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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antitumor and antioxidant evaluation of some new thiazole and thiophene derivatives incorporated coumarin moiety

Moustafa A. Gouda · Moged A. Berghot · Eman A. Baz · Wafaa S. Hamama

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Abstract 3-Acetylcoumarin (**1**) was utilized as a key intermediate for the synthesis of 2-aminothiazole derivative **3** *via* bromination of **1** followed by treatment of the formed acetylbromide **2** with thiourea or *via* Bignailli reaction of **1**. Treatment of **3** with **2** afforded the *bis*-coumarin **4**, whereas, cyanoacetylation of **3** followed by treatment of the formed cyanoacetamide **6** with salicyaldehyde give the *bis*-coumarin **7**. Reaction of **6** with phenyl isothiocyanate in DMF/KOH produced the potassium salt **8**, which cyclized with chloroacetyl chloride to give the thiazolidinone **9**. Acidification of **8** with HCl afforded the thiocarbamoyl **10**, which condensed with **2** in DMF to give the mercapto derivative **12**, whereas in DMF/TEA gave the thiophene derivative **13**. The thiophenes **15a–c** were achieved via treatment of the thiocarbamoyls **14a–c** with **2** in DMF/TEA, whereas, in DMF gave the corresponding thiazoles **16a–c**. Treatment of the components **17a**, **b** with carbon disulfide in DMF/KOH followed by addition of **2** afforded the dithioacetals **19a**, **b**. Cyclization of **19b** under alkaline condition gave the desired thiophene **20**. Representative compounds of the synthesized products were evaluated as antitumor and antioxidant agents.

Keywords Coumarin · Synthesis · Antitumor · Antioxidant activities

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Introduction

Coumarin derivatives constitute an important class of heterocyclic compounds with anticoagulant (e.g., warfarin, acenocoumarol) (Anderson *et al*., 2002; Tassies *et al*., 2002), anticoagulant rodenticide (e.g., brodifacoum, bromadiolone) (Stone *et al*., 1999), insecticide (e.g., coumaphos) (Weick and Thorn, 2002), and antibacterial (e.g., novobiocin, clorobiocin) (Mitscher, 2002; Lafitte *et al*., 2002), pharmacological properties. The cytotoxic activities of coumarin and its known metabolite 7-hydroxycoumarin were tested in several human tumor cell lines. Both compounds inhibited cell proliferation of a gastric carcinoma cell line, a colon-carcinoma cell line, a hepatoma-derived cell line, and a lymphoblastic cell line (Weber *et al*., 1998). On the other hand, the thiazoline derivatives have been reported to exhibit antibacterial and antifungal activities (Habib and Khali, 1984; Nofal *et al*., 2002). Furthermore, thiophene derivatives were found to possess analgesic and antiinflammatory activities (Russo *et al*., 1994). The previously reported works on the synthesis of thiazole derivatives indicated that most of the compounds showed high antimicrobial (Gouda *et al*., 2010; Khalil *et al*., 2009a, b; Khalil *et al*., 2010a, b), and analgesic (Abu-Hashem *et al*., 2010), activities. In this study the authors report the synthesis, structural determination, and in vitro antioxidant and antitumor activity of the new 3-substituted coumarins incorporated thiazole or thiophene moiety.

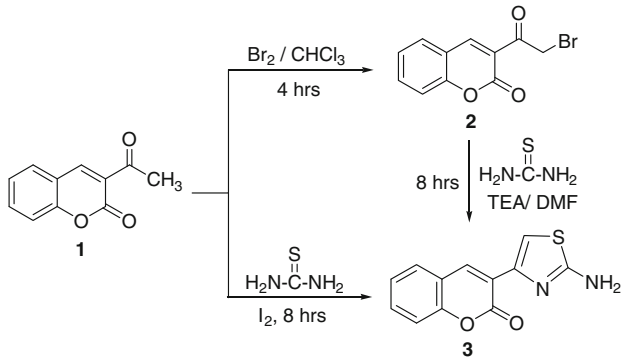
Results and discussion

The synthetic procedure adopted to obtain the target compounds are depicted in Schemes 1, 2, 3, 4, and 5. The starting compound 3-(2-bromoacetyl)-coumarin (**2**)

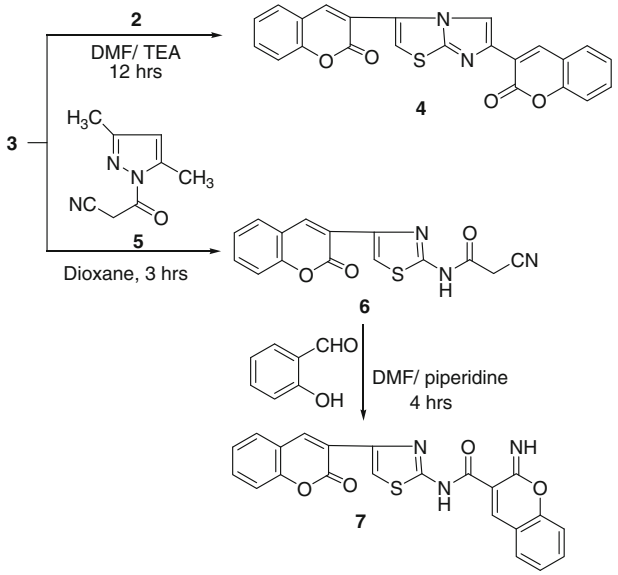
(Siddiqui *et al*., 2009), was prepared according to the reported method via bromination of 3-acetyl-coumarin (**1**) (Gürsoy and Karali, 2003). Reaction of **2** with thiourea in refluxing ethanol gave the corresponding 2-amino-4-(3-coumarinyl)thiazole **3** (Koelsch, 1950).

In another route, compound **3** was obtained in good yield *via* Biganilli Reaction of 3-acetyl coumarin, thiourea, and iodine. Treatment of **3** with 3-bromoacetyl coumarin **2** in DMF and a basic catalyst led to the corresponding *bis*-coumarins **4**, while cyanoethylation of **3** with 3,5-dimethyl-1-cyanoacetyl pyrazole (**5**) (Gorobets *et al*., 2004), gave the cyanoacetamide **6**. Knoevenagel condensation of the latter compound with salicyaldehyde in DMF catalyzed by piperidine achieved the iminocoumarin **7**.

Isothiocyanates have been used as synthetic intermediates to prepare biologically active heterocyclic compounds (Mukerjee and Ashare, 1991). The biological and physiological activities of sulfur heterocyclic may be attributed to the presence of N–C–S fragment characteristic of thiazoles,

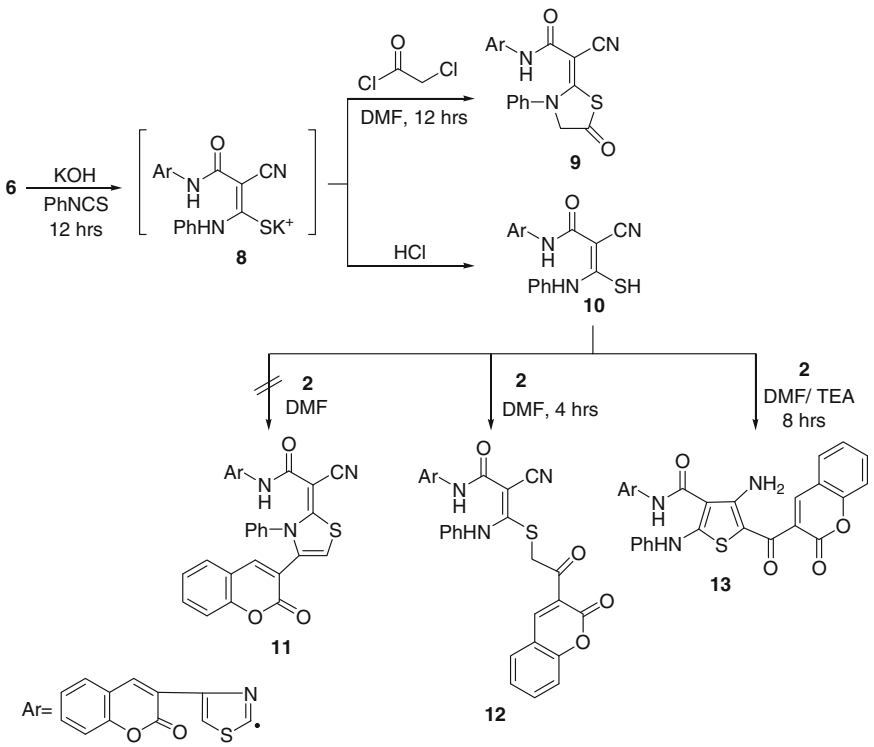


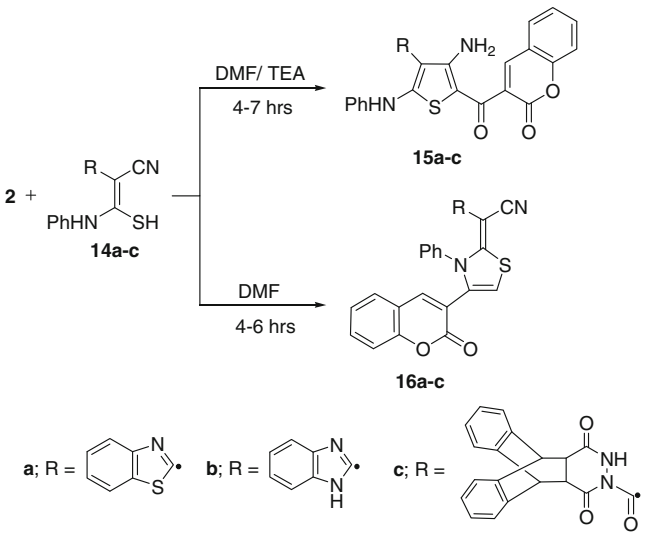
Scheme 1 Synthesis of 3-(2-aminothiazol-4-yl)-2*H*-chromen-2-one (**3**)



Scheme 2 Synthesis of *bis*-coumarin derivatives **4** and **7**

Scheme 3 Reaction of 2-cyano-*N*-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)acetamide (**6**) with phenyl isothiocyanate





Scheme 4 Reaction of 3-(2-bromoacetyl)-2*H*-chromen-2-one (**2**) with thiocarbamoyl derivatives **14a–c**

thiazolines, and thiazolidines (El-Desoky *et al*., 2002). Thus, treatment of **6** with phenyl isothiocyanate in dry DMF in the presence of KOH produced the potassium salt of thiocarbamoyl derivative **8**, which cyclized with chloroacetyl chloride in DMF to give the thiazolidinone derivative **9**, whereas acidification of such potassium salt yielded the corresponding thiocarbamoyl derivative **10**. Attempting preparation of thiazoline derivative **11** through the condensation reaction of thiocarbamoyl derivative **10** with **2** in DMF was failed and instead of **11**, the authors obtained the mercaptoacetyl coumarin derivative **12**. On continuation of this study on the synthesis of biologically interesting heterocyclic molecules containing thiophene moiety (Khalil *et al*., 2010a, b; Khalil *et al*., 2009a, b), several thiophene derivatives have been synthesized with a view of evaluation of their antitumor and antioxidant activities. Thus, refluxing of **10** with **2** in DMF catalyzed by TEA led to the formation of thiophene derivative **13** (Scheme 3).

Furthermore, condensation of **2** with the appropriate thiocarbamoyl derivatives **14a–c** (Augustin and Doelling, 1982; Dawood *et al*., 1998; Khalil *et al*., 2009a, b), in DMF catalyzed by TEA gave the corresponding thiophenes **15a–c**. On the other hand, the thiazole derivatives **16a–c** were obtained *via* refluxing of **2** with the previously thiocarbamoyl derivatives **14a–c** in DMF (Scheme 4).

Oxoketene dithioacetals, especially dimethyl thioacetal have considerable attention because of their synthetic importance for the construction of variety of alicyclic, aromatic, and heterocyclic compounds (Dieter, 1986; Junjappa *et al*., 1990). Thus, treatment of **17a**, **b** (Hauser and Reynolds, 1948; Rinehart and Forbis, 1970), with carbon disulfide and potassium hydroxide in DMF followed by addition of **2**, gave the dithioacetal derivatives **19a**, **b** instead of diacetals **18a**, **b**. Cyclization of dithioacetal derivative **19b** under alkaline conditions gave the desired thiophene **20** (Scheme 5).

Assignment of the new synthesized compounds was based on elemental analyses, IR, ^{1}H NMR, Mass Spectra data (*c.f.* Exp. Part).

Biological activity

Antitumor activity

Effect of drugs on the viability of Ehrlich ascites cells (EAC) in vitro

Ten 3-substituted coumarin derivatives were tested for cytotoxicity against well-known established model EAC in

Scheme 5 Synthesis of mercaptothiophene derivative **20**

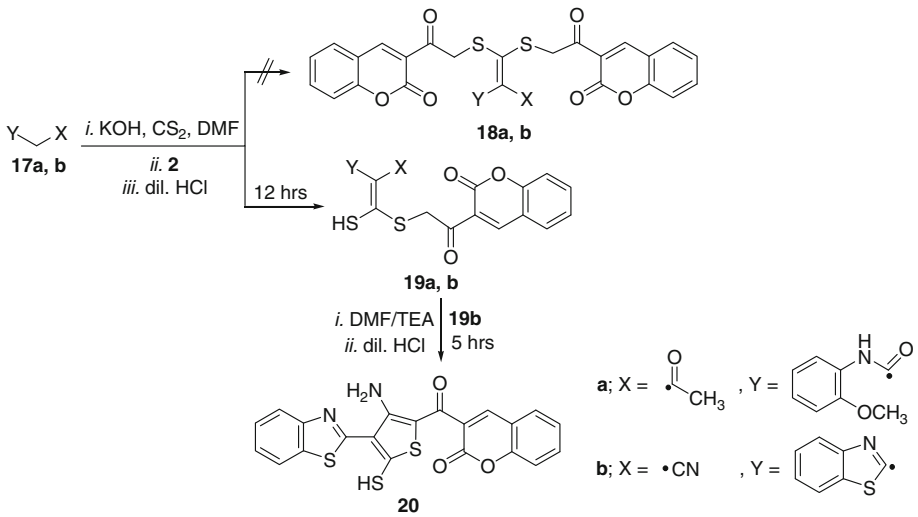


Table 1 In vitro cytotoxicity of 3-substituted coumarin derivatives (Ehrlich ascites cells dead %)

Compound No.	% Dead		
	ED_{100} μg/cm^3	ED_{50} μg/cm^3	ED_{25} μg/cm^3
5-FU	93	58	37
6	8	3.8	1.8
7	4.9	2.1	1.3
9	7.0	3.1	1.6
12	6.8	3.2	1.5
13	7.1	3.0	1.7
15a	5.7	2.9	1.6
15b	5.6	2.6	1.4
15c	6.1	3.0	1.6
16a	5.7	3.0	1.5
16c	5.4	2.6	1.1

Where, ED_{100}, ED_{50}, and ED_{25} are the effective doses at 25, 50, and 100 μl, respectively, of the compounds used. The dead % refers to the % of the dead tumor cells and **5**-FU is **5**-fluorouracil as a well-known cytotoxic agent

Table 2 Antioxidant activity assay of 3-substituted coumarin derivatives

Compound	No. ABTS	
	Absorbance of samples	%Inhibition
Control of ABTS	0.506	0.0
Ascorbic Acid	0.081	83.99
1	0.18	63.58
2	0.248	50.2
3	0.128	74.29
6	0.15	70.29
7	0.195	61.38
9	0.359	28.91
12	0.385	23.76
13	0.326	35.44
15a	0.475	5.94
15b	0.504	0.19
15c	0.436	13.66
16a	0.484	4.15
16c	0.476	5.74
20	0.113	75.64

vitro (Karrer and Rubini, 1965). Results for the ED_{100}, ED_{50}, and ED_{25} values of the active compounds are summarized in Table 1. The data showed clearly that most of compounds have weak activities except compound **20** which have moderate antitumor activity because of the presence of both thiazole and thiophene moieties.

Antioxidant activity assay

The antioxidant activity of the synthesized compounds was evaluated by Lissi *et al.,* (1999). Some of the 3-substituted coumarin derivatives exhibited antioxidant activities as shown in Table 2. Compared with the control (Ascorbic Acid), the antioxidant potency of compounds **3**, **6**, and **20** was found to be the highest activities, while compounds **1**, **2**, and **7** showed a moderate antioxidant activities and the rest of the tested compounds showed weak activities. On the other hand, compounds **3**, **6**, and **20** exhibited high-antioxidant activities compared with the starting material **1**. Thus, it would appear that introducing of thiazole, thiophene moieties at the 3-position of the coumarin enhanced the antioxidant activity, also, the presence of both thiphene and thiazole groups increases the antioxidant activity as in the case of compounds **20** (Fig. 1). By comparing the results obtained from the antioxidant activity of the investigated compounds with their structures the authors found that, the following structure activity relation ships (SARs) were postulated: (i) 2-Aminothiazole derivative **3** is more potent than compounds **1** and **2** which may be attributed to the replacement of acetyl or bromoacetyl with the aminothiazole moiety. (ii) *N*-Substituted-2-aminothiazole **6** is less potent than compound **3** because of the decrease in aromaticity and conjugation. (iii)

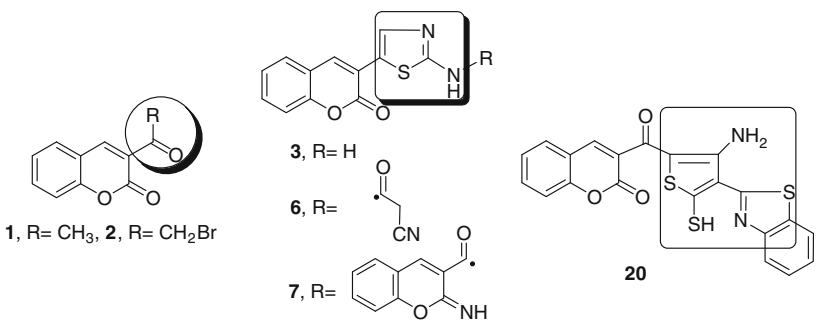


Fig. 1 Comparison of the results obtained from antioxidant activity of the investigated compounds with their structures (SARs)

Bis-coumarin **7** is less potent than compound **3** which may be attributable to slightly solubility. (iv) Compound **20** is more potent than compound **3** which may be because of the presence of both thiazole and thiophene moieties or may be because of oxidation of S–H to S–S.

Experimental

General

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. Elemental analyses were carried out in The Microanalytical Center (Faculty of Science; Cairo University). IR spectra were recorded (KBr) on a Mattson 5000 FTIR Spectrophotometer in The Microanalytical Center (Faculty of Science; Mansoura University). ^{1}H NMR spectra of compounds **9**, **10**, **13**, **15b**, **15c**, **16a**, **16c**, and **19a** were determined on a Varian XL 200 MHz (Faculty of Science, Cairo University), while, for compounds **6**, **7**, **12**, and **16a** and ^{13}C NMR spectra were acquired on a JEOL ECX-400 spectrometer Chemistry department, School of Engineering and Science University of Jacobs, Bremen, Germany, operating at 400 MHz. The mass spectra were recorded on (Kratos, 70 eV) MS equipment and/or a Varian MAT 311 A Spectrometer, in The Microanalytical Center; (Faculty of Science; Cairo University). High-Resolution Mass Spectra (HRMS) were recorded using both a Bruker HCT ultra and a high-resolution (Bruker Daltonics micrOTOF) instruments from methanol or dichloromethane solutions using the positive Electrospray Ionization Mode (ESI). Biological activities were carried in Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

3,3′-(Imidazo[2,1-b]thiazole-3,6-diyl)bis (2H-chromen-2-one) (**4**)

A mixture of 3-(2-aminothiazol-4-yl)-2H-chromen-2-one (**3**) (0.49 g, 2 mmol), and 3-(2-bromoacetyl)-2H-chromen-2-one (**2**) (0.53 g, 2 mmol) in DMF (15 mL) catalyzed by TEA (0.5 ml), was heated under reflux for 12 h. The reaction mixture was left to cool and poured into ice-cold water. The resulting precipitate was filtered off, dried, and recrystallized from DMF/MeOH (1:2) to furnish **4**; dark brown powder; 78.3% yield, mp 235°C. Analysis: Calcd. for $C_{23}H_{12}N_2O_4S$ (412.42): C, 66.98; H, 2.93; N, 6.79; Found C, 67.06; H, 2.97; N, 6.85. IR (cm^{-1}) (KBr): 1716 (br, 2CO), 1608 (C=N). MS (*m/z*) (I%): 442 ($M^+-[CO_2$, H]), 352 (M^+-[coumarin + 2H]), 356 (31.6), 244 (100.0), 211 (29.1), 145 (38.0), 102 (72.2), 51 (81.0). ^{1}H NMR (DMSO-d_6) δ: 6.80–8.18 (m, 10H, Ar–H), 8.40 (s, 1H, C_4–H, coumarin).

2-Cyano-*N*-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl)acetamide (**6**)

A mixture of **3** (0.98 g, 4 mmol) and 3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropanenitrile (**5**) (0.65 g, 4 mmol) in dioxane (20 ml) was refluxed for 3 h, then cooled and poured into ice-cold water. The resulting precipitate was filtered off, washed with water, dried, and recrystallized from DMF/MeOH (1:3) to give **6**; brown crystals; 85.4% yield, mp 256°C. Analysis: Calcd. for $C_{15}H_9N_3O_3S$ (311.32): C, 57.87; H, 2.91; N, 13.50; Found C, 57.90; H, 2.97; N, 13.59. IR (cm^{-1}) (KBr): 3382 (NH), 2022 (CN), 1700, 1643 (2C=O), 1604 (C=N). The (–) ESI mass spectrum showed one quasi-molecular ion peak at 310 ($[M–H]^-$ pointing 311 as the molecular mass of the compound. MS (*m/z*) (I%): 311 (M^+, 51.3), 244 (56.6), 211 (42.1), 172 (38.2), 102 (25.0), 68 (100.0). ^{1}H NMR (DMSO-d_6) δ: 4.05 (s, 2H, CH_2), 7.35–8.03 (m, 5H, Ar–H), 8.47 (s, 1H, C_4–H, coumarin), 8.57 (s, 1H, NH).

2-Imino-*N*-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-2H-chromene-3-carboxamide (**7**)

A mixture of **6** (0.31 g, 1 mmol), and salicylaldehyde (0.12 g, 1 mmol) in dry DMF (10 ml) catalyzed by piperidine (0.5 ml) was refluxed for 4 h, then cooled and poured into ice-cold water. The resulting precipitate was filtered off, dried, and crystallized from DMF/EtOH (3:1) to afford **7**; brown crystals; 80.0% yield, mp 260°C. Analysis: Calcd. for $C_{22}H_{13}N_3O_4S$ (415.42): C, 63.61; H, 3.15; N, 10.12; Found C, 63.65; H, 3.21; N, 10.18. IR (cm^{-1}) (KBr): 3378, 3342 (2NH), 1714 (br, 2C=O), 1606 (C=N). The (+) ESI mass spectrum showed three quasi-molecular ion peaks at 414 $([M + H])^+$, 436 $([M + Na])^+$ and 871 $([2 M + 2Na-H])^+$ pointing 311 as the molecular mass of the compound. MS (*m/z*) (I%): 244 (M^+-[coumarin + CO], 28.6), 212 (11.9), 156 (11.9), 129 (9.5), 102 (38.1), 120 (23.8), 63 (100.0). ^{1}H NMR (DMSO-d_6) δ: 7.06–7.63 (m, 11H, Ar–H, NH), 8.42 (s, 1H, C_4–H, coumarin), 10.35 (br, 1H, NH-C=O).

2-Cyano-*N*-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-2-(5-oxo-3-phenyl-thiazolidin-2-ylidene)acetamide (**9**)

To a cold suspension of finely divided KOH (0.28 g, 5 mmol) in dry DMF (15 ml), cyanoacetamide derivative **6** (1.56 g, 5 mmol) followed by phenyl isothiocyanate

(0.68 g, 5 mmol) were added. The mixture was stirred at room temperature for 12 h, then cooled again to 0°C, treated with chloroacetyl chloride (0.56 g, 5 mmol) and left to stir at room temperature for 12 h. The mixture was poured into ice-cold water. The resulting precipitate was filtered off, dried, and crystallized from DMF/EtOH (5:2) to afford **9**; dark brown crystals; 65.0% yield, mp 340°C. Analysis: Calcd. for $C_{24}H_{14}N_4O_4S_2$ (486.52): C, 59.25; H, 2.90; N, 11.52; Found C, 59.37; H, 2.94; N, 11.56. IR (cm^{-1}) (KBr): 3423 (NH), 2206 (CN), 1714, 1647 (br, 3C=O), 1606 (C=N). MS (*m/z*) (I%): 486 (M^+, 1.71), 468 (2.28), 446 (2.24), 395 (8.13), 356 (7.74), 270 (12.35), 244 (100.0), 242 (9.23), 211 (14.27), 174 (55.03), 145 (39.60), 135 (5.85), 119 (51.60), 76 (75.10), 50 (73.88). ^{1}H NMR (DMSO-d_6) δ: 3.67 (s, 2H, CH_2–C=O), 6.95-8.10 (m, 10H, Ar–H), 8.34 (br, s, C_4–H, coumarin), 10.23 (br, s, NH–C=O).

2-Cyano-3-mercapto-*N*-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl)-3-(phenylamino) acrylamide (**10**)

To a cold suspension of finely divided KOH (0.28 g, 5 mmol) in dry DMF (15 ml), cyanoacetamide derivative **6** (1.56 g, 5 mmol) followed by phenyl isothiocyanate (0.68 g, 5 mmol) were added. The mixture was stirred at room temperature for 12 h, and then poured into ice-cold water and then acidified with 0.1 N HCl to a pH 3–4. The formed precipitate was filtered off, dried, and crystallized from benzene/EtOH (3:2) to afford **10**; yellow crystals; 70.3% yield, mp 128°C. Analysis: Calcd. for $C_{22}H_{14}N_4O_3S_2$ (446.5): C, 59.18; H, 3.16; N, 12.55; Found C, 59.22; H, 3.25; N, 12.63. IR (cm^{-1}) (KBr): 3197, 3176 (2NH), 2533 (SH), 2187 (CN), 1712 (br, 2C=O). MS (*m/z*) (I%): 442 (M^+−4H, 2.14), 409 (2.38), 341 (2.32), 293 (4.60), 261 (4.36), 244 (5.17), 228 (9.04), 191 (8.00), 176 (16.09), 164 (11.63), 149 (25.83), 145 (8.01), 135 (58.53), 119 (6.36), 92 (46.70), 85 (78.83), 63 (81.79), 56 (100.0). ^{1}H NMR (DMSO-d_6) δ: 6.69 (s, 1H, C_5–H, thiazole), 7.45–8.08 (m, 9H, Ar–H), 8.53 (s, 1H, C_4–H, coumarin), 9.56 (br, 1H, NH), 10.21 (br, 1H, NH–C=O), 11.10 (br, s, 1H, SH).

Synthesis of coumarin derivatives **12** and **16a–c**

General procedure

To a suspension of 3-(2-bromoacetyl)-2H-chromen-2-one (**2**) (0.27 g, 1 mmol) in DMF (15 ml), and thiocarbamoyl derivatives namely; **10** (0.45 g, 1 mmol), **14a** (0.31 g, 1 mmol), **14b** (0.29 g, 1 mmol) or **14c** (0.52 g, 1 mmol) was added. The reaction mixture was refluxed for 4–6 h, then left to cool, poured into ice-cold water and neutralized with sodium acetate solution. The formed precipitate was filtered off, dried, and crystallized from benzene/EtOH (3:1) to give **16a**, **b** and from DMF/EtOH (2:1) to give **12**, **16c**.

*2-Cyano-3-(2-oxo-2-(2-oxo-2H-chromen-3-yl) ethylthio)-N-(4-(2-oxo-2H-chromen-3-yl) thiazol-2-yl)-3-(phenylamino)acrylamide (**12**)*

Dark yellow crystals; 68% yield, mp 303–306°C. Reaction time 4 h; Analysis: Calcd. for $C_{33}H_{20}N_4O_6S_2$ (632.67): C, 62.65; H, 3.19; N, 8.86; Found C, 62.73; H, 3.22; N, 8.93. IR (cm^{-1}) (KBr): 3318, 3195 (2NH), 2179 (CN), 1726 (br, 4C=O), 1608 (C=N). The (+) ESI mass spectrum displayed two quasi-molecular ion peaks at 633 $([M + H])^+$, 656$([M + Na])^+$, and the (–)ESI spectrum showed one quasi-molecular ion peak at 631 pointing 632 as the molecular mass. ^{1}H NMR (DMSO-d_6) δ: 2.47 (s, 2H, CH_2–C=O), 6.64 (s, 1H, NH–Ph), 6.96 (s, 1H, C_5–H,), 7.33–8.17 (m, 13H, Ar–H), 8.45 (s, 1H, C_4–H, coumarin), 8.47 (s, 1H, C_4–H, coumarin), 10.20 (br, 1H, NH–C=O).

*2-(Benzo[d]thiazol-2-yl)-2-(4-(2-oxo-2H-chromen-3-yl) -3-phenylthiazol-2(3H)-ylidene) acetonitrile (**16a**)*

Brown crystals; 84% yield, mp 242°C. Reaction time 4.5 h; Analysis: Calcd. for $C_{27}H_{15}N_3O_2S_2$ (477.56): C, 67.91; H, 3.17; N, 8.80; Found C, 67.96; H, 3.25; N, 8.83. IR (cm^{-1}) (KBr): 2184 (CN), 1718 (C=O), 1602 (C=N). MS (*m/z*) (I%): 479 (M^+ + 2, 17.71), 477 (M^+, 100.0), 460 (1.01), 400 (1.14), 342 (12.28), 248 (46.45), 145 (36.71), 134 (9.97), 130 (0.80), 76 (43.80). ^{1}H NMR (DMSO-d_6) δ: 7.32–7.38 (m, 10H, Ar–H, C_5–H, thiazole), 8.22 (s, 1H, C_4–H, coumarin). ^{13}C NMR (DMSO-d_6) δ: 166.03 (C=O), 162.08, 158.90, 154.07, 147.74, 137.02, 136.80, 134.04, 133.20, 131.44, 130.43, 129.69, 126.90, 126.74, 123.90, 122.22, 121.80, 120.90, 118.20, 118.46, 117.20, 117.02, 112.70, 69.20.

*2-(1H-Benzo[d]imidazol-2-yl)-2-(4-(2-oxo-2H-chromen-3-yl)-3-phenylthiazol-2(3H)-ylidene)acetonitrile (**16b**)*

Dark green crystals; 90% yield, mp 320°C. Reaction time 4 h; Analysis: Calcd. for $C_{27}H_{16}N_4O_2S$ (460.51): C, 70.42; H, 3.50; N, 12.17; Found C, 70.48; H, 3.57; N, 12.23. IR (cm^{-1}) (KBr): 3264 (NH), 2184 (CN), 1718 (C=O), 1600 (C=N). MS (*m/z*) (I%): 462 (M^+ +2, 11.73), 461 (M^+ +1, 37.33), 460 (M^+, 100.0), 443 (2.37), 432 (2.16), 415 (1.62), 372 (1.92), 342 (2.54), 288 (2.01), 258 (3.63), 230 (8.16), 205 (2.29), 174 (3.53), 156 (1.87), 145 (5.6), 129 (1.85), 115 (2.68), 102 (9.99), 90 (2.93), 77 (18.55). ^{1}H NMR (DMSO-d_6) δ: 6.93–7.46 (m, 9H, Ar–H), 8.20 (s, 1H, C_4–H, coumarin), 9.35 (s, 1H, NH).

*3-[1,4-Dioxo-3,4,4a,5,10,10a-hexahydro-1H-5,10-benzenobenzo[g]-phthalazin-2-yl]-2-[4-(2-oxo-2H-chromen-3-yl)-3-phenyl-1,3-thiazol-2-(3H)ylidine]-3-oxo-propiononitrile (**16c**)*

Reddish brown powder; 89% yield, mp 320°C. Reaction time 6 h; Analysis: Calcd. for $C_{39}H_{24}N_4O_5S$ (660.7): C, 70.90; H, 3.66; N, 8.48; Found C, 70.84; H, 3.76; N, 8.55. IR (cm^{-1}) (KBr): 3445 (NH), 2191 (CN), 1726, 1668, 1613 (4C=O). MS (*m/z*) (I%): 371 (M^+-[hexahydrobenzenobenzophthalazine moiety], 20.5), 178 (100.0, anthracene), 77 (24.32). ^{1}H NMR (DMSO-d_6) δ: 3.4 (s, 2H, C_{11}–H, C_{12}–H), 4.8 (s, 2H, C_9–H, C_{10}–H), 6.96–7.82 (m, 13H, Ar–H), 8.17 (s, 1H, C_4–H, coumarin), 11.61 (br, 1H, NH).

Synthesis of 4-amino-*N*-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-5-(2-oxo-2H-chromene-3-carbonyl)-2-(phenylamino)thiophene-3-carboxamide (**13**) and 3-(3-amino-4-aryl-5-(phenylamino)thiophene-2-carbonyl)-2H-chromen-2-ones **15a–c**

General procedure

To a suspension of 3-(2-bromoacetyl)-2H-chromen-2-one (**2**) (0.27 g, 1 mmol) in DMF (15 ml) catalyzed by TEA (0.5 ml), and thiocarbamoyl derivatives namely; **10** (0.45 g, 1 mmol), **14a** (0.31 g, 1 mmol), **14b** (0.29 g, 1 mmol) or **14c** (0.52 g, 1 mmol) was added. The reaction mixture was refluxed for 4–8 h, then cooled and poured into ice-cold water. The formed precipitate was collected by filtration, dried, and crystallized from benzene/EtOH (1:4) to give **13** and **15a–c**.

*4-Amino-N-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-5-(2-oxo-2H-chromene-3-carbonyl)-2-(phenylamino)thiophene-3-carboxamide (**13**)*

Reaction time 8 h; brown crystals; 63% yield, mp 303–306°C. Analysis: Calcd. for $C_{33}H_{20}N_4O_6S_2$ (632.67): C, 62.65; H, 3.19; N, 8.86; Found C, 62.73; H, 3.24; N, 8.95. IR (cm^{-1}) (KBr): 3334, 3264, 3180 (NH_2, 2NH), 1720, 1687, 1639 (3C=O), 1600 (C=N). MS (*m/z*) (I%): 599 ([M^+−SH], 1.3), 504 (1.3), 386 [M^+-(aminothiazole, NH_2), 50.3], 272 (2.3), 244 (100.0), 211 (17.3), 174 (22.9), 102 (35.9), 76 (26.8), 51 (43.1). ^{1}H NMR (DMSO-d_6) δ: 6.95–8.13 (m, 16H, NH_2, Ar–H), 8.50 (br, 2H, C_4–H, coumarin), 9.25 (br, 1H, NH–Ph), 10.82 (br, 1H, C_4–H, NH–C=O).

*3-(3-Amino-4-(benzo[d]thiazol-2-yl)-5-(phenylamino)thiophene-2-carbonyl)-2H-chromen-2-one (**15a**)*

Reaction time 7 h; green crystals; 66% yield, mp 320°C. Analysis: Calcd. for $C_{27}H_{17}N_3O_3S_2$ (495.57): C, 65.44; H, 3.46; N, 8.48; Found C, 65.48; H, 3.52; N, 8.56. IR (cm^{-1}) (KBr): 3463, 3434 (NH_2, NH), 1735 (br, C=O), 1606 (C=N). MS (*m/z*) (I%): 496 (M^+ +1, 2.97), 495 (M^+, 12.21), 493 (76.19), 477 (2.27), 460 (8.22), 389 (8.32), 358 (6.08), 329 (2.00), 301 (1.65), 247 (2.5), 230 (4.58), 146 (5.45), 145 (3.7), 108 (7.74), 89 (11.42), 77 (100.0), 69 (13.53). ^{1}H NMR (DMSO-d_6) δ: 4.99 (br, s, 2H, NH_2), 7.15–8.09 (m, 14H, Ar–H, NH), 8.43 (s, 1H, C_4–H, coumarin).

*3-(3-Amino-4-(1H-benzo[d]imidazol-2-yl)-5-(phenylamino)thiophene-2-carbonyl)-2H-chromen-2-one (**15b**)*

Reaction time 5.5 h; pale green powder; 65% yield, mp 320°C. Analysis: Calcd. for $C_{27}H_{18}N_4O_3S$ (478.52): C, 67.77; H, 3.79; N, 11.71; Found C, 67.83; H, 3.86; N, 11.75. IR (cm^{-1}) (KBr): 3342 (br, NH_2, 2NH), 1673 (C=O, coumarin), 1691 (C=O). MS (*m/z*) (I%): 478 (M^+, 5.74), 476 (51.2), 460 (9.09), 443 (2.7), 372 (2.76), 238 (14.64), 210 (2.39), 181 (3.34), 156 (3.38), 145 (2.56), 129 (3.88), 118 (4.16), 102 (9.23), 89 (12.15), 65 (13.05), 51 (30.64). ^{1}H NMR (DMSO-d_6) δ: 5.78 (br, 2H, NH_2), 7.12–8.18 (m, 14H, Ar–H, NH), 8.42 (s, 1H, C_4–H, coumarin), 9.75 (s, 1H, NH–Ph).

*2-[(4-Amino-2-anilino-5-(2-oxo-2H-chromen-3-oyl)-3-thienyl)carbonyl]-3,4,4a,5,10,10a-hexahydro-1H-5,10-benzenobenzo[g]-phthalazine-1,4-dione (**15c**)*

Reaction time 4 h; pale green crystals; 83% yield, mp 320°C. Analysis: Calcd. for $C_{39}H_{26}N_4O_6S$ (678.71): C, 69.02; H, 3.86; N, 8.25; Found C, 69.13; H, 3.94; N, 8.18. IR (cm^{-1}) (KBr): 3443, 3425 (NH_2, H), 1716, 1683, 1642 (4C=O). MS (*m/z*) (I%): 677 ([M^+–H], 0.6), 386 (M^+–[hexahydrobenzenobenzophthalazine moiety], 2.5), 329 (0.6), 275 (0.9), 252 (0.5), 234 (0.7), 225 (1.2), 220 (0.6), 204 (1.1), 195 (0.8), 189 (0.9), 178 (100.0), 76 (20.7). ^{1}H NMR (DMSO-d_6) δ: 3.31 (s, 2H, C_{11}–H, C_{12}–H), 4.85 (s, 2H, C_9–H, C_{10}–H), 5.95 (br, 2H, NH_2), 7.15–7.80 (m, 13H, Ar–H), 8.27 (s, 1H, C_4–H, coumarin), 9.67 (s, 1H, NH–Ph), 11.82 (br, 1H, NH–C=O).

Synthesis of dithioacetal derivatives **19a**, **b**

General procedure

To a cold suspension of finely divided KOH (0.11 g, 2 mmol) in DMF (20 mL), *N*-(2-methoxyphenyl)-3-oxobutanamide (**17a**) (0.21 g, 1 mmol) or 2-(benzo[d]thiazol-2-yl)acetonitrile (**17b**) (0.17 g, 1 mmol) followed by carbon disulfide (0.61 g, 8 mmol) were added. The reaction mixture was stirred for 12 h at 0–5°C, then 3-(2-bromoacetyl)-2H-chromen-2-one (**2**) (0.27 g, 1 mmol) was added and left to stirred at room temperature for 12 h. The mixture was poured into ice-cold water acidified with dil. HCl. The obtained precipitate was collected by filtration, dried, and crystallized from benzene/EtOH (4:3) to give **19a**, **b**.

*2-(Mercapto(2-oxo-2-(2-oxo-2H-chromen-3-yl)ethylthio)methylene)-N-(2-methoxyphenyl)-3-oxobutanamide (**19a**)*

Yellow powder; 68% yield, mp 212°C. Analysis: Calcd. for $C_{23}H_{19}NO_6S_2$ (469.53): C, 58.83; H, 4.08; N, 2.98; Found C, 58.87; H, 4.11; N, 3.07. IR (cm^{-1}) (KBr): 2535 (SH), 1725, 1668 (3CO), 1606 (C=N). MS (*m/z*) (I%): 435.6 (M^+–H_2S, 0.45), 356 (0.6), 303 (2.85), 249 (4.82), 173 (48.72), 122 (77.93), 108 (100), 88 (62.29). ^{1}H NMR (DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 4.30 (s, 2H, CH_2), 5.35 (br, s, 1H, NH), 7.16–8.18 (m, 8H, Ar–H), 8.40 (s, 1H, C_4–H), 9.75 (s, 1H, SH).

*2-(Benzo[d]thiazol-2-yl)-3-mercapto-3-(2-oxo-2-(2-oxo-2H-chromen-3-yl)ethylthio) acrylonitrile (**19b**)*

Orange powder; 73% yield, mp 233–235°C. Analysis: Calcd. for $C_{21}H_{12}N_2O_3S_3$ (436.53): C, 57.78; H, 2.77; N, 6.42; Found C, 57.84; H, 2.84; N, 6.52. IR (cm^{-1}) (KBr): 2520 (SH), 2120 (CN), 1731, 1668 (2CO), 1606 (C=N). MS (*m/z*) (I%): 434 (M^+–2H, 0.14), 324 (2.41), 282 (1.73), 220 (25.28), 173 (100), 146 (13.57), 89 (30.16), 76 (24.25). ^{1}H NMR (DMSO-d_6) δ: 2.47 (s, 2H, CH_2), 7.19–7.93 (m, 8H, Ar–H), 8.45 (s, 1H, C_4–H, coumarin), 9.03 (br, s, 1H, SH).

*3-(3-Amino-4-(benzo[d]thiazol-2-yl)-5-mercaptothiophene-2-carbonyl)-2H-chromen-2-one (**20**)*

A suspension of **19b** (0.44 g, 1 mmol) in DMF (15 ml) catalyzed by TEA (0.5 ml) was refluxed for 5 h. The reaction mixture was left to cool at room temperature, poured into ice-cold water and treated with conc. HCl. The produced precipitate was filtered off, dried, and crystallized from benzene/EtOH (1:2) to afford **20**.

Dark brown powder; 76% yield, mp 180°C. Analysis: Calcd. for $C_{21}H_{12}N_2O_3S_3$ (436.53): C, 57.78; H, 2.77; N, 6.42; Found C, 57.84; H, 2.81; N, 6.39. IR (cm^{-1}) (KBr): 3324, 3290 (NH_2), 2550 (SH), 1670, 1644 (2CO). MS (*m/z*) (I%): 439 (M^+ +3, 0.27), 438 (M^+ +2, 1.17), 437 (M^+ +1, 4.16), 436 (M^+, 15.22), 434 (81.39), 433 (100.0), 401 (12.96), 391 (0.87), 369 (5.07), 356 (1.70), 345 (1.42), 334 (0.94), 317 (1.84), 282 (2.30), 272 (1.74), 246 (2.55), 235 (1.37), 217 (11.37), 200 (3.29), 174 (6.59), 159 (2.04), 138 (1.82), 123 (2.50), 108 (3.77), 86 (7.27), 76 (9.48). ^{1}H NMR (DMSO-d_6) δ: 3.71 (br, s, 2H, NH_2), 7.22–8.42 (m, 9H, Ar–H, C_4–H, coumarin), 9.45 (br, s, 1H, SH).

Biological activity

Antitumor activity

Different concentrations of the tested compounds were prepared (ED_{100}, ED_{50}, and ED_{25} μg/ml DMSO). The amount of DMSO was adjusted to give a final concentration of 0.1%. Ascites fluid was obtained from the peritoneal cavity of the donor animal from (National Cancer Institute, Cairo, Egypt) contain Ehrlich cell was as aseptically aspirated. The cells were grown partially floating and attach in a suspension culture (RPMI 1660 medium, Sigma Chemical Co. St. Louis, USA), supplemented with 10% foetal bovine serum (GIBCO, UK). They were maintained at 37°C in humidified atmosphere with 5% CO_2 for 2 h. The viability of the cell used in control experiments (DMSO only without drug) exceeded 95% as determined by microscopically examination using a hemocytometer and trypan blue stain (stain only the dead cells).

Antioxidant assay

Antioxidant activity determinations were evaluated from the bleaching of ABTS derived radical cations. The radical cation was derived from ABTS [2,2′-azino-*bis* (3-ethyl benzothiazoline-6-sulfonic acid)] was prepared by reaction of ABTS (60 μl) with MnO_2 (3 ml, 25 mg/ml) in (5 ml) aqueous buffer solution (pH 7). After shaking the solution for a few minutes, it was centrifuged and filtered.

The Absorbance (A control) of the resulting green–blue solution (ABTS radical solution) was recorded at λ_{max} 734 nm. The absorbance (A test) was measured upon the addition of (20 μl of 1 mg/ml) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The decrease in the absorbance is expressed as % inhibition which calculated from the Eq. 1.

$$\% \text{ Inhibition} = [\text{A (control)} - \text{A (test)}/\text{ A (control)}] \times 100 \quad (1)$$

Ascorbic acid (20 µl, 2 mM) solution was used as standard antioxidant (positive control). Blank sample was run using solvent without ABTS (Table 2).

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