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EFFICIENT SYNTHESIS AND ANTICANCER ACTIVITIES OF SOME NOVEL FUNCTIONALIZED (4-OXO-4*H*-CHROMEN-3-YL)-2-SELENOXO-1,2-DIHYDROPYRIMIDINES

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Abstract – Some novel functionalized 2-selenoxo-1,2-dihydropyrimidines bearing a chromone ring **2a-h** were synthesized in excellent yields *via* a simple one-pot reaction. The simple method depended on one-pot three-component the reaction of 3-formylchromone (**1**) and selenourea with some active methylene compounds in the presence of sodium benzoate as a basic catalyst in a mixture of ethanol and water. The reactions were completed in 1.5–2.5 h in 88-94% yields. Structures of the synthesized compounds were based on elemental analysis, IR, ^{1}H- and ^{13}C-NMR and mass spectrometry. The anticancer properties of the synthesized compounds were evaluated against five cancer cell lines. Compounds **2a-d** displayed the most potent anticancer activities against A549, MCF-7 and HepG2 cell lines in comparison with doxorubicin as the standard drug.

INTRODUCTION

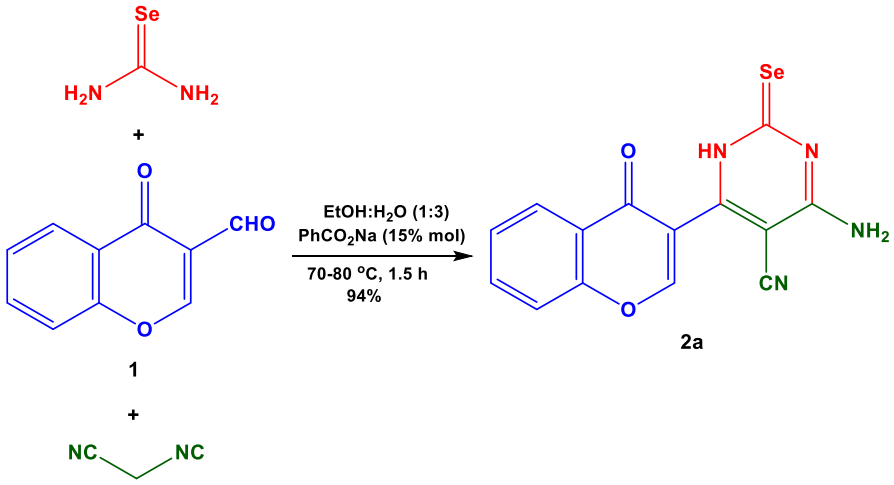
In 1817, the selenium element was discovered. It is a red amorphous powder.[1] Although some organoselenium compounds are less stable and toxic, but their distinctive chemical and biological

properties make the chemistry of selenium-containing heterocycles more interesting.[2–5] Organoselenium compounds are well known for their antioxidant activities with the ability to mimic selenoenzyme glutathione peroxidase (GPx-like activity).[6] Also, these compounds are well known to inhibit cell proliferation and induce cell death in human cancer cells by apoptosis.[7,8] In addition, some of selenium compounds showed anti-inflammatory,[9] anticancer,[10,11] neuroprotective[12] and antimelanogenesis properties.[13] Furthermore, selenourea derivatives have found applications as a chalcogenizing agent,[14] high potential dechloroacetylation reagent,[15] and scavenger of superoxide radicals.[16] Selenoureas represent important building blocks for the synthesis of pharmacologically relevant selenium heterocycles especially 1,3-selenazoles and 1,3,4-selenadiazines.[17-19] To the best of our knowledge, the selenoxopyrimidine derivatives have few reports in the literature. For example, Klein and co-workers reported the synthesis of a single seleno-analogue of Monastrol and evaluated its ability to inhibit the kinesin Eg5 enzyme; however, it caused a two-fold decrease in the activity when compared to Monastrol.[20] Kolb and colleagues[21] evaluated a pyrimidinyl selenourea and its selenazolopyrimidine derivative as phosphatase inhibitors. Also, some selenoxopyrimidines recorded antiproliferative effects and cell death by apoptosis[22] and excellent antifungal activity against *Aspergillius janus* and *Penicillium glabrum*.[23] On the other hand, chromone compounds are a group of bioactive molecules, found substantially in nature with a wide range of structural modifications.[24] Chromones are a privileged oxygen heterocycles widely distributed in various species of plants as well as animals and microbial metabolite, playing an important role in the agricultural and pharmaceutical industries.[25] They exhibited antitumor,[26] anti-inflammatory,[27] antioxidant,[28] anti-HIV properties.[29] Considering the above facts and our program research on the development of new biologically active polyheterocyclic,[30-32] the present work aimed to obtain some novel functionalized 2-selenoxo-1,2-dihydropyrimidines bearing a chromone ring in a single molecule, especially there is no any previous report of this molecular frame. The methodology depended on the reaction of 3-formylchromone (**1**) and selenourea with different active methylene compounds in one-pot in the presence of a basic catalyst. As well as the anticancer activities of the synthesized compounds against different human cancer cell lines including lung A594, breast MCF-7, liver HepG2, colon HCT116 and prostate PC3 cancer cell lines were evaluated. Additionally, the toxic effect of these compounds against the normal cells was evaluated against human normal melanocyte HFB4.

RESULTS AND DISCUSSION

In order to optimize the reaction conditions, the reaction of 3-formylchromone (**1**), selenourea and malononitrile was chosen as a model reaction (Scheme 1). This combination in the absence of any catalyst and solvent-free condition at 80 °C provided the desired product **2a** after 1 h in 24% yield (Table 1, entry 1). Next, the same reaction mixture stirred at reflux temperature in water to give the desired

product **2a** in 44% yield after 3 h (Table 1, entry 2). Ethanol is coincidently a better solvent from an environmental perspective. However, studies indicated that ethanol–water mixtures are more environmentally favorable compared to pure alcohol.[33,34] Using aqueous medium as a solvent in organic reaction is not only inexpensive and environmentally benign, but also offers advantages over those occurring in organic solvents.[35,36] Thus, the model reaction was performed again in absolute ethanol and mixtures of ethanol-water (in ratio EtOH:H_2O = 1:1, 1:2 and 1:3) at 75 °C. We observed that excellent yield of product was afforded after 2 h by using a mixture of EtOH:H_2O in ratio 1:3 (Table 1, entry 6). With this fascinating result of the model reaction, we examined catalysts to shorten the reaction time as well as increase the yield further.



Scheme 1

Table 1. Effect of solvent on the synthesis of 4-amino-6-(4-oxo-4*H*-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidine-5-carbonitrile (**2a**)[a]

Entry	Solvent	Time (h)	Yields %
1	-----	1	24
2	H_2O	3	44
3	EtOH	5	34
4	EtOH:H_2O (1:1)	3	56
5	EtOH:H_2O (1:2)	2.5	62
6	EtOH:H_2O (1:3)	2	76

[a] Reactions are performed on 2 mmol scale of the reactants.

To select the best catalyst, we carried out the reaction between 3-formylchromone (**1**), selenourea and malononitrile in the presence of 10 mol% of different basic catalysts such as K_2CO_3, Et_3N, piperidine, NaOH and $PhCO_2Na$ (Table 2). We found that K_2CO_3 afforded the product in good yield and reaction time; similar results were obtained with piperidine and triethylamine (Table 2). The yield of the desired

product was a very less extent when NaOH was used as a basic catalyst and the product was a mixture and a sticky mass. When the same reaction was carried out in the presence of $PhCO_2Na$, the product was obtained in very high yield (90%) within 1.5 h (Table 2). It may due to its freely solubility in the used water as solvent. The presence of water as solvent increased the reactivity of $PhCO_2Na$ as a catalyst. On contrary to other catalysts that are less effective in the presence of water. In addition, $PhCO_2Na$ has not influence on chromone ring that may be sensitive toward the basic regents. For example, NaOH gave the bad results due to its reaction with position 2 of this ring. We have also varied the amount of $PhCO_2Na$ from 5, 10, and 15 to 20 mol% and the results revealed that 15 mol% gave excellent yield of the product in a short duration as shown in Table 3.

Table 2. Influence of various catalysts on the synthesis of 4-amino-6-(4-oxo-4*H*-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidine-5-carbonitrile (**2a**)[a]

Entry	Catalyst (10 mol%)	Time (h)	Yield%
1	K_2CO_3	2	83
2	Et_3N	3	82
3	piperidine	4	81
4	NaOH	3	62
5	$PhCO_2Na$	1.5	90

[a] Reactions are performed on 2 mmol scale of the reactants.

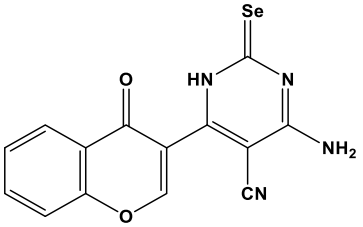
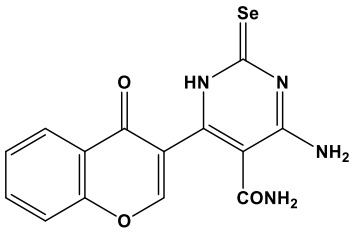
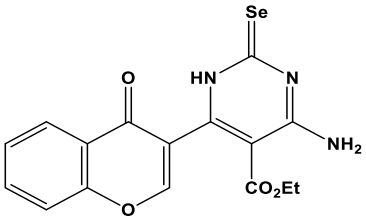
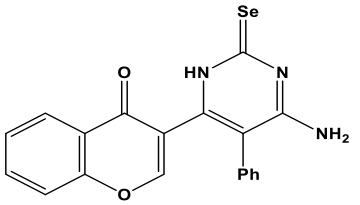
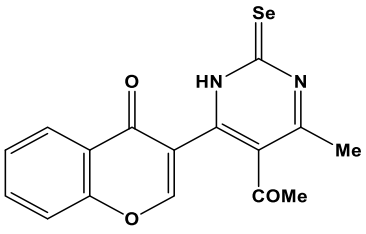
Table 3. Effect of the amount of $PhCO_2Na$ on the synthesis of 4-amino-6-(4-oxo-4*H*-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidine-5-carbonitrile (**2a**)[a]

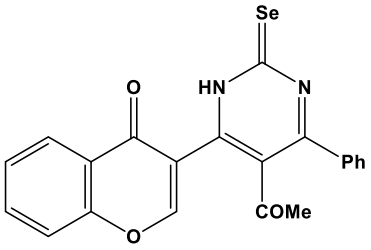
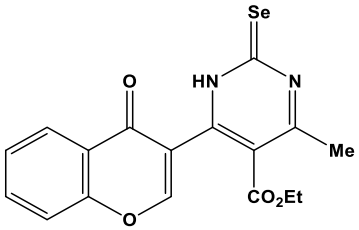
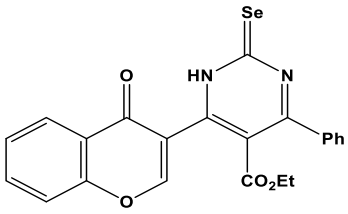
Entry	$PhCO_2Na$ (mol%)	Yield%
1	5	86
2	10	90
3	15	94
4	20	94

[a] Reactions are performed on 2 mmol scale of the reactants.

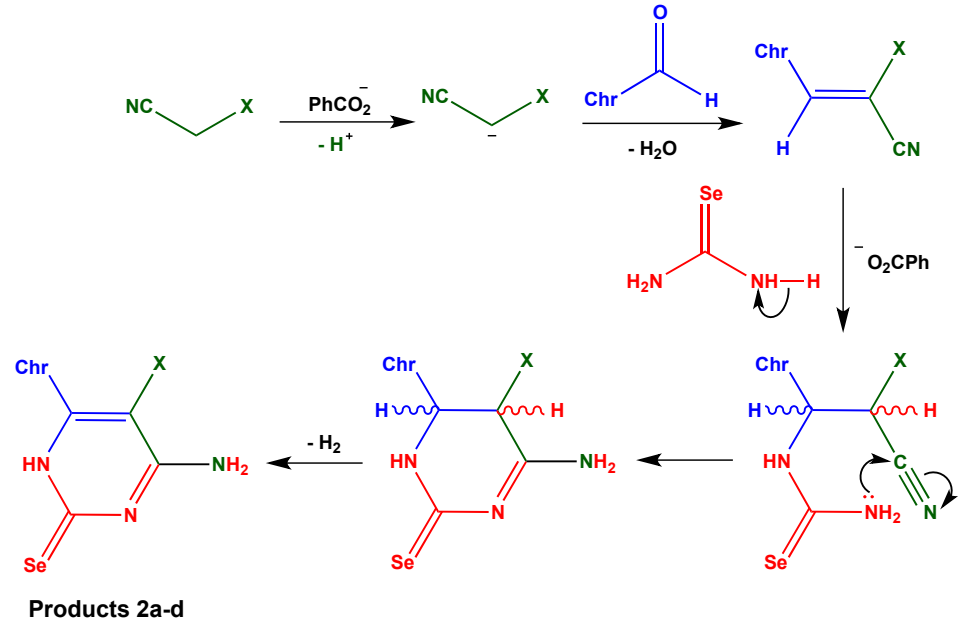
After optimizing the conditions, the generality of this method was examined by the reaction of other different active methylene compounds such as cyanoacetamide, ethyl cyanoacetate, benzyl cyanide, acetylacetone, benzoyl acetone, ethyl acetoacetate and ethyl benzoylacetate with 3-formylchromone (**1**) and selenourea in the presence of 15 mol% $PhCO_2Na$ in a mixture of ethanol and water (ratio 1:3) under reflux at 70-80 ^oC. The corresponding novel functionalized chromonyl selenoxopyrimidines **2b-h** were isolated in yields 88-94% and reaction times between 1.5 to 2.5 h (Table 4).

Table 4. Synthesis of functionalized (4-oxo-4*H*-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidines **2a-h**

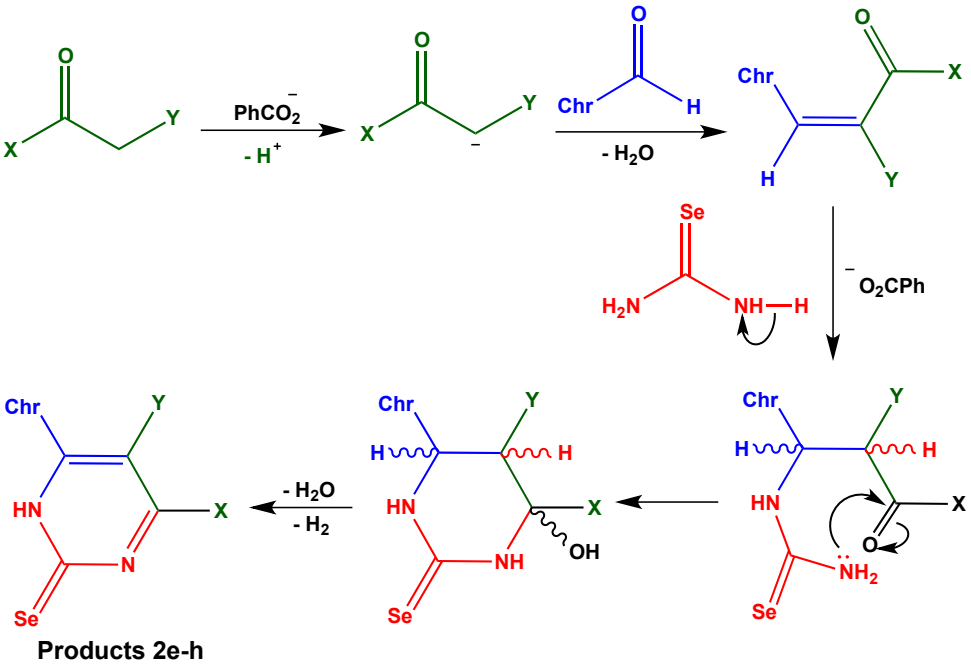
Entry	Active methylene	Product	Time (h)	Yield (%)
1	**$NCCH_2CN$**	 **2a**	1.5	94
2	**$NCCH_2CONH_2$**	 **2b**	1.5	92
3	**$NCCH_2CO_2Et$**	 **2c**	2	90
4	**$NCCH_2Ph$**	 **2d**	1.5	92
5	**$MeCOCH_2COMe$**	 **2e**	2.5	88

6	**$PhCOCH_2COMe$**	 **2f**	1.5	90
7	**$MeCOCH_2CO_2Et$**	 **2g**	1.5	89
8	**$PhCOCH_2CO_2Et$**	 **2h**	1.5	92

The formation of the products **2a-h** in the present reaction is expected to involve the following tandem reaction mechanism: Knoevenagel condensation 3-formylchromone (**1**) and active methylene compound to give the corresponding arylidene. *Michael* addition of selenourea with the arylidene intermediate followed by an intramolecular cyclization by addition or dehydration, then removal of a molecule of hydrogen in subsequent step to give the final products (Schemes 2 and 3).[37,38] The structures of all the synthesized compounds were established by elemental analysis and spectroscopic tools (See experimental section).



Scheme 2



Scheme 3

In summary, we have demonstrated a simple, efficient, and a novel one-pot three-component protocol for the synthesis of some novel chromonyl 2-selenoxo-1,2-dihydropyrimidine derivatives in a mixture of ethanol and water using sodium benzoate as a readily available, inexpensive, and efficient catalyst. The advantages offered by this method are simple reaction condition, short reaction time, ease of product isolation, and excellent yields.

ANTICANCER ACTIVITIES

The anticancer activities of the synthesized compounds against five human cancer cell lines namely lung cancer cells (A594), breast cancer cells (MCF-7), liver cancer cells (HepG2) colon carcinoma cells (HCT116) and prostate cancer cells (PC3) as well as human normal melanocyte (HFB4) were measured in *vitro* by MTT method.[39] The anticancer activities were expressed by median growth inhibitory concentration (IC_{50}) as shown in Table 5. The results revealed that the synthesized compounds did not exert any activity against colon carcinoma cells (HCT116) and prostate cancer cells (PC3). Compounds **2e-2g** displayed weak anticancer properties comparing with the drug doxorubicin against lung cancer cells (A594), breast cancer cells (MCF-7) and liver cancer cells (HepG2). Moreover, compound **2h** revealed moderate anticancer activity against these three cells with IC_{50} values 8.85 ± 1.12 ($IC_{50\ drug}$= 3.41 ± 0.38), 9.80 ± 1.20 ($IC_{50\ drug}$= 3.11 ± 0.33), and 10.30 ± 1.23 ($IC_{50\ drug}$= 2.90 ± 0.32) μg/mL, respectively. On the other hand, within the series of the synthesized compounds, we found that compounds **2a-d** displayed the most promising anticancer activities against lung cancer cells (A594), breast cancer cells (MCF-7) and liver cancer cells (HepG2) as compared to doxorubicin as the standard drug, with no toxic effect on the normal cells. The IC_{50} values of the compound **2c** were 4.72 ± 0.60 ($IC_{50\ drug}$= 3.41 ± 0.38), 3.3 2 ± 0.29 ($IC_{50\ drug}$= 3.11 ± 0.33) and 3.72 ± 0.43 ($IC_{50\ drug}$= 2.90 ± 0.32) μg/mL against these three cell cancer lines, respectively, while the IC_{50} values of compound **2d** were 4.65 ± 0.38 ($IC_{50\ drug}$= 3.41 ± 0.38), 3.29 ± 0.33 ($IC_{50\ drug}$= 3.11 ± 0.33) and 3.10 ± 0.41 ($IC_{50\ drug}$= 2.90 ± 0.32) μg/mL, respectively. In addition, the IC_{50} values of compound **2b** against these three cell lines were 4.30 ± 0.60, 3.65 ± 0.48 and 3.96 ± 0.48 μg/mL, respectively. Compound **2a** exhibited the best anticancer properties would be the most promising for further development as anticancer agents whereas their IC_{50} were 4.05 ± 0.60 μg/mL for lung cancer cells (A594), 3.13 ± 0.42 μg/mL for breast cancer cells (MCF-7) and 3.50 ± 0.39 μg/mL for liver cancer cells (HepG2) in comparing with the drug doxorubicin. The screening results indicated the combination of chromone ring with substituted selenoxopyrimidines showed variable anticancer activities in comparing with reference drug. Moreover, the introduction of amino group to chromonyl selenoxopyrimidine frame caused the excellent anticancer properties. In addition, the presence of the nitrile or phenyl group adjacent to amino group in compounds **2a**,**d** displayed the highest anticancer effectives.

Table 5. The anticancer activities of the synthesized compounds **2a-h** against different cell lines using MTT method.

Compound	IC_{50} (µg/mL)					
	A594	MCF-7	HepG2	HCT116	PC3	HFB4
2a	4.05 ± 0.60	3.13 ± 0.42	3.50 ± 0.39	65.22 ± 8.10	38.19 ± 4.80	86.45 ± 9.16
2b	4.30 ± 0.60	3.65 ± 0.48	3.96 ± 0.48	41.19 ± 5.86	36.20 ± 4.11	82.65 ± 9.18
2c	4.72 ± 0.60	3.32 ± 0.29	3.72 ± 0.43	28.66 ± 4.06	49.70 ± 6.00	84.11 ± 9.28
2d	4.65 ± 0.38	3.29 ± 0.33	3.10 ± 0.41	37.61 ± 5.16	41.72 ± 5.80	85.33 ± 8.76
2e	16.80±1.93	18.96 ± 2.11	17.88 ± 2.74	32.60 ± 4.81	48.89 ± 6.00	52.60 ± 7.11
2f	16.80 ± 1.86	18.11 ± 2.00	22.13 ± 2.70	62.14 ± 7.62	57.37 ± 6.38	45.38 ± 4.80
2g	14.65 ± 1.60	19.64 ± 1.79	15.62 ± 1.75	32.60 ± 4.19	24.39 ± 3.90	60.17 ± 7.24
2h	8.85 ± 1.12	9.80 ± 1.20	10.30 ± 1.23	44.00 ± 5.11	37.20 ± 4.72	75.00 ± 8.23
Doxorubicin	3.41 ± 0.38	3.11 ± 0.33	2.90 ± 0.32	4.22 ± 0.60	4.86 ± 0.73	88.00 ± 8.91

Data were expressed as Mean ± Standard error (S.E.) of three independent experiments; N.A. is no activity.

EXPERIMENTAL

The melting points were determined in an open capillary tube on a digital Stuart SMP-3 apparatus. Infrared spectra were measured on FT-IR (Nicolet iS10) spectrophotometer using KBr disks and Perkin-Elmer 293 spectrophotometer using KBr disks. ^{1}H- and ^{13}C-NMR spectra were measured on Gemini-300BB spectrometer (400 and 100 MHz), using DMSO-d_6 as a solvent and TMS (δ) as an internal standard. Mass spectra were recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 ev and 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.

General procedure for reaction of 3-formylchromone (1), selenourea and active methylene compounds: Synthesis of the target products 2a-h.

A mixture of 3-formylchromone (**1**) (0.35 g, 2 mmol), selenourea (0.24 g, 2 mmol) and active methylene compounds (2 mmol) in EtOH (7 mL) and distilled H_2O (21 mL) containing a catalytic amount of $PhCO_2Na$ (0.042 g, 15 mol%), was stirred and heated under reflux at 70-80 ^oC for the appropriate time. The formed solids were filtered off, washed with water, and crystallized from diluted EtOH.

4-Amino-6-(4-oxo-4H-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidine-5-carbonitrile (**2a**)**:** Beige solid; mp 240–242 ^oC. IR (KBr), (*v* max, cm^{-1}): 3364, 3201 (NH_2), 3105 (NH), 2189 (C≡N), 1648 ($C=O_{pyrone}$), 1611 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.84 (s, 2H, NH_2), 7.35 (t, 1H, *J*=6.8 Hz, H–$6_{chromone}$), 7.46 (d, 1H, *J*=7.2 Hz, H–$8_{chromone}$), 7.68 (t, 1H, *J*=6.8 Hz, H–$7_{chromone}$), 8.03 (d, 1H, *J*=7.6 Hz, H–$5_{chromone}$), 8.51 (s, 1H, H–$2_{chromone}$), 10.98 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-d_6): 65.2, 112.3,

115.3, 118.4, 122.3, 123.6, 125.7, 134.6, 150.2, 153.6, 156.2, 158.1, 174.3, 181.0. MS (*m/z*, I%): 344 (M^+, 15%). Anal. Calcd for $C_{14}H_8N_4O_2Se$ (343.98): C, 49.00%; H, 2.35%; N, 16.32%. Found: C, 48.68%; H, 2.24%; N, 16.09%.

4-Amino-6-(4-oxo-4H-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidine-5-carboxamide **(2b):** Pale yellow solid; mp 236–237 ºC. IR (KBr), (*v* max, cm^{-1}): 3395, 3311, 3206, 3184 (NH_2), 3112 (NH), 1668 ($C=O_{amide}$), 1642 ($C=O_{pyrone}$), 1605 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.53 (s, 2H, NH_2), 7.32 (t, 1H, *J*=8.0 Hz, H–$6_{chromone}$), 7.49 (d, 1H, *J*=7.2 Hz, H–$8_{chromone}$), 7.74 (t, 1H, *J*=7.2 Hz, H–$7_{chromone}$), 8.01 (d, 1H, *J*=8.0 Hz, H–$5_{chromone}$), 8.43 (s, 1H, H–$2_{chromone}$), 9.06 (br, 2H, NH_2), 11.06 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-d_6): 72.3, 115.9, 119.3, 121.8, 123.9, 126.1, 135.3, 150.4, 152.9, 155.8, 157.9, 168.4, 175.2, 180.6. MS (*m/z*, I%): 362 (M^+, 12%). Anal. Calcd for $C_{14}H_{10}N_4O_3Se$ (361.99): C, 46.55%; H, 2.79%; N, 15.51%. Found: C, 46.19%; H, 2.53%; N, 15.28%.

Ethyl 4-amino-6-(4-oxo-4H-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidine-5-carboxylate **(2c):** Yellow solid; mp 203–204 ºC. IR (KBr), (*v* max, cm^{-1}): 3315, 3216 (NH_2), 3180 (NH), 1690 ($C=O_{ester}$), 1639 ($C=O_{pyrone}$), 1606 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6): 1.12 (t, 3H, *J*=7.2 Hz, CH_3), 3.98 (q, 2H, *J*=7.2 Hz, CH_2), 7.10 (s, 2H, NH_2), 7.39 (t, 1H, *J*=6.4 Hz, H–$6_{chromone}$), 7.53 (d, 1H, *J*=7.6 Hz, H–$8_{chromone}$), 7.76 (t, 1H, *J*=7.6 Hz, H–$7_{chromone}$), 8.08 (d, 1H, *J*=7.2 Hz, H–$5_{chromone}$), 8.42 (s, 1H, H–$2_{chromone}$), 10.46 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-d_6): 15.1, 59.6, 85.7, 117.8, 119.3, 123.6, 125.4, 126.9, 133.9, 151.2, 153.4, 158.2, 159.6, 164.3, 174.8, 179.5. MS (*m/z*, I%): 391 (M^+, 10%). Anal. Calcd for $C_{16}H_{13}N_3O_4Se$ (391.01): C, 49.24%; H, 3.36%; N, 10.77%. Found: C, 49.02%; H, 3.11%; N, 10.49%.

6-Amino-4-(4-oxo-4H-chromen-3-yl)-5-phenyl-2-selenoxo-2,3-dihydropyrimidine **(2d):** Yellow solid; mp 262–263 ºC. IR (KBr), (*v* max, cm^{-1}): 3260, 3195 (NH_2), 3119 (NH), 1638 ($C=O_{pyrone}$), 1606 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.02 (s, 2H, NH_2), 7.13–7.39 (m, 4H, Ph–H and H–$6_{chromone}$), 7.49–7.60 (m, 3H, Ph–H and H–$8_{chromone}$), 7.72 (t, 1H, *J*=7.2 Hz, H–$7_{chromone}$), 8.06 (d, 1H, *J*=6.8 Hz, H–$5_{chromone}$), 8.49 (s, 1H, H–$2_{chromone}$), 11.21 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-d_6): 95.2, 116.3, 119.8, 123.2, 124.1, 126.3, 131.2, 135.7, 127.9, 129.1, 128.2, 148.1, 150.3, 154.1, 157.2, 173.8, 179.4. MS (*m/z*, I%): 395 (M^+, 18%). Anal. Calcd for $C_{19}H_{13}N_3O_2Se$ (395.02): C, 57.88%; H, 3.32%; N, 10.66%. Found: C, 57.53%; H, 3.16%; N, 10.32%.

5-Acetyl-6-methyl-4-(4-oxo-4H-chromen-3-yl)-2-selenoxo-2,3-dihydropyrimidine **(2e):** white solid; mp 222–224 ºC. IR (KBr), (*v* max, cm^{-1}): 3202 (NH), 1658 ($C=O_{acetyl}$), 1637 ($C=O_{pyrone}$), 1612 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6): 2.03 (s, 3H, CH_3), 2.34 (s, 2H, CH_3), 7.23 (t, 1H, *J*=6.8 Hz, H–$6_{chromone}$), 7.42 (d, 1H, *J*=7.2 Hz, H–$8_{chromone}$), 7.68 (t, 1H, *J*=7.2 Hz, H–$7_{chromone}$), 8.11 (d, 1H, *J*=7.6 Hz, H–$5_{chromone}$), 8.53 (s, 1H, H–$2_{chromone}$), 10.92 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-d_6): 19.3, 28.4, 83.7, 117.6, 120.1, 122.9, 124.3, 125.9, 134.6, 148.3, 151.2, 153.6, 159.2, 171.3, 176.2, 181.8. MS (*m/z*, I%): 360 (M^+, 11%). Anal. Calcd for $C_{16}H_{12}N_2O_3Se$ (360.00): C, 53.49%; H, 3.37%; N, 7.80%. Found: C,

53.21%; H, 3.16%; N, 7.59%.

5-Acetyl-4-(4-oxo-4H-chromen-3-yl)-6-phenyl-2-selenoxo-2,3-dihydropyrimidine **(2f):** pale grey solid; mp 257–259 °C. IR (KBr), (*v* max, cm^{-1}): 3310 (br, NH), 1659 ($C=O_{acetyl}$), 1643 ($C=O_{pyrone}$), 1611 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 2.42 (s, 2H, CH_3), 7.11 (t, 1H, *J*=6.4 Hz, H–$6_{chromone}$), 7.39–7.54 (m, 3H, Ph–H and H–$8_{chromone}$), 7.72 (t, 1H, *J*=7.6 Hz, H–$7_{chromone}$), 7.80–8.19 (m, 4H, Ph–H and H–$5_{chromone}$), 8.36 (s, 1H, H–$2_{chromone}$), 11.43 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-*d*$_6$): 29.3, 87.4, 118.3, 119.8, 122.5, 123.9, 125.4, 127.1, 129.2, 130.1, 132.5, 135.4, 149.8, 154.0, 156.2, 158.6, 172.4, 176.8, 182.1. MS (*m/z*, I%): 422 (M^+, 21%). Anal. Calcd for $C_{21}H_{14}N_2O_3Se$ (422.02): C, 59.87%; H, 3.35%; N, 6.65%. Found: C, 59.56%; H, 3.18%; N, 6.34%.

Ethyl 4-methyl-6-(4-oxo-4H-chromen-3-yl)-2-selenoxo-1,2-dihydropyrimidine-5-carboxylate **(2g):** white solid; mp 215–217 °C. IR (KBr), (*v* max, cm^{-1}): 3142 (NH), 1684 ($C=O_{ester}$), 1636 ($C=O_{pyrone}$), 1598 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 1.16 (t, 3H, *J*=7.2 Hz, CH_3), 2.31 (s, 3H, CH_3), 4.03 (q, 2H, *J*=7.2 Hz, CH_2), 7.13 (t, 1H, *J*=8.0 Hz, H–$6_{chromone}$), 7.33 (d, 1H, *J*=7.6 Hz, H–$8_{chromone}$), 7.59 (t, 1H, *J*=7.6 Hz, H–$7_{chromone}$), 8.13 (d, 1H, *J*=7.2 Hz, H–$5_{chromone}$), 8.48 (s, 1H, H–$2_{chromone}$), 10.72 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-*d*$_6$): 13.9, 22.1, 60.3, 96.2, 118.5, 119.8, 123.4, 125.1, 126.9, 134.4, 149.3, 150.6, 152.3, 156.1, 166.4, 174.8, 179.7. MS (*m/z*, I%): 390 (M^+, 16%). Anal. Calcd for $C_{17}H_{14}N_2O_4Se$ (390.01): C, 52.45%; H, 3.63%; N, 7.20%. Found: C, 52.23%; H, 3.34%; N, 6.92%.

Ethyl 6-(4-oxo-4H-chromen-3-yl)-4-phenyl-2-selenoxo-1,2-dihydropyrimidine-5-carboxylate **(2h):** Pale yellow solid; mp 219–220 °C. IR (KBr), (*v* max, cm^{-1}): 3154 (NH), 1678 ($C=O_{ester}$), 1634 ($C=O_{pyrone}$), 1603 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d*$_6$): 1.03 (t, 3H, *J*=6.8 Hz, CH_3), 3.83 (q, 2H, *J*=6.8 Hz, CH_2), 7.06 (t, 1H, *J*=6.8 Hz, H–$6_{chromone}$), 7.19–7.48 (m, 3H, Ph–H and H–$8_{chromone}$), 7.66 (t, 1H, *J*=7.2 Hz, H–$7_{chromone}$), 7.73–8.08 (m, 4H, Ph–H and H–$5_{chromone}$), 8.56 (s, 1H, H–$2_{chromone}$), 11.12 (s, 1H, NH). ^{13}C-NMR (δ ppm, DMSO-*d*$_6$): 14.4, 61.3, 99.2, 119.1, 120.3, 123.6, 124.8, 126.4, 127.4, 129.3, 130.1, 133.6, 135.4, 151.1, 153.6, 155.7, 158.1, 167.3, 174.8, 181.5. MS (*m/z*, I%): 452 (M^+, 9%). Anal. Calcd for $C_{22}H_{16}N_2O_4Se$ (452.03): C, 58.55%; H, 3.57%; N, 6.21%. Found: C, 58.24%; H, 3.38%; N, 6.02%.

ANTICANCER ACTIVITY ASSAY

The anticancer activity of the tested compounds was measured *in vitro* using MTT assay according to Mosmann.[39] Briefly, cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg/mL immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds and doxorubicin were added to the cells. Three wells were prepared for each individual dose. Monolayer cells were incubated

with the compounds for 24 h at 37 °C and in atmosphere of 5% CO_2. After 24 h cells were fixed, washed, and for staining 40 μL MTT (2.5 mg/mL) were added to each well and incubated for further 4 h at 37 ºC under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 μL 10% sodium dodecyl sulphate in deionized water was added to each well and incubated overnight at 37 ºC. Color intensity was measured in an ELISA reader at 570 nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 5. The results were compared to the effect of the reference drug, doxorubicin.[40-42]

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REFERENCES

1. D. R. Garud, M. Koketsu, and H. Ishihara, *Molecules*, 2007, **12**, 504.
2. W. D. Pfeiffer, H. Roßberg, N. Kelzhanova, A. T. Saginayev, A. Villinger, and P. Langer, *Heterocycles*, 2014, **88**, 1397.
3. A. Bodtke, M. Kandt, W. D. Pfeiffer, and P. Langer, *Phosphorus*, *Sulfur Silicon Relat. Elem.*, 2007, **182**, 209.
4. H. Below, W. D. Pfeiffer, K. Geisler, M. Lalk, and P. Langer, *Eur. J. Org. Chem.*, 2005, 3637.
5. K. Geisler, A. Künzler, H. Below, E. Bulka, W. D. Pfeiffer, and P. Langer, *Synthesis*, 2004, 97.
6. K. P. Bhabak and G. Mugesh, *Chem. Res.*, 2010, **43**, 1408.
7. R. Sinha and K. El-Bayoumy, *Curr. Cancer Drug Targets*, 2004, **4**, 13.
8. H. Rikiishi, *J. Bioenerg. Biomembr.*, 2007, **39**, 91.
9. S. Y. Choi, Y. O. Jo, M. Koketsu, H. Ishihara, S. H. Kim, and S. Y. Kim, *J. Korean Soc. Appl. Biol. Chem.*, 2009, **52**, 371.
10. E. Block, S. Bird, J. F. Tyson, P. C. Uden, X. Zhang, and E. Denoyer, *Phosphorus*, *Sulfur Silicon Relat. Elem.*, 1998, **136**, 1.
11. H. J. Ahn, M. Koketsu, E. M. Yang, Y. M. Kim, H. Ishihara, and H. O. Yang, *J. Cell. Biochem.*, 2006, **99**, 807.
12. A. Nishina, A. Sekiguchi, R. Fukumoto, M. Koketsu, and S. Furukawa, *Biochem. Biophys. Res. Commun.*, 2007, **352**, 360.
13. E. H. Lee, Y. J. Lim, S. K. Ha, T. H. Kang, M. Koketsu, C. H. Kang, S. Y. Kim, and J. H. Park, *J.*

Pharm. Pharmacol., 2010, **62**, 352.

14. S. S. Tulenin, V. F. Markov, L. N. Maskaeva, and M. V. Kuznetsov, *Russ. J. Inorg. Chem*., 2016, **61**, 488.
15. S. Sogabe, H. Ando, M. Koketsu, and H. Ishihara, *Tetrahedron Lett*., 2006, **47**, 6603.
16. H. Takahashi, A. Nishina, R. H. Fukumoto, H. Kimura, M. Koketsu, and H. Ishihara, *Life Sci.*, 2005, **76**, 2185.
17. J. I. Olsen, G. B. Plata, J. M. Padrón, Ó. López, M. Bols, and J. G. Fernández-Bolaños, *Eur. J. Med. Chem.*, 2016, **123**, 155.
18. L. L. Romero-Hernandez, P. Merino-Montiel, S. Montiel-Smith, S. Meza-Reyes, J. L. Vega-Báez, I. Abasolo, S. Schwartz, O. López, and J. G. Fernández-Bolaños, *Eur. J. Med. Chem.*, 2015, **99**, 67.
19. W. D. Pfeiffer, K. D. Ahlers, A. Falodun, A. Villinger, and P. Langer, *Phosphorus*, *Sulfur Silicon Relat. Elem.*, 2014, **189**, 324.
20. E. Klein, S. De Bonis, B. Thiede, D. Skoufias, F. Kozielski, and L. Lebeau, *Bioorg. Med. Chem.*, 2007, **15**, 6474.
21. S. Kolb, O. Mondesert, M. L. Goddard, D. Jullien, B. O. Villoutreix, B. Ducommun, C. Garbay, and E. Braud, *ChemMedChem*, 2009, **4**, 633.
22. F. A. R. Barbosa, T. Siminski, R. F. S. Canto, G. M. Almeida, N. S. R. S. Mota, F. Ourique, R. C. Pedrosa, and A. L. Braga, *Eur. J. Med. Chem*., 2018, **155**, 503.
23. V. Samdhiana, S. K. Bhatiaa, and B. Kaura, *Russ. J. Org. Chem*., 2019, **55**, 1041.
24. J. D. Hepworth, A. J. Boulton, and A. McKillop, *Comprehensive Heterocyclic Chemistry 3*, Pergamon Press, Oxford, 1984, pp. 835–840.
25. R. S. Keri, S. Budagumpi, R. K. Pai, and R. G. Balakrishna, *Eur. J. Med. Chem.*, 2014, **78**, 340.
26. Y. D. Duan, Y.Y Jiang, F. X. Guo, L. X. Chen, L. L Xu, W. Zhang, and B. Liu, *Fitoterapia*, 2019, **135**, 114.
27. 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, *Inter. Immunopharm.*, 2019, **72**, 31.
28. M. Kuroda, S. Uchida, K. Watanabe, and K. Mimaki, *Phytochemistry*, 2009, **70**, 288.
29. T. Zhou, Q. Shi, and K. H. Lee, *Tetrahedron Lett.*, 2010, **51**, 4382.
30. T. E. Ali, M. A. Assiri, and I. S. Yahia, *Heterocycles*, 2019, **98**, 1265.
31. T. E. Ali, M. A. Assiri, H. M. El-Shaaer, A. M. Fouda, M. M. Hassan, and N. M. Hassanin, *Heterocycles*, 2019, **98**, 681.
32. A. M. Fouda, M. A. Assiri, and T. E. Ali, *Phosphorus*, *Sulfur Silicon Relat. Elem.*, 2020, **195**, 324.
33. T. Sugino and K. Tanaka, *Chem. Lett.*, 2001, **30**, 110.

34. F. Vitório, T. M. Pereira, R. N. Castro, G. P. Guedes, C. S. Graebin, and A. E. Kümmerle, *New J. Chem.*, 2015, **39**, 2323.
35. U. M. Lindström, *Chem. Rev.*, 2002, **102**, 2751.
36. C. Capello, U. Fischer, and K. Hungerbühler, *Green Chem.*, 2007, **9**, 927.
37. S. Karami, A. R. Momeni, and J. Albadi, *Res. Chem. Intermed*., 2019, **45**, 3395.
38. A. Omar and K. Ablajan, *Green Chem. Lett. Rev*., 2019, **12**, 1.
39. T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.
40. M. M. Ali, A. Mahmoud, A. Abdel-Halim, and A. Fyiad, *Asian J. Pharm. Clin. Res.*, 2014, **7**, 168.
41. A. F. M. Ismail, M. M. Ali, and L. F. M. Ismail, *J. Photochem. Photobiol. B*, 2014, **138**, 99.
42. T. El-Malah, H. F. Nour, O. Dehbi, F. M. E. Abdel-Megeid, E. A. Ishak, R. M. Shaker, A. E. Mahmoud, M. M. Ali, and S. M. Soliman, *Curr. Org. Chem.*, 2018, **22**, 2300.