Synthesis and cytotoxic activity of some novel benzocoumarin derivatives under solvent free conditions

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ABSTRACT
In the present study, a rapid, less expensive, clean and environmental friendly route to synthesis new pyrazoles, pyrazolopyridazines and condensed pyrimidines was developed via grinding of 2-(3-(dimethylamino)acryloyl)-3H-benzo[f]chromen-3-one (1) with different reagents. All the new compounds were characterized and established using elemental analysis and spectral data. Eight compounds were selected for in vitro antiproliferative against different human cancer cell lines entitled melanoma, cancers of the lung, leukemia, breast, brain, colon, prostate, ovary and kidney by the USA NCI.

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Introduction

The organic solvents are volatile and harmful, causing risks to peoples who inhale them as well as the environment. Thus, development of less hazardous synthetic methods for organic reactions is one of our objectives in current research. The grinding method is one of green chemistry techniques as it is carried out in the absence of solvent (1–4). Moreover, the solvent-free reactions have numerous advantages: reduced pollution, low costs, high yields and purities of products (5, 6). Otherwise, many compounds which contain coumarin nucleus exhibit antimicrobial (7), analgesic (8), ulcerogenic (9), anticoagulant (10), antiviral (11) and antimalarial (12), anti-inflammatory (13, 14) anti-leishmanial (15) as well as antioxidant (16, 17) activities. The antitumor activity of coumarins received a considerable interest owing to their cytotoxic activity against various types of cancer cells, including gastric cancer, liver cancer, colon cancer, breast cancer, prostate cancer, Periodontal Ligament, fibroblast, nasopharyngeal carcinoma, and normal fibroblast cell lines (18–22). In addition, coumarin derivatives can inhibit growth in human cancer cell lines (23) such as renal (ACHN), lung (A549, H727), leukemia (HL-60), breast (MCF7) and renal cell carcinoma (24). Coumarin and its derivatives are important components among the molecules in the drug industry. Warfarin, acenocoumarol and phenprocoumon are a derivatives of coumarin used as anticoagulant drugs. These are vitamin K antagonist which inhibits the coagulation via blocking of the coagulation factors I, VII, IX and X (25–27). Moreover, warfarin reduced metastases from intestinal cancer to a wide extent (28) and is also used beside the surgical treatment of cancer masses (29) (Figure 1).

In addition, scopoletin (30, 31) and esculetin (32) were found in nature and they have antiproliferative, antiarthritic, antioxidant, and anti-inflammatory activities. Ensaculin (KA-672) is a drug belong to coumarin family

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and acts on a number of receptor systems, being both a weak NMDA antagonist and a 5HT1A agonist (33, 34) (Figure 1). Furthermore, combining coumarins with different bioactive molecules like: pyrazole, pyrazolo[1,5-a]pyrimidine (35), have recently been reported to exhibit significant anticancer activities. In continuation of our previous studies on solvent free condition (36, 37), we report here in a facial, eco-friendly, high yielding approach to synthesis new coumarin derivatives containing different bioactive moieties by grinding and evaluate their anticancer activity.

**Results and discussion**

Due to the pharmaceutical importance of coumarins, pyrazoles and pyrazolopyridazines, we are interested in this part of the work to integrate these biologically active molecules. Thus, grinding of 2-(3-(dimethylamino)acryloyl)-3H-benzo[f]chromen-3-one (1) with the appropriate of hydrazonoyl halides 2a-e in moist sodium carbonate afforded pyrazoles 4a-e, respectively.

In grindstone technique, the grinding process is performed at room temperature and the reaction occurs due to heat generated by frictions between substrate and reagent. Products 4a-e were assumed to formed through 1,3-dipolar cycloaddition reaction of the nitrilimines 2′ (synthesized in situ from reaction of 2a-e with triethylamine) to the activated double bond in compound 1 affording cyclo adduct 3a-e which undergoes loss of dimethylamine molecule furnishing the final product (route A). Another route (B), producing 7 does not operate based on spectral data. In 1H NMR spectrum of 4a as an example, the ester group was resonated as triplet and quartet signals at δ 1.28 and 4.31 ppm, respectively and methyl protons was observed at δ 2.42 ppm. The H-5 of pyrazole and H-4 of coumarin were appeared as two singlet signals at δ 8.45 and 9.21 ppm, respectively. Other signals owing to aromatic protons was observed at δ 7.27–8.39 ppm (Scheme 1). 13C NMR showed signals at 13.5 ppm due to –CH3 of ester group and at 24.2 ppm for –CH3 of p-tolyl, 63.5 ppm for –OCH2 of ester beside that signals appeared at 104.6–152.5 ppm for Ar-C. Finally, three signals related to 3 C=O at δ 159.3, 160.5, 165.2 ppm. The mass spectrum of all products 4a-e exhibited a molecular ion peak for the respective compound (see Experimental).

Formation of products 4a-e rather than product 7 were further confirmed chemically by grinding pyrazoles 4a-e with hydrazine hydrate affording pyrazolo[3,4-d]pyridazines 5a-e in a good yield (Scheme 1). Based on the above results, we exclude pyrazoles 7 and indicated that the isolable products are pyrazoles 4a-e. Structure 5a-e is well supported by spectral data as well as elemental analyses. IR spectrum of 10 showed a characteristic band at $\nu$ 3424, 3304 cm$^{-1}$ related to $\text{–NH and } \text{–NH}_2$ groups, 1689 for carbonyl group, and C=N and C=S groups was shown at 1621 and 1265 cm$^{-1}$, respectively.

**Figure 1.** Naturally and synthetic coumarins used as drugs.
The $^1$H NMR spectrum for 10 as an example, displayed signals at $\delta$ 7.32–9.18 ppm assigned to nine aromatic protons, and two singlet signals at $\delta$ 10.76, 11.12 ppm corresponding for -NH and –NH$_2$ groups which were exchangeable by D$_2$O. Compound 9 was assumed to be formed via nucleophilic addition of hydrazine hydrate to β-carbon atom of enaminone 1 followed by elimination of dimethylamine yielded intermediate 8 which undergoes cyclocondensation affording 9 as outlined in Scheme 2.

Besides, the reaction of enaminone 1 with some heterocyclic amine was also examined. Thus, condensation of enaminone 1 with 2-amino thiazole by grinding with few drops of acetic acid afforded thiazole derivative 11. Analogously, grinding of enaminone 1 with the appropriate of 3-amino-4-cyanopyrazole (12a), 3-amino-4-phenylpyrazole (12b), 3-aminotriazole (12c), 2-amino benzimidazole (12d) or 4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine (12e) yielded condensed pyrimidine derivatives 15a-e, respectively (Scheme 3). Compounds 15a-e may be formed via Michael addition of the exo-amino group of amine to the double bond of enaminone 1 followed by elimination of dimethylamine molecule to yield the non-isolable intermediate 13 which undergoes intramolecular cyclization via loss of water molecule affording compound 15 (Scheme 3).

Moreover, our present study was extended to investigate the chemical behavior of enaminone 1 towards some sulphadrugs. Thus, condensation of enaminone 1 with sulphadrugs, namely: [sulphadiazine, sulphademidine, sulphadimethoxazine or sulphisoxazole] 16a-d by grinding with few drops of acetic acid afforded benzene sulphonamide derivatives 17a-d, respectively in a good yield (Scheme 4). Chemical structure of compounds 17a-d assigned on the basis of the elemental analysis and spectra. IR spectrum of 17b showed a broad band at 3410 cm$^{-1}$ corresponds to 2NH groups and at 1724 cm$^{-1}$, 1665 cm$^{-1}$ due to 2 C=O functional groups. $^1$H NMR spectrum revealed singlet signal at $\delta$ 2.27 ppm attributed to two methyl groups, beside that of aromatic protons at $\delta$ 6.60–9.25 ppm and two singlet signals at $\delta$ 10.60 and 11.28 ppm assignable to two NH protons exchangeable by D$_2$O.

Pharmacological screening

In vitro cytotoxic assay

The cytotoxic activity of eight compounds was determined by USA National Cancer Institute against different human cancer cell lines, using Adriamycin and 5-Flourouracil as positive controls. The results are cited...
Scheme 2. Synthesis of pyrazoles 9 and 10.

Scheme 3. Synthesis of thiazole 13 and pyrimidines 15a-e.
in Table 1 and revealed that pyrazole 4e exhibited powerful growth inhibition activity against Leukemia K-562 (48.45%), Melanoma MDA-MB-435 (47.79%) as well as Renal Cancer UO-31 (40.70%). On the other hand, compounds 5a and 5c showed promising anticancer potency against Renal Cancer UO-31, Leukemia CCRF-CEM and Breast Cancer cell lines. However, compound 5d showed remarkable growth inhibitory activity against all of Leukemia cell lines specially K-562 (65.42%) and SR (75.26%). In addition to a pronounced anticancer activity towards Non-Small Cell Lung Cancer NCI-H460 (47.97%), Melanoma MDA-MB-231/ATCC (39.65%), UO-31 (32.76), UACC-62 (30.19), SNB-75 (35.43), Melanoma: LOX IMVI (28.29), Renal Cancer: UO-31 (38.49), Breast Cancer: MCF-7 (28.18), MDA-MB-231/ATCC (39.19), T-47D (32.65).

Table 1. The delta values and growth inhibition percent of the selected compounds against some subpanel cell lines.

| Cpd. No | NSC. NO. | Delta | Panel and subpanel cell lines (growth inhibition percent) |
|---------|---------|-------|---------------------------------------------------------|
| 4a      | 795392  | 30.00 | Non-Small Cell Lung Cancer: HOP-92 (30.46), NCI-H522 (27.47), Melanoma: LOX IMVI (23.27), UACC-62 (33.19), Renal Cancer: UO-31 (36.39), Breast Cancer: MCF7 (39.65), MDA-MB-231/ATCC (28.67), T-47D (26.69). |
| 4c      | 795409  | 29.39 | Leukemia: SNB-75 (33.60), Melanoma: LOX IMVI (34.81), Breast Cancer: MDA-MB-231/ATCC (29.95). |
| 4e      | 795393  | 35.16 | Leukemia: K-562 (48.45), Non-Small Cell Lung Cancer: HOP-92 (27.44), NCI-H522 (39.44), Colon Cancer: KM12 (26.42), CNS Cancer: SNB-75 (30.65), Melanoma: MDA-MB-435 (37.79), UACC-62 (32.42), Ovarian Cancer: GROVI (31.81), Renal Cancer: UO-31 (40.70). |
| 5a      | 795389  | 28.14 | Leukemia: CCRF-CEM (40.52), MOLT-4 (27.88), SR (26.83), CNS Cancer: SNB-75 (35.43), Melanoma: LOX IMVI (28.29), Renal Cancer: UO-31 (38.49), Breast Cancer: MCF-7 (28.18), MDA-MB-231/ATCC (39.19), T-47D (32.65). |
| 5c      | 795408  | 32.63 | Leukemia: CCRF-CEM (49.40), MOLT-4 (38.57), SR (46.91), Colon Cancer: HCT-15 (40.08), CNS Cancer: SF-268 (28.01), SNB-75 (37.36), U251 (27.25), Melanoma: LOX IMVI (37.44), Ovarian Cancer: GROVI (37.36), OVCA3-3 (31.19), OVCA4-4 (40.02), Renal Cancer: UO-31 (47.97). |
| 5d      | 795400  | 45.85 | Leukemia: CCRF-CEM (52.87), HL-60(TH) (37.76), K-562 (65.42), MOLT-4 (44.29), RPMI-826 (34.46), SR (75.26), Non-Small Cell Lung Cancer: HOP-92 (52.71), NCI-H522 (76.17), Colon Cancer: HCT-116 (54.04), HCT-15 (35.24), KM12 (34.9), CNS Cancer: SF-268 (37.42), SF-295 (39.17), SNB-75 (40.39), Melanoma: LOX IMVI (42.81), M14 (47.4), MDA-MB-435 (67.65), UACC-62 (40.51), Ovarian Cancer: GROVI (48.41), OVCA3-3 (54.06), OVCA4-4 (30.19), NCI/ADR-RES (33.83), Renal Cancer: UO-31 (53.78), Prostate Cancer: PC-3 (46.56), Breast Cancer: MCF7 (64.38), MDA-MB-231/ATCC (40.68), T-47D (36.41). |
| 5e      | 795390  | 39.98 | Leukemia: CCRF-CEM (33.71), K-562 (62.22), MOLT-4 (30.19), RPMI-826 (27.33), SR (64.48), Non-Small Cell Lung Cancer: HOP-92 (38.94), NCI-H522 (62.84), Colon Cancer: HCT-116 (28.02), HCT-15 (28.48), KM12 (28.34), CNS Cancer: SF-268 (34.92), SF-295 (32.83), SNB-75 (42.58), U251 (30.54), Melanoma: LOX IMVI (37.99), M14 (47.4), MDA-MB-435 (35.15), SKMEL-2 (60.45), UACC-62 (39.30), Ovarian Cancer: GROVI (41.66), OVCA3-3 (39.91), OVCA4-4 (33.25), Renal Cancer: UO-31 (47.03), Prostate Cancer: PC-3 (32.37), Breast Cancer: MCF7 (53.04), MDA-MB-231/ATCC (38.84), T-47D (35.61). |
| 9       | 795395  | 30.55 | Leukemia: SR (34.25), Non-Small Cell Lung Cancer: NCI-H460 (34.96), Renal Cancer: UO-31 (37.82), Breast Cancer: MCF7 (37.46), T-47D (30.15). |

Experimental

Melting points were determined on an electrothermal apparatus and are uncorrected. IR spectra were recorded (KBr discs) on a Shimadzu FT-IR 8201 PC spectrophotometer. 1H and 13CNMR spectra were recorded in CDCl3 and (CD 3)2SO solutions on a Varian Gemini 300 MHz and JNM-LA 400 FT-NMR system spectrometer and chemical shifts are expressed in ppm units using.
TMS as an internal reference. Mass spectra were recorded on a GC-MS QP1000 EX Shimadzu. Elemental analyses were carried out at the Microanalytical Center of Cairo University. Anticancer activity was performed at Development Therapeutic Program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), Bethesda, Maryland, USA. Enaminone 1 (38), Hydrazonoyl halides 2a-e (39–44) were prepared as reported in the literature.

Synthesis of pyrazoles 4a-e

A mixture of enaminone 1 (0.293 g, 1 mmol), the appropriate hydrazonoyl halides 2a-e (1 mmol each) and moist sodium carbonate (0.2 g, 2 mmol) was thoroughly ground with a pestle in an open mortar at room temperature until the mixture turned into a melt. The initial syrupy reaction mixture solidified within 3–10 min. After completion of the reaction as monitored by TLC, the mixture was diluted with water, filtered and recrystallized from a DMF/EtOH affording 4a-e, respectively.

2-(3-Ethoxycarbonyl-1-p-tolyl-1H-pyrazol-4-yl)-carbonyl-3H-benzo[f]chromen-3-one (4a)

Yellow crystals; yield: 90%; m.p. 180–181°C; FT-IR (KBr, cm⁻¹): ν 3059, 2921 (CH), 1727, 1634 (C=O), 1594 (C=N), 1563 (C=C); ¹H NMR (300 MHz, CDCl₃, δ, ppm): 1.28 (t, 3H, J = 7 Hz, CH₂CH₃), 2.42 (s, 3H, Ar-CH₃), 4.31 (q, 2H, J = 7 Hz, CH₂CH₃), 7.27–7.32 (m, 2H), 7.49 (d, 1H, J = 8 Hz), 7.62–7.76 (m, 4H), 7.93 (d, 1H, J = 8 Hz), 8.09 (d, 1H, J = 8 Hz), 8.39 (d, 1H, J = 8 Hz), 8.45 (s,1H), 9.21 (s,1H); ¹³C NMR (300 MHz, CDCl₃, δ, ppm): 13.5 (CH₃), 24.2 (CH₃), 63.5 (OCH₂),104.6,152.5 (Ar-C), 160.3, 165.1 (3C=O). Anal. calcd. for C₂₆H₁₈N₂O₄ (422.43): C, 73.92; H, 4.29; N, 6.63%; found: C, 74.05; H, 4.38; N, 6.76%.

2-(3-Benzofuran-2-yl-carbonyl)-1-p-tolyl-1H-pyrazol-4-yl)-carbonyl-3H-benzo[f]chromen-3-one (4d)

Yellow powder; yield: 86%; m.p.: 284–285°C; FT-IR (KBr, cm⁻¹): ν 3135, 3056, 2924 (CH), 1725, 1640 (C=O), 1601 (C=N), 1553 (C=C); ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.43 (s, 3H, CH₃), 7.28–7.79 (m, 12H, ArH’s), 7.90 (d, 1H, J = 8 Hz, ArH), 8.12 (d, 1H, J = 8 Hz, ArH), 8.34 (d, 1H, J = 8 Hz, ArH), 8.45 (s,1H, ArH), 9.24 (s,1H, ArH); MS (m/z): 523 (M⁺–1). Anal. calcd. for C₂₉H₂₉N₂O₄ (524.52): C, 75.56; H, 3.84; N, 5.34%; found: C, 75.67; H, 3.78; N, 5.22%.

General method for synthesis of pyrazolo[3,4-d]pyridazines 5a-e

A mixture of pyrazoles 4a-e (1 mmol each) and hydrazine hydrate (0.1 mL, 2 mmol) was ground with a pestle in a mortar at room temperature for 10–15 min. (monitored by TLC). The reaction mixture was poured into water and the solid product was collected by filtration washed with ethanol, dried and recrystallized from ethanol affording pyrazolo[3,4-d]pyridazines 5a-e, respectively.

2-(7-Hydroxy-2-p-tolyl-2H-pyrazolo[3,4-d]pyridazin-4-yl)-3H-benzo[f]chromen-3-one (5a)

Brown crystals; yield: 90%; m.p. 145–146°C; FT-IR (KBr, cm⁻¹): ν 3425 (OH), 3060, 2925 (CH), 1715 (C=O), 1606 (C=N), 1532 (C=C); ¹H NMR (300 MHz, DMSO-d₆, δ ppm): 2.46 (s, 3H, CH₃), 7.02 (d, 1H, J = 8 Hz), 7.07–7.49 (m, 4H, A), 7.61 (d, 1H, J = 8 Hz), 7.71–7.88 (m, 2H), 7.98 (d, 1H, J = 8 Hz), 8.06 (d, 1H, J = 8 Hz), 8.81 (d, 1H, J = 8 Hz), 8.97 (s,1H), 9.88 (s,1H, OH); ¹³C NMR (300 MHz, DMSO-d₆, δ, ppm): 24.6 (CH₃),106.1–152.5 (Ar-C), 160.3
2-(7-Methyl-2-(4-nitrophenoxy)-2H-pyrazolo[3,4-d]pyridazin-4-yl)-3H-benzo[f]chromen-3-one (5b)

Brown crystals; yield: 88%; m.p. 230–232°C; FT-IR (KBr, cm⁻¹): ν 3087, 2921 (CH), 1722, 1689 (C=O), 1605 (C=N), 1530 (C=C); ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.40 (3H, CH₃), 7.23–7.30 (m, 3H), 7.46 (d, 1H, J = 8 Hz), 7.62–7.77 (m, 3H), 7.90 (d, 1H, J = 8 Hz), 8.06 (d, 1H, J = 8 Hz), 8.42 (d, 1H, J = 8 Hz), 8.46 (s, 1H), 9.22 (s, 1H); ¹³C NMR (300 MHz, CDCl₃, δ, ppm): 20.8 (CH₃), 104.7–152.7 (Ar-C), 162.4 (C=O); MS (m/z): 499 (M⁺). Anal. calcd. for C₃₂H₁₇N₄O₃ (449.42): C, 66.81; H, 3.36; N, 15.58%; found: C, 66.73; H, 3.29; N, 15.49%.

2-(7-Methyl-2-p-tolyl-2H-pyrazolo[3,4-d]pyridazin-4-yl)-3H-benzo[f]chromen-3-one (5c)

Brown powder; yield: 88%; m.p. 230–232°C; FT-IR (KBr, cm⁻¹): ν 3087, 2921 (CH), 1722, 1692, 1636 (C=O), 1619 (C=N), 1512 (C=C); ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.35 (3H, CH₃), 2.42 (3H, CH₃), 7.25–7.32 (m, 3H), 7.45 (d, 1H, J = 8 Hz), 7.57–7.76 (m, 3H), 7.94 (d, 1H, J = 8 Hz), 8.09 (d, 1H, J = 8 Hz), 8.42 (d, 1H, J = 8 Hz), 8.46 (s, 1H), 9.21 (s, 1H); ¹³C NMR (300 MHz, CDCl₃, δ, ppm): 21.3 (CH₃), 25.2 (CH₃), 107.2–150.1 (Ar-C), 161.7 (C=O); MS (m/z): 418 (M⁺). Anal. calcd. for C₃₂H₁₇N₄O₃ (418.45): C, 74.63; H, 4.34; N, 13.38%; found: C, 74.52; H, 4.26; N, 13.45%.

2-(7-Benzofuran-2-yl)-2-p-tolyl-2H-pyrazolo[3,4-d]pyridazin-4-yl)-3H-benzo[f]chromen-3-one (5d)

Brown powder; yield: 89%; m.p. 130–132°C; FT-IR (KBr, cm⁻¹): ν 3130, 3060, 2920 (CH), 1722 (C=O), 1602 (C=N), 1525 (C=C); ¹H NMR (300 MHz, CDCl₃, δ, ppm): 2.40 (3H, CH₃), 7.28–7.79 (m, 12H), 7.90 (d, 1H, J = 8 Hz), 8.12 (d, 1H, J = 8 Hz), 8.34 (d, 1H, J = 8 Hz), 8.45 (s, 1H), 9.24 (s, 1H); MS (m/z): 520 (M⁺). Anal. calcd. for C₃₅H₂₀N₄O₃ (520.54): C, 76.14; H, 3.87; N, 10.76%; found: C, 76.04; H, 3.95; N, 10.68%.

2-(7-Benzofuran-2-yl)-2-phenyl-2H-pyrazolo[3,4-d]pyridazin-4-yl)-3H-benzo[f]chromen-3-one (5e)

Brown powder; yield: 91%; m.p. 184–186°C; FT-IR (KBr, cm⁻¹): ν 3070, 2924 (CH), 1720 (C=O), 1600 (C=N), 1530 (C=C); ¹H NMR (300 MHz, CDCl₃, δ, ppm): 7.21–7.75 (m, 13H), 7.85 (d, 1H, J = 8 Hz), 8.03 (d, 1H, J = 8 Hz), 8.10 (d, 1H, J = 8 Hz), 8.55 (s, 1H), 9.22 (s, 1H). Anal. calcd. for C₃₂H₁₈N₄O₃ (506.51): C, 75.88; H, 3.58; N, 11.06%; found: C, 75.79; H, 3.70; N, 11.17%.

Synthesis of 2-(1H-pyrazol-3-yl)-3H-benzo[f]chromen-3-one (9)

A mixture of enaminone 1 (0.293 g, 1 mmol) and hydrazine hydrate (0.1 mL, 2 mmol) was ground with a pestle in a mortar at room temperature. After few seconds the mixture became sticky, the grinding process was continued for 10–15 min. (monitored by TLC). The formed product was washed with ethanol, filtered and recrystallized from ethanol to give the desired product 9.

Synthesis of 3-(3-oxo-3H-benzo[f]chromen-2-yl)-1H-pyrazole-1-carbothiohydrazide (10)

A mixture of enaminone 1 (0.293 g, 1 mmol), thiocarbohydrazide (0.106 gm, 1 mmol) and few drops of acetic acid was ground with a pestle in a mortar at room temperature with for 5–10 min. (monitored by TLC). The formed product was washed with ethanol, filtered and recrystallized from ethanol affording pyrazole 10.

Red powder; yield: 89%; m.p. 320–322°C; FT-IR (KBr, cm⁻¹): ν 3424, 3304 (NH₂, NH), 3059, 3051, 2920 (CH), 1689 (C=O), 1621 (C=N), 1565 (C=C), 1265 (C=S); ¹H NMR (300 MHz, DMSO-d₆, δ, ppm): 7.32–7.96 (m, 4H), 8.06 (d, 1H, J = 8 Hz), 8.22 (d, 1H, J = 8 Hz), 8.36 (d, 1H, J = 8 Hz), 8.63 (d, 1H, J = 8 Hz), 9.18 (s, 1H), 10.76 (s, 1H, NH-D₃O exchangeable), 11.12 (s, 2H, NH₂-D₂O exchangeable); MS (m/z): 336 (M⁺). Anal. calcd. for C₁₇H₁₄N₄O₂S (336.37): C, 60.70; H, 3.60; N, 16.66; S, 9.53%; found: C, 60.82; H, 3.48; N, 16.54; S, 9.41%.

Synthesis of thiazole derivative 11 and pyrimidines 15a-e

To a mixture of enaminone 1 (0.293 g, 1 mmol) and 2-aminothiazole, 3-amino-4-cyanopyrazole (12a), 3-amino-4-phenylpyrazole (12b), 3-aminothiazole (12c), 2-aminobenzimidazole (12d) or 4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-amine (12e) (1 mmol each), few drops of acetic acid was added and the mixture was ground with a pestle in a mortar at room temperature for 5–10 min. until the mixture turned into a melt, during which time a color change occurred (the change is colorless to yellow). After completion of the reaction as indicated by TLC, the reaction mixture was poured into water, the solid product was filtered, washed with ethanol, dried and recrystallized from ethanol affording the desired products 11 and 15a-e, respectively.
2-(E)-3-(thiazol-2-ylimino)propanoyl)-3H-benzo [f]chromen-3-one (11)
Brown powder; yield: 90%; m.p. 270–272°C; FT-IR (KBr, cm⁻¹): ν 3424 (NH), 3067, 2920 (CH), 1726, 1659 (C=O), 1617 (C=N), 1561 (C=C); ¹H NMR (300 MHz, DMSO-d₆, δ, ppm): 7.12–7.60 (m, 4H), 7.68–8.49 (m, 5H, ArH’s + NH), 8.65 (d,1H, J = 8 Hz), 8.89 (d,1H, J = 8 Hz), 9.70 (s,1H); MS (m/z): 349 [M⁺]+. Anal. calcd. for C₁₉H₁₂N₂O₂S (348.38): C, 65.51; H, 3.47; N, 9.20%; found: C, 65.63; H, 3.60; N, 8.14; S, 9.32%.

7-(3-oxo-3H-benzo[f]chromen-2-yl)pyrazolo[1,5-alpyrimidine-3-carbonitrile (15a)
Yellow crystals; yield 90%; m.p. 348–349°C (lit. m.p. 350°C (45)).

2-(3-Phenylpyrazolo[1,5-alpyrimidin-7-yl)-3H-benzo [f]chromen-3-one (15b)
Red crystals; yield: 91%; m.p. 311–312°C (lit. m.p. 310°C (45)).

2-({1,2,4}triazolo[4,3-alpyrimidin-5-yl)-3H-benzo [f]chromen-3-one (15c)
Brown crystals; yield: 90%; m.p. 232–234°C (lit. m.p. 232°C (45)).

2-Benzof[4,5]imidazo[1,2-alpyrimidin-4-yl-benzo [f]chromen-3-one (15d)
Brown crystals; yield: 90%; m.p. 280–282°C (lit. m.p. 280°C (45)).

2-(8,10-dimethylpyrido(2’,3’:3,4)pyrazolo[1,5-alpyrimidin-4-yl]-3H-benzo[f]chromen-3-one (15e)
Orange powder; yield: 91%; m.p. >360°C; FT-IR (KBr, cm⁻¹): ν 3078, 2922 (CH), 1724, 1665 (C=O), 1613 (C=N), 1550 (C=C); ¹H NMR (300 MHz, DMSO-d₆, δ, ppm): 2.31 (s, 3H, CH₃), 2.61 (s, 3H, CH₃), 6.66 (d,1H, J = 8 Hz), 7.08 (s,1H), 7.56–7.68 (m, 3H), 7.70–8.57 (m, 3H), 8.89 (d,1H, J = 8 Hz), 9.70 (s,1H). Anal. calcd. for C₂₉H₂₈N₄O₂ (392.410): C, 73.46; H, 4.11; N, 14.28%; found: C, 73.58; H, 4.43; N, 14.16%.

Reaction of enamino 1 with sulphadruge
A mixture of enamino 1 (0.293 g, 1 mmol), sulphaduugs 16a-d (1 mmol each), and few drops of acetic acid was ground with a pestle in a mortar at room temperature for 5–10 min. until the mixture turned into a melt, during which time a color change occurred (the change is colorless to yellow to orange). After completion of the reaction as monitored by TLC. The mixture was poured into water and the solid product was filtered, washed with ethanol, dried and recrystallized from DMF affording the desired products 17a-d, respectively.

4-[3-Oxo-3-(3-oxo-3H-benzo[f]chromen-2-yl)-proplylimino]-N-pyrimidin-2-yl-benzenesulfonamide (17a)
Orange crystals; yield: 92%; m.p. >360°C; FT-IR (KBr, cm⁻¹): 3427 broad (2NH), 3076, 2925 (CH), 1724, 1681 (C=O), 1604 (C=N), 1525 (C=C); ¹H NMR (300 MHz, DMSO-d₆, δ, ppm): 6.69 (d, 1H, J = 8 Hz), 7.20 (d, 1H, J = 8 Hz), 7.49 (d, 1H, J = 8 Hz), 7.65–8.07 (m, 4H), 8.12 (d, 1H, J = 8 Hz), 8.20–8.34 (m, 5H), 8.53 (d, 1H, J = 8 Hz), 9.24 (s,1H), 9.36 (s,1H), 10.71 (s,1H, NH-D₂O exchangeable), 11.57 (s,1H, NH-D₂O exchangeable); MS (m/z): 498 (M⁺). Anal. calcd. for C₂₉H₁₈N₄O₂S (498.51): C, 62.64; H, 3.64; N, 11.24; S, 6.43%; found: C, 62.52; H, 3.76; N, 11.12; S, 6.31%.

N-(4,6-Dimethyl-pyrimidin-2-yl)-4-[3-oxo-3-(3-oxo-3H-benzo[f]chromen-2-yl)-propynylamino]-benzenesulfonamide (17b)
Orange crystals; yield: 91%; m.p. 290–291°C; FT-IR (KBr, cm⁻¹): 3410 broad (2NH), 3078, 2956, 2920 (CH), 1724, 1665 (C=O), 1580 (C=N), 1541 (C=C); ¹H NMR (300 MHz, DMSO-d₆, δ, ppm): 2.27 (s, 6H, 2CH₃), 6.60 (d, 1H, J = 8 Hz), 6.74 (d, 1H, J = 8 Hz), 7.30 (d, 1H, J = 8 Hz), 7.52 (d, 1H, J = 8 Hz), 7.61–8.03 (m, 4H), 8.18 (d, 1H, J = 8 Hz), 8.22–8.32 (m, 2H), 8.57 (d, 1H, J = 8 Hz), 9.25 (s,1H), 9.39 (s,1H), 10.60 (s,1H, NH-D₂O exchangeable), 11.28 (s,1H, NH-D₂O exchangeable); MS (m/z): 526 (M⁺). Anal. calcd. for C₃₂H₂₂N₆O₅S (526.56): C, 63.87; H, 4.21; N, 10.64; S, 6.09%; found: C, 63.75; H, 4.33; N, 10.76; S, 6.20%.

N-(2,6-Dimethoxy-pyrimidin-4-yl)-4-[3-oxo-3-(3-oxo-3H-benzo[f]chromen-2-yl)-propynylamino]-benzenesulfonamide (17c)
Orange crystals; yield: 92%; m.p. 260–261°C; FT-IR (KBr, cm⁻¹): 3438 broad (2NH), 3058, 2966, 2922 (CH), 1726, 1659 (C=O), 1598 (C=N), 1551 (C=C); ¹H NMR (300 MHz, DMSO-d₆, δ, ppm): 3.60 (s, 6H, 2OCH₃), 6.58 (d, 1H, J = 8 Hz), 6.74 (d, 1H, J = 8 Hz), 7.27 (d, 1H, J = 8 Hz), 7.47 (d, 1H, J = 8 Hz), 7.60–7.81 (m, 4H), 7.88 (d, 1H, J = 8 Hz), 7.94–8.32 (m, 2H), 8.57 (d, 1H, J = 8 Hz), 9.25 (s,1H), 9.38 (s,1H), 10.55 (s,1H, NH-D₂O exchangeable), 12.08 (s,1H, NH-D₂O exchangeable); MS (m/z): 558. Anal. calcd. for C₂₉H₂₈N₄O₂S (558.56): C, 60.21; H, 3.97; N, 10.03; S, 5.74%; found: C, 60.10; H, 3.86; N, 10.15; S, 5.86%.

N-(3,4-Dimethyl-isoxazole-5-yl)-4-[3-oxo-3-(3-oxo-3H-benzo[f]chromen-2-yl)-propynylamino]-benzenesulfonamide (17d)
Orange crystals; yield: 92%; m.p. >360 °C; FT-IR (KBr, cm⁻¹): 3434 broad (2NH), 3062, 2966, 2924 (CH), 1725,
1659 (C=O), 1617 (C=N), 1551 (C=C); $^1$H NMR (300 MHz, DMSO-$d_6$, δ, ppm): 1.59 (s, 3H, CH$_3$), 1.97 (s, 3H, CH$_3$), 7.60–7.93 (m, 15H, Ar-H + 2NH); MS (m/z): 515 (M$^+$). Anal. calcd. for C$_{27}$H$_{21}$N$_3$O$_6$S (515.54): C, 62.90; H, 7.60; N, 8.15; S, 6.22%; found: C, 62.78; H, 4.23; N, 8.28; S, 6.34%.

**Cytotoxic screening**

**NCI-60 screening methodology**

The cytotoxicity against cancer cell lines were conducted at USA National Cancer Institute according to reported standard procedure (Sulphorhodamine B assay) (46–48).

**Conclusions**

In this research, benzo[f]chromen-3-one moiety is introduced as new class in antitumor agent against different cancer cell lines. Briefly, target compounds were prepared via reactions of 2-(3-(dimethylamino)acryloyl)-3H-benzo[f]chromen-3-one with hydrazonoyl halides, N-nucleophiles and sulphadrugs by grinding reactants together at room temperature. The anticancer activity was performed at USA National Cancer Institute (NCI) against different human tumor cell lines called melanoma, leukemia and cancers of the brain, lung, colon, ovary, prostate, breast and kidney. The results displayed that pyrazole 4e and pyrazolo[3,4-d]pyridazines 5a, 5c–5e exhibited powerful anticancer activity against most cell lines.

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No potential conflict of interest was reported by the authors.

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**References**

[1] Bruckmann, A.; Krebs, A.; Bolm, C. Green Chem. 2008, 10, 1131–1141.
[2] Rodriguez, B.; Bruckmann, A.; Rantanen, T.; Bolm, C. Adv. Synth. Catal. 2007, 349, 2213–2233.
[3] Raston, C.L.; Scott, J.L. Green Chem. 2000, 2, 49–52.
[4] Shan, Z.-X.; Luo, X.-X.; Hu, L.; Hu, X.-Y. Sci. China Chem. 2010, 53, 1095–1101.
[5] Tanaka; Toda, F.K. Chem. Rev. 2000, 100, 1025–1074.
[6] Schmeyers, J.; Toda, F.; Boy, J.; Kaupp, G.; Chem, J. Soc. Perkin Trans. 1998, 2, 989–994.
[7] Sahooa, J.; Mekap, S.K.; Kumara, P.S.; Taib, J. Green Chem. 2015, 9, 187–195.
[8] Sudha, B.N.; Sastry, V.G. Int. J. Adv. Res. 2016, 4 (3), 1225–1232.
[9] Gupta, J.K.; Sharma, P.K.; Dudhe, R.; Chaudhary, A.; Verma, P.K. Anal. Univ. Bucuresti-Chim. 2010, 19, 9–21.
[10] Abdelhafiz, O.M.; Amin, K.M.; Batran, R.Z.; Maher, T.J.; Nada, S.A.; Sethumadhavan, S. Bioorg. Med. Chem. 2010, 18, 3371–3378.
[11] Neyts, J.; Clercq, E.D.; Singha, R.; Chang, Y.H.; Das, A.R.; Chakraborty, S.K.; Hong, S.C.; Tsay, S.C.; Hsu, M.H.; Hwu, J.R. J. Med. Chem. 2009, 52, 1486–1490.
