Full Paper

Synthesis and Antioxidant Evaluation of Some New 3-Substituted Coumarins

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3-Acetylcoumarin (**1**) was utilized as a key intermediate for the synthesis of 2-aminothiazole derivative **3** *via* bromination of **1** to afford acetylbromide **2** followed by treatment with thiourea or *via* Biginelli reaction of **1**. Treatment of **3** with 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde, 2-methyl-4*H*-benzo[*d*][1,3]oxazin-4-one, furo[3,4-*b*]pyrazine-5,7-dione or 2-methyl-5,6,7,8-tetrahydro-4*H*-benzothieno[2,3-*d*][1,3]oxazin-4-one afforded diazine derivatives **4**–**7**. Also, pyridopyrimidine **8** was obtained *via* a one pot reaction of 6-aminothiouracil, *p*-chlorobenzaldehyde and 3-acetylcoumarin. Moreover, refluxing of 6-aminothiouracil with one equivalent amount of **2** afforded the thiazolopyrimidine **9**, while the pyrrolothiazolopyrimidine **10** was revealed when two equivalent amounts of **2** was used. Furthermore, treatment of enamine **11** with 2-aminobenzothiazole or 6-aminothiouracil afforded the pyrimidine derivatives **12** and **13**, respectively. Transamination of enamine **11** with *m*-anisidine followed by cyclization of the resulting enaminone **14** gave the desired quinoline **15**. Also, treatment of **11** with thiophenol in dioxane gave the mercapto derivative **16**. Moreover, coupling of **11** with 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl-diazonium chloride, followed by complete cyclization of the resulting product afforded the pyridopyrazolothiazine **19** *via* the intermediate **18**. Furthermore, the pyrazolopyrimidine **20** was revealed *via* a one pot condensation of **11**, 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one and ammonium acetate. The thiadiazine derivatives **21**–**23** were obtained *via* treatment of **2** with the corresponding *o*-aminothiols. Desulphonation of **23** afforded the pyrazolotriazine **24**. Finally, reaction of **2** with 2-hydroxybenzaldehyde gave benzofuran derivative **25**. Representative compounds of the synthesized products were evaluated as antioxidant agents.

Keywords: Antioxidant activity / Coumarin / Pyridopyrimidine / Thiadiazine / Thienopyrimidine

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Introduction

Coumarin derivatives constitute an important class of heterocyclic compounds with anticoagulant (*e.g.*, warfarin, acenocoumarol) [1, 2], anticoagulant rodenticide (*e.g.*, brodifacoum, bromadiolone) [3], insecticide (*e.g.*, coumaphos) [4], antibacterial (*e.g.*, novobiocin, clorobiocin) [5, 6], and pharmacological properties. The cytotoxic activities of the 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 [7]. In view of the above mentioned findings and as continuation of our effort [8, 9], to identify new candidates that may be valuable designing new, potent, selective and less toxic antioxidant agents, we report in the present work the synthesis of some new 3-substituted coumarin starting from 3-acetyl-2*H*-chromen-2-one (**1**) [10].

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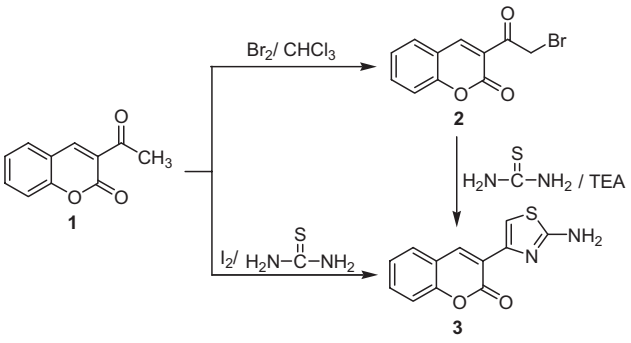
Results and discussion

Chemistry

The synthetic procedure adopted to obtain the target compounds are depicted in Schemes 1–6. The starting compound 3-(2-bromoacetyl)-2*H*-chromen-2-one (**2**) [11] was prepared



Scheme 1. Synthesis of 2-amino-4-(3-coumarinyl)thiazole (**3**).

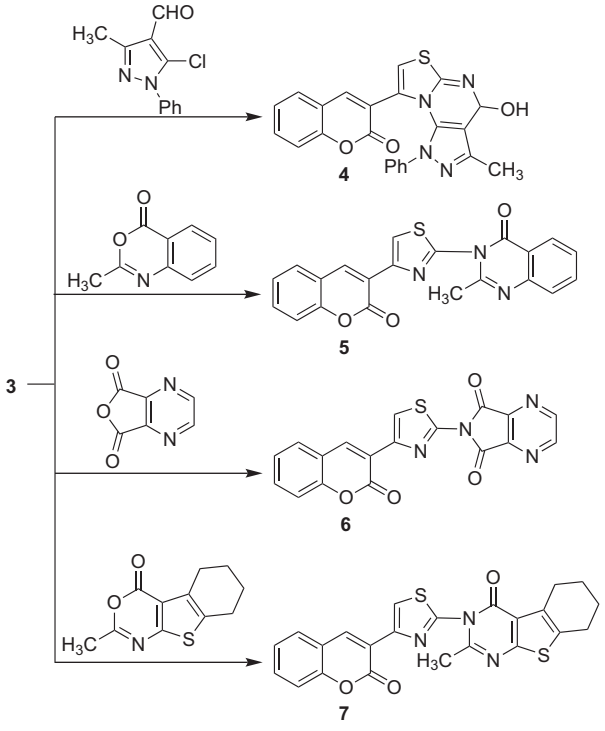
according to the reported method *via* bromination of 3-acetyl-2*H*-chromen-2-one (**1**) [10]. Reaction of **2** with thiourea in refluxing ethanol gave the corresponding 2-amino-4-(3-coumarinyl)thiazole (**3**) [12] (see Scheme 1).

In another route, compound **3** was obtained in a good yield *via* Biginelli reaction of 3-acetylcoumarin with thiourea and iodine. It is evident from the literature that quinazolines and condensed quinazolines exhibited potent central nervous system (CNS) activities, *e.g.* analgesic, anti-inflammatory [13], also, azolopyrimido-quinolines, pyrimidoquinazolines exhibited good antioxidant, anti-inflammatory and analgesic activities [14]. Furthermore, thienopyrimidines (bioisostere of quinazoline and condensed quinazoline) possess potent CNS and antibacterial activities [15, 16].

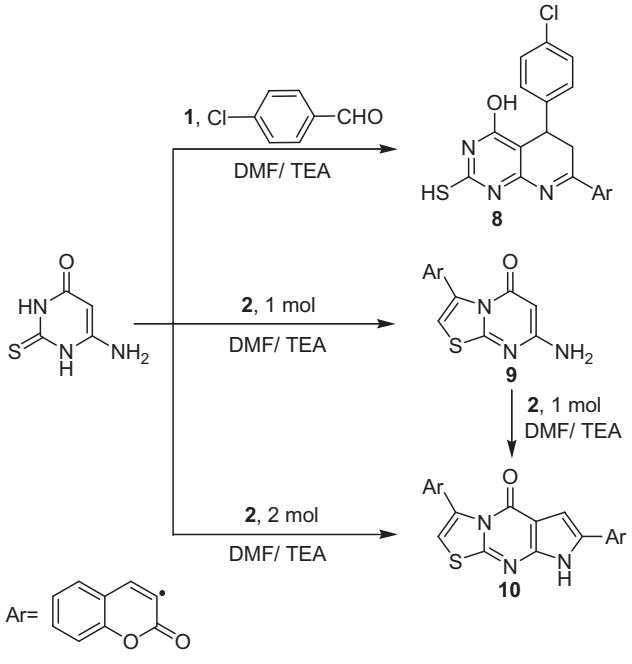
Thus, compound **3** was condensed with 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde [17] in dimethylformamide catalyzed by triethylamine to give thiazolopyrimidine derivative **4** (bioisostere of quinazoline). Furthermore, compound **3** reacted with 2-methyl-4*H*-benzo[*d*][1,3]oxazin-4-one [18], furo[3,4-*b*]pyrazine-5,7-dione or 2-methyl-5,6,7,8-tetrahydro-4*H*-benzothieno[2,3-*d*][1,3]oxazin-4-one [19] to give the quinazoline **5**, pyrrolopyrazine **6** (bioisostere of quinazoline), and thienopyrimidine **7** (bioisostere of quinazoline) derivatives, respectively (see Scheme 2).

Also, pyridopyrimidine **8** was obtained *via* a one pot reaction of 6-aminothiouracil, *p*-chlorobenzaldehyde, and 3-acetyl coumarin in dimethylformamide catalyzed by triethylamine. Moreover, the reaction of 6-aminothiouracil with one equivalent amount of **2** in dimethylformamide catalyzed by triethylamine afforded the thiazolopyrimidine **9** (bioisostere of quinazoline), while the pyrrolothiazolo-pyrimidine **10** was afforded when two equivalent amounts of **2** were used. The latter was obtained in another route *via* the reaction of **9** with another one equivalent amount of **2** in dimethylformamide catalyzed by triethylamine (see Scheme 3).

Furthermore, treatment of enamine **11** [20] (obtained through refluxing of **1** with dimethylformamide-dimethyl-



Scheme 2. Synthesis of diazine derivatives **4–7**.

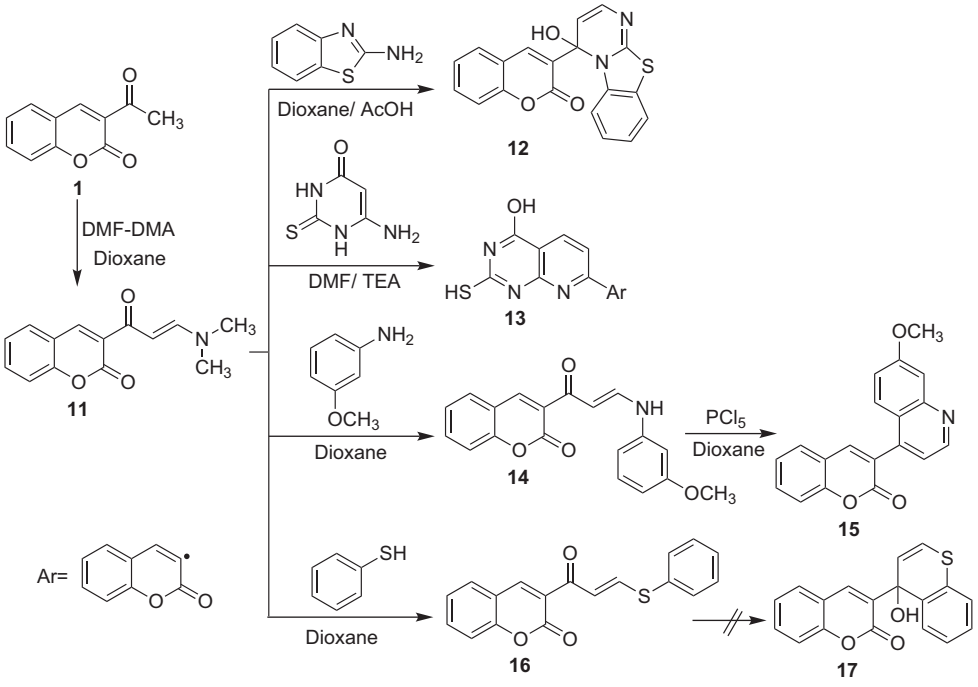


Scheme 3. Synthesis of pyrimidine derivatives.

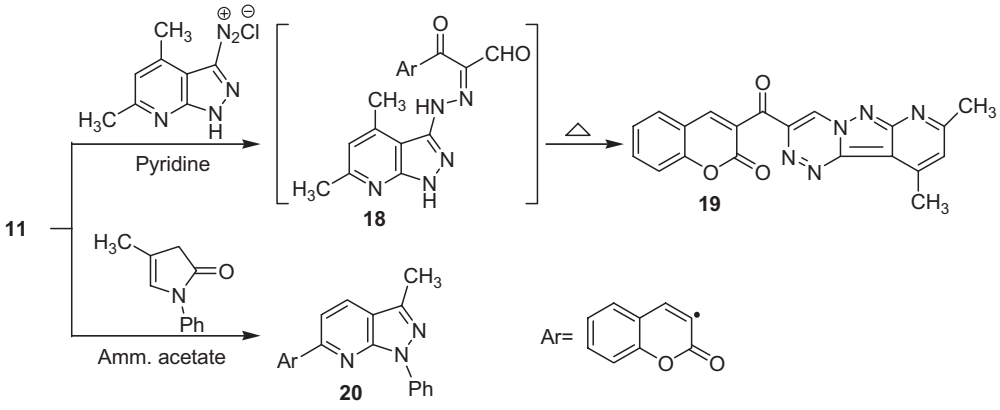
acetal (DMF-DMA) in dioxane) with 2-aminobenzothiazole in dioxane and acetic acid mixture or 6-aminothiouracil in dimethylformamide catalyzed by triethylamine afforded the desired thiazolopyrimidine **12** and pyridopyrimidine **13** (bioisostere of quinazoline), respectively. Transamination of enamine **11** with *m*-anisidine, followed by cyclization of the resulting enaminone **14** under acidic condition gave the desired quinoline **15**. Also, treatment of **11** with thiophenol in dioxane gave the mercapto derivative **16**. Attempting for the preparation of thiane **17** through cyclization of **16** under influence of conc. H_2SO_4 failed (see Scheme 4).

Moreover, coupling of **11** with 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl-diazonium chloride [21] in pyridine, followed by complete cyclization of the resulting product afforded the pyridopyrazolothiazine **19** *via* the intermediate **18**. Furthermore, the pyrazolopyrimidine **20** was revealed *via* a one pot condensation of **11**, 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one [22] and ammonium acetate (see Scheme 5).

The thiadiazines **21–23** were obtained *via* treatment of **2** with 4-amino-5-phenyl-4*H*-1,2,4-triazolo-3-thiol [23], 4-(pyridin-2-yl)thiosemicarbazide [24] or 4-amino-6-benzyl-3-mercapto-1,2,4-triazin-5(4*H*)-one [25] in dimethylformamide catalyzed by triethylamine. Desulphonation of **23** under



Scheme 4. Synthesis of thiazolopyrimidine **12**, pyridopyrimidine **13**, quinoline **15**, and mercapto **16** derivatives.



Scheme 5. Synthesis of pyrazolopyridine derivatives **19** and **20**.

the influence of acetic anhydride gave the pyrazolotriazine **24**. The biological activities of benzofuran ring system [26] promoted us to synthesize the benzofuran incorporated coumarin moiety. Thus, compound **2** was reacted with 2-hydroxybenzaldehyde in dimethylformamide in the presence of potassium carbonate to afford benzofuran derivative **25** (see Scheme 6).

Assignment of the new synthesized compounds was based on elemental analyses, IR, ^{1}H-NMR, mass spectral data (*C.f.* Exp. Part).

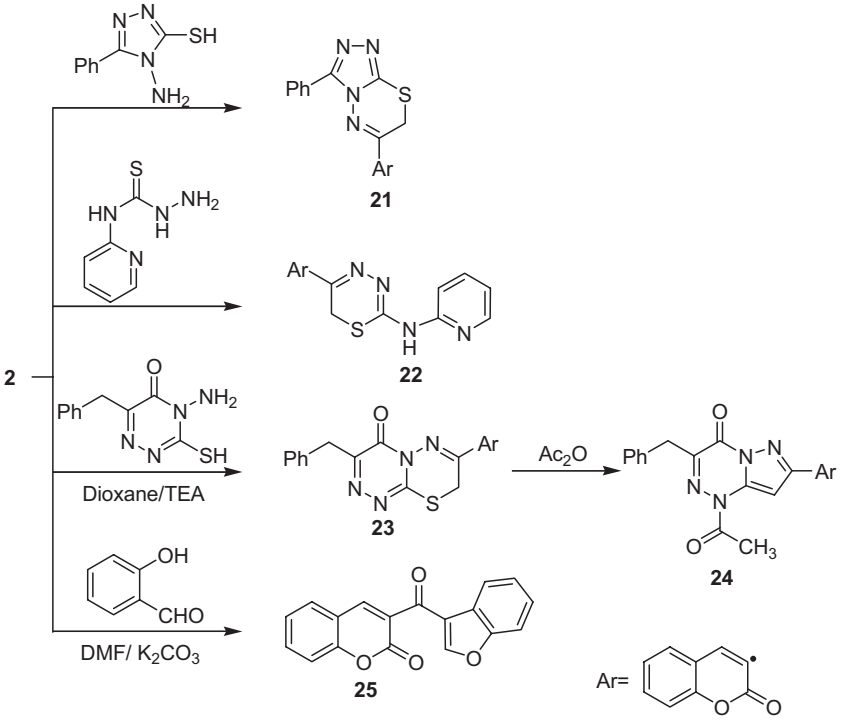
Biological activity

ABTS Antioxidant assay

The antioxidant activity of the synthesized compounds was evaluated by the ABTS antioxidant activity method described by Lissi *et al*. [27]. Some of the 3-substituted coumarin derivatives exhibited an antioxidant effect as shown in Table 1. Compared with the control (L-ascorbic acid), the antioxidant potency of compounds **8**, **13**, and **21** was found to be highest, while compounds **1**, **2**, **3**, **4**, **9**, **10**, **14**, **22**, **23**, and **25** showed a moderate antioxidant activity and the rest tested compounds showed a weak activity. On the other hand, compounds 7-hydroxycoumarin, **3**, **4**, **8**, **9**, **13**, **14**, **21**, and **22** exhibited a high antioxidant activity compared to the starting material **1**. From the structure–activity relationship (SAR) point of view, the presence of thienopyrimidine, pyridopyrimidine, enaminone, triazole or thiadiazine moieties at the 3-position

Table 1. ABTS Antioxidant activity assay.

Compound No.	Absorbance of samples	Compound No. %Inhibition
Control of ABTS	0.498	0.0
Ascorbic acid	0.057	88.55
7-Hydroxycoumarin	0.153	68.58
1	0.180	63.58
2	0.248	50.20
3	0.128	74.29
4	0.163	67.26
5	0.260	47.79
6	0.352	29.31
7	0.328	34.13
8	0.089	82.12
9	0.100	79.91
10	0.224	55.02
11	0.331	33.53
12	0.298	40.16
13	0.082	83.53
14	0.169	66.06
15	0.327	34.33
16	0.332	33.33
19	0.276	46.38
20	0.200	59.83
21	0.068	86.34
22	0.123	75.30
23	0.129	61.44
24	0.322	35.34
25	0.225	54.81



Scheme 6. Synthesis of thiadiazole **21–23**, pyrazolotriazine **24**, and benzofuran **25** derivatives.

of the coumarin enhanced the antioxidant activity, also, the presence of thiolopyrimidine group increases the antioxidant activity as in the case of compounds **8** and **1** (Table 1).

The compounds 7-hydroxycoumarin, **1**, **3**, **4**, **8**, **9**, **13**, **14**, **21**, and **22** were selected to test for Bleomycin-dependent DNA damage (Table 2). Damage to DNA in the presence of a Bleomycin–Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents [28]. If the samples to be tested are able to reduce the Bleomycin–Fe^{3+} to Bleomycin–Fe^{2+}, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. DNA degradation is accompanied by the formation of a product similar to malondialdehyde (MDA). L-Ascorbic acid as a reducing agent can reduce Fe^{3+} to Fe^{2+}.

Table 2 shows that compounds **3**, **8**, **9**, **13**, and **21** have an ability to protect DNA from the induced damage by Bleomycin. Also, compounds **3**, **4**, **8**, **9**, **13**, and **21** exhibited a high antioxidant activity compared to the starting materials. On the other hand, compounds **3**, **8**, **9**, and **13** exhibited more potent antioxidant activity compared with 7-hydroxycoumarin. Elstner *et al.* [29], reported that coumarin and 7-hydroxycoumarin have been used for the scavenging of free radicals. It is noteworthy that 7-hydroxycoumarin examined in the present investigation also possesses a hydroxyl group attached to an aromatic ring, and it is effective in inhibiting lipid peroxidation. Similarly, the presence of a hydroxyl group attached to an aromatic ring in the molecule of erianin accounts for its antioxidative activity. The tertiary amine structure present in the alkaloid tetrahydropalmatine contributes to its antioxidative activity.

Table 2. Pro-oxidant effects of 3-substituted coumarins on ferric Bleomycin-induced DNA damage.

Compound No.	Bleomycin-dependent DNA damage
	Absorbance of samples
Control	0
Ascorbic acid	0.098 ± 0.001
7-Hydroxycoumarin	0.104 ± 0.002
1	0.126 ± 0.001
3	0.094 ± 0.002
4	0.115 ± 0.002
8	0.098 ± 0.001
9	0.099 ± 0.003
13	0.101 ± 0.002
14	0.216 ± 0.003
21	0.108 ± 0.004
22	0.127 ± 0.001

All compounds were dissolved in DMSO/MeOH (1:1) and tested at the final concentration of (0.1 mL of 1 mg/mL). The extent of DNA damage is expressed by increase in absorbance at 520 nm. The values are mean ± SD ($n = 3$).

From the structure activity relationship (SAR), it is noteworthy that compounds **3**, **8**, **9**, and **13** have SH, OH, and NH_2 groups attached to aromatic ring (thiazole, tiazolopyrimidine, and pyridopyrimidine) which is effective in inhibiting Bleomycin from DNA damage.

Conclusion

The newly prepared ring systems seem to be interesting for biological studies. Furthermore, the present investigation offers rapid and effective new procedures for the synthesis of a new class of 3-substituted coumarin. The new compounds were investigated for antioxidant activity. Compounds **8**, **13**, and **21** exhibited a high antioxidant activity when compared to the ascorbic acid; these compounds manifested the best protective effect against DNA damage induced by Bleomycin.

Experimental

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. 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 were determined on a Varian XL 200 MHz (Faculty of Science, Cairo University), while, for compounds **8** and **12** ^{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). Elemental analyses (C, H, and N) were carried out at the Microanalytical Center of Cairo Univ., Giza, Egypt. Biological activities were carried in the Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

*Synthesis of 3-(4-hydroxy-3-methyl-1-phenyl-1,4-dihydropyrazolo[4,3-e]thiazolo[3,2-a]pyrimidin-8-yl)-2H-chromen-2-one (**4**)*

A mixture of **3** (0.5 g, 2 mmol), 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde (0.44 g, 2 mmol), and triethylamine (0.42 mL, 3 mmol) in dimethylformamide (20 mL) was refluxed for 48 h. The reaction mixture was left to cool and poured into ice cold water (50 mL). The formed precipitate was filtered off, dried and recrystallized from benzene/ethanol mixture to furnish **4**.

Reddish brown powder, yield, 75%, mp: 202–204°C; IR (KBr): ν_{max}, cm^{-1}: 3384 (OH), 1698 (C=O), 1639 (C=N), 1604 (C=C). ^{1}H-NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 4.50 (s, 1H, C_8-H), 5.85 (s, 1H, C_2-H), 7.36–8.21 (m, 9H, Ar-H), 8.58 (s, 1H, C_4-H of coumarin), 9.70 (br, s, OH). MS: *m/z* (%) = 413 ($M^+ + 1$, 3.7), 412 (14.4), 411 ($M^+ - OH$, 4.88), 402 (0.94), 359 (3.82), 343 (7.37), 341 (30.45), 327 (4.63), 299 (1.13), 272 (9.44), 266 (1.44), 244 (100.00), 242 (2.38), 216 (18.08), 211 (29.53), 201 (4.69), 186 (3.40), 174 (10.07), 172 (27.26), 146

(8.77), 145 (43.99), 128 (16.90), 113 (11.98), 110 (1.36), 109 (1.31), 101 (64.13), 99 (5.61), 97 (1.80), 77 (78.14), 74 (31.00), 50 (54.61). Anal. calcd. for $C_{23}H_{16}N_4O_3S$ (428.46): C, 64.47; H, 3.76; N, 13.08%. Found: C, 64.03; H, 3.82; N, 13.81%.

*Synthesis of 2-methyl-3-(4-(2-oxo-2H-chromen-3-yl)-thiazol-2-yl)quinazolin-4(3H)-one (**5**), 6-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-5H-pyrrolo[3,4-b]pyrazine-5,7(6H)-dione (**6**), and 2-methyl-3-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl-5,6,7,8-tetrahydro-4H-benzothieno[2,3-d] [1,3]quinazolin-4(3H)-one (**7**)*

A mixture of **3** (1.22 g, 5 mmol), 2-methyl-4*H*-benzo[*d*][1,3]oxazin-4-one (0.64 g, 4 mmol), furo[3,4-*b*]pyrazine-5,7-dione (0.3 g, 2 mmol) or 2-methyl-5,6,7,8-tetrahydro-4*H*-benzothieno[2,3-*d*] [1,3]oxazin-4-one (0.44 g, 2 mmol), and freshly fused sodium acetate (1.0 g, 12 mmol) was fused in a sand bath at 200–210°C for 5 h, then acetic acid (20 mL) was added. The reaction mixture was refluxed for another 3 h, poured into ice cold water, the resulting precipitate was filtered off, dried and recrystallized from dimethylformamide/methanol mixture to afford **5–7**, respectively.

Compound **5**; grey crystals, yield: 82%, mp: 321°C, IR (KBr): ν_{max}, cm^{-1}: 1714 (brs, 2 CO). ^{1}H-NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 7.32–8.19 (m, 9H, Ar-H), 8.50 (s, 1H, C_4-H, coumarin). MS: *m/z* (%) = 372 (M^+ – CH_3, 1.4), 356 (24.3), 328 (4.9), 300 (2.5), 286 (15.5), 255 (2.6), 244 (100.0), 216 (18.3), 211 (31.6), 186 (3.5), 183 (24.0), 160 (31.0), 145 (21.2), 137 (43.5), 119 (85.1), 102 (27.3), 92 (38.1), 76 (23.7). Anal. calcd. for MS: *m/z* (%) = 373 (M^+, 1.1): C, 65.11; H, 3.38; N, 10.85%. Found: C, 65.13; H, 3.36; N, 10.92%.

Compound **6**; black powder, Yield, 71%, mp: 320°C, IR (KBr): ν_{max}, cm^{-1}: 1706, 1645 (3 CO). MS: *m/z* (%) = 374 (M^+ – 2, 11.1), 327 (11.6), 269 (13.1), 244 (21.1), 241 (13.8), 226 (11.1), 210 (10.6), 201 (10.2), 178 (18.2), 165 (15.95), 150 (15.1), 145 (11.6), 143 (27.8), 127 (32.8), 104 (30.6), 96 (36.5), 82 (95.9), 69 (47.3), 57 (100.0). Anal. calcd. for $C_{18}H_8N_4O_4S$ (376.35): C, 57.45; H, 2.14; N, 14.89%. Found: C, 57.49; H, 2.23; N, 14.96%.

Compound **7**; brown powder, yield: 85%, mp: 320°C, IR (KBr): ν_{max}, cm^{-1}: 1716, 1644 (2 CO). MS: *m/z* (%) = 446 (M^+ – 1, 21.1), 212 (18.1), 171 (37.8), 91 (59.5). Anal. calcd. for $C_{23}H_{17}N_3O_3S_2$ (447.53): C, 61.73; H, 3.83; N, 9.39%. Found: C, 61.77; H, 3.87; N, 9.43%.

*Synthesis of 3-(5-(4-chlorophenyl)-4-hydroxy-2-mercapto-5,6-dihydropyrido[2,3-d]pyrimidin-7-yl)-2H-chromen-2-one (**8**)*

A solution of **1** (0.56 g, 3 mmol) in dimethylformamide (50 mL), *p*-chlorobenzaldehyde (0.42 g, 3 mmol), and catalytic amounts of triethylamine (0.5 mL, 3 mmol) were added. The reaction mixture was refluxed for 1 h and then 6-aminothiouracil (0.43 g, 3 mmol) was added. Further, whole reaction mixture was allowed to reflux for another 10 h. The resulting solution was cooled at room temperature and poured into crushed ice. The solid obtained was filtered off, washed with water, dried and crystallized from benzene/ethanol to afford **8**.

Yellowish brown crystals, yield: 73%, mp: 264–266°C, IR (KBr): ν_{max}, cm^{-1}: 3384 (OH), 2923, 2865 (C–H, aliphatic), 2510 (SH), 1677 (C=O), 1606 (C=N). ^{1}H-NMR (DMSO-d_6): δ 2.95–3.0 (m, 2H, CH_2), 5.25–5.27 (m, 1H, CH), 7.0–7.66 (m, 8H, Ar), 8.45 (s, 1H, C_4-H, coumarin), 11.8 (s, 1H, OH), 12.0 (s, 1H, SH). MS: *m/z* (%); the (–) ESI mass spectrum showed one quasi-molecular ion peak at 434 (M–H), pointing 435 as the molecular mass of the compound. Anal. calcd. for $C_{22}H_{14}ClN_3O_3S$ (435.88): C, 60.62; H, 3.24; N, 9.64%. Found: C, 60.58; H, 3.21; N, 9.61%.

*Synthesis of 7-amino-3-(2-oxo-2H-chromen-3-yl)-5H-thiazolo[3,2-a]pyrimidin-5-one (**9**) and 3,3'-(5-oxo-5,8-dihydropyrrolo[2,3-d]thiazolo[3,2-a]pyrimidine-3,7-diyl)-bis(2H-chromen-2-one) (**10**)*

General Procedure

A mixture of 6-aminothiouracil (0.14 g, 1 mmol), triethylamine (0.42 mL, 3 mmol), and compound **2** (0.27 g, 1 mmol) or (0.53 g, 2 mmol) in dimethylformamide (20 mL) was refluxed for 12 h. The resulting solution was cooled at room temperature and poured into crushed ice. The solid obtained was filtered off, washed with water, dried and crystallized from benzene/ethanol to give **9** and **10**, respectively.

In another route, compound **10** was obtained *via* the reaction of **9** (0.31 g, 1 mmol) with **2** (0.27 g, 1 mmol) under the same previous conditions.

Compound **9**; brown crystals, yield: 88%, mp: 276–280°C, IR (KBr): ν_{max}, cm^{-1}: 3403, 3388 (NH_2), 1718, 1635 (2 CO), 1625 (C=N). HRMS (micrOTOF): *m/z* for $C_{15}H_8N_3O_3S$, Calcd.: 311.3200 (M^+). Found: 310.0300 (M^+ – H). Anal. calcd. for $C_{15}H_9N_3O_3S$ (311.32): C, 57.87; H, 2.91; N, 13.50%. Found: C, 57.93; H, 2.97; N, 13.58%.

Compound **10**; brown powder, yield: 89, 78%, mp: 320°C, IR (KBr): ν_{max}, cm^{-1}: 3386 (NH), 1716, 1646 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 6.85–8.55 (m, Ar-H, C_4-H, coumarin, NH). MS: *m/z* (%) = 479 (M^+, 0.5), 454 (0.5), 386 (9.4), 356 (15.8), 329 (7.99), 300 (3.99), 271 (4.8), 227 (5.4), 200 (11.3), 173 (4.96), 150 (7.4), 126 (13.1), 115 (18.8), 91 (21.5), 76 (53.2), 62 (72.4), 55 (100.0). Anal. calcd. for $C_{26}H_{13}N_3O_5S$ (479.46): C, 65.13; H, 2.73; N, 8.76%. Found: C, 65.21; H, 2.75; N, 8.80%.

*Synthesis of 3-(4a,9-dihydro-4H-fluoren-4-ol-9-thia-1,4a-diaza)-chromen-2-one (**13**), 3-(4-hydroxy-2-mercaptopyrido[2,3-d]pyrimidin-7-yl)-2H-chromen-2-one(**13**), (E)-3-(3-(3-methoxyphenylamino)acryloyl)-2H-chromen-2-one (**14**), and (E)-3-(3-(phenylthio)acryloyl)-2H-chromen-2-one (**16**)*

To a solution of enaminone **11** (0.24 g, 1 mmol) in a mixture of dioxane and acetic acid mixture (20 mL) 2-aminobenzothiazole (0.15 g, 1 mmol) was added. The reaction mixture was refluxed for 12 h and the resulting solution was cooled at room temperature and poured into crushed ice. The solid obtained was filtered off, washed with water, dried and crystallized from benzene/ethanol mixture to afford **12**.

Similarly, a solution of enaminone **11** (0.24 g, 1 mmol), 6-aminothiouracil (0.29 g, 2 mmol), and triethylamine (0.42 mL, 3 mmol) in dimethylformamide (20 mL) was refluxed for 15 h and crystallized from benzene/ethanol mixture to afford **13**. Also, a solution of enaminone **11** (0.24 g, 1 mmol) and *m*-anisidine (0.49 mL, 4 mmol) or thiophenol (0.11 mL, 1 mmol) in dioxane (20 mL) was refluxed for 8 h and 6 h and crystallized from benzene/ethanol mixture to furnish **14** and **16**, respectively.

Compound **12**, yellow powder, yield: 75%, mp: 300–303°C, IR (KBr): ν_{max}, cm^{-1}: 3335 (OH), 1715 (C=O), 1614 (C=N). ^{1}H-NMR (DMSO-d_6): δ 5.32 (br, d, 1H, pyrimidine), 5.79 (br, d, 1H, pyrimidine), 7.1–8.16 (m, 8H, Ar-H), 8.52 (s, 1H, C_4-H, coumarin), 8.70

(s, 1H, OH). MS: m/z (%) = 331 (M^+ − OH, 4.36), 316 (2.1), 300 (1.95), 288 (1.9), 271 (2.8), 259 (2.2), 243 (3.5), 214 (3.6), 203 (14.6), 189 (4.9), 173 (100.0), 161 (5.9), 145 (13.5), 131 (12.98), 127 (7.3), 102 (11.96), 89 (2.8), 74 (10.3), 62 (28.99). Anal. calcd. for $C_{19}H_{12}N_2O_3S$ (348.38): C, 65.51; H, 3.47; N, 8.04%. Found: C, 68.71; H, 3.69; N, 8.51%.

Compound **13**, green powder, yield: 71%, mp: 244°C, IR (KBr): ν_{max}, cm^{-1}: 3378 (OH), 2525 (SH), 1713 (C=O), 1606 (C=N). ^{1}H-NMR (DMSO-d_6): δ 6.53–8.32 (m, 6H, Ar-H), 8.45 (s, 1H, C_4-H, coumarin), 11.95 (brs, 1H, OH), 12.40 (brs, 1H, SH). MS: m/z (%) = 323 (M^+, 60.8), 311 (100), 280 (15.9), 242 (9.4), 193 (47.3), 173 (37.3), 142 (53.4), 115 (50.1), 94 (61.96), 88 (68.2). Anal. calcd. for $C_{16}H_9N_3O_3S$ (323.33): C, 59.44; H, 2.81; N, 13.0%. Found: C, 59.39; H, 2.78; N, 12.97%.

Compound **14**, brown crystals, yield: 72%, mp: 286–288°C, IR (KBr): ν_{max}, cm^{-1}: 3274 (NH), 1722, 1643 (2 C=O), 1606 (C=N). ^{1}H-NMR (DMSO-d_6): δ 3.8 (s, 3H, OCH_3), 5.90 (br, 1H, α-olefinic-H), 6.58–7.90 (m, 9H, Ar-H, β-olefinic-H), 8.45 (s, 1H, C_4-H, coumarin), 10.15 (brs, 1H, NH). MS: m/z (%) = 322 (M^+ + 1, 9.8), 321 (100), 173 (63.1), 148 (61.0), 132 (47.8), 116 (14.6), 88 (15.5), 76 (18.2). Anal. calcd. for $C_{19}H_{15}NO_4$ (321.33): C, 71.02; H, 4.71; N, 4.36%. Found: C, 71.14; H, 4.76; N, 4.44%.

Compound **16**, yellow crystals, yield: 73%, mp: 209–210°C, IR (KBr): ν_{max}, cm^{-1}: 1718, 1641 (2 CO), 1606 (C=C). ^{1}H-NMR (DMSO-d_6): δ 5.90 (br, 1H, α-olefinic-H), 7.41–8.05 (m, 10H, Ar-H, β-olefinic-H), 8.47 (br, s, 1H, C_4-H, coumarin). Anal. calcd. for $C_{18}H_{12}O_3S$ (308.35): C, 70.11; H, 3.92%. Found: C, 70.21; H, 3.95%.

*Synthesis of 3-(7-methoxyquinolin-4-yl)-2H-chromen-2-one (**15**)*

To compound **14** (0.32 g, 1 mmol) in dioxane (20 mL), phosphorus pentachloride (1 g, 0.21 mmol) was added and the reaction mixture was refluxed for 6 h. The resulting solution was cooled at room temperature and poured into crushed ice and treated with conc. ammonia. The solid obtained was filtered off, washed with water, dried and crystallized from benzene/ethanol mixture to give **15**.

Black powder, yield: 70%, mp: 320°C, IR (KBr): ν_{max}, cm^{-1}: 1722 (C=O), 1606 (C=N). ^{1}H-NMR (DMSO-d_6): δ 3.94 (s, 3H, OCH_3), 7.24–8.31 (m, 9H, Ar-H), 8.48 (brs, 1H, C_4-H, coumarin). Anal. calcd. for $C_{19}H_{13}NO_3$ (303.31): C, 75.24; H, 4.32; N, 4.62%. Found: C, 75.18; H, 4.30; N, 4.57%.

*Attempting for preparation of 3-(4-hydroxy-4H-thiochromen-4-yl)-2H-chromen-2-one (**17**)*

A suspension of compound **16** (0.5 g, 2 mmol) in H_2SO_4 (5 mL) was heated on water bath at 80°C for 5 h. The resulting solution was cooled at room temperature and poured into crushed ice and treated with conc. ammonia. The solid obtained was filtered off, washed with water, dried and crystallized from benzene/ethanol mixture.

*Synthesis of 2,4-dimethyl-1,5,6,8a,9-pentaaza-fluorene-7-yl(2H-chromen-2-on-3-yl)ketone (**19**)*

A well stirred solution of 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine-3-diazonium chloride (0.42 g, 2 mmol) in conc. HCl (2 mL) and water (2 mL) was cooled in an ice bath and diazotized with the solution of $NaNO_2$ (0.14 g, 2 mmol) in water (2 mL). The cold diazonium solution was added slowly to a well stirred solution of **11** (0.48 g, 2 mmol) in pyridine (10 mL). The reaction mixture was stirred for another 2 h. The crude product was filtered off, dried well, and heated in dioxane (20 mL) containing acetic acid (20 mL) for 7 h. The resulting solution was cooled at room temperature and poured into crushed ice and treated with conc. ammonia. The solid obtained was filtered off, washed with water, dried, and crystallized from benzene/ethanol mixture to furnish **19**.

Brown powder, yield: 76%, mp: 320°C, IR (KBr): ν_{max}, cm^{-1}: 1724, 1644 (2 CO), 1608 (C=N). MS: m/z (%) = 356 (M^+ − CH_3, 100.0), 328 (15.6), 224 (15.6), 147 (26.7), 85 (28.9), 52 (66.7). Anal. calcd. for $C_{20}H_{13}N_5O_3$ (371.35): C, 64.69; H, 3.53; N, 18.86%. Found: C, 64.74; H, 3.58; N, 18.93%.

*Synthesis of 3-(3-methyl-1-phenyl-1H-pyrazolo-[3,4-b]pyridin-6-yl)-2H-chromen-2-one (**20**)*

A mixture of **11** (0.48 g, 2 mmol), 4-methyl-1-phenyl-1*H*-pyrrol-2(3*H*)-one (0.35 g, 2 mmol), and ammonium acetate (1 g, 12 mmol) was fused for 5 h in a sand bath at 170°C. Further, whole reaction mixture was allowed to reflux for another 3 h in glacial acetic acid (20 mL), cooled, and then poured into crushed ice. The solid obtained was filtered off, washed with water, dried and crystallized from dimethylformamide/ethanol mixture to afford **20**.

Brown crystals, yield: 73%, mp: 200°C, IR (KBr): ν_{max}, cm^{-1}: 1724 (CO), 1644 (C=N). ^{1}H-NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 7.4–8.2 (m, 11H, Ar-H), 8.42 (brs, 1H, C_4-H, coumarin). MS: m/z (%) = 355 (M^++ 2, 13.90), 341 (M^+ − CH_3, 26.26), 328 (4.85), 315 (4.85), 304 (10.98), 287 (2.57), 267 (5.54), 252 (8.68), 238 (3.64), 224 (12.18), 197 (3.95), 183 (11.88), 170 (576), 149 (7.33), 131 (8.79), 128 (24.21), 106 (13.21), 91 (41.38), 77 (100.0), 64 (31.53). Anal. calcd. for $C_{22}H_{15}N_3O_2$ (353.37): C, 74.78; H, 4.28; N, 11.89%. Found: C, 74.75; H, 4.24; N, 11.92%.

*Synthesis of 3-(3-phenyl-7H-[1,2,4]triazolo-[3,4-b][1,3,4]thiadiazin-6-yl)-2H-chromen-2-one (**21**), 3-(2-(pyridin-2-ylamino)-6H-1,3,4-thiadiazin-5-yl)-2H-chromen-2-one (**22**), and 3-benzyl-7-(2-oxo-2H-chromen-3-yl)-[1,2,4]triazino[3,4-b][1,3,4]thiadiazin-4(8H)-one (**23**)*

General Procedure

A mixture of 3-(2-bromoacetyl)-2*H*-chromen-2-one (**2**) (0.8 g, 3 mmol), triethylamine (0.42 mL, 3 mmol), and 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (0.58 g, 3 mmol), *N*-(pyridin-2-yl)hydrazinecarbothioamide (0.34 g, 2 mmol) or 4-amino-6-benzyl-3-mercapto-1,2,4-triazin-5(4*H*)-one (0.94 g, 4 mmol) in dimethylformamide (20 mL) was refluxed for 12, 8, and 9 h, respectively. The resulting solution was cooled, poured into ice-cold water, filtered off, dried, and crystallized from dimethylformamide/ethanol mixture to furnish **21–23**, respectively.

Compound **21**; brown powder, yield: 69%, mp: 182°C, IR (KBr): ν_{max}, cm^{-1}: 1720 (CO), 1606 (C=N). ^{1}H-NMR (DMSO-d_6): δ 2.9 (s, 2H, CH_2), 6.9–7.9 (m, 9H, Ar-H), 8.45 (s, 1H, C_4-H, coumarin). Anal. calcd. for $C_{19}H_{12}N_4O_2S$ (360.39): C, 63.32; H, 3.36; N, 15.55%. Found: C, 63.38; H, 3.41; N, 15.59%.

Compound **22**, dark brown crystals, yield: 66%, mp: 280°C, IR (KBr): ν_{max}, cm^{-1}: 3343 (NH), 1714 (CO), 1604 (C=N). ^{1}H-NMR (DMSO-d_6): δ 3.33 (s, 2H, CH_2), 6.71–8.14 (m, 8H, Ar-H), 8.47 (s, 1H, C_4-H, coumarin), 9.47 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6): δ 159.0, 155.4, 153.1, 147.8, 138.0, 137.8, 132.3, 129.2, 128.8,

125.4, 121.4, 121.3, 119.6, 116.5, 116.4, 114.7, 110.2, 24.4. Anal. calcd. for $C_{17}H_{12}N_4O_2S$ (336.37): C, 60.70; H, 3.60; N, 16.66%. Found: C, 60.76; H, 3.64; N, 16.68%.

Compound **23**, green crystals, yield: 89%, mp: 217–220°C, IR (KBr): ν_{max}, cm^{-1}: 1712 (br, 2 CO), 1608 (C=N). MS: *m/z* (%) = 402 (M^+, 7.8), 370 (M^+ – S, 8.6), 268 (7.7), 253 (8.1), 226 (8.8), 198 (13.6), 176 (8.5), 165 (13.2), 145 (12.7), 144 (22.5), 142 (32.9), 116 (100.0), 115 (90.5), 88 (82.8), 76 (71.4), 63 (39.1). Anal. calcd. for $C_{21}H_{14}N_4O_3S$ (402.43): C, 62.68; H, 3.51; N, 13.92%. Found: C, 62.72; H, 3.54; N, 13.99%.

*Synthesis of 1-acetyl-3-benzyl-7-(2-oxo-2H-chromen-3-yl)pyrazolo[5,1-c][1,2,4] triazin-4(1H)-one (**24**)*

Compound **23** (0.8 g, 2 mmol) in acetic anhydride (10 mL) was refluxed for 3 h at 100°C. The resulting solution was cooled, poured into ice-cold water, filtered off, dried, and crystallized from dimethylformamide/ethanol mixture to give **24**.

Black crystals, yield: 84–88%, mp: 320°C, IR (KBr): ν_{max}, cm^{-1}: 1727 (br, 2 CO), 1604 (C=N). ^{1}H-NMR (DMSO-d_6): δ 1.95 (s, 3H, CH_3), 2.73 (s, 2H, CH_2), 6.80–8.45 (m, 11H, Ar-H, C_4-H, coumarin). MS: *m/z* (%) = 412 (M^+, 0.7), 369 (0.7), 336 (11.6), 320 (5.3), 244 (12.6), 217 (11.5), 188 (9.2), 174 (19.2), 156 (2.4), 144 (20.5), 119 (11.9), 115 (19.7), 97 (42.4), 76 (33.6), 73 (38.4). Anal. calcd. for $C_{23}H_{16}N_4O_4$ (412.4): C, 66.99; H, 3.91; N, 13.59%. Found: C, 67.04; H, 3.96; N, 13.67%.

*Synthesis of 3-(benzofuran-3-carbonyl)-2H-chromen-2-one (**25**)*

A mixture of **2** (0.27 g, 1 mmol), salicylaldehyde (0.2 mL, 1.88 mmol), and potassium carbonate (0.5 g, 3.62 mmol) in dimethylformamide (20 mL) was refluxed for 4 h. The resulting solution was cooled, poured into ice-cold water, filtered off, dried, and crystallized from dimethylformamide/ethanol mixture to furnish **25**.

Yellowish white powder, yield: 78%, mp: 263°C, IR (KBr): ν_{max}, cm^{-1}: 1722 (br, 2 CO). ^{1}H-NMR (DMSO-d_6): δ 6.8–8.45 (m, 10H, Ar-H). MS: *m/z* (%) = 291 (M^+ + 1, 4.09), 290 (M^+, 10.5), 286 (26.6), 274 (7.9), 244 (11.6), 231 (18.3), 201 (18.1), 187 (32.9), 173 (89.2), 165 (18.5), 145 (99.3), 131 (46.4), 118 (78.9), 115 (47.6), 89 (94.3), 86 (41.5), 62 (100.0). Anal. calcd. for $C_{18}H_{10}O_4$ (290.27): C, 74.48; H, 3.47%. Found: C, 74.52; H, 3.49%.

Antioxidant screening

Antioxidant activity screening assay; ABTS method [27]

Antioxidant activity determinations were evaluated from the bleaching of ABTS derived radical cations. The radical cation 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 inhibition ratio (%) was calculated using the following formula:

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

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

Bleomycin-dependent DNA damage

The assay was done according to Aeschlach *et al.* [30] and Chan & Tang [31], with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg/mL), Bleomycin sulfate (0.05 mg/mL), and $MgCl_2$ (5 mM), $FeCl_3$ (50 mM) and the samples were dissolved in DMSO to be tested at concentration (20 μL of 1 mg/mL). L-Ascorbic acid was used as a positive control. The mixture was incubated at 37°C for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding thiobarbituric acid (TBA) (0.5 mL) (1%, w/v) and HCl (0.5 mL) (25%, v/v) followed by heating at 80°C for 10 min. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm (Table 2).

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