Facile Synthesis of Pyrazolo[3,4-*c*]pyrazoles Bearing Coumarine Ring as Anticancer Agents

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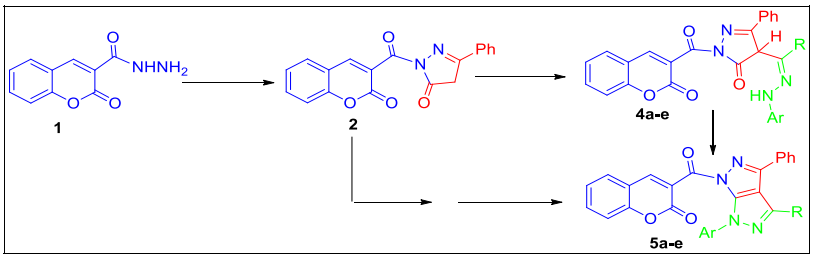
Received February 6, 2018
DOI 10.1002/jhet.3235
Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com).



In the present study, 2-(2-oxo-2*H*-chromene-3-carbonyl)-5-phenyl-2,4-dihydro-3*H*-pyrazol-3-one was prepared and reacted with various hydrazonoyl halides to give a series of 2-(2-oxo-2*H*-chromene-3-carbonyl)-5-phenyl-4-((2-phenylhydrazono)methyl)-2,4-dihydro-3*H*-pyrazol-3-one in good yield. Cyclization of the latter hydrazone with $POCl_3$ yielded the respective 3-(3-phenyl- 4,6-disubstituted-1,6-dihydropyrazolo[3,4-c]pyrazole-1-carbonyl)-2*H*-chromen-2-ones. The structures of the newly synthesized compounds were established on the basis of spectroscopic evidences and their alternative syntheses. The newly synthesized compounds were evaluated for their antitumor activities against hepatocellular carcinoma (HepG2) cell line and the results revealed promising activities of compounds **4e**, **4c**, and **4d** with IC_{50} equal 0.92 ± 0.22, 1.43 ± 0.19, and 2.17 ± 0.21 μM, respectively.

J. Heterocyclic Chem., **00,** 00 (2018).

INTRODUCTION

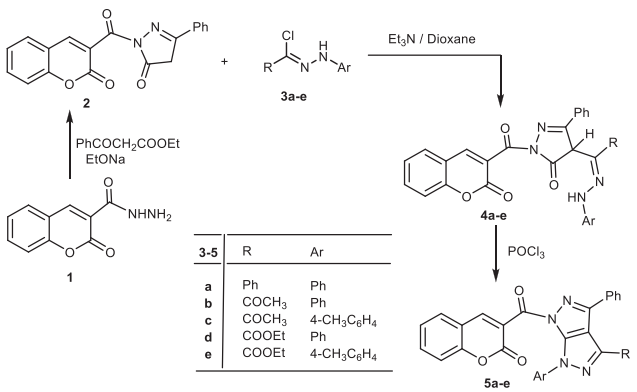
In continuation of our studies dealing with the utility of hydrazonoyl halides for synthesis of various bridgehead nitrogen polyheterocycles [1–10], the synthesis of coumarins and their derivatives have attracted considerable attention from organic and medicinal chemists for many years because of their wide range of medicinal applications such as antitumoral, anti-inflammatory, antiviral, CNS active, anti-HIV, and antioxidant activities [11–16]. Pyrazole derivatives are characterized by their biological and pharmacological activities as potential inhibitors of HIV-1, pesticides, antihypertensive, fungicides, analgesic, antipyretic, antidepressant, antirheumatic, antiviral, and anticancer agents [17–23]. They are also important and useful precursors for the synthesis of other fused heterocyclic systems, among these pyrazolo[3,4-*d*] pyrimidine derivatives [24], which have a considerable chemical and pharmacological importance as purine analogues [25–27]. Also, the pyrazolo[3,4-*b*] pyridine derivatives represent important building blocks in both natural and synthetic bioactive compounds [28]. They show anxiolytic activity along with xanthine oxidase inhibitors, cholesterol formation inhibitor, and anti-Alzheimer [29]. On the other hand, it has been found that pyranopyrazoles possess a multiplicity of pharmacological properties including anticancer, antimicrobial, anti-inflammatory, insecticidal, and molluscicidal activities [30–32]. Moreover, the pyrazolo[3,4-*c*]pyrazole nucleus was proven to constitute the active part of several biologically active compounds [33–38]. On the other hand, the literature reveals that several methods have been described for the elaboration of substituted pyrazolo [3,4-*c*]pyrazoles [39] as reaction of 4-arylidenepyrazol-5-ones with hydrazines and hydrazides [40–43], Vilsmeier reaction of hydrazonopyrazol-5-ones, reaction of thiocarbohydrazides with 4-acetyl/benzoyl-pyrazol-5-ones [44], hydrazinolysis of aminocyanopyrazoles [45], hydrazinolysis of 5-oxo-N-phenyl-4,5-dihydro-1*H*-pyrazole-4-carbothioamides [46], reaction of amino and hydroxy pyrazoles with hydrazonyl halides [47], reaction of 5-chloro-1*H*-pyrazole-4-carbaldehydes with hydrazines in the presence of *p*-TsOH [48,49], and cyclocondensation of 4,5-dihydro-3-phenyl-5-[(2-propenyl)thio]-1*H*-1,2,4-triazole with ethyl 2-chloro-2-(2-p-tolylhydrazono) acetate [50]. In view of the aforementioned facts, our efforts were directed towards a new facile synthesis of various functionalized derivatives of pyrazolo[3,4-*c*] pyrazole bearing coumarine moiety that have not been reported hitherto with the hope of augmentation in biological activities.

RESULTS AND DISCUSSION

The new starting compound, namely, 2-(2-oxo-2*H*-chromene-3-carbonyl)-5-phenyl-2,4-dihydro-3*H*-pyrazol-3-one **2** was prepared by refluxing a mixture of 2-oxo-2*H*-chromene-3-carbohydrazide **1** [51] and ethyl benzoylacetate in ethanol in the presence of a catalytic amount of sodium ethoxide for 10 h (Scheme 1). The structure **2** was established based on elemental and spectral data (IR, ^{1}H NMR, mass). For example, the IR spectrum of compound **2** revealed the stretching bands at υ = 1742, 1684, and 1668 cm^{-1} that are assigned to the three carbonyl groups. ^{1}H NMR spectrum of compound **2** displayed one signal at δ = 3.86 ppm attributed to the CH_2 protons, in addition to the expected signals of the aromatic protons. The mass spectrum revealed a molecular ion peak at m/z = 332 that is consistent with the molecular formula $C_{19}H_{12}N_2O_4$.

Next, our study was extended to investigate the reactivity of compound **2** towards hydrazonoyl halides [52] aiming to synthesize new heterocyclic compounds containing pyrazolopyrazole ring. Thus, reaction of compound **2** with hydrazonoyl chlorides **3a–e** in dioxane in the presence of triethylamine at reflux afforded the corresponding 2-(2-oxo-2*H*-chromene-3-carbonyl)-5-phenyl-4-((2-phenylhydrazono)methyl)-2,4-dihydro-3*H*-pyrazol-3-one**s 4a–e** (Scheme 1). The structures of the compounds **4a–e** were established on the basis of spectroscopic and microanalyses data. For example, the IR of the compound **4a**, taken as typical example, showed absorption bands at ν = 3336 (NH), 1700, 1684, 1658 (3C=O) cm^{-1}, its ^{1}H NMR spectrum revealed signals at δ 5.66 (s, 1H, pyrazole-H4), 6.79–7.88 (m, 19H, Ar–H), 8.27 (s, 1H, Coumarine-H4), 11.30 (brs, 1H, NH) ppm. Moreover, they revealed the absence of the CH_2 proton signal present in the spectra of compounds **2** at δ 3.86. Its mass spectrum revealed a molecular ion peak at *m/z* 526.

Scheme 1. Synthesis of pyrazolopyrazoles **5a–e**.



Reflux of compounds **4a–e** with $POCl_3$ afforded, via dehydrative cyclization, a product identified as 3-(4,6-disubstituted-1,6-dihydropyrazolo[3,4-c]pyrazole-1-carbonyl)-2*H*-chromen-2-ones **5a–e** (Scheme 1). The structure assigned for each of products **5** was elucidated via elemental analysis and spectral data. The IR spectra of each of compounds **5** revealed the absence of the NH group and one carbonyl group. The ^{1}H NMR spectra of compounds **5** revealed the absence of the signals due to the NH and pyrazoline-H4 protons. The molecular ion peaks of the cyclized products **5a–e** are less than that of their respective **4a–e** by 18.

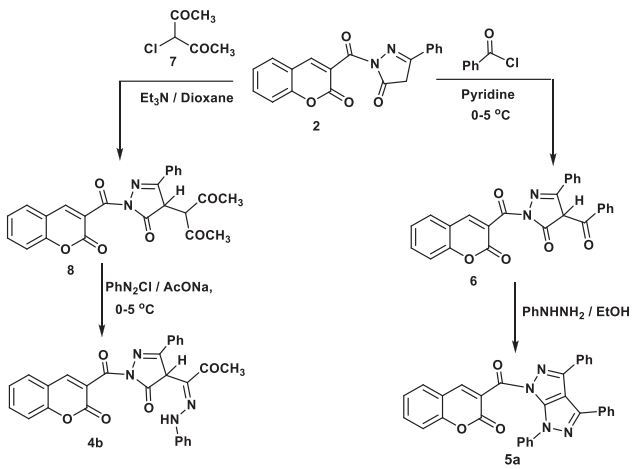
Furthermore, to provide a conclusive evidence for the assigned structure **5a–e**, we reacted compound **2** with benzoyl chloride in pyridine at 0–5°C to afford 4-benzoyl-2-(2-oxo-2*H*-chromene-3-carbonyl)-5-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (**6**) as end product according to its spectral data together with elemental analyses (Scheme 2) (Experimental).

Treatment of **6** with phenyl hydrazine in ethanol under reflux yielded product that proved identical in all respects (IR, MS, mp., and mixed mp.) with **5a.**

The structure of products **4** was further confirmed by an alternative method. Thus, reaction of compound **2** with 3-chloropentane-2,4-dione under reflux in ethanol containing equivalent amounts of TEA, led to the formation of product **8**. Compound **8** was then react with benzenediazonium salt in ethanol in the presence of sodium acetate trihydrate at 0–5°C to give a product identical in all respects (IR, mp., and mixed mp.) with **4b** that obtained from reaction of **2** with hydrazonoyl halide **3b** (Scheme 2).

Antitumor activity. The antitumor activity of compounds **4a–e** and **5a–e** was determined against a liver carcinoma cell line Hepg2, using doxorubicin as a

Scheme 2. Alternate synthesis of pyrazolinone **4b** and pyrazolopyrazole **5a**.



reference drug. Data generated were used to plot a dose–response curve of which the concentration (μM) of test compounds required to kill 50% of cell population (IC_{50}) was determined. The cytotoxic activity was expressed as the mean IC_{50} of three independent experiments (Table 1) and the results revealed that all the tested compounds showed inhibitory activity to the tumor cell lines in a concentration dependent manner. The small values of IC_{50} for the selected compounds indicate that for more anticancer effect higher concentrations can be used.

The results are represented in Table 1 and showed that

- The *in vitro* inhibitory activities of tested compounds against the human liver carcinoma (Hepg2) have the descending order as follows: **4e** > **4c** > **4d** > **5d** > **5e** > **5c** > **4b** > **5b** > **4a** > **5a**.
- The hydrazono pyrazolines **4** have *in vitro* inhibitory activity than the pyrazolopyrazoles **5** (**4a** > **5a, 4b** > **4b, 4c** > **5c, 4d** > **5d, 4e** > **5e**).
- The introduction of electron-donating group (methyl group) at the 4-position of phenyl group at position 4 in the pyrazoline ring enhances the antitumor activity.
- The tolyl group has *in vitro* inhibitory activities more than the phenyl group (**4c** > **4b, 4e** > **4d, 5c** > **5b, 5e** > **5d**).
- The ester group has *in vitro* inhibitory activities more than the acetyl group (**4d** > **4b, 4e** > **4c, 5d** > **5b, 5e** > **5c**).

CONCLUSION

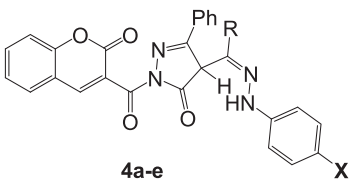
In our present work, a new series of 2-(2-oxo-2*H*-chromene-3-carbonyl)-5-phenyl-4-((2-phenylhydrazono) methyl)-2,4-dihydro-3*H*-pyrazol-3-ones were synthesized and cyclized to 3-(3-phenyl-4,6-disubstituted-1,6-dihydropyrazolo[3,4-c]pyrazole-1-carbonyl)-2*H*-chromen-2-ones. The structures of the newly synthesized compounds were established on the basis of spectroscopic evidences and their synthesis by alternative methods. The *in vitro* growth inhibitory activity of the synthesized compounds against hepatocellular carcinoma (HepG2) cell line was investigated in comparison with doxorubicin as a standard drug using MTT assay and the results revealed promising activities of compounds **4e**, **4c**, and **4d** with IC_{50} equal 0.92 ± 0.22, 1.43 ± 0.19, and 2.17 ± 0.21 μM, respectively.

EXPERIMENTAL

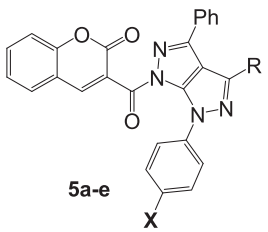
All melting points were determined on an electrothermal Gallenkamp apparatus and are uncorrected. Solvents were generally distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury VXR-300 spectrometer (300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR) and the

Table 1

The *in vitro* inhibitory activity of tested compounds against a liver carcinoma cell line Hepg2 expressed as IC_{50} values (μM) ± standard deviation from six replicates.



4a-e



5a-e

Compound no.	R	X	IC_{50} (μM)
Doxorubicin	-	-	0.72 ± 0.18
4a	Ph	H	56.3 ± 0.30
4b	COMe	H	14.6 ± 0.24
4c	COMe	4-Me	1.43 ± 0.19
4d	COOEt	H	2.17 ± 0.21
4e	COOEt	4-Me	0.92 ± 0.22
5a	Ph	H	74.19 ± 0.29
5b	COMe	H	16.17 ± 0.21
5c	COMe	4-Me	11.04 ± 0.21
5d	COOEt	H	6.80 ± 0.14
5e	COOEt	4-Me	9.83 ± 0.26

chemical shifts were related to that of the solvent DMSO-d_6. The mass spectra were recorded on a GCMSQ1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses and spectral measurements were carried out by the microanalytical center at Cairo University and the analytical laboratory of the institute of organic chemistry, Technical University of Dresden, Germany. Antitumor activity was evaluated at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Synthesis of 2-(2-oxo-2*H*-chromene-3-carbonyl)-5-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (2). To a stirred solution of (10 mmol) sodium ethoxide (prepared by adding 0.23 g sodium to 20 mL absolute ethanol) was added 2-oxo-2*H*-chromene-3-carbohydrazide (**1**) (2.04 g, 10 mmol). The solution was left stirring for about 10 min. Then the ethyl benzoylacetate (10 mmol) was added. The reaction mixture was refluxed for 10 h. The solvent was then evaporated and the solid left was collected and crystallized from ethanol to give product **2** as yellow solid (64% yield), mp. 188–190°C; IR (KBr) υ1712, 1684, 1668 (3C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ3.86 (s, 2H, CH_2), 7.11–7.84 (m, 9H, Ar–H), 8.21 (s, 1H, Coumarine-H4); MS, m/z (%) 270 (M^+, 64), 158 (100), 77 (34). *Anal*. Calcd for $C_{19}H_{12}N_2O_4$ (332.31): C, 68.67; H, 3.64; N, 8.43. Found: C, 68.53; H, 3.61; N, 8.28%.

Reaction of pyrazolone 2 with hydrazonoyl chlorides 3a–e. To a mixture of **2** (0.664 g, 2 mmol) and the appropriate hydrazonoyl chlorides **3a–e** (2 mmol) in dioxane (20 mL), triethylamine (0.14 mL, 2 mmol) was added. The reaction mixture was stirred at room temperature for about 10 h. The precipitate formed was collected by filtration and crystallized from the proper solvent to give products **4a–e**. The physical constants and spectral data of the obtained products **4a–e** are listed in the succeeding text:

2-(2-Oxo-2H-chromene-3-carbonyl)-5-phenyl-4-(phenyl (2-phenylhydrazono)methyl)-2,4-dihydro-3H-pyrazol-3-one (4a). Yellow solid (74%); mp = 203–205°C (DMF); IR (KBr): υ 3336 (NH), 1700, 1684, 1658 (3C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 5.66 (s, 1H, pyrazole-H4), 6.79–7.88 (m, 19H, Ar–H), 8.27 (s, 1H, Coumarine-H4), 11.30 (s, br, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 45.8 (CH), 118.3, 119.6, 122.1, 122.8, 124.5, 124.9, 125.1, 126.6, 127.0, 127.7, 128.3, 129.4, 131.1, 132.7, 134.3, 136.3, 137.0, 139.4, 140.6, 143.4, 147.0, 151.7 (Ar–C and C=N), 164.7, 171.3, 177.2 (3C=O); MS *m/z* (%): 526 (M^+, 16), 412 (100), 333 (49), 217 (82), 77 (80). *Anal*. Calcd. for $C_{32}H_{22}N_4O_4$ (526.54): C, 72.99; H, 4.21; N, 10.64. Found C, 72.76; H, 4.01; N, 10.49%.

4-(2-Oxo-1-(2-phenylhydrazono)propyl)-2-(2-oxo-2H-chromene-3-carbonyl)-5-phenyl-2,4-dihydro-3H-pyrazol-3-one (4b). Yellow solid (67%); mp = 169–171°C (EtOH); IR (KBr): υ 3341 (NH), 1710, 1692, 1670, 1652 (4C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 6.52 (s, 1H, pyrazole-H4), 6.70–7.86 (m, 14H, Ar–H), 8.34 (s, 1H, Coumarine-H4), 11.37 (s, br, 1H, NH); MS *m/z* (%): 492 (M^+, 18), 325 (74), 228 (49), 105 (100), 77 (73). *Anal*. Calcd. for $C_{28}H_{20}N_4O_5$ (492.48): C, 68.29; H, 4.09; N, 11.38. Found C, 68.15; H, 4.00; N, 11.24%.

4-(2-Oxo-1-(2-(p-tolyl)hydrazono)propyl)-2-(2-oxo-2H-chromene-3-carbonyl)-5-phenyl-2,4-dihydro-3H-pyrazol-3-one (4c). Yellow solid (75%); mp = 189–191°C (EtOH); IR (KBr): υ 3342 (NH), 1708, 1692, 1673, 1650 (4C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 6.47 (s, 1H, pyrazole-H4), 6.71–7.77 (m, 13H, Ar–H), 8.33 (s, 1H, Coumarine-H4), 11.32 (s, br, 1H, NH); MS *m/z* (%): 506 (M^+, 8), 388 (75), 204 (47), 118 (100), 63 (44). *Anal*. Calcd. for $C_{29}H_{22}N_4O_5$ (506.51): C, 68.77; H, 4.38; N, 11.06. Found C, 68.67; H, 4.31; N, 11.01%.

Ethyl 2-(5-oxo-1-(2-oxo-2H-chromene-3-carbonyl)-3-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-2-(2-phenylhydrazono) acetate (4d). Yellow solid (69%); mp = 171–173°C (EtOH); IR (KBr): υ 3320 (NH), 1712, 1698, 1679, 1660 (4C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.12 (t, J = 7.8 Hz, 3H, CH_2CH_3), 4.17 (q, J = 7.8 Hz, 2H, CH_2CH_3), 6.49 (s, 1H, pyrazole-H4), 6.83–7.95 (m, 14H, Ar–H), 8.38 (s, 1H, Coumarine-H4), 11.43 (s, br, 1H, NH); MS *m/z* (%): 522 (M^+, 19), 412 (73), 222 (39), 105 (63), 77 (100). *Anal*. Calcd. for $C_{29}H_{22}N_4O_6$ (522.51): C, 66.66; H, 4.24; N, 10.72. Found C, 66.47; H, 4.21; N, 10.56%.

Ethyl 2-(5-oxo-1-(2-oxo-2H-chromene-3-carbonyl)-3-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-2-(2-(p-tolyl)hydrazono) acetate (4e). Yellow solid (66%); mp = 166–168°C (EtOH); IR (KBr): υ 3322 (NH), 1730, 1698,1669, 1655 (4C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.19 (t, J = 7.8 Hz, 3H, CH_2CH_3), 2.20 (s, 3H, CH_3), 4.19 (q, J = 7.8 Hz, 2H, CH_2CH_3), 6.35 (s, 1H, pyrazole-H4), 6.72–7.91 (m, 13H, Ar–H), 8.31 (s, 1H, Coumarine-H4), 11.28 (s, br, 1H, NH); MS *m/z* (%): 536 (M^+, 11), 303 (72), 274 (38), 105 (61), 57 (100). *Anal*. Calcd. for $C_{30}H_{24}N_4O_6$ (536.54): C, 67.16; H, 4.51; N, 10.44. Found C, 67.02; H, 4.39; N, 10.29%.

Synthesis of 1-(2-oxo-2*H*-chromene-3-carbonyl)-(4,6-disubstituted-1,6-dihydropyrazolo[3,4-*c*]pyrazoles 5a–e. General method. To the appropriate 3-methyl-1-(2-oxo-2*H*-chromene-3-carbonyl)-4-(phenyl (2-substituted phenylhydrazono)methyl)-1*H*-pyrazol-5(4*H*)-ones **4** (1 mmol), phosphorus oxychloride (2 mL) was added. The mixture was refluxed for 4 h (monitored by TLC), then the excess $POCl_3$ was evaporated and the reaction mixture was poured onto crushed ice with stirring. The solid product that precipitated was filtered, washed with water, and finally crystallized from the appropriate solvent to give the respective **5**. The physical constants of the products **5a–e** isolated are listed in the succeeding text.

1-(2-Oxo-2H-chromene-3-carbonyl)-3-(3,4,6-triphenyl-1,6-dihydropyrazolo[3,4-c]pyrazole (5a). Brown solid (74%); mp = 263–265°C (DMF); IR (KBr): *v* 1698, 1662 (2C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 6.80–7.82 (m, 19H, Ar–H), 8.39 (s, 1H, Coumarine-H4); ^{13}C-NMR (DMSO-d_6): δ 113.5, 119.2, 119.6, 120.7, 120.9, 122.6, 123.2, 125.6, 125.8, 127.3, 127.5, 129.2, 129.7, 130.6, 131.5, 131.8, 133.3, 133.9, 135.4, 136.3, 137.4, 139.5, 141.8, 144.2 (Ar–C and C=N), 164.9, 173.0 (2C=O); MS *m/z* (%): 508 (M^+, 100), 362 (55), 211 (40), 106 (39), 77 (85). *Anal*. Calcd. for $C_{32}H_{20}N_4O_3$ (508.53): C, 75.58; H, 3.96; N, 11.02. Found C, 75.64; H, 3.76; N, 11.01%.

4-Acetyl-1-(2-oxo-2H-chromene-3-carbonyl)-3,6-diphenyl-1,6-dihydropyrazolo[3,4-c]pyrazole (5b). Brown solid (68%); mp = 271–273°C (DMF); IR (KBr): *v* 1703, 1688, 1660 (3C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 6.83–7.77 (m, 14H, Ar–H), 8.43 (s, 1H, Coumarine-H4); MS *m/z* (%): 474 (M^+, 100), 315 (39), 266 (28), 164 (60), 77 (53). *Anal*. Calcd. for $C_{28}H_{18}N_4O_4$ (474.47): C, 70.88; H, 3.82; N, 11.81. Found C, 70.67; H, 3.65; N, 11.71%.

4-Acetyl-1-(2-oxo-2H-chromene-3-carbonyl)-3-phenyl-6-(p-tolyl)-1,6-dihydropyrazolo[3,4-c]pyrazole (5c). Brown solid (68%); mp = 251–253°C (DMF); IR (KBr): *v* 1700, 1686, 1662 (3C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.25 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 6.91–7.83 (m, 13H, Ar–H), 8.36 (s, 1H, Coumarine-H4); MS *m/z* (%): 488 (M^+, 851), 339 (67), 270 (100), 173 (82), 77 (83). *Anal*. Calcd. for $C_{29}H_{20}N_4O_4$ (488.49): C, 71.30; H, 4.13; N, 11.47. Found C, 71.16; H, 4.09; N, 11.33%.

Ethyl 6-(2-oxo-2H-chromene-3-carbonyl)-1,4-diphenyl-1,6-dihydropyrazolo[3,4-c]pyrazole-3-carboxylate (5d). Brown solid (65%); mp = 250–252°C (DMF); IR (KBr): *v* 1724, 1686, 1662 (3C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.10 (t, *J* = 7.8 Hz, 3H, CH_2CH_3), 4.14 (q, *J* = 7.8 Hz, 2H, CH_2CH_3), 6.93–7.69 (m, 14H, Ar–H), 8.47 (s, 1H, Coumarine-H4); ^{13}C-NMR (DMSO-d_6): δ 12.8 (CH_3), 61.3 (CH_2), 109.0, 117.3, 119.0, 121.4, 122.3, 123.1, 124.5, 125.0, 126.4, 126.7, 128.5, 130.4, 132.1, 133.5, 135.4, 136.9, 137.0, 139.5, 141.6, 149.6 (Ar–C and C=N), 165.7, 170.5, 174.3 (3C=O) MS *m/z* (%): 504 (M^+, 26), 419 (73), 280 (55), 163 (37), 77 (100). *Anal*. Calcd. for $C_{29}H_{20}N_4O_5$ (504.49): C, 69.04; H, 4.00; N, 11.11. Found C, 69.01; H, 4.13; N, 11.01%.

Ethyl 6-(2-oxo-2H-chromene-3-carbonyl)-4-phenyl-1-(p-tolyl)-1,6-dihydropyrazolo[3,4-c]pyrazole-3-carboxylate (5e). Green solid (67%); mp = 236–238°C (DMF); IR (KBr): *v* 1739, 1698, 1655 (3C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.14 (t, *J* = 7.8 Hz, 3H, CH_2CH_3), 2.23 (s, 3H, CH_3), 4.16 (q, *J* = 7.8 Hz, 2H, CH_2CH_3), 7.03–7.75 (m, 13H, Ar–H), 8.44 (s, 1H, Coumarine-H4); MS *m/z* (%): 518 (M^+, 100), 412 (51), 325 (28), 239 (54), 77 (60). *Anal*. Calcd. for $C_{30}H_{22}N_4O_5$ (518.52): C, 69.49; H, 4.28; N, 10.81. Found C, 69.7; H, 4.20; N, 10.76%.

Alternate synthesis of 5a. ***Synthesis of 4-benzoyl-2-(2-oxo-2H-chromene-3-carbonyl)-5-phenyl-2,4-dihydro-3H-pyrazol-3-one (6).*** To a solution of compound **2** (0.664 g, 2 mmol) in pyridine (15 mL) was added benzoyl chloride (0.280 g, 2 mmol) and the mixture was cooled in an ice bath while being stirred. The reaction mixture was stirred for further 4 h. The formed precipitate after acidification was isolated by filtration, washed with water, dried and recrystallized from ethanol to give the benzoyl pyrazolone **6** as white solid (74%); mp = 184–186°C; IR (KBr): *v* 1712, 1692, 1672, 1660 (4C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 7.17–7.93 (m, 14H, Ar–H), 6.30 (s, 1H, pyrazole-H4), 8.37 (s, 1H, Coumarine-H4); MS *m/z* (%): 436 (M^+, 100), 306 (40), 236 (38), 173 (54), 57 (84). *Anal*. Calcd. for $C_{26}H_{16}N_2O_5$ (436.42): C, 71.56; H, 3.70; N, 6.42. Found C, 71.43; H, 3.76; N, 6.36%.

Reaction of 6 with phenyl hydrazine. A mixture of **6** (0.436 g, 1 mmol) and phenyl hydrazine (0.108 g, 1 mmol) in ethanol (10 mL) was refluxed for 8 h. The solid that precipitated was filtered off, washed with water, dried, and finally crystallized from DMF to give **5a** identical in all respects (mp., mixed mp., IR, MS, and ^{1}H NMR spectra) with that one obtained from **4a** and $POCl_3$.

Alternate synthesis of 4b. ***Synthesis of 3-(5-Oxo-1-(2-oxo-2H-chromene-3-carbonyl)-3-phenyl-4,5-dihydro-1H-pyrazol-4-yl)pentane-2,4-dione (8).*** To a mixture of equimolar quantities of **2** and 3-chloro-2,4-pentanedione (**7**) (2 mmol each) in dioxane (20 mL) was added triethylamine (2 mmol). The mixture was stirred at room temperature for 24 h, the solid that formed was collected and crystallized from ethanol to give the respective product **8** as white solid (74%); mp = 150–152°C; IR (KBr): *v* 1703, 1687, 1676, 1661, 1644 (5C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.28 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 4.04 (s, 1H, CH), 5.75 (s, 1H, pyrazole-H4), 67.17–7.92 (m, 9H, Ar–H), 8.30 (s, 1H, Coumarine-H4); MS *m/z* (%): 430 (M^+, 14), 340 (31), 237 (49), 143 (33), 77 (100). *Anal*. Calcd. for $C_{24}H_{18}N_2O_6$ (430.41): C, 66.97; H, 4.22; N, 6.51. Found C, 66.67; H, 4.13; N, 6.44%.

Coupling of 8 with benzenediazonium chloride. To a solution of compound **8** (1 mmol) in ethanol (10 mL) was added sodium acetate trihydrate (0.3 g) and the mixture was cooled in an ice bath at 0–5°C while being stirred. To the resulting cold solution was added a cold solution of benzenediazonium chloride, prepared as usual by diazotizing aniline (1 mmol) in hydrochloric acid (1 mL, 6 M) with sodium nitrite (0.07 g, 1 mmol) in water (1 mL). After all of the diazonium salt solution was added, the reaction mixture was stirred for further 30 min while being cooled in an ice bath. The solid that precipitated was filtered off, washed with water, dried, and finally crystallized from ethanol to give **4b** identical in all respects (mp., mixed mp., IR, MS, and ^{1}H NMR spectra) with that one obtained from **2** and **3b.**

Antitumor activity. The cytotoxic evaluation of the synthesized compounds was carried out at the Regional Center for Mycology and Biotechnology at Al-Azhar University, Egypt, according to the reported method [53].

REFERENCES AND NOTES

[1] Gomha, S. M. A Monatsh Chem 2009, 140, 213.

[2] Abdelhamid, A. O.; Gomha, S. M.; Abdelriheem, N. A. Synth Commun 2017, 47, 999.

[3] Abbas, I. M.; Gomha, S. M.; Elneairy, M. A. A.; Elaasser, M. M.; Mabrouk, B. K. A. J Chem Res 2015, 39, 719.

[4] Gomha, S. M.; Riyadh, S. M. ARKIVOC 2009, xi, 58.

[5] Abdallah, M. A.; Riyadh, S. M.; Abbas, I. M.; Gomha, S. M. J Chin Chem Soc 2005, 52, 987.

[6] Gomha, S. M.; Badrey, M. G.; Abdalla, M. M.; Arafa, R. K. M. Chem Commun 2014, 5, 1685.

[7] Abbas, I. M.; Riyadh, S. M.; Abdallah, M. A.; Gomha, S. M. J Heterocyclic Chem 2006, 43, 935.

[8] Dawood, K. M.; Gomha, S. M. J Heterocyclic Chem 2015, 52, 1400.

[9] Gomha, S. M.; Riyadh, S. M. Synthesis 2014, 46, 258.

[10] Gomha, S. M.; Riyadh, S. M. J Braz Chem Soc 2015, 26, 916.

[11] Curini, M.; Cravotto, G.; Epifano, F.; Giannone, G. Curr Med Chem 2006, 13, 199.

[12] Gomha, S. M.; Khalil, K. D. Molecules 2012, 17, 9335.

[13] Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. Curr Med Chem 2005, 12, 887.

[14] Dawood, D. H.; Batran, R. Z.; Farghaly, T. A.; Khedr, M. A.; Abdulla, M. M. Arch Pharm Chem Life Sci 2015, 348, 875.

[15] Xie, L.; Takeuchi, Y.; Cosentino, L. M.; McPhail, A. T.; Lee, K. H. J Med Chem 2001, 44, 664.

[16] Yang, Z. Y.; Xia, Y.; Xia, P.; Brossi, A.; Cosentino, L. M.; Lee, K. H. Bioorg Med Chem Lett 2000, 10, 1003.

[17] Gomha, S. M.; Riyadh, S. M.; Abdalla, M. M. Curr Org Synth 2015, 12, 220.

[18] Farghaly, T. A.; Hassaneen, H. M. E.; Elzahabi, H. S. A. M. Chem Res 2015, 24, 652.

[19] Min, G.; Eric, C.; Lihu, Y. Tetrahedron Lett 2006, 47, 5797.

[20] Gomha, S. M.; Farghaly, T. A.; Sayed, A. R. J Heterocyclic Chem 2016, 53, 1503.

[21] Hassan, A. E.; Abdella, M. M.; Ahmed, A. I. J Chem Res 1997, S, 322.

[22] Abdalla, M. A.; Gomha, S. M.; Abdelaziz, M.; Serag, N. Heterocycles 2016, 92, 649.

[23] Aymn, E. R.; Mohamed, I. H.; Randa, E. A. M.; Nahed, F.; Farouk, M. E. A. M. Eur J Med Chem 2009, 44, 3285.

[24] Elkhawaga, A. M.; Kamal El-Dean, A. M.; Radwan, M.; Ahmed, M. M. Bull Korean Chem Soc 2009, 30, 561.

[25] Schenone, S.; Bruno, O.; Radi, M.; Botta, M. Org Chem 2009, 6, 220.

[26] Eljazi, I. A.; Samer, A. A. Molecules 2001, 6, 621.

[27] Abdellatif, M. S.; Ana, M. F. O.; Ligia, M. R. Synth Commun 2009, 39, 1186.

[28] Mourad, C.; Elena, S.; Abdelouahid, S.; Contelles, J. M. J Heterocyclic Chem 2010, 47, 861.

[29] Zhmurenko, L. A.; Molodavkin, G. M.; Voronina, T. A.; Lezina, V. P. Pharm Chem J 2012, 46, 15.

[30] Mandha, S. R.; Siliveri, S.; Alla, M.; Bommena, V. R.; Bommineni, M. R.; Balasubramanian, S. Bioorg Med Chem Lett 2012, 22, 5272.

[31] Zaki, M. E. A.; Soliman, H. A.; Hiekal, O. A.; Rashad, A. E. Z Naturforsch 2006, 61c, 1.

[32] Abdelrazek, F. M.; Metz, P.; Metwally, N. H.; El-Mahrouky, S. F. Arch Pharm 2006, 339, 456.

[33] Rao, R. M.; Reddy, G. N.; Sreeramulu, J. Der Pharma Chem 2011, 3, 301.

[34] Ojha, S.; Bapna, A.; Talesara, G. L. ARKIVOC 2008, xi, 112.

[35] Jungheim, L. N.; Sigmund, S. K. J Org Chem 1987, 52, 4007.

[36] Jungheim, L. N.; Sigmund, S. K.; Fisher, J. W. Tetrahedron Lett 1987, 28, 285.

[37] Kosower, E. M.; Radkowsky, A. E.; Fairlamb, A. H.; Croft, S. L.; Nea, R. A. Eur J Med Chem 1995, 30, 659.

[38] Taylor, E. C.; Patel, H.; Kumar, H. Tetrahedron 1992, 48, 8089.

[39] Khidre, R. E.; Mohamed, H. A.; Abdel-wahab, B. F. Turk J Chem 2013, 37, 1.

[40] Sharma, C.; Thadhaney, B.; Pemawat, G.; Talesara, G. L.; Indian, J. Chem 2008, 47B, 1892.

[41] Abdel Hafez, A. A.; Awad, I. M. A. Phosphorus Sulfur Silicon Rel Elem 1992, 71, 253.

[42] Bhaskar, V. H.; More, V. S.; Kumar, M. Asian J Chem 2008, 20, 5474.

[43] Othman, E. S. Acta Chim Slov 2003, 50, 15.

[44] Awad, I. M. A. Monatsh Chem 1990, 121, 1023.

[45] Deeb, A.; El-Mobayed, M.; Essawy, A.; Abd El-Hamid, A.; Abd El-Hamid, A. M. Coll Czech Chem Commun 1990, 55, 728.

[46] Sayed, G. H.; Kassab, R. R. Bull Fac Pharm Cairo Uni 1998, 36, 53.

[47] Elfahham, H. A.; Sadek, K. U.; Elgemeie, G. E. H.; Elnagdi, M. H. Chem Lett 1982, 1, 119.

[48] Ahluwalia, V. K.; Dahiya, A.; Bala, M. Indian J Chem 1996, 35B, 848.

[49] Mogilaiah, K.; Kavitha, S.; Reddy, G. R.; Indian, J. Heterocycl Chem 2002, 12, 113.

[50] Kumar, P. S.; Nagoji, K. E. V.; Kumar, B. V. V. R. Asian J Chem 2003, 15, 515.

[51] Gomha, S. M.; Abdel-aziz, H. M. Heterocycles 2012, 591, 583.

[52] Shawali, A. S.; Gomha, S. M. Adv Synth Catal 2000, 342, 599.

[53] Gomha, S. M.; Riyadh, S. M.; Mahmmoud, E. A.; Elasser, M. M. Heterocycles 2015, 91, 1227.