\text{chromone}}$). MS (m/z , I%): 335, 333, 331 (M^+ , 12%). Anal. Calcd for $\text{C}_{13}\text{H}_9\text{N}_3\text{O}_3\text{Se}$ (334.19): C, 46.72%; H, 2.71%; N, 12.57%. Found: C, 46.55%; H, 2.56%; N,

12.43%.

2-{2-[(4-Oxo-4H-chromen-3-yl)methylene]hydrazinyl}-1,3-selenazole-4,5-dione (7): Yellow solid in 56% yield; mp 158–160 °C. IR (KBr), (ν max, cm^{-1}): 3111 (NH), 1675 ($\text{C}=\text{O}_{\text{amide}}$), 1662 ($\text{C}=\text{O}_{\text{ketone}}$), 1643 ($\text{C}=\text{O}_{\text{pyrone}}$), 1608 ($\text{C}=\text{N}$), 1580 ($\text{C}=\text{C}$). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 7.38 (t, 1H, $J=6.4$ Hz, H-6_{chromone}), 7.51 (d, 1H, $J=7.2$ Hz, H-8_{chromone}), 7.70–7.75 (m, 1H, H-7_{chromone}), 8.23 (d, 1H, $J=6.4$ Hz, H-5_{chromone}), 8.53 (s, 1H, H-2_{chromone}), 8.73 (s, 1H, CH=N), 10.42 (s, 1H, NH). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): 114.1 (C-3_{chromone}), 117.6 (C-8_{chromone}), 121.2 (C-4_achromone), 124.5 (C-6_{chromone}), 126.6 (C-5_{chromone}), 135.8 (C-7_{chromone}), 155.7 (C-2_{chromone}), 156.6 (C-8_achromone), 158.6 (CH=N), 165.6 (C-2_{selenazole}), 172.1 ($\text{C}=\text{O}_{\text{selenazole}}$), 175.1 ($\text{C}=\text{O}_{\text{pyrone}}$), 183.5 ($\text{C}=\text{O}_{\text{selenazole}}$). MS (m/z , I%): 349, 347, 345 (M^+ , 14%). Anal. Calcd for $\text{C}_{13}\text{H}_7\text{N}_3\text{O}_4\text{Se}$ (348.18): C, 44.85%; H, 2.03%; N, 12.07%. Found: C, 44.67%; H, 1.94%; N, 11.87%.

4-Methyl-2-{2-[(4-oxo-4H-chromen-3-yl)methylene]hydrazinyl}-1,3-selenazole (8): Yellow solid in 69% yield; mp 202–204 °C. IR (KBr), (ν max, cm^{-1}): 3186 (NH), 1646 ($\text{C}=\text{O}_{\text{pyrone}}$), 1613 ($\text{C}=\text{N}$), 1582 ($\text{C}=\text{C}$). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 2.43 (s, 3H, CH_3), 6.98 (s, 1H, H-5_{selenazole}), 7.45 (t, 1H, $J=6.4$ Hz, H-6_{chromone}), 7.61 (d, 1H, $J=7.2$ Hz, H-8_{chromone}), 7.72–7.78 (m, 1H, H-7_{chromone}), 8.18 (d, 1H, $J=6.4$ Hz, H-5_{chromone}), 8.54 (s, 1H, H-2_{chromone}), 8.89 (s, 1H, CH=N), 10.13 (s, 1H, NH). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): 17.8 (CH_3), 114.2 (C-3_{chromone}), 119.3 (C-8_{chromone}), 120.4 (C-5_{selenazole}), 121.4 (C-4_achromone), 125.3 (C-6_{chromone}), 126.8 (C-5_{chromone}), 135.2 (C-7_{chromone}), 141.2 (C-4_{selenazole}), 155.4 (C-2_{chromone}), 158.0 (C-8_achromone), 159.6 (CH=N), 169.1 (C-2_{selenazole}), 173.6 ($\text{C}=\text{O}_{\text{pyrone}}$). MS (m/z , I%): 333, 331, 329 (M^+ , 8%). Anal. Calcd for $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_2\text{Se}$ (332.22): C, 50.62%; H, 3.34%; N, 12.65%. Found: C, 50.43%; H, 3.15%; N, 12.49%.

2-{2-[(4-Oxo-4H-chromen-3-yl)methylene]hydrazinyl}-4-phenyl-1,3-selenazole (9): Pale brown solid in 54% yield; mp 210–213 °C. IR (KBr), (ν max, cm^{-1}): 3132 (NH), 1648 ($\text{C}=\text{O}_{\text{pyrone}}$), 1610 ($\text{C}=\text{N}$), 1573 ($\text{C}=\text{C}$). $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$): 7.11 (s, 1H, H-5_{selenazole}), 7.34–7.38 (m, 1H, Ph-H), 7.45–7.50 (m, 3H, Ph-H and H-6_{chromone}), 7.55–7.66 (m, 3H, Ph-H and H-8_{chromone}), 7.75–7.80 (m, 1H, H-7_{chromone}), 8.25 (d, 1H, $J=6.8$ Hz, H-5_{chromone}), 8.61 (s, 1H, H-2_{chromone}), 8.83 (s, 1H, CH=N), 10.31 (s, 1H, NH). $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$): 115.1 (C-3_{chromone}), 118.2 (C-8_{chromone}), 120.8 (C-5_{selenazole}), 122.1 (C-4_achromone), 124.9 (C-6_{chromone}), 126.5 (C-5_{chromone}), 127.3 (C-2,6_{phenyl}), 129.1 (C-3,5_{phenyl}), 130.1 (C-4_{phenyl}), 133.2 (C-1_{phenyl}), 135.7 (C-7_{chromone}), 145.2 (C-4_{selenazole}), 154.8 (C-2_{chromone}), 157.6 (C-8_achromone), 158.4 (CH=N), 167.6 (C-2_{selenazole}), 174.4 ($\text{C}=\text{O}_{\text{pyrone}}$). MS (m/z , I%): 395, 393, 391 (M^+ , 11%). Anal. Calcd for $\text{C}_{19}\text{H}_{13}\text{N}_3\text{O}_2\text{Se}$ (394.29): C, 57.88%; H, 3.32%; N, 10.66%. Found: C, 57.59%; H, 3.17%; N, 10.39%.

4-Chloromethyl-2-{2-[(4-oxo-4H-chromen-3-yl)methylene]hydrazinyl}-1,3-selenazole (10): Pale yellow solid in 65% yield; mp 178–179 °C. IR (KBr), (ν max, cm^{-1}): 3136 (NH), 1641 ($\text{C}=\text{O}_{\text{pyrone}}$), 1609 ($\text{C}=\text{N}$),

1584 (C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 4.34 (s, 2H, CH₂), 7.21 (s, 1H, H-5_{selenazole}), 7.41 (t, 1H, *J*=6.8 Hz, H-6_{chromone}), 7.58 (d, 1H, *J*=7.6 Hz, H-8_{chromone}), 7.67–7.75 (m, 1H, H-7_{chromone}), 8.21 (d, 1H, *J*=6.8 Hz, H-5_{chromone}), 8.48 (s, 1H, H-2_{chromone}), 8.81 (s, 1H, CH=N), 10.19 (s, 1H, NH). ¹³C-NMR (100 MHz, DMSO-*d*₆): 40.1 (CH₂), 115.3 (C-3_{chromone}), 118.5 (C-8_{chromone}), 121.6 (C-5_{selenazole}), 123.1 (C-4_achromone), 125.8 (C-6_{chromone}), 127.1 (C-5_{chromone}), 135.6 (C-7_{chromone}), 142.1 (C-4_{selenazole}), 154.8 (C-2_{chromone}), 158.2 (C-8_achromone), 159.2 (CH=N), 167.8 (C-2_{selenazole}), 174.0 (C=O_{pyrone}). MS (*m/z*, I%): 367, 365, 363 (M⁺, 8%). Anal. Calcd for C₁₄H₁₀ClN₃O₂Se (366.66): C, 45.86%; H, 2.75%; N, 11.46%. Found: C, 45.69%; H, 2.61%; N, 11.19%.

Ethyl 4-methyl-2-{2-[(4-oxo-4H-chromen-3-yl)methylene]hydrazinyl}-1,3-selenazole-5-carboxylate (11): Pale brown solid in 70% yield; mp 159–161 °C. IR (KBr), (*v* max, cm⁻¹): 3142 (NH), 1687 (C=O_{ester}), 1636 (C=O_{pyrone}), 1606 (C=N), 1582 (C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 1.13 (t, 3H, *J*=7.2 Hz, CH₃), 2.42 (s, 3H, CH₃), 3.99–4.03 (m, 2H, OCH₂), 7.38 (t, 1H, *J*=6.4 Hz, H-6_{chromone}), 7.54 (d, 1H, *J*=7.6 Hz, H-8_{chromone}), 7.65–7.71 (m, 1H, H-7_{chromone}), 8.19 (d, 1H, *J*=6.4 Hz, H-5_{chromone}), 8.49 (s, 1H, H-2_{chromone}), 8.83 (s, 1H, CH=N), 10.33 (s, 1H, NH). ¹³C-NMR (100 MHz, DMSO-*d*₆): 13.8 (CH₃), 17.6 (CH₃), 59.8 (OCH₂), 114.8 (C-3_{chromone}), 119.6 (C-8_{chromone}), 121.6 (C-5_{selenazole}), 123.4 (C-4_achromone), 126.0 (C-6_{chromone}), 127.1 (C-5_{chromone}), 135.8 (C-7_{chromone}), 143.6 (C-4_{selenazole}), 153.9 (C-2_{chromone}), 157.6 (C-8_achromone), 159.3 (CH=N), 166.6 (C-2_{selenazole}), 175.1 (C=O_{pyrone}), 178.4 (C=O_{ester}). MS (*m/z*, I%): 405, 403, 401 (M⁺, 11%). Anal. Calcd for C₁₇H₁₅N₃O₄Se (404.28): C, 50.51%; H, 3.74%; N, 10.39%. Found: C, 50.36%; H, 3.59%; N, 10.21%.

Cell Lines

Colon (HCT-116), human hepatocellular (Hep-G2), lung (A-549) and breast (MCF-7) carcinoma cells were obtained from VACSERA Tissue Culture Unit. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂ and were sub-cultured two times a week.

Evaluation of cytotoxicity activities

Cytotoxicity of all compounds was tested in HCT-116, Hep-G2, A-549 and MCF-7 cells. For cytotoxicity assay,^{29–31} the cells were seeded in 96-well plate at a cell concentration of 1×10⁴ cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested compounds were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO₂ for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in

the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37 °C, various concentrations of the sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated, and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates was measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as: The percentage of cell viability = $[1 - (OD_t/OD_c)] \times 100\%$, where OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration, using Graphpad Prism software (San Diego, CA. USA).

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REFERENCES

1. R. Naithani, *Mini-Rev. Med. Chem.*, 2008, **8**, 657.
2. O. Lopez, S. Maza, V. Ulgar, I. Maya, and J. G. Fernandez-Bolanos, *Tetrahedron*, 2009, **65**, 2556.
3. N. T. Akbaraly, J. Arnaud, I. Haninger-Favier, V. Gourlet, A. M. Roussel, and C. Berr, *Clin. Chem.*, 2005, **51**, 2117.
4. S. Sheikhi-Mohammareh, A. Shiri, E. H. Maleki, M. M. Matin, H. Beyzaei, P. Baranipour, F. Oroojalian, and T. Memariani, *ChemistrySelect*, 2020, **5**, 10060.
5. M. L. Al-Smadi, R. Mansour, A. Mahasneh, O. F. Khabour, M. M. Masadeh, and K. H. Alzoubi, *Molecules*, 2019, **24**, 4082.
6. J. Mlochowski, K. Kloc, R. Lisiak, P. Potaczek, and H. Wojtowicz, *ARKIVOC*, 2007, **vi**, 14.
7. H. van der Goot, J. C. Eriks, R. Leurs, and H. Timmerman, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1913.

8. D.-D. Li, J. He, and H.-H. Zeng, *Appl. Organomet. Chem.*, 2012, **26**, 619.
9. K. N. Nam, M. Koketsu, and E. H. Lee, *Eur. J. Pharm.*, 2008, **589**, 53.
10. J. D. Hepworth, A. J. Boulton, and A. McKillop, *Comprehensive Heterocyclic Chemistry 3*, Pergamon Press, Oxford, 1984, pp. 835–840.
11. R. S. Keri, S. Budagumpi, R. K. Pai, and R. G. Balakrishna, *Eur. J. Med. Chem.*, 2014, **78**, 340.
12. Y. D. Duan, Y. Y. Jiang, F. X. Guo, L. X. Chen, L. L. Xu, W. Zhang, and B. Liu, *Fitoterapia*, 2019, **135**, 114.
13. L. C. F. Opretzka, R. F. doEspírito-Santo, O. A. Nascimento, L. S. Abreu, I. M. Alves, E. Döring, M. B. P. Soares, E. da S. Velozo, S. A. Laufer, and C. F. Villarreal, *Int. Immunopharmacol.*, 2019, **72**, 31.
14. M. Kuroda, S. Uchida, K. Watanabe, and K. Mimaki, *Phytochemistry*, 2009, **70**, 288.
15. T. Zhou, Q. Shi, and K. H. Lee, *Tetrahedron Lett.*, 2010, **51**, 4382.
16. R. M. Abdel-Rahman and T. E. Ali, *Monatsh. Chem.*, 2013, **144**, 1243.
17. T. E. Ali, M. A. Assiri, N. M. Hassanin, I. S. Yahia, and M. S. A. Hussien, *J. Heterocycl. Chem.*, 2019, **56**, 1684.
18. T. E. Ali, M. A. Assiri, M. M. Ali, A. E. M. Ali, I. S. Yahia, and H. Y. Zahran, *Heterocycles*, 2020, **100**, 1831.
19. I. A. Shaaban, M. A. Assiri, T. E. Ali, and A. M. Fouda, *J. Mol. Struct.*, 2020, **1203**, 127408.
20. A. M. Fouda, M. A. Assiri, and T. E. Ali, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2020, **195**, 324.
21. J. I. Borrell, J. Teixido, E. Schuler, and E. Michelotti, *Tetrahedron Lett.*, 2001, **42**, 5331.
22. B. Liu, C. Xu, and X. Zhou, *Chem. Reagents*, 1993, **15**, 334.
23. Z. Chen, D. Li, N. Xu, J. Fang, Y. Yu, W. Hou, H. Ruan, P. Zhu, R. Ma, S. Lu, D. Cao, R. Wu, M. Ni, W. Zhang, W. Su, and B. H. Ruan, *J. Med. Chem.*, 2019, **62**, 589.
24. S. Majnooni, J. Duffield, J. Price, A. R. Khosropour, H. Zali-Boeini, and M. H. Beyzavi, *ACS Comb. Sci.*, 2019, **21**, 516.
25. K. Abou-Melha, *J. Mol. Struct.*, 2021, **1224**, 129215.
26. J. Rafique, R. F. S. Canto, S. Saba, F. A. R. Barbosa, and A. L. Braga, *Curr. Org. Chem.*, 2016, **20**, 166.
27. V. Malinauskiene, A. Kveselyte, K. Dzedulionyte, A. Bieliauskas, S. Burinskas, F. A. Sloek, and A. Sackus, *Chem. Heterocycl. Compd.*, 2018, **54**, 469.
28. M. Koketsu, Y. Takenaka, and H. Ishihara, *Synthesis*, 2001, 731.
29. T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.
30. V. Gangadevi and J. Muthumary, *Afr. J. Biotechnol.*, 2007, **6**, 1382.
31. E. Vega-Avila and M. K. Pugsley, *Proc. West. Pharmacol. Soc.*, 2011, **54**, 10.