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ONE-POT AND THREE-COMPONENT SYNTHESIS OF SOME NOVEL FUNCTIONALIZED CHROMONYL PYRIDO[2,3-*d*]PYRIMIDINES AS ANTICANCER AGENTS

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Abstract – A facile and efficient method for the construction of functionalized chromonyl pyrido[2,3-*d*]pyrimidines *via* a one-pot, three-component reaction of 6-aminothiouracil and 4,6-diaminopyrimidine-2(1*H*)-thione with 4-oxo-4*H*-chromene-3-carboxaldehyde in the presence of different nitrile active methylene compounds in distillated water at 70 ºC without using a catalyst was achieved. The methodology displayed excellent yields and simple workup procedure. The targeted compounds were assessed for their in *vitro* anticancer activity against mammary gland breast cancer cell line (MCF-7), liver cancer (HepG-2), and human colon cancer (HCT-116) by using sulphorhodamine B assay (SRB) method, while doxorubicin, was utilized as standard reference drug. Compounds **4b** and **6a** were the best potent cytotoxic agents towards liver (HepG-2) and colon (HCT-116) compared with doxorubicin as a reference drug with IC_{50} values ranging from 1.1 to 1.8 μg/mL.

INTRODUCTION

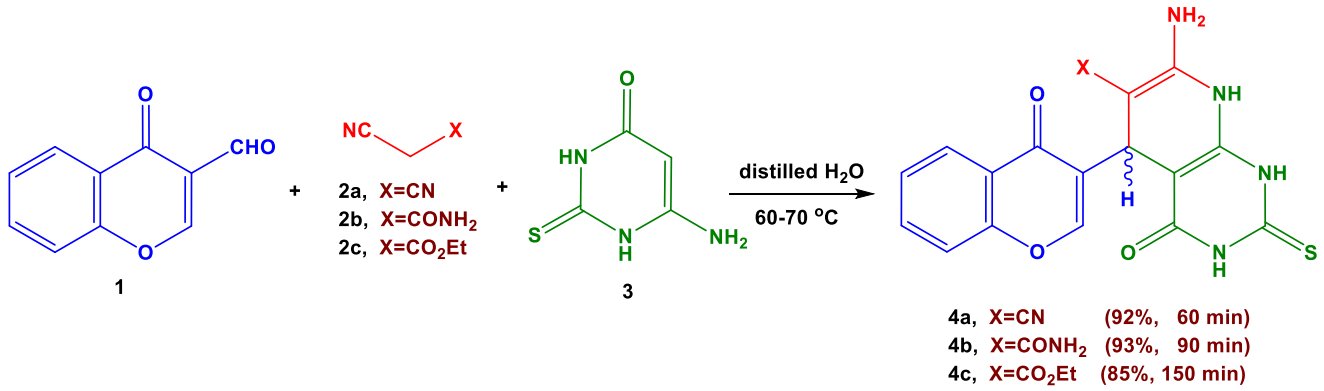
Chromones are the most important heterocyclic structure of natural compounds of plant origin and act as the base for flavonoid structures.[1] Owing to the low toxicity to mammals and the strong solubility in water, the chromone ring is considered a desirable building block for the production of pharmacologically important compounds.[2-4] On the other hand, pyridopyrimidines demonstrated valuable biological and therapeutic importance, such as antihistamine, anti-inflammatory, antihypertensive, antimicrobial, anticancer, antimalarial and CNS depressant properties.[5-13] Furthermore, some specific applications have also been mentioned for some pyrido[2,3-*d*]pyrimidines such as enzyme inhibitors of dihydrofolate reductase,[14] diarrhea,[15] cycline-dependent kinase 4,[16] and adenosine kinase.[17] In recent years, various methods have been reported for construction of pyridopyrimidines.[18] These were prepared *via* multicomponent reaction in the presence or absence of acid catalyst.[19-21] Subsequently, the development of safe formulations utilizing eco-friendly solvents and catalysts for pyrido[2,3-*d*]pyrimidine synthesis is also extremely intrigued. Thus, design of pyridopyrimidine derivatives, in which these scaffolds are merged with chromone moiety, could provide novel molecular frame that may exhibit the biological properties of each moiety. As part of our ongoing work on the synthesis of novel heterocycles bearing chromone moiety by classical and one-pot multicomponent reactions,[22-25] we report one-pot, three component method for novel functionalized pyrido[2,3-*d*]pyrimidines bearing chromone ring in high yields. The method depended on the reaction of a mixture of 4-oxo-4*H*-chromene-3-carboxaldehyde with nitrile active methylene compounds and cyclic active methylene compounds in distilled water without any catalyst.

RESULTS AND DISCUSSION

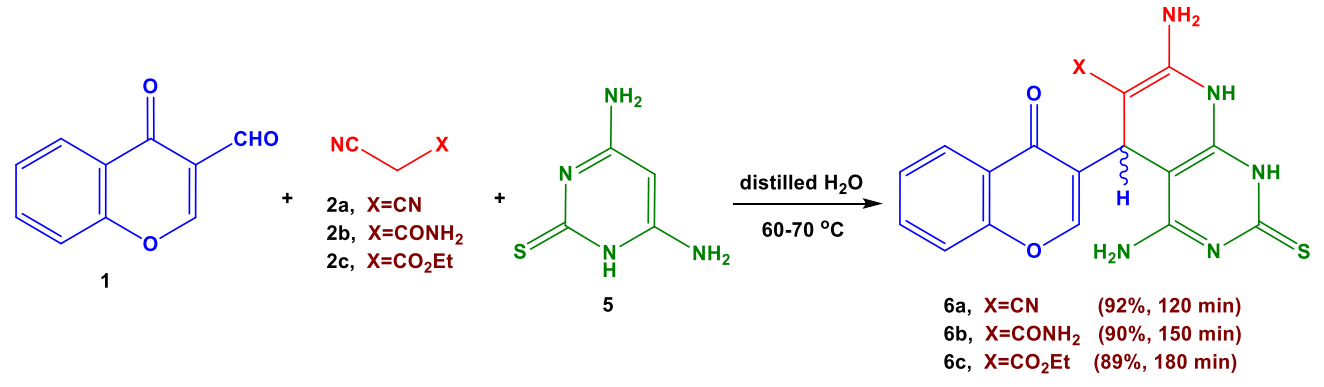
Using water as a solvent in chemical reactions is not only cheap and environmentally safe but also provides advantages over synthetic solvents.[26,27] As indicated in Scheme 1, equimolar amounts of 4-oxo-4*H*-chromene-3-carboxaldehyde (**1**), malononitrile (**2a**) and 6-aminothiouracil (**3**) underwent reaction in water at 60-70 ºC (3-formylchromone and malononitrile are mixed first for 15 minutes, and then 6-aminothiouracil was added) to produce 7-amino-4-oxo-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,3,4,5,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (**4a**) in 92% yield in 1 h.

Under similar conditions, 7-amino-4-oxo-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,3,4,5,8-hexahydro-pyrido[2,3-*d*]pyrimidines **4b-c** and 4,7-diamino-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,5,8-tetrahydro-pyrido[2,3-*d*]pyrimidines **6a-c** were separated in 85-93% yields through reaction 6-aminothiouracil (**3**) or 4,6-diaminopyrimidine-2(1*H*)-thione (**5**) with 4-oxo-4*H*-chromene-3-carboxaldehyde (**1**) in the presence of different nitrile active methylene compounds **2a-c** (Schemes 1 and 2).

The structures of the pyrido[2,3-*d*]pyrimidine derivatives **4a-c** and **6a-c** were elucidated using MS, IR, ^{1}H- and ^{13}C-NMR spectroscopy. The IR spectra of all products recorded the absorption bands of NH_2, NH and $C{=}O_{pyrone}$ groups at 3469–3120 and 1665–1636 cm^{-1}, respectively.[28] In the ^{1}H-NMR spectra, NH_2 and NH protons of the products showed singlets at δ 6.96–8.91 and 10.00–13.19 ppm, respectively. The proton H–4 of pyridine rings in **4a-c** and **6a-c** were located at around δ 6.44–6.56 ppm.[29] The proton H–2 of the chromone ring of the products appeared as singlets at δ 8.28–8.66 ppm. In the ^{13}C-NMR spectra of **4a-c** and **6a-c** signals appeared at regions δ 26.3–34.3 ppm were ascribed to C–4 of pyridine rings while that appeared at range δ 187.7–190.7 ppm due to C=S groups in all products. The signals that were showed at δ 175.9–179.9 ppm due to the carbon atoms of $C{=}O_{pyrone}$ groups.[30] The elemental analysis and spectral data of the synthesized compounds were in accordance with their proposed structures.



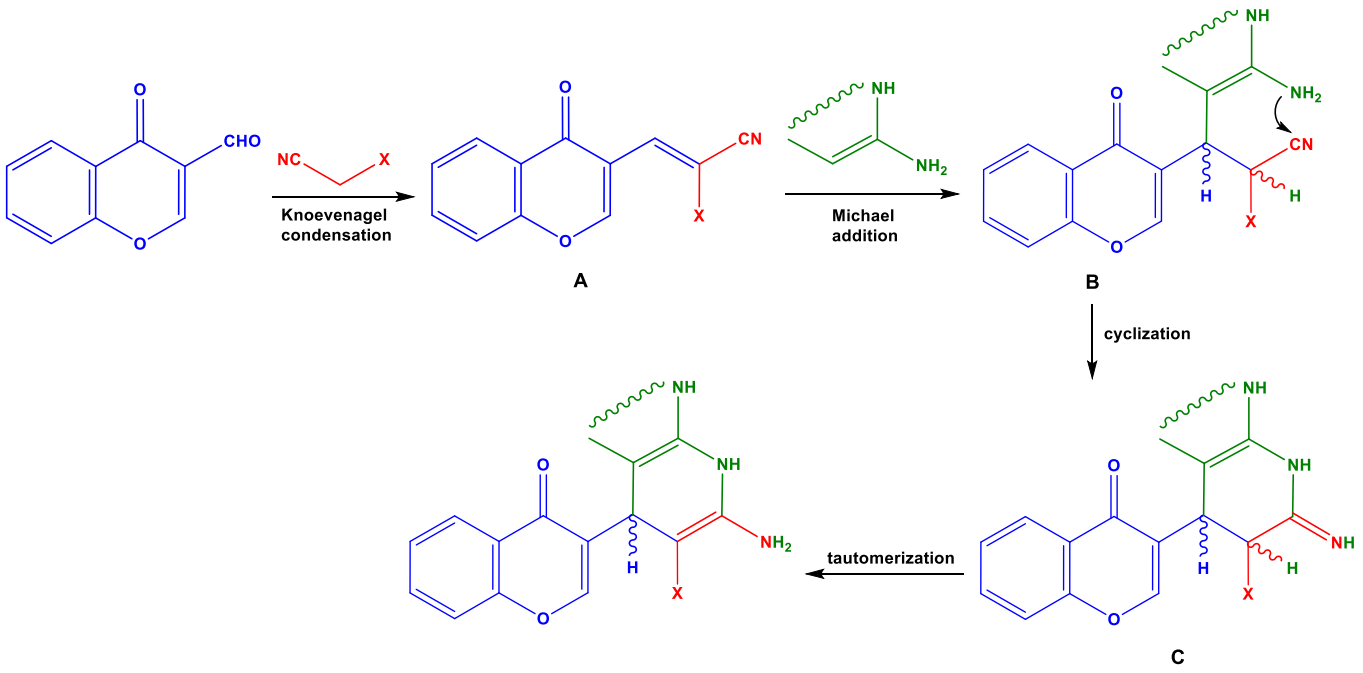
Scheme 1



Scheme 2

A suggested reaction mechanism for the synthesis of pyrido[2,3-*d*]pyrimidine derivatives by one-pot three-component technique was depicted in Scheme 3. In the first step, condensation process between 4-oxo-4*H*-chromene-3-carboxaldehyde (**1**) and nitrile active methylene compounds **2a-c** to form intermediate **A** *via* Knoevenagel condensation. Subsequently, the carbon atom of active methylene

compound **2** underwent Michael addition on the intermediate **A** to produce an acyclic intermediate **B**. The latter intermediate underwent self-cyclization followed rearrangement to furnish the desired products **4a-d** and **6a-d**, in excellent yields (Scheme 3).[31,32]



Scheme 3

ANTICANCER ACTIVITIES

The antiproliferative activities of all synthesized compounds **4a-c** and **6a-c** were evaluated *in vitro* against the three human cancer cell lines, mammary gland breast cancer (MCF-7), liver cancer (HepG-2) and human colon cancer (HCT-116) in comparison with doxorubicin as reference drug, using the standard sulphorhodamine B (SRB) assay.[33] The *in vitro* cytotoxicity evaluation was achieved using different concentrations. The results were expressed as growth inhibitory concentration (IC_{50}) values, where the necessitated concentration produced a 50% inhibition of cell growth after 72 h of incubation, compared to the untreated cell control. The IC_{50} values are summarized in Table 1. The relation between the surviving cells with different concentrations of tested compounds were plotted to get the survival curve for each type of cancer cell line after 72 h as depicted in Figure 1. The screened synthesized compounds against tumor cell lines (MCF-7, HepG-2 and HCT-116) showed variable cytotoxic activities (Table 1 and Figure 1). Compounds **4a** and **4b** have a moderate effects on MCF-7 cells while **4c** and **6c** had similar effects on HePG-2 cells as well as **4c** and **6b** against HCT-116 cells. On the other hand, both compounds **4c** and **6c** showed acceptable toxic effects towards HCT-116 cells. In addition, compounds **4a** and **6b** have good activities towards hepatocellular carcinoma (HepG-2), while both **4a** and **6c** had acceptable significant

cytotoxicity on colon cancer (HCT-116). The significantly demonstration was exhibited by the products **4b** and **6a** against HePG-2 with IC_{50} 1.5 and 1.1 µg/mL and HCT-116 cells with IC_{50} 1.8 and 1.6 µg/mL, respectively. Moreover, the products **6a** and **6b** had a promising cytotoxic effects on breast cancer (MCF-7) with IC_{50} around 3.7 µg/mL. In general, the SAR study revealed crucial structural requirements, which enhanced the potency of the chromonyl pyrido[2,3-*d*]pyrimidines **4a-c** and **6a-c** (Table 1 and Figure 1). It is cleared that 7-amino-4-oxo-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,3,4,5,8-hexahydropyrido[2,3-*d*]-pyrimidine-6-carboxamide (**4b**) and 4,7-diamino-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,5,8-tetrahydro-pyrido[2,3-*d*]pyrimidine-6-carbonitrile (**6a**) have the most significant impacts towards all the used cancer cells in comparison with the other products and doxorubicin expect in case MCF-7 cell.

Table 1. The IC_{50} (µg/mL) of the synthesized compounds against different tumor cell lines

Compound	IC_{50} (µg/mL)		
	MCF-7	**HepG-2**	**HCT-116**
4a	7.4 ± 0.8	3.4 ± 0.7	2.8 ± 0.6
4b	8.2 ± 0.5	1.5 ± 0.13	1.8 ± 0.3
4c	4.3 ± 0.1	7.5 ± 2.1	5.4 ± 0.3
6a	3.7 ± 0.4	1.1 ± 0.04	1.6 ± 0.7
6b	3.7 ± 0.5	2.8 ± 0.2	5.1 ± 0.9
6c	4.5 ± 0.7	6.8 ± 0.6	3.6 ± 0.4
Doxorubicin	1.4 ± 0.07	1.6 ± 0.04	2.0 ± 0.03

CONCLUSION

We suggested a simple and efficient eco-friendly strategy for the construction of novel functionalized chromonyl pyrido[2,3-*d*]pyrimidines *via* condensation of 6-aminothiouracil or 4,6-diaminopyrimidine-2(1*H*)-thione with 4-oxo-4*H*-chromene-3-carboxaldehyde in the presence of different nitrile active methylene compounds using distillated water. According to our knowledge, this is the first report on using water for the synthesis of this type of compounds. All the synthesized compounds were tested as anticancer agents against three human cancer cell lines: breast (MCF-7), liver (HepG-2) and colon (HCT-116). The results have shown that some of the compounds exhibited significant activity against all cancer cell lines. Especially, compounds **4b** and **6a** were the best potent cytotoxic agents towards liver (HepG-2) and colon (HCT-116) compared with doxorubicin as a reference drug.

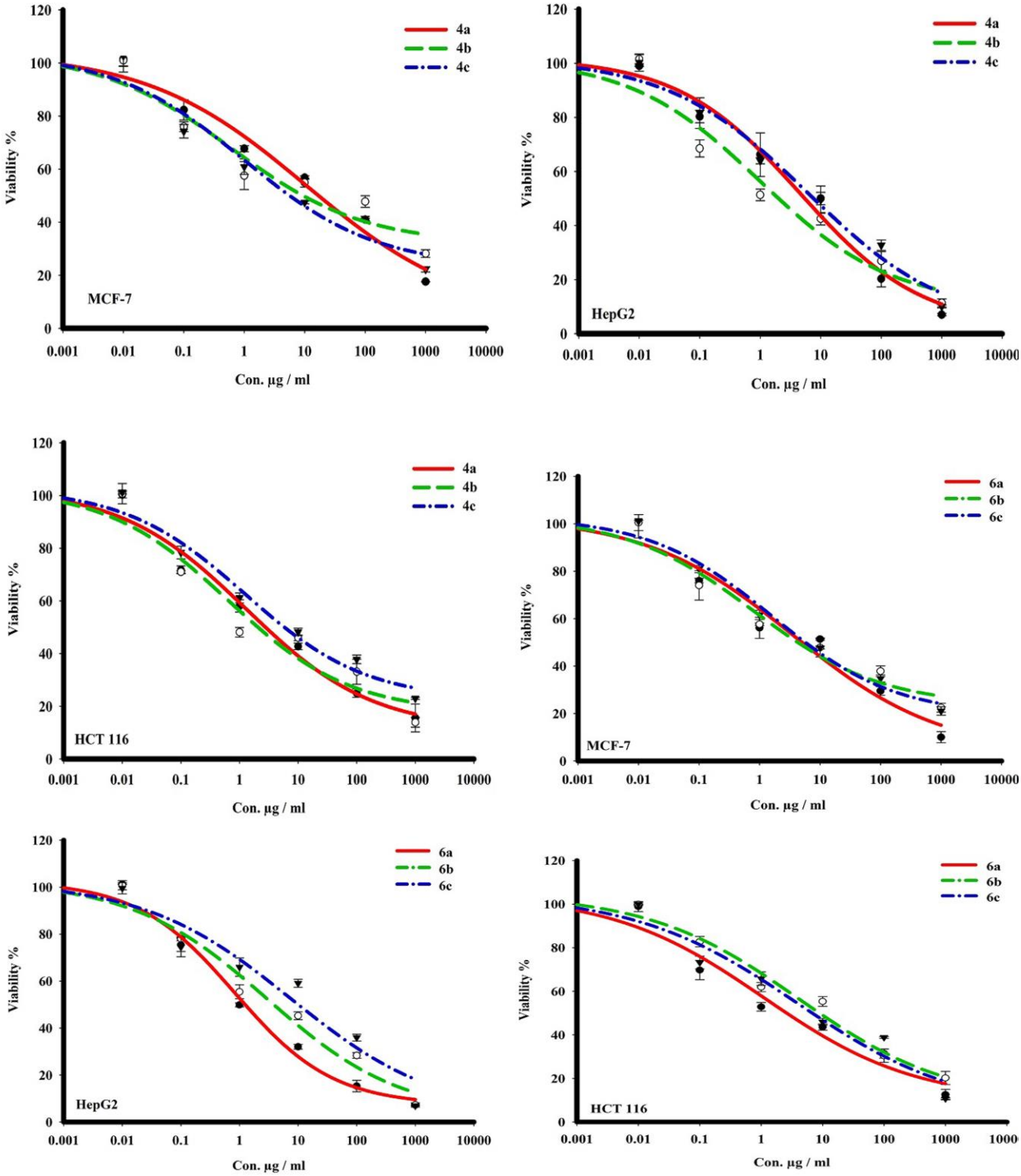


Figure 1. The dose response curve of the synthesized compounds on the cytotoxicity in MCF-7, HePG-2 and HCT-116 cancer cell lines. Cells were exposed to compounds **4a-c** and **6a-c** with different dilutions of compounds for 72 h.

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 the synthesis of products 4a-c and 6a-c.

A mixture of 4-oxo-4*H*-chromene-3-carboxaldehyde (**1**) (2.5 mmol) and nitrile active methylene compound **2a-c** (malononitrile, cyanoacetamide and ethyl cyanoacetate) (2.5 mmol) in distilled H_2O (50 mL), was stirred for 15 min at 60-70 °C. Equivalent amount of 6-aminothiouracil (**3**) or 4,6-diamino-pyrimidine-2(1*H*)-thione (**5**) (2.5 mmol) was added to the mixture, and the reaction was heated for 60-180 min at 60-70 °C. The completion of reaction was confirmed by TLC (EtOAc–petroleum ether 2:1) every 30 min. The resulting precipitate was separated by filtration and recrystallized from EtOH to afford the pure title compounds.

7-Amino-4-oxo-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,3,4,5,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (4a): Beige solid; yield 92%; mp >320 (decomp.) °C. IR (KBr), (*v* max, cm^{-1}): 3466, 3342, 3182 (br, NH_2, NH), 2225 (C≡N), 1706 (C=$O_{pyrimidine}$), 1642 (C=O_{pyrone}), 1129 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.55 (s, 1H, H–5), 7.09 (d, 1H, *J*=8.8 Hz, H–$8_{chromone}$), 7.19 (t, 1H, *J*=8.0 Hz, H–$6_{chromone}$), 7.49 (t, 1H, *J*=7.2 Hz, H–$7_{chromone}$), 7.73 (d, 1H, *J*=6.0 Hz, H–$5_{chromone}$), 8.19 (s, 2H, NH_2), 8.29 (s, 1H, H–$2_{chromone}$), 10.38 (s, 1H, NH), 12.60 (s, 1H, NH), 13.19 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 26.3 (C–5), 60.6 (C–6), 91.9 (C–4a), 111.5 (C≡N), 118.6 (C–$3_{chromone}$), 120.6 (C–$8_{chromone}$), 122.5 (C–$4a_{chromone}$), 124.4 (C–$6_{chromone}$), 125.5 (C–$5_{chromone}$), 134.9 (C–$7_{chromone}$), 144.4 (C–8a), 151.5 (C–$2_{chromone}$), 152.1 (C–7), 154.5 (C–$8a_{chromone}$), 160.0 (C=$O_{pyrimidine}$), 176.5 (C=O_{pyrone}), 187.7 (C=S). MS (*m/z*, I %): 365 (M^+, 10%). Anal. Calcd for $C_{17}H_{11}N_5O_3S$ (365.37): C, 55.89%; H, 3.03%; N, 19.17%; S, 8.78%. Found: C, 55.63%; H, 2.86%; N, 18.92%; S, 8.49%.

7-Amino-4-oxo-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,3,4,5,8-hexahydropyrido[2,3-*d*]pyrimidine-6-carboxamide (4b): Yellow solid; yield 93%; mp 289–290 °C. IR (KBr), (*v* max, cm^{-1}): 3469, 3363, 3268, 3216, 3156 (NH_2, NH), 1704 (C=$O_{pyrimidine}$), 1653 (C=O_{amide}), 1636 (C=O_{pyrone}), 1128 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.56 (s, 1H, H–5), 7.09 (d, 1H, *J*=8.4 Hz, H–$8_{chromone}$), 7.19 (t, 1H, *J*=7.6

Hz, H–$6_{chromone}$), 7.49 (t, 1H, *J*=7.2 Hz, H–$7_{chromone}$), 7.72 (d, 1H, *J*=6.4 Hz, H–$5_{chromone}$), 8.18 (s, 2H, NH_2), 8.29 (s, 1H, H–$2_{chromone}$), 8.91 (s, 2H, NH_2), 10.36 (s, 1H, NH), 12.59 (s, 1H, NH), 13.18 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 26.6 (C–5), 75.3 (C–6), 91.9 (C–4a), 118.8 (C–$3_{chromone}$), 120.6 (C–$8_{chromone}$), 122.5 (C–$4a_{chromone}$), 124.3 (C–$6_{chromone}$), 125.4 (C–$5_{chromone}$), 134.9 (C–$7_{chromone}$), 147.2 (C–8a), 149.5 (C–$2_{chromone}$), 152.1 (C–7), 154.6 (C–$8a_{chromone}$), 159.9 (C=$O_{pyrimidine}$), 167.9 (C=O_{amide}), 176.5 (C=O_{pyrone}), 190.7 (C=S). MS (*m/z*, I %): 383 (M^+, 4%). Anal. Calcd for $C_{17}H_{13}N_5O_4S$ (383.39): C, 53.26%; H, 3.42%; N, 18.27%; S, 8.36%. Found: C, 53.01%; H, 3.23%; N, 18.02%; S, 8.04%.

Ethyl 7-amino-4-oxo-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,3,4,5,8-hexahydropyrido[2,3-*d*]-pyrimidine-6-carboxylate (4c): Beige solid; yield 85%; mp 310–312 ºC. IR (KBr), (*v* max, cm^{-1}): 3469, 3276, 3187 (NH_2, NH), 1738 (C=O_{ester}), 1698 (C=$O_{pyrimidine}$), 1665 (C=O_{pyrone}), 1128 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6): 1.21 (t, 3H, *J*=7.6 Hz, CH_3), 4.13 (q, 2H, *J*=7.6 Hz, CH_2), 6.55 (s, 1H, H–5), 7.09 (d, 1H, *J*=8.0 Hz, H–$8_{chromone}$), 7.19 (t, 1H, *J*=6.8 Hz, H–$6_{chromone}$), 7.49 (t, 1H, *J*=6.8 Hz, H–$7_{chromone}$), 7.72 (d, 1H, *J*=6.4 Hz, H–$5_{chromone}$), 8.17 (s, 2H, NH_2), 8.29 (s, 1H, H–$2_{chromone}$), 10.36 (s, 1H, NH), 12.58 (s, 1H, NH), 13.18 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 15.4 (CH_3), 27.6 (C–5), 62.3 (CH_2), 70.3 (C–6), 92.0 (C–4a), 118.6 (C–$3_{chromone}$), 120.5 (C–$8_{chromone}$), 122.5 (C–$4a_{chromone}$), 124.3 (C–$6_{chromone}$), 125.5 (C–$5_{chromone}$), 134.9 (C–$7_{chromone}$), 144.9 (C–8a), 151.5 (C–$2_{chromone}$), 152.2 (C–7), 154.5 (C–$8a_{chromone}$), 159.9 (C=$O_{pyrimidine}$), 164.9 (C=O_{ester}), 176.5 (C=O_{pyrone}), 189.9 (C=S). MS (*m/z*, I %): 412 (M^+, 8%). Anal. Calcd for $C_{19}H_{16}N_4O_5S$ (412.43): C, 55.33%; H, 3.91%; N, 13.58%; S, 7.77%. Found: C, 55.09%; H, 3.72%; N, 13.33%; S, 7.49%.

4,7-Diamino-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,5,8-tetrahydropyrido[2,3-*d*]pyrimidine-6-carbonitrile (6a): Deep red solid; yield 92%; mp 299–300 ºC. IR (KBr), (*v* max, cm^{-1}): 3329, 3277, 3138 (br, NH_2, NH), 2227 (C≡N), 1653 (C=O_{pyrone}), 1197 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.18 (s, 1H, H–5), 6.76–7.01 (m, 3H, H–$8_{chromone}$ and NH_2), 7.32–7.59 (m, 2H, H–$6_{chromone}$ and H–$7_{chromone}$), 8.13 (d, 1H, *J*=8.0 Hz, H–$5_{chromone}$), 8.59 (s, 1H, H–$2_{chromone}$), 8.91 (s, 2H, NH_2), 10.00 (s, 1H, NH), 11.32 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 29.3 (C–5), 67.6 (C–6), 89.3 (C–4a), 115.6 (C≡N), 118.1 (C–$3_{chromone}$), 120.0 (C–$8_{chromone}$), 123.9 (C–$4a_{chromone}$), 124.7 (C–$6_{chromone}$), 126.9 (C–$5_{chromone}$), 134.9 (C–$7_{chromone}$), 147.7 (C–8a), 150.9 (C–$2_{chromone}$), 153.4 (C–7), 155.3 (C–$8a_{chromone}$), 156.6 (C–4), 176.6 (C=O_{pyrone}), 187.8 (C=S). MS (*m/z*, I %): 364 (M^+, 3%). Anal. Calcd for $C_{17}H_{12}N_6O_2S$ (364.39): C, 56.04%; H, 3.32%; N, 23.06%; S, 8.80%. Found: C, 55.79%; H, 3.11%; N, 22.84%; S, 8.52%.

4,7-Diamino-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,5,8-tetrahydropyrido[2,3-*d*]pyrimidine-6-carboxamide (6b): Orange solid; yield 90%; mp 272–273 ºC. IR (KBr), (*v* max, cm^{-1}): 3433, 3390, 3258, 3120 (NH_2, NH), 1691 (C=O_{amide}), 1659 (C=O_{pyrone}), 1196 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.54 (s, 1H, H–5), 6.83 (s, 2H, NH_2), 7.08 (d, 1H, *J*=7.2 Hz, H–$8_{chromone}$), 7.16–7.20 (m, 1H, H–$6_{chromone}$), 7.38–7.50 (m, 1H, H–$7_{chromone}$), 7.84 (d, 1H, *J*=8.0 Hz, H–$5_{chromone}$), 8.09 (s, 2H, NH_2), 8.33 (s, 1H,

H–$2_{chromone}$), 8.69 (s, 2H, NH_2), 10.28 (s, 1H, NH), 11.70 (s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 27.9 (C–5), 74.4 (C–6), 92.5 (C–4a), 117.1 (C–$3_{chromone}$), 119.9 (C–$8_{chromone}$), 122.6 (C–$4a_{chromone}$), 123.8 (C–$6_{chromone}$), 124.8 (C–$5_{chromone}$), 133.5 (C–$7_{chromone}$), 148.8 (C–8a), 150.8 (C–$2_{chromone}$), 152.1 (C–7), 156.0 (C–$8a_{chromone}$), 157.6 (C–4), 167.3 (C=O_{amide}), 177.7 (C=O_{pyrone}), 190.6 (C=S). MS (*m/z*, I %): 382 (M^+, 11%). Anal. Calcd for $C_{17}H_{14}N_6O_3S$ (382.40): C, 53.40%; H, 3.69%; N, 21.98%; S, 8.38%. Found: C, 53.28%; H, 3.42%; N, 21.69%; S, 8.16%.

Ethyl 4,7-diamino-5-(4-oxo-4*H*-chromen-3-yl)-2-thioxo-1,2,5,8-tetrahydropyrido[2,3-*d*]pyrimidine-6-carboxylate (6c): Orange solid; yield 89%; mp 244–245 ºC. IR (KBr), (*v* max, cm^{-1}): 3440, 3363, 3309 (NH_2, NH), 1743 (C=O_{ester}), 1643 (C=O_{pyrone}), 1167 (C=S). ^{1}H-NMR (400 MHz, DMSO-d_6): 0.99–1.05 (m, 3H, CH_3), 4.10 (q, 2H, *J*=7.2 Hz, CH_2), 6.44 (s, 1H, H–5), 6.96 (s, 2H, NH_2), 7.08 (d, 1H, *J*=8.4 Hz, H–$8_{chromone}$), 7.18 (t, 1H, *J*=7.6 Hz, H–$6_{chromone}$), 7.39–7.50 (m, 1H, H–$7_{chromone}$), 7.84 (d, 1H, *J*=6.4 Hz, H–$5_{chromone}$), 8.55 (s, 2H, NH_2), 8.66 (s, 1H, H–$2_{chromone}$), 10.37 (s, 1H, NH), 12.65(s, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): 14.1 (CH_3), 34.3 (C–5), 62.9 (CH_2), 72.9 (C–6), 92.5 (C–4a), 118.5 (C–$3_{chromone}$), 120.5 (C–$8_{chromone}$), 122.6 (C–$4a_{chromone}$), 123.6 (C–$6_{chromone}$), 125.4 (C–$5_{chromone}$), 133.6 (C–$7_{chromone}$), 151.4 (C–$2_{chromone}$), 152.2 (C–8a), 157.3 (C–4), 157.5 (C–$8a_{chromone}$), 159.3 (C–7), 165.4 (C=O_{ester}), 175.9 (C=O_{pyrone}), 188.8 (C=S). MS (*m/z*, I %): 411 (M^+, 2%). Anal. Calcd for $C_{19}H_{17}N_5O_4S$ (411.44): C, 55.47%; H, 4.16%; N, 17.02%; S, 7.79%. Found: C, 55.21%; H, 3.96%; N, 16.75%; S, 7.61%.

ANTICANCER SCREENING

Cell culture

The tumor cell lines, mammary gland breast cancer cell line (MCF-7), hepatocellular carcinoma (HepG-2) and human colon carcinoma (HCT-116) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on an RPMI-1640 medium, supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subculture two to three times a week.

Cytotoxicity evaluation using viability assay

The cytotoxic activity was appraised, using the standard sulphorhodamine B (SRB) assay, as reported previously.[33]

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