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Synthesis of Some Coumarin Hybrids and Evaluating their Anticancer Activity

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Abstract

Coumarin-chalcone hybrids **3a** and **3b** were utilized as versatile precursor for the synthesis of coumarinyl-pyridine and coumarinyl-pyrimidine hybrids. The reaction of chalcones **3** with malononitrile or ethyl cyanoacetate proceeded in hot glacial acetic acid and ammonium acetate to afford the coumarinyl 2-aminonicotinonitriles **4** and coumarinyl 2-hydroxynicotinonitriles derivatives **5**, respectively. Heating of chalcone derivatives **3** with thiourea in refluxing sodium ethoxide furnished the corresponding coumarinyl-pyrimidines **6**. Condensation of coumarinyl-acetohydrazide scaffold **7** various aromatic aldehydes afforded the corresponding acrylohydrazides **8** which underwent heterocyclization with malononitrile furnished the coumarinyl-pyridinone derivatives **9**. The constructed coumarin scaffolds were evaluated *in vitro* for their anticancer activity *via* the standard MTT method versus Hepatocellular Carcinoma (HepG2) and breast cancer (MCF-7).

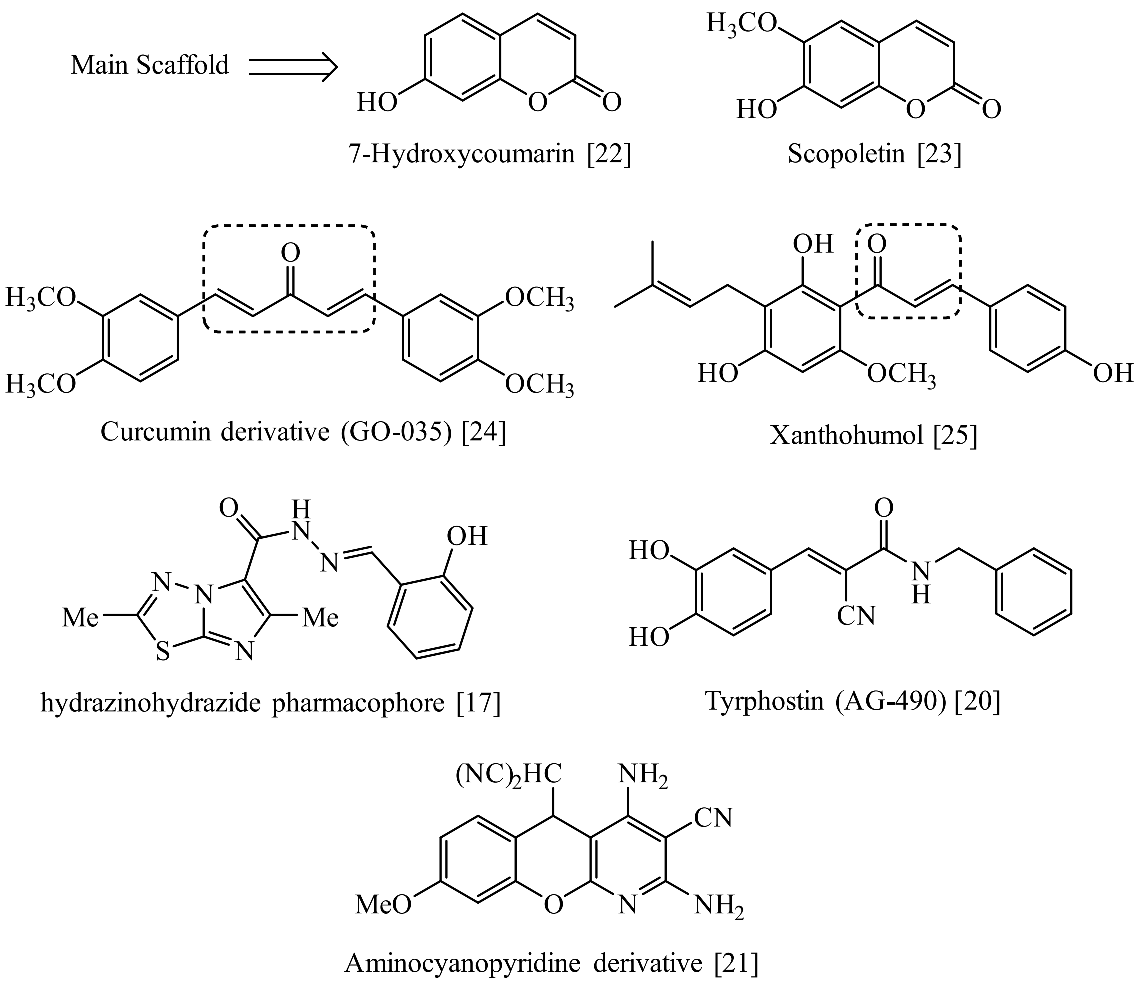
Keywords: 3-Acetylcoumarin; Breast Cancer; Coumarinyl 2-Aminonicotinonitriles; Isovanillin; Malononitrile

Introduction

Coumarin scaffolds display wide range of pharmaceutical properties with low toxicity. They were described as anticancer [1-4], antioxidant [4,5], anticoagulant [6], anti HIV [7,8], antimicrobial [9,10] and anti-inflammatory [11] agents. The reported cytotoxic activity of these coumarins versus various cell lines is attributed to the inhibition of the cellular proliferation via different mechanisms such as inhibition of the telomerase enzyme [12], inhibition of protein kinase activity and down regulation of oncogene expression [13] or by inducing the caspase-9 mediated apoptosis [14]. In addition, coumarin derivatives exhibited high ability to suppress cancer cell proliferation by arresting cell cycle in G0/G1 [12] and G2/M phases [15]. These findings make coumarin derivatives (Figure 1), promising scaffolds for cytotoxic hybrids framework.

Chalconoid compounds, as examples of α,β-unsaturated carbonyl class, are utilized to inhibit cancer and have comprehensive cytotoxic potential in several models [16] (Figure 1). It has been reported in the literature that hydrazide-hydrazone scaffolds (-CO-NH-N=CH-) [17-19], acrylonitrile [20] and/or 2-amino-nicotinonitrile moieties [21] display important antitumor activity (Figure 1).

In the present research, we focused on hybridization strategy to combine coumarin nucleus with three biologically active molecules; pyridine, pyrimidine and acryl hydrazide in an attempt to find bioactive pharmacophores. 3-Acetylcoumarin **(1)** was utilized as versatile precursor for the synthesis of chalcones and their corresponding pyridine, pyrimidine and acrylohydrazide derivatives. 3-Acetylcoumarin has been synthesized according to the reported literature procedure [26] from salicylaldehyde, and equimolar amount of ethyl acetoacetate.



7-Hydroxycoumarin [22]

Scopoletin [23]

Curcumin derivative (GO-035) [24]

Xanthohumol [25]

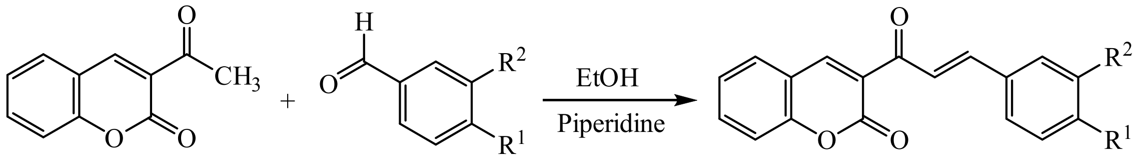
hydrazinohydrazide pharmacophore [17]

Tyrphostin (AG-490) [20]

Aminocyanopyridine derivative [21]

Figure 1: Chemical structures of anticancer scaffolds.

Thus, our strategy in this work starts by the synthesis of coumarin-chalcone hybrids containing different substituents in the aromatic rings that can potentially be used as new lead compounds in drug discovery, particularly as anticancer agents. Coumarin-chalcone hybrids **3a** [27] and **3b** were prepared by Claisen-Schmidt reaction of 3-acetylcoumarin **(1)** with substituted aromatic aldehydes **2** (namely; 4-nitrobenzaldehyde and isovanillin). The reaction has been achieved by refluxing the reactants in ethyl alcohol and piperidine as catalyst.



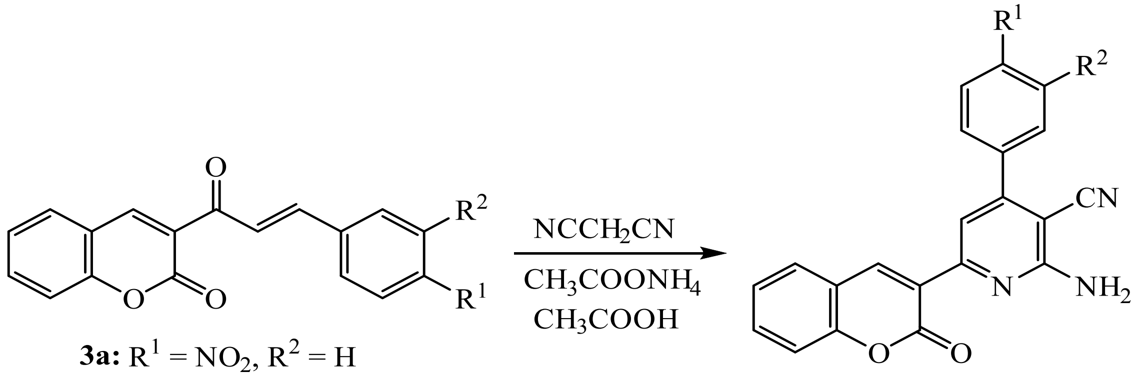
1

2

3a: $R^1 = NO_2$, $R^2 = H$
3b: $R^1 = OCH_3$, $R^2 = OH$

Scheme (1)

The 2-aminonicotinonitrile derivatives **4a** [28] and **4b** were synthesized through the reaction of chalcone scaffolds **3** with malononitrile in refluxing glacial acetic acid containing ammonium acetate (Scheme 2). Spectroscopic techniques (^{1}H NMR, IR and MS) was employed to secure the structures of these synthesized 2-aminonicotinonitrile scaffolds. (cf. experimental)



3a: $R^1 = NO_2$, $R^2 = H$
3b: $R^1 = OH$, $R^2 = OCH_3$

4a: $R^1 = NO_2$, $R^2 = H$
4b: $R^1 = OCH_3$, $R^2 = OH$

Scheme (2)

A plausible mechanism for the reaction of chalcones **3** with malononitrile is indicated in Figure (2). The reaction passes through addition of malononitrile and ammonia to the δ,β-unsaturated system to afford intermediate **4-i**, which underwent tautomerization into amino form **4-ii**. Intramolecular cyclization of **4-ii** proceeded via nucleophilic addition of the amino function to the nitrile group to form the imino-tetrahydropyridine **4-iii**, which underwent tautomerization into amino-dihydropyridine **4-iv**. Autoxidation of this dihydropyridine **4-iv** promoted the loss of hydrogen molecule with the formation of pyridine compound **4**.

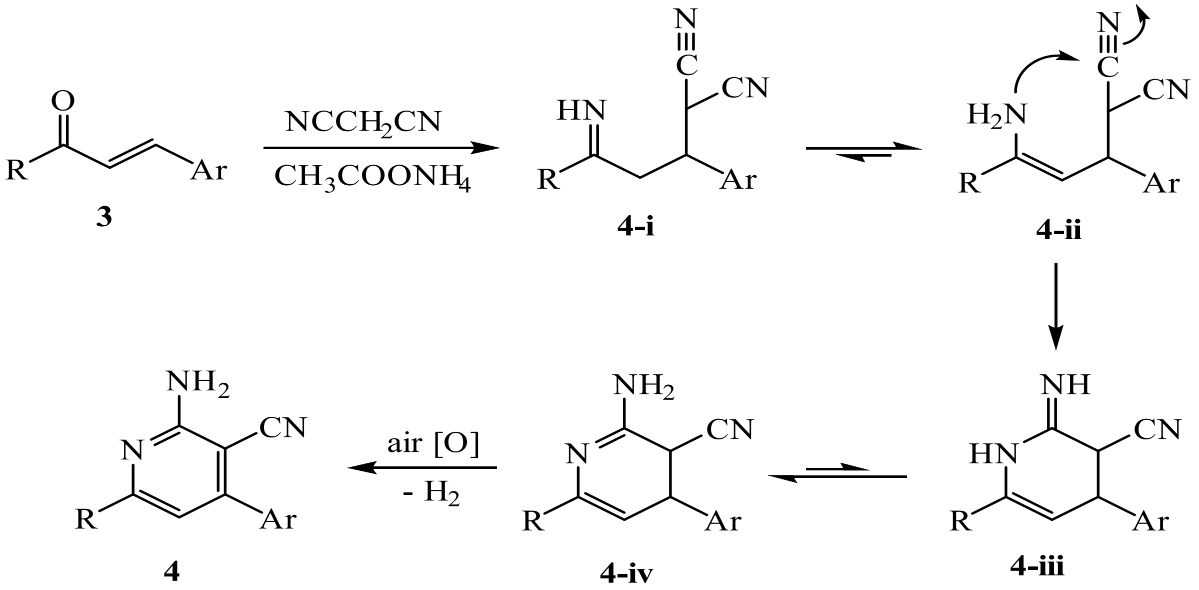
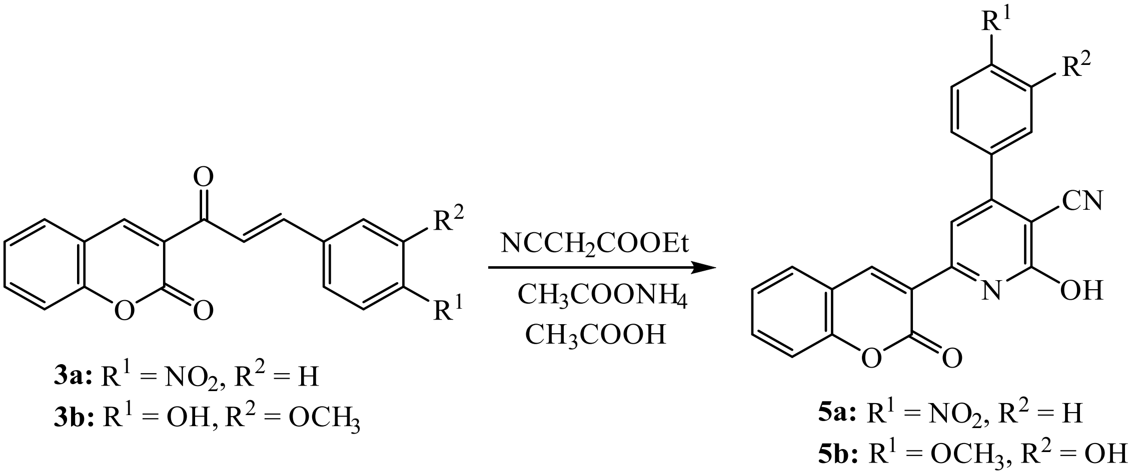


Figure 2: A plausible mechanism for the reaction of chalcones 3 with malononitrile.

The 2-hydroxynicotinonitrile derivatives **5a** and **5b** were constructed through the reaction of chalcone scaffolds **2** with ethyl cyanoacetate in refluxing glacial acetic acid containing ammonium acetate (Scheme 3). Spectroscopic techniques (IR, ^{1}H NMR and MS) was employed to secure the structures of these synthesized 2-hydroxynicotinonitrile scaffolds. The presence of infrared absorptions, in the spectrum of **5a**, at 3408 and 3376 cm^{-1} refers to the N-H and O-H functions. The stretching absorptions at 2216 and 1736 cm^{-1} clearly indicates the presence of nitrile and carbonyl functions. ^{1}H NMR spectrum of **5a** revealed, in addition to the multiplet signal of eight aromatic protons, two singlet signals at 7.24 and 8.70 ppm for the protons of pyridine C-5 and coumarin C-4, respectively. The singlet at 12.64 ppm indicated the proton of hydroxyl group.



3a: $R^1 = NO_2$, $R^2 = H$
3b: $R^1 = OH$, $R^2 = OCH_3$

5a: $R^1 = NO_2$, $R^2 = H$
5b: $R^1 = OCH_3$, $R^2 = OH$

Scheme (3)

A plausible mechanism for the reaction of chalcones **3** with malononitrile is indicated in (Figure 3). The reaction passes through addition of ethyl cyanoacetate and ammonia to the δ,β-unsaturated system to afford intermediate **5-i**, which underwent tautomerization into amino form **5-ii**. Intramolecular cyclization of **4-ii** proceeded via nucleophilic addition of the $-NH_2$ function to the C=O group followed by loss of ethanol molecule of to form the tetrahydropyridone **5-iii**, which underwent tautomerization into hydroxy-dihydropyridine **5-iv**. Autoxidation of this dihydropyridine **5-iv** promoted the loss of hydrogen molecule with the formation of pyridine compound **5**.

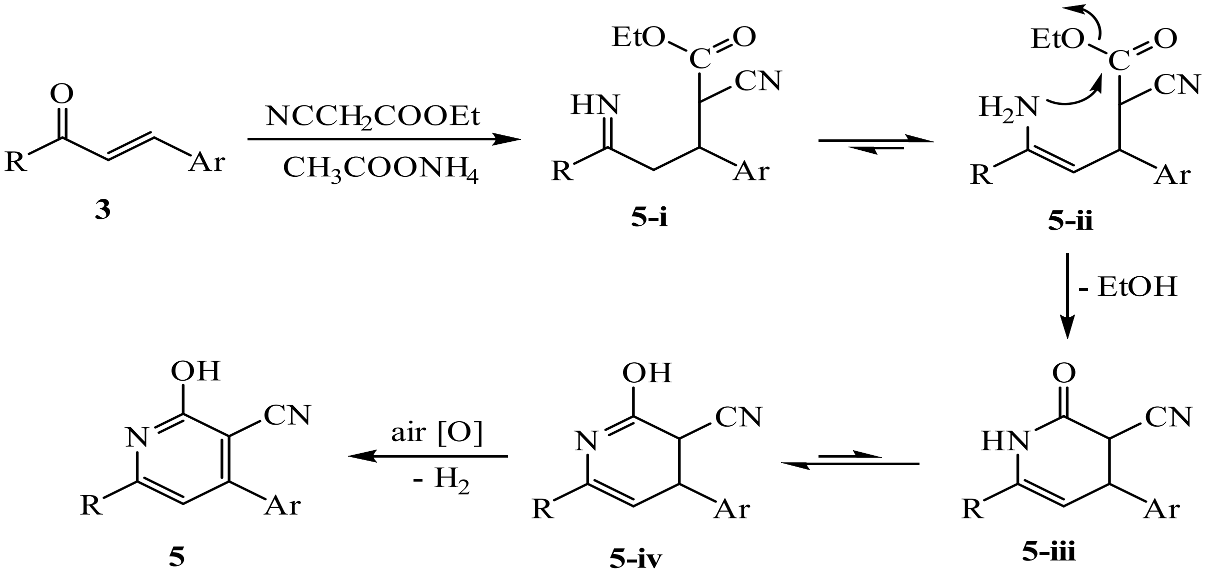
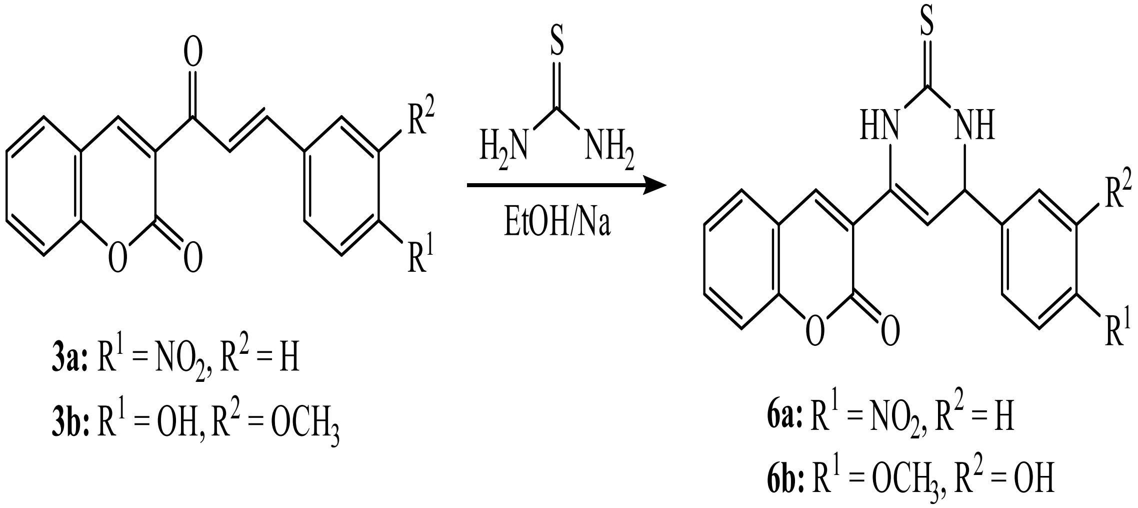


Figure 3: A plausible mechanism for the reaction of chalcones 3 with ethyl cyanoacetate.

The reaction of chalcone derivatives **3** with thiourea has been carried out by refluxing in ethanolic solution of C_2H_5ONa to furnish the corresponding aryl coumarinyl-pyrimidines **6a** and **6b** (Scheme 4). Spectroscopic techniques (IR, ^1H NMR and MS) was employed to secure the structures of these synthesized coumarinyl-pyrimidine scaffolds. The infrared spectrum of **6b** displayed absorption bands at 3415, 3394 and 3213 cm^{-1} for the (NH and OH) functions in addition to 1717 cm^{-1} for the (C=O) group. The ^1H NMR spectrum of **6b** showed signals at 3.81 ppm (s, 3H, OCH_3), 4.78 ppm (d, 1H, H-6 of pyrimidine), 6.50 ppm (d, 1H, H-5 of pyrimidine), 6.88-7.76 ppm (m, 7H, aromatic-H), 8.39 ppm (s, 1H, H-4 of coumarin), 9.10 ppm (s, 1H, NH), 9.55 ppm (s, 1H, OH) and 9.72 ppm (s, 1H, NH).



3a: $R^1 = NO_2, R^2 = H$
3b: $R^1 = OH, R^2 = OCH_3$

6a: $R^1 = NO_2, R^2 = H$
6b: $R^1 = OCH_3, R^2 = OH$

Scheme (4)

The plausible reaction mechanism was indicated in Figure (4), the reaction is initiated by base-mediated aza-Michel addition of thiourea to chalcones **3** to form the Michel adduct **6-i**. The amino group of intermediate **6-i** underwent intramolecular attacked on the carbonyl function to give the intermediate **6-ii**, followed by loss water molecule gave the target aryl coumarinyl-pyrimidine product **6**.

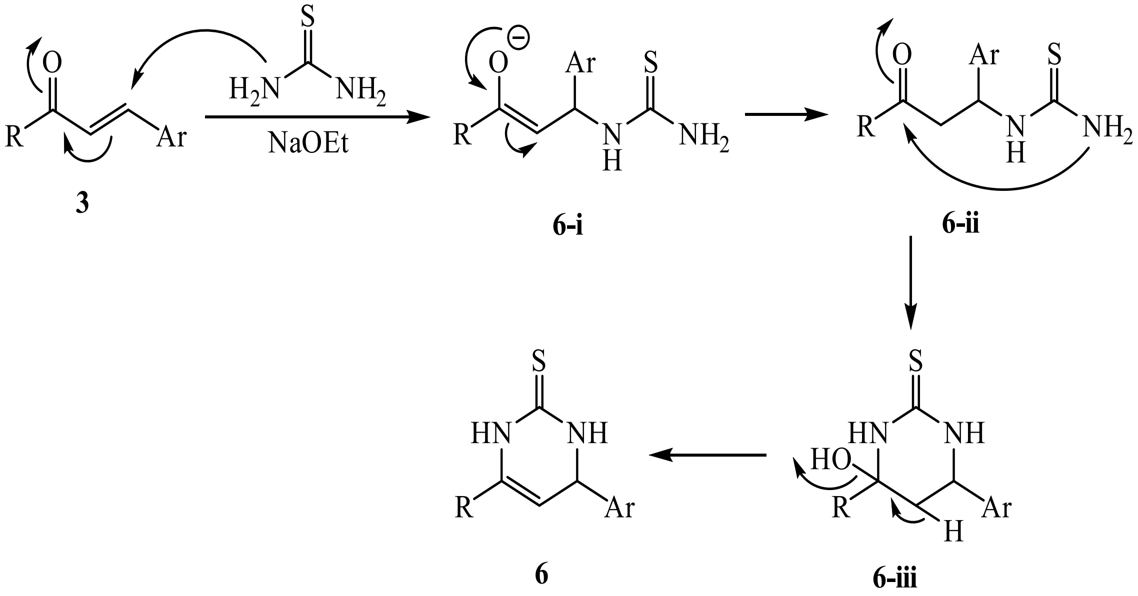
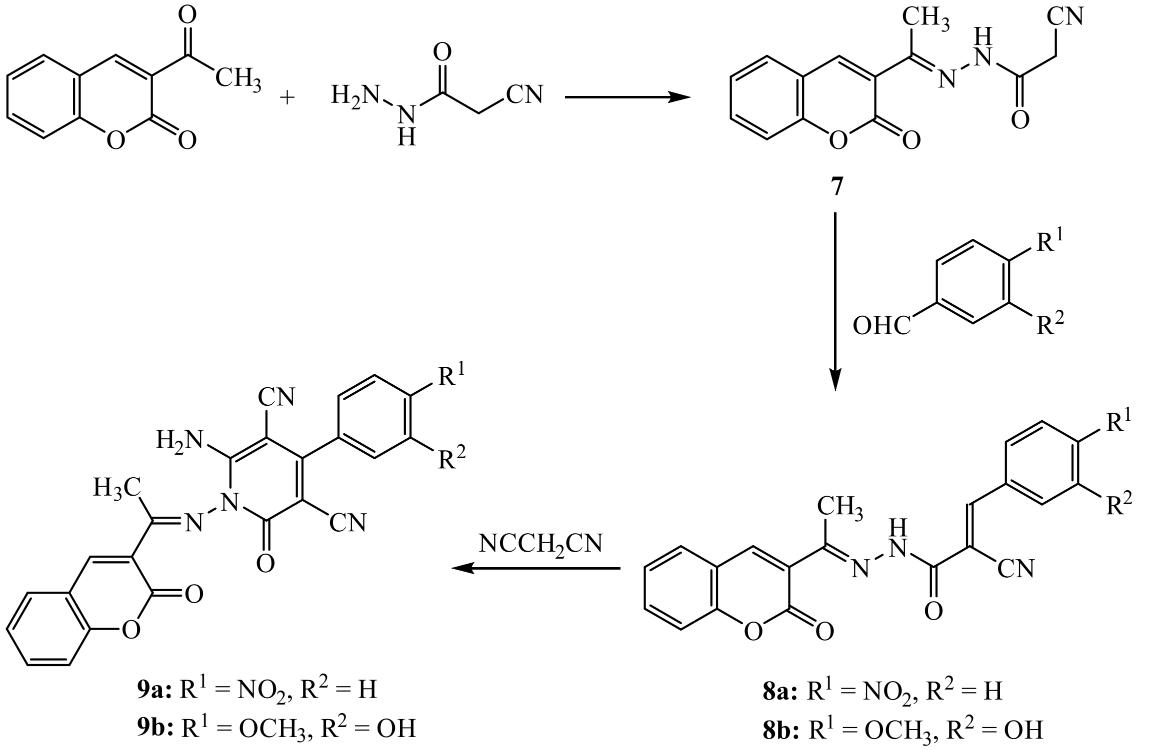


Figure 4: A plausible mechanism for the reaction of chalcones 3 with thiourea.

Condensation of **1** with cyanoacetic acid hydrazide in methyl alcohol containing drops of glacial CH_3COOH furnished the corresponding acetohydrazide scaffold **7** [29], which underwent subsequent condensation with various aromatic aldehydes by heating in ethyl alcohol containing three drops of piperidine to afford the corresponding acrylohydrazide compounds **8a** and **8b** (Scheme 5). To explore the synthetic potentially of compounds **8a** and **8b**, the reactivity of **8** towards Michael addition with malononitrile as a possible synthetic route to get pyridinone derivatives **9** was examined. Compounds **9a** and **9b** were achieved by refluxing compounds **8a** and **8b** with malononitrile and triethylamine in ethyl alcohol. Elucidation of the new synthesized derivatives **9a** and **9b** was based on elemental analysis, IR, ^{1}H NMR and mass spectroscopy (cf. Experimental Section).



9a: $R^1 = NO_2$, $R^2 = H$
9b: $R^1 = OCH_3$, $R^2 = OH$

8a: $R^1 = NO_2$, $R^2 = H$
8b: $R^1 = OCH_3$, $R^2 = OH$

Scheme (5)

Biological Evaluation (Anti-cancer activity)

The constructed seventy coumarin scaffolds were evaluated *in vitro* for their anticancer activity *via* the standard MTT method [30-32] versus two human tumor cell lines; Hepatocellular Carcinoma (HepG2) and breast cancer (MCF-7). Doxorubicin has been used as standard anticancer drug for comparison. MTT assay is a standard colorimetric assay for measuring the inhibit effects of compounds on cell growth.

Yellow MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide is reduced to purple formazan derivative by mitochondria succinate dehydrogenase of viable cells. The absorbance of this colored formazan solution in DMSO is measured and recorded at certain wave length 570 nm by using a plate reader (EXL 800, USA). the relative cell viability in percentage was calculated as (A570 of treated sample / A570 of untreated samples) x 100.

The results of anticancer activity of the chalconoid compounds are depicted in (Table 1). They revealed that compounds and revealed that compounds **7**, **8b** and **5b** exhibited the highest cytotoxic activity against the tested cell lines HepG2 and MCF-7. Their IC_{50} values (ranges from 5.81 to 11.26 µM) were very close to the reference drug (Doxorubicin, IC_{50} = 4.17-4.50 µM).

The coumarin compound **9b** showed strong cytotoxic activity with the percentage viability IC_{50} at 13.68 and 17.01 µM for the hepatocellular carcinoma (HepG2) and the breast cancer (MCF-7), respectively. The coumarin-chalconoid compound **3b**, substituted with methoxy and hydroxy groups at the benzene ring, displayed moderate inhibitory activity for both cell lines (HepG2 and MCF-7) with the percentage viability IC_{50} at 22.96 and 29.54 µM, respectively. The coumarinyl-nicotinonitrile compound **4b** displayed also moderate inhibitory activity against HepG2 and MCF-7 with the percentage viability IC_{50} at 28.60 and 24.89 µM, respectively.

Structure Activity Relation (SAR)

In an attempt to find a relationship between the Structure and their Anticancer Activity (SAR), we can assume that the presence of methoxy group in the skeleton of chalcone is responsible for the broad spectrum of anti-cancer activity towards the tested cell lines (HepG2 and MCF-7) and makes the compounds very stronger as anti-cancer agents. This assumption finds support from the results (Table 1), the anticancer activity of the chalconoid compounds substituted (**3b**, **4b**, **5b**, **6b**, **8b** and **9b**) with methoxy at the para-position and hydroxy group at the meta-position of the benzene ring is higher than its corresponding chalconoid compounds (**3b**,

4b, **5b**, **6b**, **8b** and **9b**) substituted with nitro group at the para-position of the benzene ring.

Compounds	***In vitro* Cytotoxicity IC_{50} (µM)**	
	HepG2	**MCF-7**
DOX	**4.50±0.3**	**4.17±0.2**
3a	31.23±2.4	36.91±2.6
3b	22.96±1.8	29.54±2.0
4a	63.49±3.7	61.18±4.1
4b	28.60±2.1	24.89±1.9
5a	60.48±3.6	51.85±3.5
5b	9.24±1.0	11.26±1.1
6a	56.73±3.4	42.38±3.1
6b	46.41±3.0	30.42±2.3
7	5.81±0.4	6.34±0.5
8a	39.76±2.7	59.12±3.9
8b	7.73±0.8	8.92±0.7
9a	38.12±2.6	48.50±3.3
9b	13.68±1.2	17.01±1.4

- **IC_{50} (µM)**: 1-10 (very strong), 11-20 (strong), 21-50 (moderate), 51-100 (weak) and higher than 100 (non-cytotoxic). **DOX** (Doxorubicin)

Table 1: Cytotoxic activity of the synthesized coumarin scaffolds versus HePG2 and MCF-7.

Experimental

General Remarks

Melting points (uncorrected) have been determined on Gallenkamp electric apparatus. IR spectra were registered on a Nicolet 5000 FT-IR spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were obtained by JEOL's NMR spectrometer (500 MHz) in DMSO-d_6. Mass analyses were registered on Quadrupole GC/MS Thermo Scientific Focus/DSQII at 70 eV. Elemental analyses (C, H and N) were determined on Perkin Elmer 2400 analyzer.

Synthesis of coumarin-Chalcone hybrids 3a and 3b

To a well-stirred suspension of 3-acetylcoumarin (0.02 mol) in ethyl alcohol, 4-nitrobenzaldehyde or isovanillin (0.02 mol) and few drops of piperidine were added. The mixture was refluxed for 6 hours and the resulting solid was picked up by filtration and recrystallized via boiling in MeOH.

1-(Coumarin-3-yl)-3-(4-nitrophenyl)-prop-2-en-1-one (3a)

Yellow powder, yield = 78%, m.p. 265-266°C, lit. m.p. 267°C [27]. IR (ν_{max}/cm^{-1}): 1671 (C=O, conjugated), 1723 (C=O, coumarin). ^1H NMR (DMSO-d_6): δ_{ppm} 7.34 (d, 1H, J = 8.50 Hz), 7.40-7.81 (m, 4H), 7.94 (d, 1H, J = 8.50 Hz), 8.05 (d, 2H, J = 8.80 Hz), 8.24 (d, 2H, J = 8.40 Hz), 8.60 (s, 1H). Analysis for $C_{18}H_{11}NO_5$ (321), Calcd: C, 67.29; H, 3.45; N, 4.36%. Found: C, 67.16; H, 3.49; N, 4.28%.

1-(Coumarin-3-yl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (3b)

Yellow powder, yield = 72%, m.p. 218-219°C. IR (ν_{max}/cm^{-1}): 3435 (O-H), 1676 (C=O, conjugated), 1722 (C=O, coumarin), 1608 (C=C). ^1H NMR (DMSO-d_6): δ_{ppm} 3.85 (s, 3H), 6.82 (d, 1H, J = 8.40 Hz), 7.00 (d, 1H, J = 8.60 Hz), 7.08 (d, 1H, J = 8.50 Hz), 7.18 (s, 1H), 7.41-7.82 (m, 4H), 7.84 (d, 1H, J = 8.50 Hz), 8.61 (s, 1H), 9.48 (s, 1H). Analysis for $C_{19}H_{14}O_5$ (322), Calcd: C, 70.80; H, 4.38%. Found: C, 70.96; H, 4.44%.

Synthesis of 2-amino-4-aryl-6-(coumarin-3-yl)-nicotinonitriles 4a and 4b

A solution of coumarin-chalcone hybrids **3a** or **3b** (0.003 mol), malononitrile (0.006 mol) and ammonium acetate (1.2 mol) in glacial acetic acid (15 mL) was refluxed for 6 hours. After cooling, 15 mL water was added to the reaction mixture and the solid that formed was collected by filtration. Recrystallization was carried out from AcOH/H_2O to furnish the corresponding nicotinonitriles **4a** and **4b**.

2-Amino-4-(4-nitrophenyl)-6-(coumarin-3-yl) nicotinonitrile (4a)

Yellow powder, yield = 70%, m.p. 250-251°C, lit. m.p. 252-254°C [28]. IR (ν_{max}/cm^{-1}): 3456, 3365 (NH_2), 2212 (C≡N), 1721 (C=O). ^1H NMR: δ_{ppm} 7.21 (s, 2H), 7.38-7.87 (m, 8H), 8.36 (s, 1H), 8.76 (s, 1H). MS (EI, m/z): 384 (M^+, 100%). Analysis for $C_{21}H_{12}N_4O_4$ (384), Calcd: C, 65.63; H, 3.15; N, 14.58%. Found: C, 65.46; H, 3.21; N, 14.47%.

2-Amino-4-(3-hydroxy-4-methoxyphenyl)-6-(coumarin-3-yl)-nicotinonitrile (4b)

Yellow powder, yield = 66%, m.p. 257-258°C. IR (ν_{max}/cm^{-1}): 3432, 3378 (NH_2 and O-H), 2218 (C≡N), 1727 (C=O). ^1H NMR: δ_{ppm} 3.82 (s, 3H), 6.98-7.56 (m, 9H), 7.68 (s, 1H), 8.72 (s, 1H), 9.54 (s, 1H). MS (EI, m/z): 385 (M^+, 71.6%). Analysis for $C_{22}H_{15}N_3O_4$ (385), Calcd: C, 68.57; H, 3.92; N, 10.90%. Found: C, 68.69; H, 3.96; N, 10.96%.

Synthesis of 2-hydroxy-4-aryl-6-(coumarin-3-yl)-nicotinonitriles 5a and 5b

A solution of coumarin-chalcone hybrids **3a** or **3b** (0.003 mol), ethyl cyanoacetate (0.006 mol) and ammonium acetate (1.2 mol) in glacial acetic acid (15 mL) was refluxed for 6 hours. After cooling, the reaction mixture was diluted with 15 mL water and the solid that formed was collected by filtration, dried and recrystallized from AcOH/H_2O to furnish the corresponding nicotinonitriles **5a** and **5b**.

2-Hydroxy-4-(4-nitrophenyl)-6-(coumarin-3-yl) nicotinonitrile (5a)

Brown powder, yield = 62%, m.p. 284-284°C. IR (ν_{max}/cm^{-1}): 3408, 3376 (NH and OH), 2216 (C≡N), broad centered at 1736 (C=O). ^{1}H NMR: δ_{ppm} 7.24 (s, 1H), 7.35-7.89 (m, 8H), 8.70 (s, 1H), 12.64 (s, 1H). MS (EI, m/z): 385 (M$^+$, 84.5%). Analysis for $C_{21}H_{11}N_3O_5$ (385), Calcd: C, 65.46; H, 2.88; N, 10.91%. Found: C, 65.67; H, 2.81; N, 10.79%.

2-Hydroxy-4-(3-hydroxy-4-methoxyphenyl)-6-(coumarin-3-yl) nicotinonitrile (5b)

Yellowish brown powder, yield = 68%, m.p. 240-242°C. IR (ν_{max}/cm^{-1}): 3341, 3337 (NH and OH), 2213 (C≡N), broad centered at 1726 (C=O). ^{1}H NMR: δ_{ppm} 3.85 (s, 3H), 6.92-7.62 (m, 7H), 7.74 (s, 1H), 8.70 (s, 1H), 9.58 (s, 1H), 12.64 (s, 1H). MS (EI, m/z): 386 (M$^+$, 48.3%). Analysis for $C_{22}H_{14}N_2O_5$ (386), Calcd: C, 68.39; H, 3.65; N, 7.25%. Found: C, 68.21; H, 3.58; N, 7.36%.

Synthesis of 3-(6-aryl-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-2*H*-chromen-2-ones 6a and 6b

A solution of sodium ethoxide was intended by dissolving fine pieces of sodium (0.006 mol) in 20 ml dry ethanol. To this ethoxide solution, the coumarin-chalcone hybrids **3a** or **3b** (0.003 mol) and thiourea (0.003 mol) were added. The mixture was refluxed for 4 hours, then cooled to 25°C and diluted with 20 ml cold water. The solid that formed, after acidification with dilute HCl, was filtered and purified by recrystallization from ethyl alcohol.

3-(6-(4-Nitrophenyl)-2-thioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-2*H*-chromen-2-one (6a)

Brown powder, yield = 54%, m.p. 204-205°C. IR (ν_{max}/cm^{-1}): 3394, 3213 (NH), 1706 (C=O). ^{1}H NMR: δ_{ppm} 4.82 (d, 1H, J = 7.80 Hz), 6.56 (d, 1H, J = 8.60 Hz), 7.38-8.04 (m, 8H), 8.46 (s, 1H), 9.12 (s, 1H), 9.68 (s, 1H). MS (EI, m/z): 379 (M$^+$, 62.7%). Analysis for $C_{19}H_{13}N_3O_4S$ (379), Calcd: C, 60.15; H, 3.45; N, 11.08%. Found: C, 60.01; H, 3.51; N, 11.19%.

3-(6-(3-Hydroxy-4-methoxyphenyl)-2-thioxo-1,2,3,6-tetrahydro-pyrimidin-4-yl)-2*H*-chromen-2-one (6b)

Brown powder, yield = 62%, m.p. 194-196°C. IR (ν_{max}/cm^{-1}): 3415, 3394, 3213 (OH and NH), 1717 (C=O). ^{1}H NMR: δ_{ppm} 3.81 (s, 3H), 4.78 (d, 1H, J = 7.80 Hz), 6.50 (d, 1H, *J = 8.55 Hz*), 6.88-7.76 (m, 7H), 8.39 (s, 1H), 9.10 (s, 1H), 9.55 (s, 1H), 9.72 (s, 1H). MS (EI, m/z): 380 (M$^+$, 32.8%). Analysis for $C_{20}H_{16}N_2O_4S$ (380), Calcd: C, 63.15; H, 4.24; N, 7.36%. Found: C, 63.31; H, 4.17; N, 7.46%.

Preparation of 2-cyano-*N'*-(1-(coumarin-3-yl) ethylidene)-acetohydrazide (7)

To a solution of cyanoacetic acid hydrazide (0.01 mol) in methyl alcohol (40 mL), 3-acetyl-coumarin (0.01 mol, 2.18 g) and 1 mL CH_3COOH were added. The reaction was completed after reflux for 2 hours and then the solid that formed was filtered, and recrystallized from EtOH/$CHCl_3$ mixture (1:1).

Yellow crystals, yield = 80%, m.p. 171-173°C, lit. m.p. 172°C [29]. IR (ν_{max}/cm^{-1}): 3193 (NH), 2227 (C≡N), 1691 (C=O, amide), 1722 (C=O, coumarin). ^{1}H NMR: δ_{ppm} 2.22 (s, 3H), 4.18 (s, 2H), 7.32-7.91 (m, 4H), 8.82 (s, 1H), 11.06 (s, 1H).

Preparation of 2-cyano-3-aryl-*N'*-(1-(coumarin-3-yl)-ethylidene)-acrylohydrazide 8a and 8b

To a solution of **7** (0.005 mol) and 4-nitrobenzaldehyde or isovanillin (0.005 mol) in ethyl alcohol (30 mL), three drops of piperidine were added. The reaction was completed after reflux for 2 hours. The solid that obtained while hot was picked up by filtration, dried and recrystallized from ethyl alcohol.

2-Cyano-3-(4-nitrophenyl)-*N'*-(1-(coumarin-3-yl) ethylidene)-acrylohydrazide (8a)

Yellow crystals, yield = 81%, m.p. 204-205°C. IR (ν_{max}/cm^{-1}): 3446 (NH), 2227 (C≡N), 1721 (C=O, coumarin), 1681 (C=O, amide). ^{1}H NMR: δ_{ppm} 2.17 (s, 3H), 7.45-8.14 (m, 8H), 8.34 (s, 1H), 8.62 (s, 1H), 11.48 (s, 1H). MS (EI, m/z): 402 (M$^+$, 46.7%). Analysis for $C_{21}H_{14}N_4O_5$ (402), Calcd: C, 62.69; H, 3.51; N, 13.92%. Found: C, 62.51; H, 3.58; N, 13.81%.

2-Cyano-3-(3-hydroxy-4-methoxyphenyl)-*N'*-(1-(coumarin-3-yl)-ethylidene) acrylohydrazide (8b)

Yellow crystals, yield = 77%, m.p. 158-160°C. IR (ν_{max}/cm^{-1}): 3439 (NH and OH), 2221 (C≡N), 1722 (C=O, coumarin), 1675 (C=O, amide). ^{1}H NMR: δ_{ppm} 2.19 (s, 3H), 3.82 (s, 3H), 6.98-7.80 (m, 7H), 8.18 (s, 1H), 8.68 (s, 1H), 9.58 (s, 1H), 11.64 (s, 1H). MS (EI, m/z): 403 (M$^+$, 26.1%). Analysis for $C_{22}H_{17}N_3O_5$ (403), Calcd: C, 65.50; H, 4.25; N, 10.42%. Found: C, 65.28; H, 4.32; N, 10.54%.

Synthesis of 6-amino-4-aryl-2-oxo-1-((1-(coumarin-3-yl)-ethylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile 9a and 9b

To a well-stirred suspension of acrylohydrazide **8** (0.002 mol) in 30 mL ethyl alcohol, malononitrile (0.002 mol) and 0.5 mL of triethylamine were added. The reaction was completed after refluxing the reactants for 4 hours and then cooled to 25°C. The solid that formed was picked up by filtration and recrystallized from ethyl alcohol.

6-Amino-4-(4-nitrophenyl)-2-oxo-1-((1-(coumarin-3-yl)-ethylidene) amino)-1,2-dihydropyridine-3,5-dicarbonitrile (9a)

Brown powder, yield = 68%, m.p. > 300°C. IR (ν_{max}/cm^{-1}): 3438, 3347 (NH_2), strong broad at 2200 (C≡N), 1724 (C=O, coumarin), 1687 (C=O, pyridone). ^{1}H NMR: δ_{ppm} 2.08 (s, 3H), 6.54 (s, 2H), 7.35-8.05 (m, 8H), 8.41 (s, 1H). MS (EI, m/z): 466 (M^+, 86.3%). Analysis for $C_{24}H_{14}N_6O_5$ (466): Calcd: C, 61.80; H, 3.03; N, 18.02%. Found: C, 61.91; H, 3.07; N, 18.10%.

6-Amino-4-(3-hydroxy-4-methoxyphenyl)-2-oxo-1-((1-(coumarin-3-yl)-ethylidene)-amino)-1,2-dihydropyridine-3,5-dicarbonitrile (9b)

Brown powder, yield = 73%, m.p. >300°C. IR (ν_{max}/cm^{-1}): 3430, 3348, 3237 (NH_2 and OH), strong broad at 2205 (C≡N), 1721 (C=O, coumarin), 1688 (C=O, pyridone). ^{1}H NMR: δ_{ppm} 2.11 (s, 3H), 3.84 (s, 3H), 6.63 (s, 2H), 6.91-7.65 (m, 7H), 8.47 (s, 1H), 9.61 (s, 1H). MS (EI, m/z): 467 (M^+, 69.4%). Analysis for $C_{25}H_{17}N_5O_5$ (467): Calcd: C, 64.24; H, 3.67; N, 14.98%. Found: C, 64.36; H, 3.62; N, 14.88%.

Conclusion

The target of this work involved the preparation and evaluation the anti-cancer activity of some coumarinyl-pyridine and coumarinyl-pyrimidine scaffolds with the hope of discovering new structure leads serving as anticancer agents. Compounds **7** and **8b** proved to be the most important compounds in this study; they displayed very strong activity against the human HepG2 and MCF-7. The other synthesized coumarin scaffolds displayed different degrees of anti-cancer effect, compounds substituted with methoxy and hydroxy groups in benzene ring (derived from isovanillin) showed stronger anticancer activity than their corresponding compounds substituted with nitro group (derived from 4-nitrobenzaldhyde), which displayed moderate and/or weak anti-cancer effect.

References

1. Riveiro ME, Moglioni A, Vazquez R, Gomez N, Facorro G, et al. (2008) Structural insights into hydroxycoumarin-induced apoptosis in U-937 cells. Bioorg Med Chem 16: 2665-2675.
2. Feuer G, Kellen JA, Kovacs K (1976) Suppression of 7,12-Dimethylbenz (α) anthracene-induced breast carcinoma by coumarin in the rat. Oncology 33: 35-39.
3. Paul K, Bindal S, Luxami V (2013) Synthesis of new conjugated coumarin-benzimidazole hybrids and their anticancer activity. Bioorg Med Chem Lett 23: 3667-3672.
4. Salem MAI, Marzouk MI, El-Kazak AM (2016) Synthesis and characterization of some new coumarins with *in vitro* antitumor and antioxidant activity and high protective effects against DNA damage. Molecules, 21: 249.
5. Kontogiorgis CA, Hadjipavlou-Litina LD (2004) Synthesis and biological evaluation of novel coumarin derivatives with a 7-azomethine linkage. Bioorg Med Chem Lett 14: 611-614.
6. Abdelhafez OM, Amin KM, Batran RZ, Maher TJ, Nada SA, et al. (2010) Synthesis, anticoagulant and PIVKA-II induced by new 4-hydroxycoumarin derivatives. Bioorg Med Chem 18: 3371-3378.
7. Kashman Y, Gustafson KR, Fuller RW, Cardellina JH, McMahon JB, et al. (1993) HIV inhibitory natural products. Part 7. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. J Med Chem 36:1110-1110.
8. Shikishima Y, Takaishi Y, Honda G, Ito M, Takeda Y, et al. (2001) Chemical constituents of *Prangos tschimganica*; structure elucidation and absolute configuration of coumarin and furanocoumarin derivatives with anti-HIV activity. Chem Pharm Bull 49: 877-880.
9. Ostrov DA, Hernández Prada JA, Corsino PE, Finton KA, Le N, et al. (2007) Discovery of novel DNA gyrase inhibitors by high-throughput virtual screening. Antimicrob Agents Chemother 51: 3688-3698.
10. Gormley NA, Orphanides G, Meyer A, Cullis PM (1996) The interaction of coumarin antibiotics with fragments of the DNA gyrase B protein. Maxwell A. Biochemistry 35: 5083-5092.
11. Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Nicolaides DN (2004) Natural and synthetic coumarin derivatives with anti-inflammatory/antioxidant activities. Curr Pharm Des 10: 3813-3833.
12. Wu XQ, Huang C, Jia YM, Song BA, Li J, et al. (2014) Novel coumarin-dihydropyrazole thio-ethanone derivatives: design, synthesis and anticancer activity. Eur J Med Chem 74: 717-725.
13. Vianna DR, Hamerski L, Figueiró F, Bernardi A, Visentin LC, et al. (2012) Selective cytotoxicity and apoptosis induction in glioma cell lines by 5-oxygenated-6,7-methylenedioxycoumarins from Pterocaulon species. Eur J Med Chem 57: 268-274.
14. Nasr T, Bondock S, Youns M (2014) Anticancer activity of new coumarin substituted hydrazide-hydrazone derivatives. Eur J Med Chem 76: 539-548.
15. Chen Y, Liu HR, Liu HS, Cheng M, Xia P, et al. (2012) Antitumor agents 292. Design, synthesis and pharmacological study of S- and O-substituted 7-mercapto- or hydroxy-coumarins and chromones as potent cytotoxic agents. Eur J Med Chem 49: 74-85.
16. Ahn BZ, Sok DE (1996) Michael acceptors as a tool for anticancer drug design. Curr Pharm Des 2: 247-262.
17. Terzioglu N, Gürsoy A (2003) Synthesis and anticancer evaluation of some new hydrazone derivatives of 2,6-dimethylimidazo[2,1-b] [1,3,4] thiadiazole-5-carbohydrazide. Eur J Med Chem 38: 781-786.
18. Abadi AH, Eissa AAH, Hassan GS (2003) Synthesis of novel 1,3,4-trisubstituted pyrazole derivatives and their evaluation as antitumor and antiangiogenic agents. Chem Pharm Bull 51: 838-844.
19. Kumar D, Kumar NM, Ghosh S, Shah K (2012) Novel bis (indolyl) hydrazide-hydrazones as potent cytotoxic agents. Bioorg Med Chem Lett 22: 212-215.
20. Gilbert J, De Iuliis GN, Tarleton M, McCluskey A, Sakoff JA (2018) (Z)-2-(3,4-Dichlorophenyl)-3-(1*H*-pyrrol-2-yl) acrylonitrile exhibits selective antitumor activity in breast cancer cell lines via the aryl hydrocarbon receptor pathway. Mol Pharmacol 93: 168-177.

21. Anderson DR, Hegde S, Reinhard E, Gomez L, Vernier WF, et al. (2005) Aminocyanopyridine inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2). Bioorg Med Chem Lett 15: 1587-1590.

22. Lacy A, Kennedy RO (2004) Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. Curr Pharm Des 10: 3797-3811.

23. Kim EK, Kwon KB, Shin BC, Seo EA, Lee YR, et al. (2005) Scopoletin induces apoptosis in human promyeloleukemic cells, accompanied by activations of nuclear factor κB and caspase-3. Life Sci 77: 824-836.

24. Ohori H, Yamakoshi H, Tomizawa M, Shibuya M, Kakudo Y, et al. (2006) Synthesis and biological analysis of new curcumin analogues bearing an enhanced potential for the medicinal treatment of cancer. Mol Cancer Ther 5: 2563-2571.

25. Tatsuzaki J, Bastow KF, Goto KN, Nakamura S, Itokawa H, et al. (2006) Dehydrozingerone, chalcone, and isoeugenol analogues as *in vitro* anticancer agents. J Nat Prod 69: 1445-1449.

26. Gursoy A, Karali N (2003) Synthesis, characterization and primary antituberculosis activity evaluation of 4-(3-coumarinyl)-3-benzyl-4-thiazolin-2-one benzylidene-hydrazones. Turk J Chem 27: 545-551.

27. Van den Goorbergh JAM, Van der Steeg M, Van der Gen A (1984) A convenient synthesis of 3-(1-oxo-2-alkenyl)-coumarins. Synthesis 859-860.

28. Zhou JF, Song YZ, Lv JS, Gong GX, Tu S (2009) Facile one-pot, multicomponent synthesis of pyridines under microwave irradiation. Synthetic Communications 39: 1443-1450.

29. Gomha SM, Khalil KD (2012) A convenient ultrasound-promoted synthesis of some new thiazole derivatives bearing a coumarin nucleus and their cytotoxic activity. Molecules 17: 9335-9347.

30. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

31. Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival: Modifications to the tetrozolium dye procedure giving improved sensistivity and reliability. J Immunol Methods 89: 271-277.

32. Mauceri HJ, Hanna NN, Beckett MA, Gorski DH, Staba MJ, et al. (1998) Combined effects of angiostatin and ionizing radiation in antitumour therapy. Nature 394: 287-291.