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Synthesis, reactions, antioxidant and anticancer evaluation of some novel coumarin derivatives using ethyl 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetate as a starting material

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ABSTRACT

We report on the synthesis of new coumarin derivatives coupled with heterocyclic and bifunctionalized moieties at position 7. The proposed structures were confirmed by correct elemental analysis and spectral data (IR, MS, ^{1}H NMR and ^{13}C NMR). Some selected derivatives were also screened for their antioxidant and anticancer activities. The results indicated that 7-{[6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl]methoxy}-4-phenyl-*H*-chromen-2-one has the highest antioxidant and anticancer activity in tested compounds.

1. Introduction

The coumarin nucleus is frequently found in bioactive compounds and plays an important role in biochemical processes. The cytotoxic activities of the coumarin derivatives were tested in several human tumor cell lines [1-7]. In addition, the antimicrobial, antitumor, anti-inflammatory, antimalarial and anti-HIV activities of hydrazide compounds on tumor cell lines have been observed and recently reported [8-14], Moreover, some synthetic oxadiazole derivatives exhibit a range of pharmacological activities [15-18]. The biological and medicinal activities of triazole moieties have stimulated considerable interest in the synthesis of derivatives of this ring system [19-25]. In view of the above observations and in continuation of our previous works in heterocyclic chemistry, we report herein the synthesis of some new derivatives of these ring systems incorporated with 4-phenylcoumarin moiety, using ethyl 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetate (**1**) [26] as a key starting material. A selected series of these compounds were investigated for their antitumor activities.

2. Experimental

2.1. Instrumentation

All melting points, antioxidant and anticancer activities are uncorrected. IR spectra (KBr) were recorded on FT-IR 5300 spectrometer and Perkin Elmer spectrum RXIFT-IR system (ν, cm^{-1}). The ^{1}H NMR spectra were recorded in (DMSO-d_6) at 300 MHz on a Varian Mercury VX-300 NMR spectrometer (δ, ppm) using TMS as an internal standard. ^{13}C NMR spectra were recorded on Varian Mercury VX 300 NMR using DMSO-d_6 as solvent and TMS as an internal standard. Mass spectrum was obtained on GC MS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out by the Microanalytical Research Center, Faculty of Science, Cairo University and Al-Azhar University.

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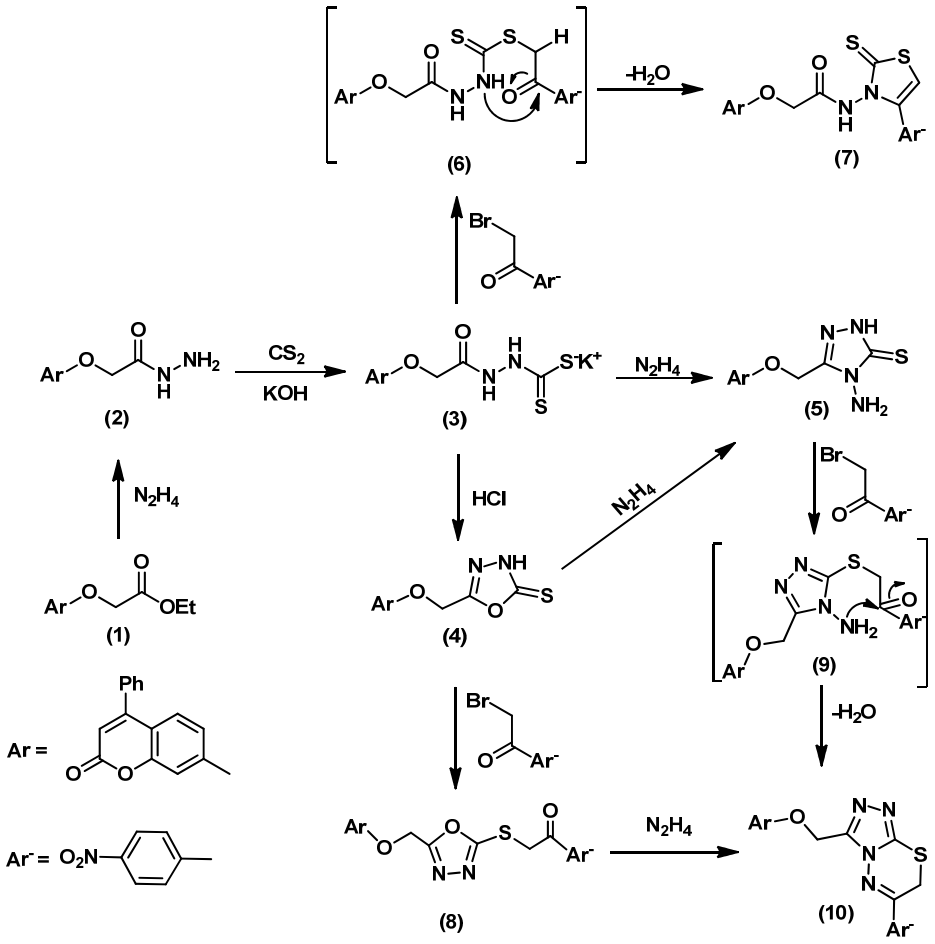


Scheme 1

2.2. Synthesis

2.2.1. Synthesis of 2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetohydrazide **(2)**

A mixture of compound **1** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethanol (30 mL) was refluxed for 8 h. The reaction mixture was concentrated and left to cool (Scheme 1). Color: Colorless. Yield: 80%. M.p.: 188-190 °C. FT-IR (KBr, ν, cm^{-1}): 3340, 3254 (NH_2, NH), 1716 (C=O, lactone), 1690 (C=O, amide). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 9.43 (s, 1H, NH exchangeable with D_2O), 6.96-7.58 (m, 8H, Ar-H), 6.25 (s, 1H, H-3), 4.63 (s, 2H, OCH_2), 4.36 (s, 2H, NH_2 exchangeable with D_2O). MS (EI, *m/z* (%)): 310 (M^+, 77). Anal. calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03. Found: C, 65.74; H, 4.43; N, 8.91%.

2.2.2. Synthesis of potassium 2-[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetyl]hydrazinecarbodithioate **(3)**

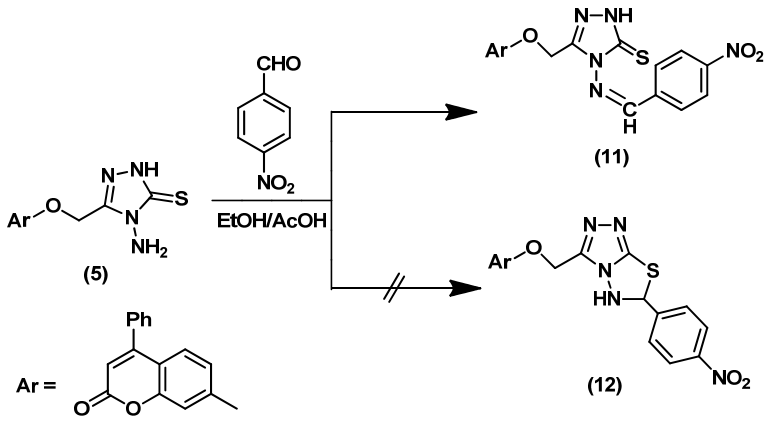
To a solution of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)-acetohydrazide (**2**, 0.01 mol) in ethanol (30 mL), a solution of potassium hydroxide (0.01 mol) in water (5 mL) and carbon disulfide (0.01 mol) were added. The reaction mixture was heated under reflux for 3 h, the resulting solid on heating was collected by filtration, washed with ether and dried to afford 2.8 g of the potassium salt **3** (Scheme 1). Color: yellow. Yield: 90%. M.p.: >360 °C. FT-IR (KBr, ν, cm^{-1}): 3320, 3266 (NH), 1698 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.57-7.58 (m, 10H, Ar-H and 2NH), 6.25 (s, 1H, H-3), 5.19 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 424 (M^+, 61). Anal. calcd. for $C_{18}H_{13}KN_2O_4S_2$: C, 50.92; H, 3.09; N, 6.60. Found: C, 50.85; H, 3.00; N, 6.51%.

2.2.3. Synthesis of 7-[(5-thioxo-1,3,4-oxadiazol-2-yl)methoxy]-4-phenyl-2H-chromen-2-one **(4)**

The potassium salt **3** (0.01 mol) was dissolved in aqueous potassium hydroxide solution (0.01 mol) in water (10 mL) and refluxed for 2 h then cooled. The resulting reaction mixture was treated with dilute hydrochloric acid till pH=~4. The resulting solid was collected by filtration, washed with water, and recrystallized from ethanol to give compound **4** (Scheme 1). Color: yellow. Yield: 76%. M.p.: 185-187 °C. FT-IR (KBr, ν, cm^{-1}): 1686 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 14.23 (s, 1H, NH), 7.02-7.58 (m, 8H, Ar-H), 6.28 (s, 1H, H-3), 5.42 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 352 (M^+, 51). Anal. calcd. for $C_{18}H_{12}N_2O_4S$: C, 61.35; H, 3.43; N, 7.95. Found: C, 61.28; H, 3.35; N, 7.87%.

2.2.4. Synthesis of 7-[(4-amino-5-thioxo-4, 5-dihydro-1H-1, 2, 4-triazol-3-yl) meth-oxy]-4-phenyl-2H-chromen-2-one **(5)**

Method A: To a solution of potassium salt **3** (0.01 mol) in ethanol (20 mL) and water (10 mL), hydrazine hydrate (0.2 mL) was added. The reaction mixture was heated under reflux for 2 h, then left to cool, then poured into crushed ice. The resulted reaction mixture was acidified with dilute hydrochloric acid.



Scheme 2

The resulting solid was collected by filtration, washed with water and recrystallized from dioxane (Scheme 1). Color: White. Yield: 77%. M.p.: 222-224 °C. FT-IR (KBr, ν, cm^{-1}): 1686 (C=O), 3384, 3298 (NH_2), 3182 (NH), 1698 (C=O). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.84 (s, 1H, NH exchangeable with D_2O), 6.99-7.56 (m, 8H, Ar-H), 6.25 (s, 1H, H-3), 5.67 (b, 2H, NH_2 exchangeable with D_2O), 5.27(s, 2H, OCH_2). ^{13}C NMR (300 MHz, DMSO-d_6, δ, ppm): 166.79, 160.61, 159.79, 155.17, 154.93, 147.49, 134.83, 129.61, 128.79, 128.35, 127.85, 112.80, 112.48, 111.78, 102.35 and 59.73. MS (EI, *m/z* (%)): 366 (M^+, 25). Anal. calcd. for $C_{18}H_{14}N_4O_3S$: C, 59.01; H, 3.85; N, 15.29. Found: C, 58.89; H, 3.78; N, 15.18%.

Method B: A mixture of 7-[(5-thioxo-1,3,4-oxadiazol-2-yl)methoxy]-4-phenyl-2*H*-chromen-2-one (**4**) (0.01 mol) in ethanol (20 mL) and hydrazine hydrate (0.2 mL) was heated under reflux for 2 h, then left to cool and poured onto crushed ice. The reaction mixture was acidified with dilute hydrochloric acid till pH = ~4. The resulting solid was collected by filtration, washed with water and recrystallized from dioxane, to give compound **5**.

2.2.5. Synthesis of N-(4-(4-nitrophenyl)-2-thioxothiazol-3(2H)-yl)-2-((2-oxo-4-phenyl-2H-chromen-7-yl)oxy) acetamide (7)

A mixture of potassium salt **3** (0.01 mol) and *p*-nitro phenacyl bromide (0.01 mol) in *N*,*N*-dimethylformamide (DMF) (15 mL) was heated under reflux for 3 h, then left to cool and poured onto crushed ice. The precipitated solid was collected by filtration, washed with ethanol, dried and recrystallized from dioxane (Scheme 1). Color: Yellow. Yield: 54%. M.p.: 170-172 °C. FT-IR (KBr, ν, cm^{-1}): 3315(NH), 1716, 1700 (C=O). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.62 (s, 1H, NH), 6.80-8.38 (m, 13H, Ar-H and CH-thiazole), 6.29 (s, 1H, H-3), 5.18 (s, 2H, OCH_2). Anal. calcd. for $C_{26}H_{17}N_3O_6S_2$: C, 58.75; H, 3.22; N, 7.91. Found: C, 58.70; H, 3.14; N 7.82%.

2.2.6. Synthesis of 7-{[5-(2-(4-nitrophenyl)-2-oxoethylthio)-1,3,4-oxadiazol-2-yl]methoxy}-4-phenyl-2H-chromen-2-one (8)

A mixture of 7-[(5-thioxo-1,3,4-oxadiazol-2-yl)methoxy]-4-phenyl-2*H*-chromen-2-one (**4**) (0.01 mol), *p*-nitrophenacyl bromide (0.01 mol) and anhydrous potassium carbonate (0.01 mol) in dry acetone (15 mL) was refluxed for 6 h. The formed precipitate was filtered off and washed with acetone. The filtrate was distilled under reduced pressure till dryness then the residue was treated with water. The solid formed was collected by filtration, washed with water, dried and recrystallized from dioxane to give compound **8** (Scheme 1). Color: Yellow. Yield: 64%. M.p.: 110-112 °C. FT-IR (KBr, ν, cm^{-1}): 1710 (C=O). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.51-8.39 (m, 12H, Ar-H), 6.14 (s, 1H, H-3), 5.21 (s, 2H, OCH_2), 4.60 (s, 2H, CH_2). MS (EI, *m/z* (%)): 515 (M^+, 25). Anal. calcd. for $C_{26}H_{17}N_3O_7S$: C, 60.58; H, 3.32; N, 8.15. Found: C, 60.48; H, 3.20; N, 8.03%.

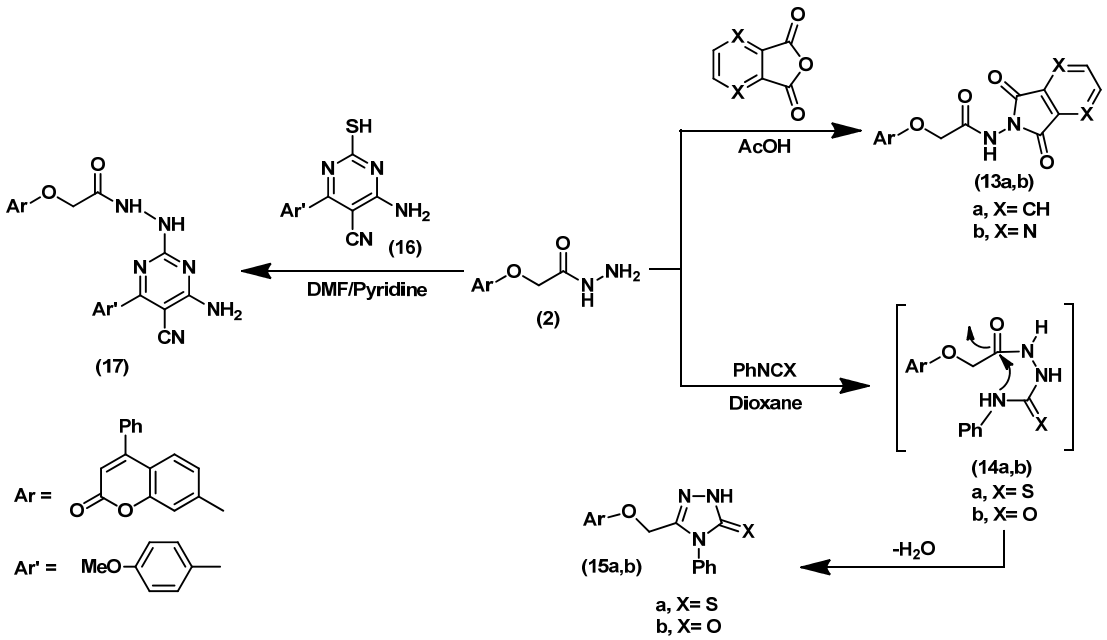
2.2.7. Synthesis of 7-((6-(4-nitrophenyl)-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazin-3-yl)methoxy)-4-phenyl-2H-chromen-2-one (10)

Method A: A mixture of 7-[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]-4-phenyl-2*H*-chromen-2-one (**5**) (0.01 mol), *p*-nitrophenacyl bromide (0.01 mol) and triethylamine (TEA) (0.2 mL) in DMF (15 mL) was heated under reflux for 2 h, then left to cool and poured onto crushed ice. The reaction mixture was acidified with dilute hydrochloric acid till pH = ~4. The resulting solid was collected by filtration, washed with water and recrystallized from dioxane (Scheme 1). Color: Brown. Yield: 56%. M.p.: 176-178 °C. FT-IR (KBr, ν, cm^{-1}): 1712 (C=O). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.76-8.35 (m, 12H, Ar-H), 6.23 (s, 1H, H-3), 5.22 (s, 2H, OCH_2), 4.81 (s, 2H, CH_2). MS (EI, *m/z* (%)): 511 (M^+, 36). Anal.calcd. for $C_{26}H_{17}N_5O_5S$: C, 61.05; H, 3.35; N, 13.69. Found: C, 60.91; H, 3.23; N, 13.60%.

Method B: To 7-{[5-(2-(4-nitrophenyl)-2-oxoethylthio)-1,3,4-oxadiazol-2-yl]methoxy}-4-phenyl-2*H*-chromen-2-one (**8**) (0.01 mol) in ethanol (20 mL), hydrazine hydrate (0.2 mL) was added. The reaction mixture was heated under reflux for 3 h, then left to cool. The resulting solid was collected by filtration, washed with water, and recrystallized from dioxane, to give compound **10**.

2.2.8. Synthesis of 7-{[4-(4-nitrobenzylideneamino)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]methoxy}-4-phenyl-2H-chromen-2-one (11)

A mixture of *p*-nitrobenzaldehyde (0.01 mol) and 7-[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]-4-phenyl-2H-chromen-2-one (**5**) (0.01 mol) in ethanol (20 mL) in the presence of acetic acid (1 mL) was refluxed for 4 h, then left to cool. The solid product was collected by filtration, washed with ethanol, dried and recrystallized from dioxane (Scheme 2). Color: Brown. Yield: 70%. M.p.: 238-240 °C. FT-IR (KBr, ν, cm^{-1}): 3190 (NH), 1700(C=O). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.39 (s, 1H, NH exchangeable with D_2O), 8.86 (s, 1H, CH=N), 7.03-8.38 (m, 12H, Ar-H), 6.28 (s, 1H, H-3), 5.40 (s, 2H, OCH_2). Anal. calcd. for $C_{25}H_{17}N_5O_5S$: C, 60.11; H, 3.43; N, 14.02. Found: C, 60.02; H, 3.30; N, 13.92%.



Scheme 3

2.2.9. General procedure for preparation of N-(substituted)-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamide (13a,b)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)aceto hydrazide (**2**) (0.01 mol) and phthalic anhydride orpyrazine-2,3-dicarboxylic anhydride (0.01 mol) in glacial acetic acid (20 mL) was refluxed for 6 h, then the reaction mixture left to cool. The white solid was filtered off and recrystallized from dioxane to give compounds **13a**,**b** (Scheme 3).

N-(1,3-Dioxoisoindolin-2-yl)-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)-acetamide (**13a**): Color: White. Yield: 80%. M.p.: 262-264 °C. FT-IR (KBr, ν, cm^{-1}): 3176 (NH), 1715, 1708 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.09 (s, 1H, NH), 7.04-8.01 (m, 12H, Ar-H), 6.29 (s, 1H, H-3), 5.01 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 440 (M^+, 20). Anal. calcd. for $C_{25}H_{16}N_2O_6$: C, 68.18; H, 3.66; N, 6.36. Found: C, 68.10; H, 3.58; N, 6.29%.

N-(5,7-Dioxo-5H-pyrrolo[3,4-b]pyrazin-6(7H)-yl)-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamide (**13b**): Color: White. Yield: 82%. M.p.: 158-160 °C. FT-IR (KBr, ν, cm^{-1}): 3276 (NH), 1720, 1714 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.78 (s, 1H, NH), 8.76, 8.90 (d, 2H, 2CH pyrazine), 6.93-7.55 (m, 8H, Ar-H), 6.24 (s, 1H, H-3), 4.84(s, 2H, OCH_2). ^{13}CNMR (300 MHz, DMSO-d_6, δ, ppm): 166.06, 162.02, 160.75, 159.84, 155.19, 154.99, 147.96, 144.00, 143.67, 143.52, 134.87, 129.62, 128.81, 128.35, 127.76, 120.79, 112.93, 112.35, 111.68, 102.16 and 66.36. MS (EI, *m/z* (%)): 442 (M^+, 50). Anal. calcd. for $C_{23}H_{14}N_4O_6$: C, 62.45; H, 3.19; N, 12.66. Found: C, 62.38; H, 3.10; N, 12.52%.

2.2.10. General procedure for the preparation of 4-phenyl-7-((4-phenyl-5-substituted-4,5-dihydro-1H-1,2,4-triazol-3-yl) methoxy)-2H-chromen-2-one (15a,b)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)aceto hydrazide (**2**) (0.01 mol) and phenylisothiocyanate or phenyliso-cyanate (0.01 mol) in dioxane (15 mL) was refluxed for3 h, the resulting solid on heating was collected by filtration, washed with ethanol several times and recrystallized from DMF to give compounds **15a**,**b** (Scheme 3).

4-Phenyl-7-((4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methoxy)-2H-chromen-2-one (**15a**): Color: White. Yield: 76%. M.p.: 224-226 °C. FT-IR (KBr, ν, cm^{-1}): 3345 (NH), 1720 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 14.08 (s, 1H, NH), 6.78-7.56 (m, 13H, Ar-H), 6.24 (s, 1H, H-3), 5.13 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 427 (M^+, 10). Anal. calcd. for $C_{24}H_{17}N_3O_3S$: C, 67.43; H, 4.01; N 9.83. Found: C, 67.37; H, 3.93; N, 9.75%.

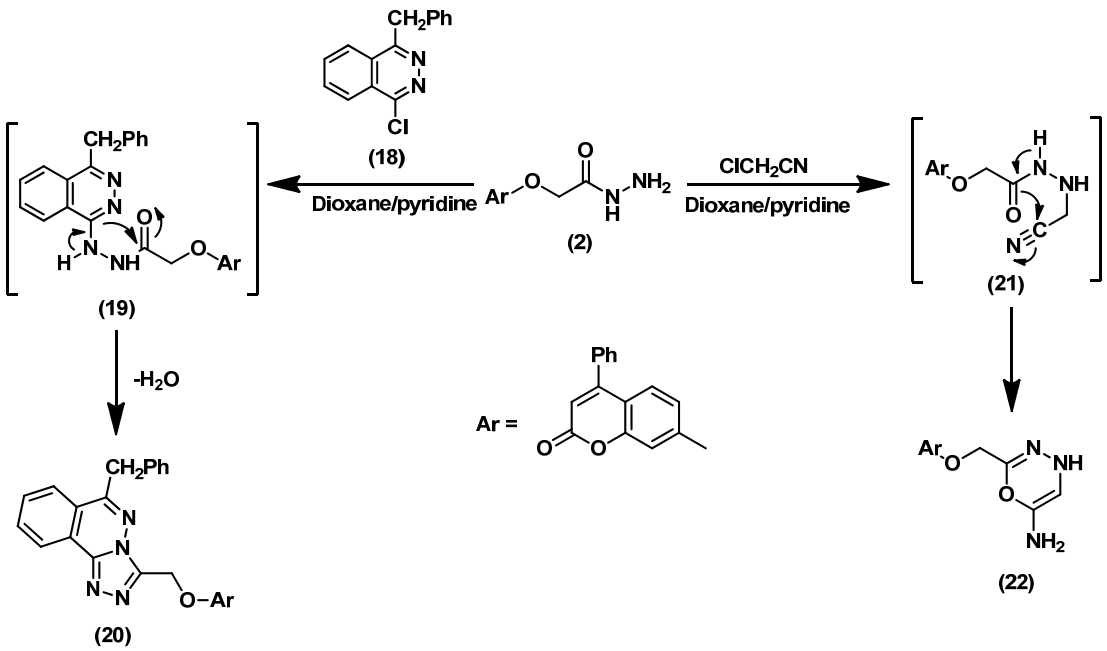
3-((2-Oxo-4-phenyl-2H-chromen-7-yloxy)methyl)-4-phenyl-1H-1,2,4-triazol-5(4H)-one (**15b**): Color: White. Yield: 82%. M.p.: 220-222 °C. FT-IR (KBr, ν, cm^{-1}): 3302 (NH), 1692 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.06 (s, 1H, NH), 6.95-8.74 (m, 13H, Ar-H), 6.27 (s, 1H, H-3), 4.78 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 411 (M^+, 75). Anal. calcd. for $C_{24}H_{17}N_3O_4$: C, 70.07; H, 4.16; N, 10.21. Found: C, 69.89; H, 4.08; N, 10.10%.

2.2.11. Synthesis of N'-(4-amino-5-cyano-6-(4-methoxy phenyl) pyrimidin-2-yl)-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetohydrazide (17)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)aceto hydrazide (**2**) (0.01 mol),4-amino-2-mercapto-6-(4-methoxy-phenyl)pyrimidine-5-carbonitrile (**16**) (0.01 mol) and pyridine (0.2 mL) in DMF (15 mL) was heated under reflux for 4 h, then left to cool and poured onto crushed ice. The reaction mixture was acidified with dilute hydrochloric acid till pH = ~4. The resulting solid was collected by filtration, washed with water and recrystallized from dioxane (Scheme 3). Color: Yellow. Yield: 92%. M.p.: 98-100 °C. FT-IR (KBr, ν, cm^{-1}): 3325 (NH_2), 3230 (NH), 2200 (CN), 1712 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.03 (s, 1H, NH), 10.29 (s, 1H, NH), 8.02 (s, 2H, NH_2), 7.01-7.81 (m, 12H, Ar-H), 6.24 (s, 1H, H-3), 4.74 (s, 2H, OCH_2), 3.79 (s, 3H, OCH_3). Anal. calcd. for $C_{29}H_{22}N_6O_5$: C, 65.16; H, 4.15; N, 15.72. Found: C, 65.08; H, 4.07; N, 15.65%.

2.2.12. Synthesis of 7-[(6-benzyl-[1,2,4]triazolo[3,4-a] phthalazin-3-yl)methoxy]-4-phenyl-2H-chromen-2-one (20)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) aceto hydrazide (**2**) (0.01 mol) and 4-benzyl-1-chlorophthalazine (**18**) (0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for 4 h. The solid product which obtained after cooling was collected and recrystallized from dioxane to give compound **20** (Scheme 4).



Scheme 4

Color: Yellow. Yield: 84%. M.p.: 170-172 °C. FT-IR (KBr, ν, cm^{-1}): 3012 (CH-aromatic), 2902 (CH-aliphatic), 1712 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.96-8.26 (m, 17H, Ar-H), 6.25 (s, 1H, H-3), 4.63 (s, 2H, OCH_2), 4.35 (s, 2H, CH_2). MS (EI, *m/z* (%)): 510 (M^+, 29). Anal. calcd. for $C_{32}H_{22}N_4O_3$: C, 75.28; H, 4.34; N, 10.97. Found: C, 75.20; H, 4.23; N, 10.90%.

2.2.13. Synthesis of 7-[(6-amino-4H-1,3,4-oxadiazin-2-yl) methoxy]-4-phenyl-2H-chromen-2-one (22)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)aceto hydrazide (**2**) (0.01 mol) and chloroacetonitrile (0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for 3 h, then the reaction mixture left to cool. The solid product was filtered off, washed with ethanol several times and recrystallized from dioxane (Scheme 4). Color: White. Yield: 56%. M.p.: 240-242 °C. FT-IR (KBr, ν, cm^{-1}): 3245 (NH_2), 3175 (NH), 1712 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.29 (s, 1H, NH), 7.07-7.53 (m, 11H, Ar-H, CH-oxadiazin and NH_2), 6.23 (s, 1H, H-3), 4.76 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 349 (M^+, 16). Anal. calcd. for $C_{19}H_{15}N_3O_4$: C, 65.32; H, 4.33; N, 12.03. Found: C, 65.19; H, 4.12; N, 11.83%.

2.2.14. Synthesis of 7-[(4,5-dihydro-1H-imidazol-2-yl) methoxy]-4-phenyl-2H-chromen-2-one (24)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetic acid (**23**) (0.01 mol) and ethylenediamine (0.01 mol) was heated without solvent at 180-200 °C for 30 min. Then the mixture was left to cool. The solid product that formed was recrystallized from dioxane (Scheme 5). Color: Brown. Yield: 58%. M.p.: 266-268 °C. FT-IR (KBr, ν, cm^{-1}): 3376 (NH), 1712 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.24 (s, 1H, NH), 6.91-7.56 (m, 8H, Ar-H), 6.22 (s, 1H, H-3), 4.59 (s, 2H, OCH_2), 3.28 (s, 4H, $2CH_2$). MS (EI, *m/z* (%)): 320 (M^+, 17). Anal. calcd. for $C_{19}H_{16}N_2O_3$: C, 71.24; H, 5.03; N, 8.74. Found: C, 71.17; H, 4.91; N, 8.69%.

2.2.15. General procedure for preparation of 7-(substituted-2-ylmethoxy)-4-phenyl-2H-chromen-2-one (25a-c)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetic acid (**23**) (0.01 mol) and 1,2-phenylenediamine, or 2-aminophenol, or 2-aminothiophenol (0.01 mol) was heated without solvent at 180-200°C for 30 min. Then the mixture was left to cool. The solid products that formed were recrystallized from dioxane to give compounds **25a-c** (Scheme 5).

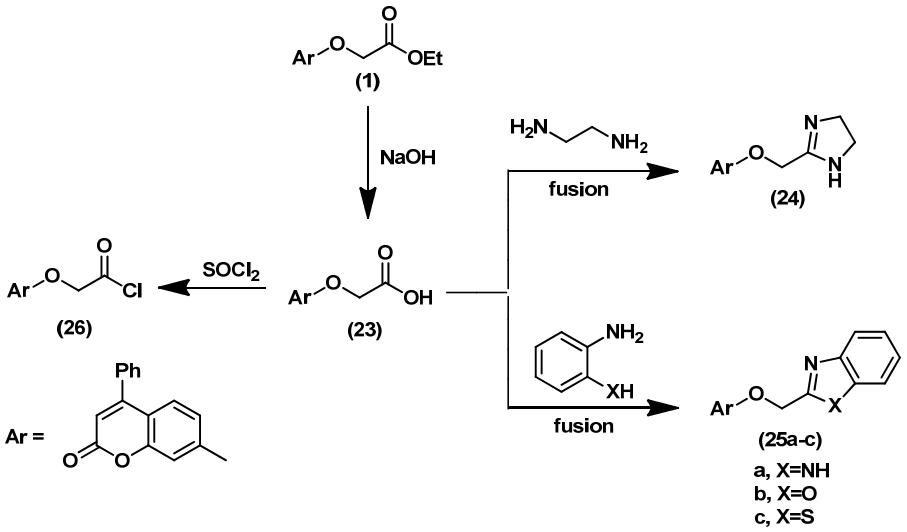
7-[(1H-Benzo[d]imidazol-2-yl)methoxy]-4-phenyl-2H-chromen-2-one (**25a**): Color: Brown. Yield: 64%. M.p.: 242-244 °C. FT-IR (KBr, ν, cm^{-1}): 3320 (NH), 1706 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.72 (s, 1H, NH), 7.05-7.65 (m, 12H, Ar-H), 6.25 (s, 1H, H-3), 5.46 (s, 2H, OCH_2). Anal. calcd. for $C_{23}H_{16}N_2O_3$: C, 74.99; H, 4.38; N, 7.60. Found: C, 74.91; H, 4.30; N, 7.52%.

7-(Benzo[d]oxazol-2-ylmethoxy)-4-phenyl-2H-chromen-2-one (**25b**): Color: Reddish brown. Yield: 52%. M.p.: 170-172 °C. FT-IR (KBr, ν, cm^{-1}): 1730 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.05-7.80 (m, 12H, Ar-H), 6.26 (s, 1H, H-3), 5.63 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 369 (M^+, 32). Anal. calcd. for $C_{23}H_{15}NO_4$: C, 74.79; H, 4.09; N, 3.79. Found: C, 74.68; H, 4.00; N, 3.68%.

7-(Benzo[d]thiazol-2-ylmethoxy)-4-phenyl-2H-chromen-2-one (**25c**): Color: Yellow. Yield: 78%. M.p.: 138 °C. FT-IR (KBr, ν, cm^{-1}): 1716 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.08-8.15 (m, 12H, Ar-H), 6.27 (s, 1H, H-3), 5.76 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 385 (M^+, 26). Anal. calcd. for $C_{23}H_{15}NO_3S$: C, 71.67; H, 3.92; N, 3.63. Found: C, 71.58; H, 3.83; N, 3.57%.

2.2.16. Synthesis of 2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetyl chloride (26)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetic acid (**23**) (0.01 mol) and thionyl chloride (15 mL) was fused for 1 h. Excess of thionyl chloride removed under pressure to obtain the pure acyl chloride (Scheme 5). Color: Pale yellow. Yield: 95%. M.p.: 140-142 °C. FT-IR (KBr, ν, cm^{-1}): 1700 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 6.76-7.58 (m, 8H, Ar-H), 6.15 (s, 1H, H-3), 4.84 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 314 (M^+, 25). Anal. calcd. for $C_{17}H_{11}ClO_4$: C, 64.88; H, 3.52. Found: C, 64.80; H, 3.44%.



Scheme 5

2.2.17. General procedure for preparation of N-(4-acetyl phenyl)-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamide, 2-{4-[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamido] phenyl} acetic acid and 4-[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamido]benzoic acid (27a-c)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetyl chloride (**26**) (0.01 mol) and aniline derivatives namely (4-amino-acetophenone, 4-aminophenylacetic acid and 4-aminobenzoic acid) (0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for1 h, the resulting solid on heating was collected by filtration, washed with ethanol several times and recrystallized from DMF to give compounds **27a-c** (Scheme 6).

N-(4-Acetylphenyl)-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetamide (**27a**): Color: White. Yield: 97%. M.p.: 222-224 °C. FT-IR (KBr, ν, cm^{-1}): 3344 (NH), 1718, 1682 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.48 (s, 1H, NH), 7.01-7.96 (m, 12H, Ar-H), 6.26 (s, 1H, H-3), 4.91 (s, 2H, OCH_2), 2.48 (s, 3H, CH_3). Anal. calcd. for $C_{25}H_{19}NO_5$: C, 72.63; H, 4.63; N, 3.39. Found: C, 72.51; H, 4.58; N, 3.28%.

2-{4-[2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetamido] phenyl}acetic acid (**27b**): Color: Yellow. Yield: 90%. M.p.: 216-218 °C. FT-IR (KBr, ν, cm^{-1}): 3432 (OH), 3334 (NH), 1720 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.22 (s, 1H, OH exchangeable with D_2O), 10.12 (s, 1H, NH exchangeable with D_2O), 6.80-7.62 (m, 12H, Ar-H), 6.25 (s, 1H, H-3), 4.85 (s, 2H, OCH_2), 3.56 (s, 2H, CH_2). MS (EI, *m/z* (%)): 429 (M^+, 10). Anal. calcd. for $C_{25}H_{19}NO_6$: C, 69.92; H, 4.46; N, 3.26. Found: C, 69.84; H, 4.38; N, 3.18%.

4-(2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetamido) benzoic acid (**27c**): Color: White. Yield: 95%. M.p.: 316-318 °C. FT-IR (KBr, ν, cm^{-1}): 3435 (OH), 3352 (NH), 1698 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 12.71 (s, 1H, OH exchangeable with D_2O), 10.45 (s, 1H, NH exchangeable with D_2O), 7.01-7.93 (m, 12H, Ar-H), 6.26 (s, 1H, H-3), 4.91 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 415 (M^+, 16). Anal. calcd. for $C_{24}H_{17}NO_6$: C, 69.39; H, 4.12; N, 3.37. Found: C, 69.30; H, 4.06; N, 3.26%.

2.2.18. Synthesis of 4-[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamido]benzenesulfonic acid (28)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetyl chloride (**26**) (0.01 mol) and 4-aminobenzenesulfonic acid(0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for1 h, the resulting solid on heating was collected by filtration, washed with ethanol several times and recrystallized from DMF to give compound **28** (Scheme 6). Color: White. Yield: 70%. M.p.: 344-346 °C. FT-IR (KBr, ν, cm^{-1}): 3430(OH), 3235(NH), 1700(C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 14.01 (s, 1H, OH), 9.16 (s, 1H, NH), 7.04-7.70 (m, 12H, Ar-H), 6.26 (s, 1H, H-3), 4.92 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 451 (M^+, 22). Anal. calcd. for $C_{23}H_{17}NO_7S$: C, 61.19; H, 3.80; N, 3.10. Found: C, 61.08; H, 3.73; N, 3.02%.

2.2.19. General procedure for preparation of 2-[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamido] benzoic acid and Methyl-2-[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetamido] benzoate (29a,b)

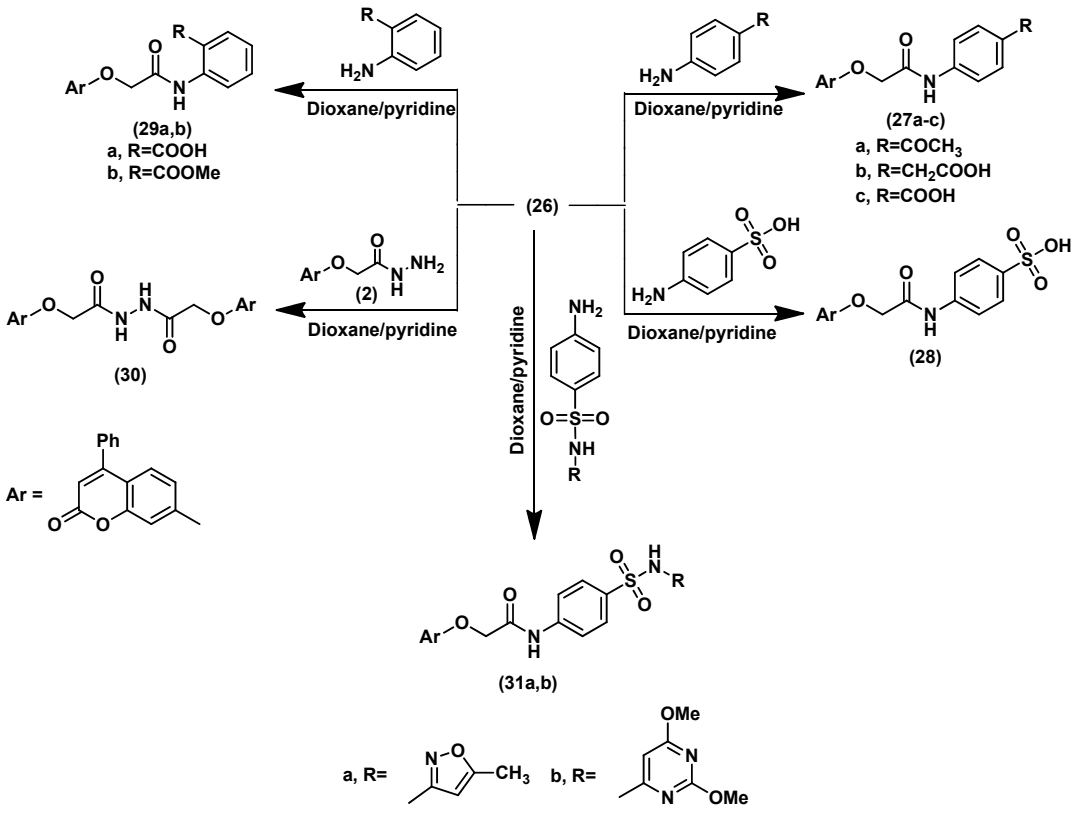
A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetyl chloride (**26**) (0.01 mol) and anthranilic acid or methyl anthranilate (0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for1 h, the resulting solid on heating was collected by filtration, washed with ethanol several times and recrystallized from DMF to give compounds **29a**,**b** (Scheme 6).

2-[2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetamido] benzoicacid (**29a**): Color: White. Yield: 90%. M.p.: 280-282 °C. FT-IR (KBr, ν, cm^{-1}): 3258(NH), 1732, 1686 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 13.90 (s, 1H, OH), 12.12 (s, 1H, NH), 7.07-8.68 (m, 12H, Ar-H), 6.28 (s, 1H, H-3), 4.90 (s, 2H, OCH_2). MS (EI, *m/z* (%)): 415 (M^+, 10). Anal. calcd. for $C_{24}H_{17}NO_6$: C, 69.39; H, 4.12; N, 3.37. Found: C, 69.30; H, 4.05; N, 3.27%.

Methyl-2-[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetamido]benzoate (**29b**): Color: White. Yield: 87%. M.p.: 202-204 °C. FT-IR (KBr, ν, cm^{-1}): 3244(NH), 1726, 1694 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 11.65 (s, 1H, NH), 7.10-8.58 (m, 12H, Ar-H), 6.28 (s, 1H, H-3), 4.90 (s, 2H, OCH_2), 3.95 (s, 3H, OCH_3). Anal. calcd. for $C_{25}H_{19}NO_6$: C, 69.92; H, 4.46; N, 3.26. Found: C, 69.85; H, 4.38; N, 3.19%.

2.2.20. Synthesis of N,N′-bis [2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetyl] hydrazine (30)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetyl chloride (**26**) (0.01 mol) and 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetohydrazide (**2**) (0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for 2 h.



Scheme 6

The precipitate formed was filtered off hot, washed with ethanol several times, dried and recrystallized from DMF to give compound **30**. Color: White. Yield: 80%. M.p.: 246-248 °C. FT-IR (KBr, ν, cm^{-1}): 3220 (NH), 1715(C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.29 (s, 2H, 2NH), 6.99-7.58 (m, 16H, Ar-H), 6.26 (s, 2H, 2H-3), 4.79 (s, 4H, $2OCH_2$). MS (EI, *m/z* (%)): 588 (M^+, 27). Anal. calcd. for $C_{34}H_{24}N_2O_8$: C, 69.38; H, 4.11; N, 4.76. Found: C, 69.28; H, 4.03; N, 4.69%.

2.2.21. General procedure for preparation of N-{4-[N-(substituted) sulfamoyl] phenyl}-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetamide (31a,b).

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetyl chloride (**26**) (0.01 mol) and benzenesulfonamide derivatives namely (4-amino-*N*-(5-methylisoxazol-3-yl) benzene sulfonamide and 4-amino-*N*-(2,6-dimethoxy pyrimidin-4-yl)benzenesulfonamide) (0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for1 h. Then the mixture was left to cool. The solid product that formed was recrystallized from dioxane to give compounds **31a**,**b** (Scheme 6).

N-{4-[N-(5-Methylisoxazol-3-yl)sulfamoyl]phenyl}-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamide (**31a**): Color: White. Yield: 64%. M.p.: 248-250 °C. FT-IR (KBr, ν, cm^{-1}): 3352, 3230 (NH), 1706 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.56 (s, 1H, 1NH), 11.31 (s, 1H, 1NH), 6.99-7.82 (m, 12H, Ar-H), 6.25 (s, 1H, H-3), 6.11 (s, 1H, CH-oxazole), 4.90 (s, 2H, OCH_2), 2.28 (s, 3H, CH_3). Anal. calcd. for $C_{27}H_{21}N_3O_7S$: C, 61.01; H, 3.98; N, 7.91. Found: C, 60.90; H, 3.91; N, 7.85%.

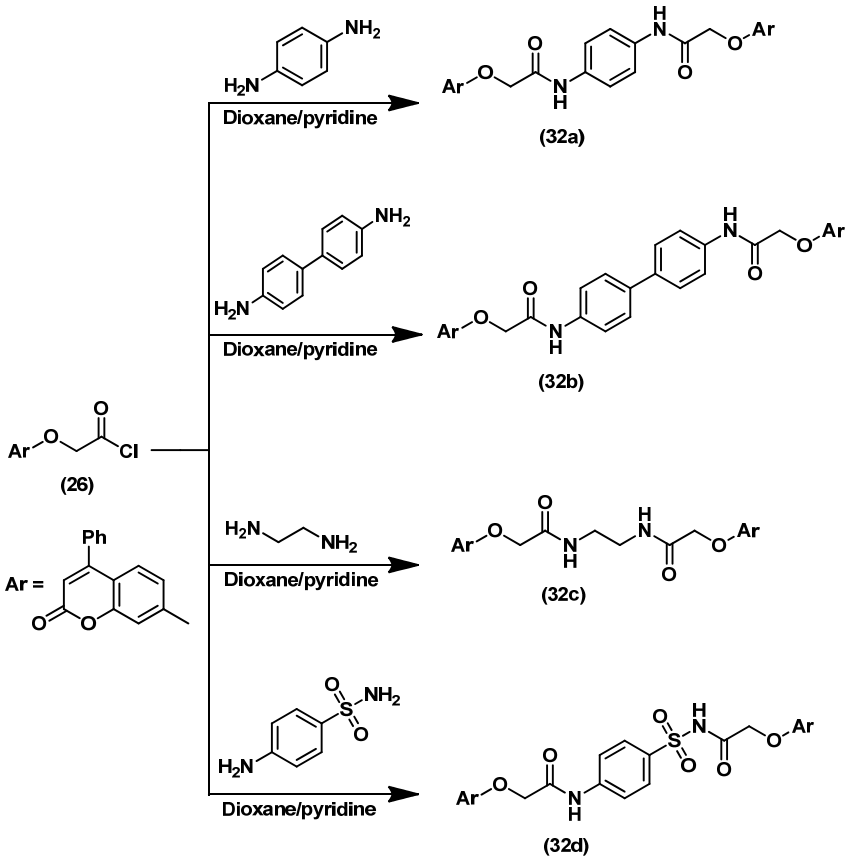
N-{4-[N-(2,6-Dimethoxypyrimidin-4-yl)sulfamoyl]phenyl}-2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetamide (**31b**): Color: White. Yield: 64%. M.p.: 240-242 °C. FT-IR (KBr, ν, cm^{-1}): 3336 (NH), 1706 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.56 (s, 1H, 1NH exchangeable with D_2O), 11.48 (s, 1H, 1NH exchangeable with D_2O), 6.99-7.92 (m, 12H, Ar-H), 6.25 (s, 1H, H-3), 5.94 (s, 1H, CH-pyrimidine), 4.90 (s, 2H, OCH_2), 3.86 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3). Anal. calcd. for $C_{29}H_{24}N_4O_8S$: C, 59.18; H, 4.11; N, 9.52. Found: C, 59.10; H, 4.03; N, 9.44%.

2.2.22. General procedure for preparation of N,N'-bis[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetyl]p-phenylene diamine, N,N'-bis[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetyl] benzidine, N,N'-bis[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy) acetyl]ethylenediamine and N,N'-bis[2-(2-oxo-4-phenyl-2H-chromen-7-yloxy)acetyl]4-aminobenzenesulfonamide (32a-d)

A mixture of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetyl chloride (**26**) (0.02 mol) and bifunctional amines namely (1,4-phenylenediamine, benzidine, ethylenediamine and 4-aminoben-zenesulfonamide) (0.01 mol) in dry dioxane (10 mL) and a catalytic amount of pyridine was refluxed for2 h. The precipitate formed was filtered off hot, washed with ethanol several times, dried and recrystallized from DMF to give compounds **32a**-**d** (Scheme 7).

N,N'-bis[2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetyl]p-phenylene-diamine (**32a**): Color: Grey. Yield: 83%. M.p.: 306-308 °C. FT-IR (KBr, ν, cm^{-1}): 3434, 3348 (NH), 1714 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.13 (s, 2H, 2NH exchangeable with D_2O), 7.01-7.58 (m, 20H, Ar-H), 6.25 (s, 2H, 2H-3), 4.84 (s, 4H, $2OCH_2$). Anal. calcd. for $C_{40}H_{28}N_2O_8$: C, 72.28; H, 4.25; N, 4.21. Found: C, 72.20; H, 4.13; N, 4.12%.

N,N'-bis[2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetyl] benzidine (**32b**): Color: Pale yellow. Yield: 85%. M.p.: 268-270 °C. FT-IR (KBr, ν, cm^{-1}): 3300 (NH), 1714 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.28 (s, 2H, 2NH), 6.76-7.74 (m, 24H, Ar-H), 6.26 (s, 2H, 2H-3), 4.89 (s, 4H, $2OCH_2$).



Scheme 7

MS (EI, *m/z* (%)): 740 (M^+, 56). Anal. calcd. for $C_{46}H_{32}N_2O_8$: C, 74.59; H, 4.35; N, 3.78. Found: C, 74.50; H, 4.27; N, 3.67%.

N,N'-bis[2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetyl] ethylenediamine (**32c**): Color: White. Yield: 80%. M.p.: 280-282 °C. FT-IR (KBr, ν, cm^{-1}): 3380 (NH), 1716, 1668 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.26 (s, 2H,2NH), 6.92-7.56 (m, 16H, Ar-H), 6.27 (s, 1H, H-3), 6.22 (s, 1H, H-3), 4.66 (s, 2H, OCH_2), 4.60 (s, 2H, OCH_2), 3.56 (s, 2H, CH_2), 3.39 (s, 2H, CH_2). MS (EI, *m/z* (%)): 616 (M^+, 8). Anal. calcd. for $C_{36}H_{28}N_2O_8$: C, 70.12; H, 4.58; N, 4.54. Found: C, 70.06; H, 4.50; N, 4.47%.

N,N'-bis[2-(2-Oxo-4-phenyl-2H-chromen-7-yloxy)acetyl]4-aminobenzene-sulfonamide (**32d**): Color: White. Yield: 73%. M.p.: 252-254 °C. FT-IR (KBr, ν, cm^{-1}): 3392, 3336 (NH), 1698(C=O). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 10.48 (s, 2H, 2NH), 7.04-7.79 (m, 20H, Ar-H), 6.26 (s, 2H, 2H-3), 4.91 (s, 4H, $2OCH_2$). MS (EI, *m/z* (%)): 728 (M^+, 27). Anal. calcd. for $C_{40}H_{28}N_2O_{10}S$: C, 65.93; H, 3.87; N, 3.84. Found: C, 65.87; H, 3.80; N, 3.77%.

2.3. Pharmacology

2.3.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

In the DPPH assay, antioxidants reduce the free radical 2,2-diphenyl-1- picrylhydrazyl. In the presence of an antioxidant, the purple color of DPPH• fades and the change of absorbance can be followed spectrophotometrically at 515 nm [27].

Test compounds are dissolved in *N,N*-dimethylformamide (DMF) solution to specific concentrations, and each sample was mixed with DPPH in DMF solution, the blank sample contains the same amount of DMF and DPPH. The mixtures were left for 15 min at 25 °C then the absorbance measured at 517 nm using the UV-vis spectrophotometer. Generally, the results are reported as the percent of inhibition, i.e. the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%. The percentage of DPPH radical scavenger was calculated using the equation (1), [28].

$$\text{The percentage of DPPH radical scavenger} = [(A_0-A_t)/A_0]\times 100 \quad (1)$$

Where, A_0 is the absorbance value of blank sample, at a particular time and A_t is the absorbance value of the tested sample. The IC_{50} (concentration causing 50% inhibition of DPPH) values of each test compound are determined. Ascorbic acid can be used as positive controls.

2.3.2. Cytotoxicity assay in-vitro

The cytotoxicity of the drugs will be tested against human liver cancer cell line (HepG2) by SRB assay as described by Sumangala *et al.* [29]. Exponentially growing cells will be collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells will be exposed to the drugs for 72 h and subsequently fixed with TCA (10%) for 1 h at 4 °C. After several washings, cells will be exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl will be used to dissolve the SRB-stained cells and color intensity will be measured at 492 and 630 nm (for the reference wavelength) with the enzyme linked immune sorbent assay (ELISA) reader. All of the compounds were tested in twice in each of the cell lines.

3. Results and discussion

3.1. Synthesis

Hydrazinolysis of ethyl 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetate (**1**)with hydrazine hydrate in ethanol at refluxing temperature,2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)aceto hydrazide (**2**)was obtained in good yield (Scheme 1).

Interaction of acetohydrazide**2** with carbon disulfide in refluxing ethanol in the presence of potassium hydroxide resulted in the formation of the potassium salt of hydrazinecarbodithioate,**3**. Which on treatment with aqueous potassium hydroxide solution under reflux then neutralized with hydrochloric acid afforded a product identified as 7-[(5-thioxo-1,3,4-oxadiazol-2-yl)-methoxy]-4-phenyl-2*H*-chromen-2-one (**4**). Treatment of the potassium salt **3** or **4** with hydrazine hydrate in aqueous ethanol yielded the corresponding 7-[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]-4-phenyl-2*H*-chromen-2-one (**5**).

Interaction of the potassium salt **3** with 4-nitrophenacyl bromide in refluxing DMF furnished the expected product that identified as *N*-[4-(4-nitrophenyl)-2-thioxothiazol-3(2*H*)-yl)-2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy] acetamide (**7**),the formation of this compound was shown through the proposed mechanism outline in Scheme 1. Treatment of compound**4**with 4-nitrophenacyl bromide in refluxing acetone in the presence of anhydrous potassium carbonate afforded a single product which identified as 7-{[5-(2-(4-nitrophenyl)-2-oxoethylthio)-1,3,4-oxadiazol-2-yl]methoxy}-4-phenyl-2*H*-chromen-2-one (**8**). Compound **10** was obtained by treatment of compound **5** with *p*-nitrophenacyl bromide in refluxing DMF in the presence of triethylamine. The formation of compound **10** was explained by the nucleophilic transformation into acyclic non-isolable intermediate (**9**) that undergoes intramolecular ring closure to the final product through nucleophilic addition of amino (NH_2) to the carbonyl group followed by elimination of water molecule to furnish the expected product7-{[6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadia-zin-3-yl]methoxy}-4-phenyl-*H*-chromen-2-one (**10**). The structure of this compound was further confirmed by its alternative synthesis from the reaction of the 1,3,4-oxadiazole derivative **8** with hydrazine hydrate in refluxing ethanol afforded a product which identical in all aspects (M.p., mixed m.p. and spectral data) with compound **10**, (Scheme 1).

Condensation of 7-[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]-4-phenyl-2*H*-chromen-2-one(**5**)with 4-nitrobenzaldehyde in refluxing ethanol containing a few drops from acetic acid gave the corresponding imine **11**, rather than the expected product triazolothiadiazolyl derivative**12**, (Scheme 2).

Also, the acetohydrazide**2** was reacted with some anhydrides such as phthalic anhydride and pyrazine-2,3-dicarboxylicanhydride in refluxing glacial acetic acid to give the corresponding imides which formulated as *N*-(substituted)-2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetamide (**13a**,**b**), respectively. Treatment of compound **2** with phenylisothio cyanate and phenylisocyanate in refluxing dioxane gave 4-phenyl-7-[(4-phenyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methoxy]-2*H*-chromen-2-one (**15a**) and 3-[(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)methyl]-4-phenyl-1*H*-1,2,4-triazol-5(4*H*)-one (**15b**), respectively. The formation of these compounds were assumed to proceed via nucleophilic addition of amino group in compound **2** to isothiocyanate and/or isocyanate to give acyclic non-isolable semicarbazide intermediate **14a**,**b** which underwent intramolecular cyclization by elimination of water molecule to furnish the final products **15a**,**b**, respectively. Acetohydrazide **2** reacted with 4-amino-2-mercapto-6-(4-methoxyphenyl) pyrimidine-5-carbonitrile (**16**) [30] in refluxing DMF in the presence of a few drops from pyridine to give *N*'-[4-amino-5-cyano-6-(4-methoxyphenyl) pyrimidin-2-yl]-2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)aceto hydrazide (**17**), (Scheme 3).

The reaction of hydrazide**2** with 4-benzyl-1-chloro phthalazine (**18**)[31] in boiling dioxane in the presence of pyridine furnished 7-[(6-benzyl-[1,2,4]triazolo[4,5-a] phthalazin-3-yl)methoxy]-4-phenyl-2*H*-chromen-2-one (**20**) as the only isolable product. The formation of this compound was explained by the nucleophilic transformation into acyclic non-isolable intermediate **19** which undergoes intramolecular ring closure to the final product via elimination of water molecule, while treatment of hydrazide**2** with chloroacetonitrile in dry dioxane-pyridine under reflux yielded a product which identified as 7-[(6-amino-4*H*-1,3,4-oxadiazin-2-yl)methoxy]-4-phenyl-2*H*-chromen-2-one (**22**). The formation of this compound was assumed by nucleophilic substitution to form acyclic non-isolable intermediate **21** followed by intra-molecular attack at the cyanide group to form the cyclized product **22** (Scheme 4).

On the other hand, when ethyl 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetate (**1**) was treated with sodium hydroxide in refluxing ethanol yielded the corresponding acid which identified as 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetic acid (**23**) [26].

7-((4,5-Dihydro-1*H*-imidazol-2-yl)methoxy)-4-phenyl-2*H*-chromen-2-one (**24**) was prepared by fusion of ethylene diamine with compound **23**, while cyclocondensation of compound **23** with (1,2-phenylenediamine, 2-aminophenol and 2-aminothiophenol) under fusion conditions yielded products which identified as 7-[(substituted)methoxy]-4-phenyl-2*H*-chromen-2-one (**25a**-**c**). Treatment of compound **23** with thionyl chloride gave the corresponding acid chloride as 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetyl chloride (**26**) (Scheme 5).

The acid chloride **26** was allowed to react with different aromatic amines in dry dioxane containing a few drops of pyridine under reflux afforded products which identified as: *N*-(4-acetylphenyl)-2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetamide (**27a**), 2-{4-[2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetamido]-phenyl}acetic acid (**27b**) and 4-[2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetamido]benzoic acid (**27c**), respectively. Furthermore, whencompound **26** was subjected to the reaction with 4-amino benzenesulfonic acid in refluxing dioxane-pyridine yielded asingle product which identified as: 4-[2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetamido] benzenesulfonic acid (**28**), while compound **26** reacted with anthranilic acid and methyl anthranilate in boiling dioxane-pyridineto give the products which were found to be 2-[2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetamido] benzoic acid (**29a**) and methyl 2-[2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetamido]benzoate (**29b**), respectively. Also, reaction of acid chloride **26** with 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy) acetohydrazide (**2**) in dry dioxane containing a few drops from pyridine at reflux temperature afforded bis acyl hydrazine derivative **30**. The acid chloride **26** was reacted with sulfa drug derivatives under the same reaction conditions to produce *N*-{4-[*N*-(substituted)sulfamoyl] phenyl}-2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetamide (**31a**,**b**) (Scheme 6).

Finally, this investigation was extended to include the reactivity of 2-(2-oxo-4-phenyl-2*H*-chromen-7-yloxy)acetyl chloride (**26**) towards bifunctional isolated amines to furnish the corresponding bis compounds (**32a**-**d**) (Scheme 7).

3.2. Pharmacology

3.2.1. Antioxidant activity

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases. The role of antioxidant is to remove free radicals, by donating hydrogen to free radicals in its reduction to produce non-reactive species.

Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects; therefore attention has been paid to naturally occurring antioxidants. Test compounds were screened for *in vitro* antioxidant activity using DPPH.

The effect of the different synthetic compounds on DPPH radical scavenging was compared to ascorbic acid using as positive control and appreciated by the determination of the IC_{50} values. The results are listed in Table 1.

DPPH test is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance.

The ratio of antioxidant/DPPH required to decrease the concentration of DPPH to 50% of its initial value, denoted as IC_{50} or Efficient Concentration (EC_{50}), is an indicator of antiradical activity [32]. It is clear from the tabulated results that compound **10**showed the best antioxidant activity against DPPH intested compounds, while compounds **29b**, **31a** and **32a** showed no activity. In concentration 1000 µg/mL, we noted that derivative **10** has most potent scavenging behavior among test compounds and also more potent than ascorbic acid. Similarly, **28** and **32b** have scavenging activity more than ascorbic acid (Figure 1). However, compound **4** have scavenging activity similar to ascorbic acid.

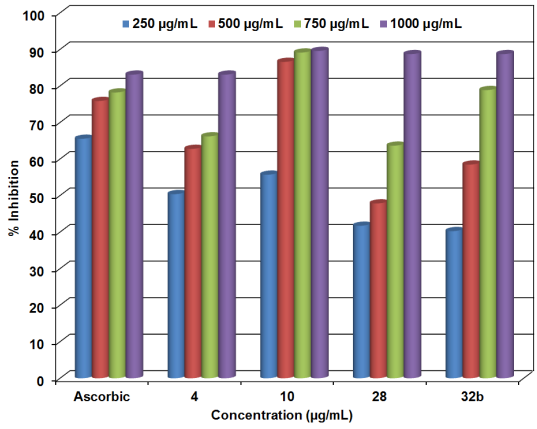


Figure 1. Effect of compounds **4**, **10**, **28** and **32b** toward 2,2-diphenyl-1-picrylhydrazyl (DPPH).

3.2.2. Cytotoxicity assay in vitro

The results expressed as IC_{50} (inhibitory concentration of 50%) were the averages of two determinations and were calculated by using igmoidal concentration-response curve fitting models (Sigma Plot software). The results are listed in Table 2.

This study revealed that 7-{[6-(4-nitrophenyl)-7*H*-[1,2,4]tria-zolo[3,4-b][1,3,4]thiadiazin-3-yl]methoxy}-4-phenyl-*H*-chromen-2-one (**10**) derivative is the most potent (IC_{50} (µg/mL) = 9.067), while derivatives **28** and **32b** are similar (IC_{50} (µg/mL) = 18.56).

4. Conclusions

Our interest in synthesis of heterocyclic compounds is to focus on their biological activity as a part of our program, which aimed at the development of new and more potent antioxidant and anticancer agents. Thus, in this paper, we revealed the synthesis of some coumarin derivatives and biological evaluation of some novel compounds.

Table 1. Values of IC_{50} exhibited by test compounds.

Entry	Compound no	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
1	4	250	50.40	263
		500	62.78	
		750	66.21	
		1000	83.10	
2	7	250	31.17	6118
		500	34.16	
		750	36.56	
		1000	39.33	
3	10	250	55.72	213
		500	86.60	
		750	89.15	
		1000	89.58	
4	13b	250	19.83	1473
		500	24.61	
		750	35.30	
		1000	43.18	
5	15b	250	26.02	1433
		500	36.99	
		750	42.19	
		1000	43.32	
6	24	250	45.88	123
		500	38.60	
		750	37.80	
		1000	36.56	
7	25a	250	37.44	114
		500	34.89	
		750	28.84	
		1000	19.30	
8	25c	250	52.66	317
		500	44.65	
		750	38.02	
		1000	31.54	
9	27b	250	37.36	5380
		500	39.40	
		750	39.62	
		1000	44.21	
10	27c	250	39.40	683792
		500	39.55	
		750	40.13	
		1000	41.51	
11	28	250	41.73	397
		500	47.85	
		750	63.66	
		1000	88.71	
12	29b	250	39.04	Inactive
		500	38.82	
		750	38.09	
		1000	37.73	
13	31a	250	40.79	Inactive
		500	40.93	
		750	41.88	
		1000	42.61	
14	31b	250	39.91	11663
		500	40.57	
		750	41.81	
		1000	43.99	
15	32a	250	39.48	Inactive
		500	39.11	
		750	37.58	
		1000	36.13	
16	32b	250	40.28	344
		500	58.49	
		750	78.88	
		1000	88.71	
17	32d	250	32.77	2590
		500	36.57	
		750	41.35	
		1000	42.33	
Std.	Ascorbic acid	250	65.55	91.45
		500	75.89	
		750	78.22	
		1000	83.10	

The structures of the newly compounds were elucidated on the basis of IR, ^{1}H NMR, ^{13}C NMR and MS spectral data. A series of novel coumarin derivatives were prepared. The antioxidant

and anticancer activity of some compounds were evaluated. 7-{[6-(4-Nitrophenyl)-7*H*-[1,2,4]tria-zolo[3,4-b][1,3,4]thiadiazin-3-yl] methoxy}-4-phenyl-*H*-chromen-2-one (**10**) has the highest antioxidant and anticancer activity in screened compounds.

Table 2. Cytotoxicity assay in vitro for some synthesized compounds.

Entry	Compounds	IC_{50} (µg/mL)
1	4	14.48
2	10	9.067
3	28	18.56
4	32b	18.56

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