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An efficient synthesis of some new isolated and fused 2-oxo-2*H*-chromene derivatives as antimicrobial and antitumor agents

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ABSTRACT

An efficient synthesis of the biologically active novel systems derived from the reaction of 4-methyl-2-oxo-2*H*-chromene-3-carbonitrile (1) with sodium hydroxide and/or DMF-DMA and cyclocondensation reactions of 4-[(*E*)-2-(dimethylamino)ethenyl]-2-oxo-2*H*-chromene-3-carbonitrile (5) with nitrogen nucleophilic reagents afforded the corresponding 4-iminochromeno[3,4-c]pyridine derivatives (6-14). The structures of the prepared compounds have been proved by elemental analysis, IR, ^{1}H and ^{13}C NMR and mass spectra. Significant antitumor activities in planta were observed for some of the prepared compounds.

1. Introduction

Fused and isolated 2-oxo-2*H*-chromenes (coumarins) comprise a very interesting class of compounds due to their significant antibacterial [1], antifungal [2-4], antimycobecterial [5,6], anticoagulants [7,8], inhibition of some enzymes [9,10] and antitumor [11-13] activities. With the expectations to find biological activity, we decided to investigate the synthesis of some novel systems of 2-oxo-2H-chromene derivatives bearing fused and isolated moieties. Recently, the synthesis [14], photochemical [15], and theoretical [2,16-18] properties of chromene derivatives were investigated.

The aim of the present paper is to investigate an efficient synthesis of fused and isolated 2-oxo-2*H*-chromene derivatives containing active methyl and cyano groups and study their cyclocondensation reactions with nitrogen nucleophilic reagents such as hydroxylamine hydrochloride, urea, semicarbazide, methylamine hydrochloride, 4-amino-1,2,4-triazine derivative, 2-aminoethanol, ethane-1,2-diamine and thiosemicarbazide. The antimicrobial and antitumor activities for the prepared compounds were investigated.

2. Experimental

2.1. Instrumentation

The uncorrected melting point was determined in an open capillary tube on a digital Stuart SMP-3 apparatus. ^{1}H NMR/^{13}C NMR spectra were obtained on a 500/125 MHz Jeol Eca or on a 300/75.46 MHz Varian Mercury VX-300 NMR spectrometer in DMSO-d6 with tetramethylsilane as an internal standard. Elemental analyses were performed on Vario El Elementar apparatus. IR spectra were recorded on FT-IR Nicolet IS10 spectrophotometer (cm^{-1}), using KBr disks. Mass spectra recorded on a Gas chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 eV. 4-Methyl-2-oxo-2*H*-chromene-3-carbonitrile (**1**) [2], 4-amino-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one [19] were prepared by previously reported procedures. All other chemicals used in this study were commercially available.

2.2. Synthesis

2.2.1. 4-Methyl-2-oxo-2H-chromene-3-carboxylic acid (2)

A solution of compound **1** (0.5 g, 0.27 mmol) in ethanol (2 cm^3), sodium hydroxide solution (0.09 g sodium hydroxide, 2 cm^3 ethanol) was added and the reaction mixture was refluxed with stirring for 0.5 h, cooled to room temperature and acidified with dilute hydrochloric acid. The solid obtained was filtered off, and recrystallized from ethanol to give compound **2** as brown crystals (Scheme 1). Yield: 72%. M.p.: 152-153 °C. FT-IR (KBr, ν, cm^{-1}): 3149 (br, OH), 3006 (CH_{arom}), 1709 ($C{=}O_{acid}$), 1684 ($C{=}O_{coumarin}$). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 2.46 (s, 3H, CH_3), 7.29 (bs, 1H, OH), 7.38-7.47 (m, 2H, 6-H and 8-H), 7.65 (dd, *J* = 8.4 Hz, 6.8 Hz, 1H, 7-H), 7.85 (d, *J* = 8.4 Hz, 1H, 5-H). MS (EI, *m/z* (%)): 202.15 (M^+-2, 1.1), 203.15 (M^+-1, 2.5), 204.10 (M^+, 22.4), 205.10 (M^++1, 3.1), 206.10 (M^++2, 0.6). Anal. calcd. for $C_{11}H_8O_4$: C, 64.71; H, 3.95. Found: C, 64.51; H, 3.60%.

2.2.2. N-[Imino(4-methy-2-oxo-2H-chromen-3-yl)methyl]-4-methyl-2-oxo-2H-chromene-3-carboxamide (3)

A mixture of compound **1** (0.5 g, 0.27 mmol), sodium hydroxide solution (0.09 g sodium hydroxide, 4 cm^3 water) was refluxed with stirring for 0.5 h, then ethanol (2 cm^3) was added and the mixture was refluxed for 3 h, cooled to room temperature and filtered off. The solid obtained was acidified with dilute hydrochloric acid, stirred at room temperature for 5 min, poured over water (10 cm^3), filtered off and recrystallized from ethanol to give compound **3** as white crystals (Scheme 1).

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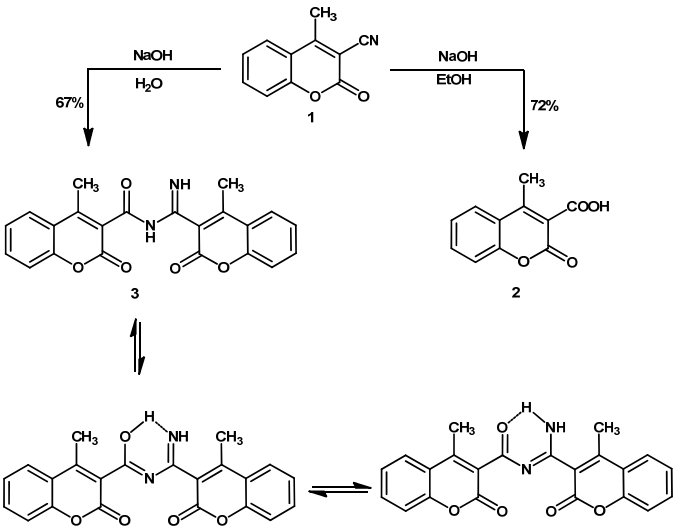
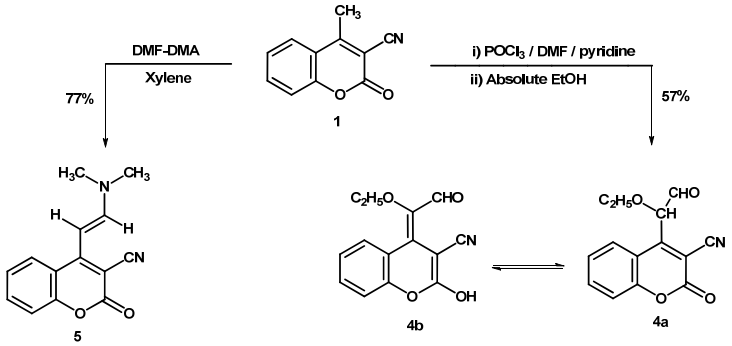


Scheme 1



Scheme 2

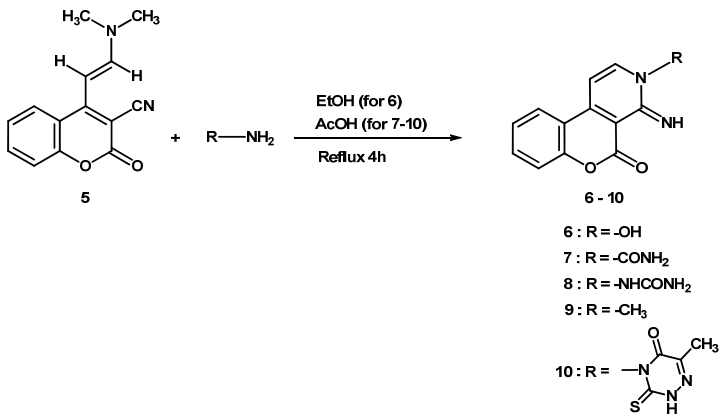
Yield: 67%. M.p.: 225-226 °C. FT-IR (KBr, ν, cm^{-1}): 3424 (NH), 3365 (NH), 3068 (CH_{arom}), 2986, 2900 (CH_{aliph}), 1728 (br, $C=O_{coumarin}$), 1651 ($C=O_{amide}$). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 2.46 (bs, 6H , C*H₃*), 6.99 (bs, 1H, Ar-*H*), 7.37-7.43 (m, 3H, Ar-*H*), 7.59-7.64 (m, 1H, Ar-*H*), 7.77-7.82 (m, 1H, Ar-*H*), 8.21 (bs, 1H, Ar-*H*), 8.28 (bs, 1H, Ar-*H*), 11.06 (s, 1H, =N*H*) (NH amide proton was displaced due to hydrogen bonding). MS (EI, *m/z* (%)): 387.80 (M^+, 75.0), 388.80 (M^++1, 54.1). Anal. calcd. for $C_{22}H_{16}N_2O_5$: C, 68.04; H, 4.15; N, 7.21. Found: C, 68.30; H, 4.01; N, 7.45%.

2.2.3. 4-(1-Ethoxy-2-oxoethyl)-2-oxo-2H-chromene-3-carbonitrile (4)

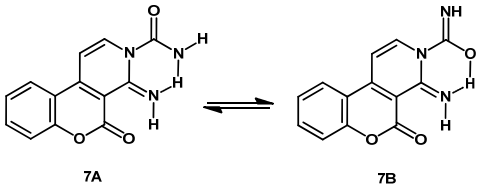
Phosphorus oxychloride (1.2 cm^3) was added dropwise to DMF (2.7 cm^3) with stirring at 30-35 °C, after the addition was completed, the solution was stirred at 50-60 °C for 30 min. A solution of compound **1** (0.5 g, 0.27 mmol) in dry pyridine (2 cm^3) was added to the above mixture dropwise at 30-35 °C and after the addition was completed, the mixture was stirred at 50-60 °C for 3 h, cooled to room temperature. The mixture was poured over cold water (2 cm^3), acidified with hydrochloric acid to give a semisolid product. The product was refluxed with absolute ethanol (2 cm^3) for 1 h, cooled, filtered, and recrystallized from ethanol to give compound **4** as pale-gray crystals (Scheme 2). Yield: 57%. M.p.: 230-232 °C. FT-IR (KBr, ν, cm^{-1}): 3371 (OH), 2975 (CH_{aliph}), 2211 (C≡N), 1704 (s, $C=O_{aldehyde}$), 1685 (w, $C=O_{coumarin}$), 1637 (exocyclic C=C). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.12 (t, *J* = 6.9 Hz, 3H, *CH₃)*, 2.71 (s, 1H, -CH-), 4.15 (q, *J* = 6.9 Hz, 2H, CH_2), 7.44-7.48 (m, 2H, 6-H and 8-H), 7.73 (m, 1H, 7-H), 7.94 (m, 1H, 5-H), 8.95, 9.19 (ss, 1H, CHO). MS (EI, *m/z* (%)): 256.10 (M^+-1, 3.3), 257.10 (M^+, 1.7), 258.10 (M^++1, 30.9), 259.10 (M^++2, 5.0). Anal. calcd. for $C_{14}H_{11}NO_4$: C, 65.37; H, 4.31; N, 5.44. Found: C, 65.01; H, 4.11; N, 5.12%.

2.2.4. 4-[(E)-2-(Dimethylamino)ethenyl]-2-oxo-2H-chromene-3-carbonitrile (5)

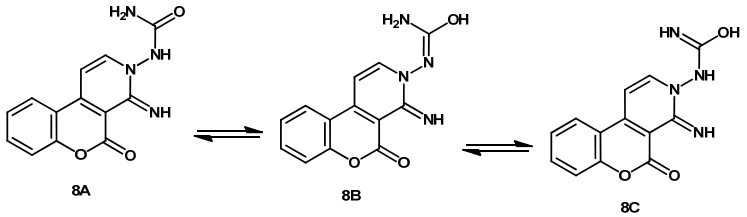
A solution of compound **1** (1.0 g, 0.54 mmol) in dry xylene (10 cm^3), and then DMF-DMA (0.7 cm^3, 0.58 mmol) was added and the mixture was refluxed for 0.5 h, cooled to room temperature. The solid obtained was filtered off, and recrystallized from cyclohexane to give compound **5** as pale-green crystals (Scheme 2). Yield: 77%. M.p.: 209-210 °C. FT-IR (KBr, ν, cm^{-1}): 3079, 3001 (CH_{arom}), 2927 (CH_{aliph}), 2197 (C≡N), 1682 ($C=O_{coumarin}$), 1616 (C=C). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 3.12 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 5.73 (d, *J* = 12.2 Hz, 1H, 9-H), 7.26-7.30 (m, 2H, 6-H and 8-H), 7.60 (dd, *J* = 12.2 Hz, 6.8 Hz, 1H, 7-H), 8.15 (dd, *J* = 7.6 Hz, 4.5 Hz, 1H, 5-H), 8.47 (d, *J* = 12.2 Hz, 1H, 10-H). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 39.69, 40.52, 78.26, 88.70, 117.82, 118.08, 120.25, 124.94, 126.33, 133.95, 152.79, 155.63, 160.19. MS (EI, *m/z* (%)): 240.00 (M^+, 100.0), 241.00 (M^++1, 16.8), 242.00 (M^++2, 4.8). Anal. calcd. for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 70.21; H, 5.23; N, 11.42%.



Scheme 3



Hydrogen bonding of compound 7



Keto-enol tautomerism of compound 8

Scheme 4

2.2.5. 3-Hydroxy-3,4-dihydro-4-imino-5H-chromeno[3,4-c] pyridin-5-one (6)

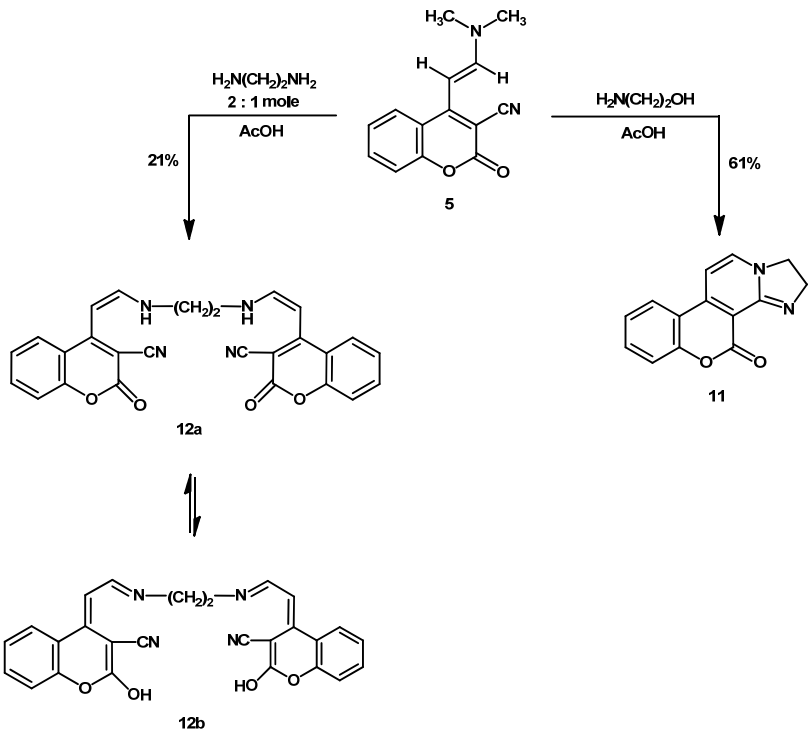
A mixture of compound **5** (0.5 g, 0.21 mmol), hydroxylamine hydrochloride (0.15 g, 0.21 mmol), and ethanol (15 cm^3) was refluxed for 4 h. After cooling the solid obtained was filtered off, and recrystallized from DMF to give compound **6** as pale-yellow crystals (Scheme 3). Yield: 85%. M.p.: 306-307 °C. FT-IR (KBr, ν, cm^{-1}): 3412 (OH), 3241 (br, NH), 3096 (CH_{arom}), 1704 ($C=O_{coumarin}$), 1612 (exocyclic C=N). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.39-7.64 (m, 4H, Ar-H), 8.00 (bs, 1H, NH or OH exchangeable with D_2O), 8.25 (dd, *J* = 8.4 Hz, 1.5 Hz, 1H, 10-H), 8.27 (bs, 1H, NH or O*H* exchangeable with D_2O), 8.54 (d, *J* = 6.9 Hz, 1H, 2-H). ^{13}C NMR (75.46 MHz, DMSO-d_6, δ, ppm): 69.94, 71.99, 104.22, 116.28, 117.08, 120.71, 124.29, 125.09, 131.85, 141.38, 151.23, 218.03. MS (EI, *m/z* (%)): 227.20 (M^+-1, 3.4), 227.60 (M^+, 2.8). Anal. calcd. for $C_{12}H_8N_2O_3$: C, 63.16; H, 3.53; N, 12.28. Found: C, 63.33; H, 3.71; N, 12.01%.

2.2.6. 3,4-Dihydro-4-imino-5H-chromeno[3,4-c]pyridin-5-one derivatives (7-10)

A mixture of compound **5** (0.5 g, 0.21 mmol), amine derivatives (0.21 mmol) in glacial acetic acid (5 cm^3) was refluxed for 4 h, cooled to room temperature. The solid obtained was filtered, and crystallized from the proper solvent to give compounds **7**-**10** (Scheme 3).

4-Imino-5-oxo-4H-chromeno[3,4-c]pyridine-3(5H)-carboxamide (**7**): Obtained from urea (0.13 g), crystallized from DMF as pale-blue crystals. Yield: 56%. M.p.: > 350 °C. FT-IR (KBr, ν, cm^{-1}): 3440 (br, NH_2), 3173 (br, NH), 3072 (CH_{arom}), 1729 (s, $C=O_{coumarin}$), 1653 (w, $C=O_{amide}$), 1613 (exocyclic C=N). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.07 (d, *J* = 6.9 Hz, 1H, 7-H), 7.32-7.41 (m, 2H, 1-*H* and 9-H), 7.68 (dd, *J* = 8.4, 7.5 Hz, 1H, 8-H), 7.83 (d, *J* = 6.9 Hz, 1H, 10-H), 8.16 (d, *J* = 8.1 Hz, 1H, 2-H), 12.10 (bs, 1H, NH exchangeable with D_2O) (NH_2 protons were displaced due to hydrogen bonding (Scheme 4)). ^{13}C NMR (75.46 MHz, DMSO-d_6, δ, ppm): 80.69, 98.13, 107.74, 115.76, 116.75, 124.22, 125.56, 133.55, 142.20, 150.29, 153.23, 155.70, 158.94. MS (EI, *m/z* (%)): 252.00 (M^+-3, 5.9), 253.00 (M^+-2, 5.9), 254 (M^+-1, 4.7). Anal. calcd. for $C_{13}H_9N_3O_3$: C, 61.18; H, 3.55; N, 16.46. Found: C, 61.34; H, 3.11; N, 16.21%.

1-(4-Imino-5-oxo-4H-chromeno[3,4-c]pyridin-3(5H)-yl)urea (**8**): Obtained from semicarbazide (0.16 g), crystallized from acetic acid as pale-yellow crystals. Yield: 71%. M.p.: > 350 °C. FT-IR (KBr, ν, cm^{-1}): 3447, 3093 (br, NH, NH_2), 1736 ($C=O_{coumarin}$), 1647 ($C=O_{amide}$), 1607 (C=C).



Scheme 5

^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 7.45-7.55 (m, 2H, 1-H and 9-H), 7.66 (d, *J* = 6.9 Hz, 1H, 7-H), 8.02 (d, *J* = 6.9 Hz, 1H, 8-H), 8.42 (d, *J* = 7.6 Hz, 1H, 10-H), 9.02 (d, *J* = 7.6 Hz, 1H, 2-H), 12.46 (bs, 2H, 2NH exchangeable with D_2O) (NH_2 protons were displaced due to *keto-enol* tautomerism (Scheme 4)). MS (EI, *m/z* (%)): 269.00 (M^+-1, 16.9), 270.00 (M^+, 15.7), 271.00 (M^++1, 15.1). Anal. calcd. for $C_{13}H_{10}N_4O_3$: C, 57.78; H, 3.73; N, 20.73. Found: C, 57.51; H, 3.45; N, 20.45%.

4-Imino-3-methyl-3,4-dihydrochromeno[3,4-c]pyridin-5-one (**9**): Obtained from methylamine hydrochloride (0.2 g, 0.29 mmol), crystallized from acetic acid as gray crystals. Yield: 21%. M.p.: above 350 °C. FT-IR (KBr, ν, cm^{-1}): 3170 (br, NH), 3071 (CH_{arom}), 1727 ($C{=}O_{coumarin}$), 1614 (exocyclic C=N). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 2.50 (s, 3H, CH_3), 7.05 (d, *J* = 6.9 Hz, 1H, 1-H), 7.32-7.37 (m, 2H, 7-H and 9-H), 7.66 (dd, *J* = 7.6, 7.6 Hz, 1H, 8-H), 7.86 (d, *J* = 6.9 Hz, 1H, 2-H), 8.21 (d, *J* = 8.4 Hz, 1H, 10-H), 12.14 (bs, 1H, NH exchangeable with D_2O). MS (EI, *m/z* (%)): 225.10 (M^+-1, 9.4), 226.10 (M^+, 10.7), 227.10 (M^++1, 8.9). Anal. calcd. for $C_{13}H_{10}N_2O_2$: C, 69.02; H, 4.46; N, 12.38. Found: C, 68.82; H, 4.21; N, 12.01%.

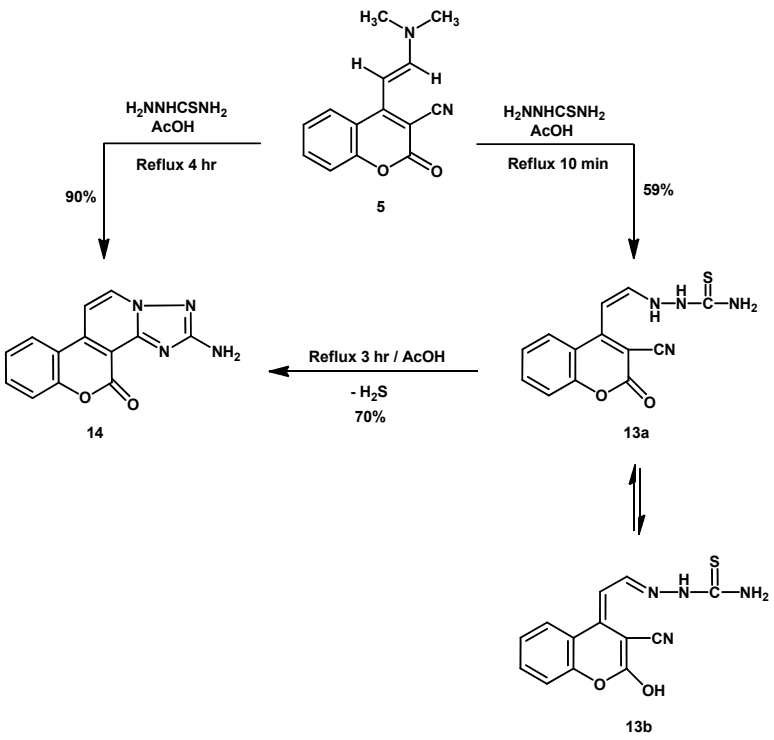
4-Imino-3-(6-methyl-5-oxo-3-thioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)-3,4-dihydro-chromeno[3,4-c]pyridin-5-one (**10**): Obtained from 4-amino-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one (0.33 g), crystallized from acetic acid as yellow crystals. Yield: 54%. M.p.: 183-184 °C. FT-IR (KBr, ν, cm^{-1}): 3176 (br, NH), 3064 (CH_{arom}), 1727 ($C{=}O_{coumarin}$), 1653 ($C{=}O_{triazine}$), 1614 (exocyclic C=N). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 2.47 (s, 3H, CH_3), 7.06 (d, *J* = 6.1 Hz, 1H, 1-H), 7.33-7.37 (m, 2H, 7-H and 9-H), 7.66 (dd, 1H, *J* = 8.4, 6.8 Hz, 8-H), 7.86 (d, *J* = 6.1 Hz, 1H, 2-H), 8.21 (d, *J* = 7.6 Hz, 1H, 10-H), 12.19 (s, 1H, NH),14.10 (bs, 1H, NH). MS (EI, *m/z* (%)): 353.10 (M^+, 0.9), 354.10 (M^++1, 1.2), 355.10 (M^++2, 0.9). Anal. calcd. for $C_{16}H_{11}N_5O_3S$: C, 54.38; H, 3.14; N, 19.82; S, 9.07. Found: C, 54.10; H, 2.98; N, 19.61; S, 8.81%.

2.2.7. 1H-Chromeno[3,4-c]imidazo[1,2-a]pyridin-4(2H)-one (11)

A mixture of compound **5** (0.5 g, 0.21 mmol), 2-aminoethanol (0.13 cm^3, 0.21 mmol), and glacial acetic acid (2 cm^3) was refluxed for 5 h, cooled to room temperature. The solid obtained was filtered, and crystallized from acetic acid to give compound **11** as pale-yellow crystals (Scheme 5). Yield: 61%. M.p.: 180-182 °C. FT-IR (KBr, ν, cm^{-1}): 3092 (CH_{arom}), 2949 (CH_{aliph}), 1736 ($C{=}O_{coumarin}$), 1648 (C=N), 1609 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.19-4.29 (m, 4H, 2 CH_2), 7.11 (d, *J* = 5.15 Hz, 1H, 6-H), 7.31-7.35 (m, 2H, 8-H and 9-H), 7.63 (d, *J* = 6.3 Hz, 1H, 10-H), 8.16-8.19 (m, 2H, 5-H and 7-H). MS (EI, *m/z* (%)): 238.10 (M^+, 6.6), 239.10 (M^++1, 2.8), 240.10 (M^++2, 11.5), 241.10 (M^++3, 1.9). Anal. calcd. for $C_{14}H_{10}N_2O_2$: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.26; H, 4.01; N, 11.44%.

2.2.8. 4,4'-(Ethane-1,2-diylbis[imino(z)ethene-2,1-diyl]) bis(2-oxo-2H-chromene-3-carbonitrile) (12)

A mixture of compound **5** (0.5 g, 0.21 mmol), ethane-1,2-diamine 80% (0.07 cm^3, 0.11 mmol), and glacial acetic acid (5 cm^3) was refluxed for 2 h, cooled to room temperature. The solid obtained was filtered off, and recrystallized from acetic acid to give compound **12** as gray crystals (Scheme 5). Yield: 21%. M.p.: 183-185 °C. FT-IR (KBr, ν, cm^{-1}): 3434 (br, OH), 2225 (C≡N), 1719 (br, $C{=}O_{coumarin}$). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 2.70-2.72 (m, 4H, 2 CH_2), 6.83-6.88 (m, 1H, =CH), 7.11-7.14 (m, 1H, =CH), 7.44-7.49 (m, 4H, Ar-H), 7.67-7.78 (m, 2H, Ar-H), 7.92-7.96 (m, 2H, Ar-H), 8.23 (d, *J* = 7.6 Hz, 1H, =CH), 8.52 (d, *J* = 8.6 Hz, 1H, =CH), 9.24-9.45 (m, 1H, NH exchangeable with D_2O), 10.89 (bs, 1H, NH exchangeable with D_2O). MS (EI, *m/z* (%)): 450.00 (M^+, 14.7), 451.00 (M^++1, 17.4). Anal. calcd. for $C_{26}H_{18}N_4O_4$: C, 69.33; H, 4.03; N, 12.44. Found: C, 69.52; H, 3.88; N, 12.11%.



Scheme 6

2.2.9. 2-[2-(3-Cyano-2-oxo-2H-chromen-4-yl)ethenyl] hydrazinecarbothioamide (13)

A mixture of compound **5** (0.5 g, 0.21 mmol), thiosemicarbazide (0.2 g, 0.21 mmol), and glacial acetic acid (5 cm^3) was refluxed for 10 min, cooled to room temperature. The solid obtained was filtered, and crystallized from dilute DMF to give compound **13** as yellow crystals. Yield: 59%. M.p.: 173-174 °C. FT-IR (KBr, ν, cm^{-1}): 3377, 3257, 3180 (OH, NH, NH_2), 2238 (C≡N), 1726 ($C=O_{coumarin}$). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.01-4.07 (m, 2H, NH_2 exchangeable with D_2O), 7.39-7.47 (m, 2H, Ar-H and =CH), 7.57 (bs, 1H, Ar-H), 7.72-7.75 (m, 1H, Ar-H), 7.92-7.96 (m, 2H, Ar-H and =CH), 11.25 (bs, 1H, NH exchangeable with D_2O) (NH proton was displaced due to *keto-enol* tautomerism (Scheme 6)). MS (EI, *m/z* (%)): 286.00 (M^+, 80.9), 287.00 (M^++1, 65.4). Anal. calcd. for $C_{13}H_{10}N_4O_2S$: C, 54.54; H, 3.52; N, 19.57; S, 11.20. Found: C, 54.23; H, 3.25; N, 19.15; S, 10.91%.

2.2.10. 2-amino-4H-chromeno[3,4-c][1,2,4]triazolo[1,5-a] pyridin-4-one (14)

Method A: A mixture of compound **5** (0.5 g, 0.21 mmol), thiosemicarbazide (0.2 gm, 0.21 mmol), and glacial acetic acid (5 cm^3) was refluxed for 4 h, cooled to room temperature and poured over water (10 cm^3). The solid obtained was filtered, and crystallized from ethanol to give compound **14** as purple crystals (Scheme 6). Yield: 90%. M.p.: 199-200 °C. FT-IR (KBr, ν, cm^{-1}): 3434 (br, NH_2), 1719 ($C=O_{coumarin}$), 1600 (C=N). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.32 (bs, 2H, NH_2 exchangeable with D_2O), 7.45-7.50 (m, 4H, Ar-H), 7.79 (dd, *J* = 6.9, 5.3 Hz, 1H, Ar-H), 7.96 (d, *J* = 7.6 Hz, 1H, Ar-H). MS (EI, *m/z* (%)): 252.00 (M^+, 4.1), 253.00 (M^++1, 5.2). Anal. calcd. for $C_{13}H_8N_4O_2$: C, 61.90; H, 3.20; N, 22.21. Found: C, 61.65; H, 3.01; N, 21.91%.

Method B: A mixture of compound **13** (0.2 g, 0.069 mmol) and glacial acetic acid (2 mL) was refluxed for 3 h, cooled to room temperature and poured over water (2 mL). The solid obtained was filtered, and crystallized from ethanol to give compound **14** as purple crystals, Yield: 70%, M.p.: 199-200 °C.

2.2.11. 4-Imino-4H,5H-pyrano[3,4-c]chromen-5-one (16) and (Z)-2-(3-cyano-2-oxo-2H-chromen-4-yl)vinyl 2-oxo-4-(2-oxoethyl)-2H-chromene-3-carbimidate (17)

A mixture of compound **5** (0.5 g, 0.21mmol), acetic acid (4 cm^3) and water (1 cm^3) was refluxed for 5 h, cooled to room temperature and filtered off. The solid obtained was crystallized from acetic acid to give compound **17** as pale-yellow crystals (Scheme 7). Yield: 34%, M.p.: 340-342 °C. The filtrate was diluted with water (5 cm^3) and filtered off. The solid obtained was crystallized from ethanol to give compound **16** as yellow crystals (Scheme 7). Yield: 22%, M.p.: 231-232 °C.

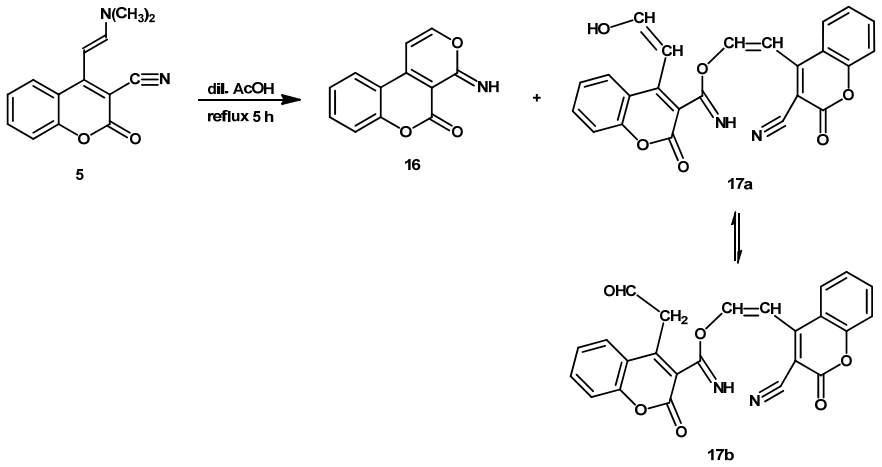
4-Imino-4H,5H-pyrano[3,4-c]chromen-5-one (**16**): FT-IR (KBr, ν, cm^{-1}): 3173 (br, NH), 3069, 3031 (CH_{arom}), 1732 ($C=O_{coumarin}$), 1614 (exocyclic C=N). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 7.05 (d, *J* = 6.9 Hz, 1H, 1-H), 7.32-7.37 (m, 2H, 7-H and 9-H), 7.66 (dd, *J* = 7.6, 7.6 Hz, 1H, 8-H), 7.85 (d, *J* = 6.5 Hz, 1H, 2-H), 8.20 (d, *J* = 7.6 Hz, 1H, 10-H), 12.17 (s, 1H, NH). MS (EI, *m/z* (%)): 213.10 (M^+, 100.0), 214.10 (M^++1, 14.7), 215.10 (M^++2, 1.6), 216.10 (M^++3, 0.1). Anal. calcd. for $C_{12}H_7NO_3$: C, 67.61; H, 3.31; N, 6.57. Found: C, 67.50; H, 3.21; N, 6.44%.

(Z)-2-(3-cyano-2-oxo-2H-chromen-4-yl)vinyl 2-oxo-4-(2-oxoethyl)-2H-chromene-3-carbimidate (**17**): FT-IR (KBr, ν, cm^{-1}): 3370 (br, OH), 3194 (br, NH), 3075 (CH_{arom}), 2984 (CH_{aliph}), 2211 (C≡N), 1727, 1654 ($C=O_{coumarin}$), 1612 (C=N). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 2.46 (d, *J* = 1.5 Hz, 2H, CH_2), 4.14 (d, *J* = 6.9 Hz, 1H, =CH), 7.06 (d, *J* = 6.9 Hz, 1H, -O-CH=), 7.33-7.37 (m, 3H, Ar-H), 7.46 (dd, *J* = 8.4, 7.6 Hz, 1H, Ar-H), 7.65-7.73 (m, 2H, Ar-H), 7.89 (dd, *J* = 8.4, 6.1 Hz, 1H, Ar-H), 8.21 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.94, 9.19 (ss, 1H, -CHO), 12.19 (s, 1H, NH).

Table 1. Antimicrobial activities data of compounds (**2-17**).

Sample	Gram-positive bacteria				Gram-negative bacteria				Yeasts [b]			
	Staphylococcus aureus (ATCC 25923)		*Bacillus subtilis* (ATCC 6635)		*Salmonella typhimurium* (ATCC 14028)		*Escherichia coli* (ATCC 25922)		*Candida albicans* (ATCC 10231)		*Aspergillus fumigatus*	
	Concentration [f]		Concentration		Concentration		Concentration		Concentration		Concentration	
	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
	Mean of zone diameter [a], mm											
2	8 L [d]	7 L	10 L	9 I	- [c]	-	8 L	7 L	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	14 I [e]	12 I	-	-	8 L	7 L	11 L	7 L	21 I	16 I
5	-	-	12 I	8 L	-	-	-	-	9 L	7 L	-	-
6	-	-	-	-	-	-	-	-	-	-	10 L	7 L
7	-	-	-	-	-	-	-	-	-	-	10 L	7 L
8	-	-	-	-	-	-	8 L	7 L	-	-	-	-
9	-	-	-	-	12 I	7 L	8 L	7 L	-	-	9 L	7 L
10	-	-	-	-	11 L	10 I	-	-	-	-	8 L	7 L
11	-	-	10 L	7 L	11 L	9 L	8 L	7 L	-	-	8 L	7 L
13	9 L	7 L	-	-	-	-	-	-	-	-	9 L	7 L
14	-	-	8 L	7 L	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-
17	9 L	7 L	-	-	-	-	-	-	-	-	-	-
Control [g]	35	26	35	25	36	28	38	27	35	28	37	26

[a] Calculate from 3 values.
[b] Identified on the basis of routine cultural, morphological and microscopical characteristics.
[c] - : No effect.
[d] L: Low activity = Mean of zone diameter ≤ 1/3 of mean zone diameter of control.
[e] I: Intermediate activity = Mean of zone diameter ≤ 2/3 of mean zone diameter of control.
[f] Concentration, mg/mL.
[g] Chloramphencol in the case of Gram-positive bacteria, Cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi.



Scheme 7

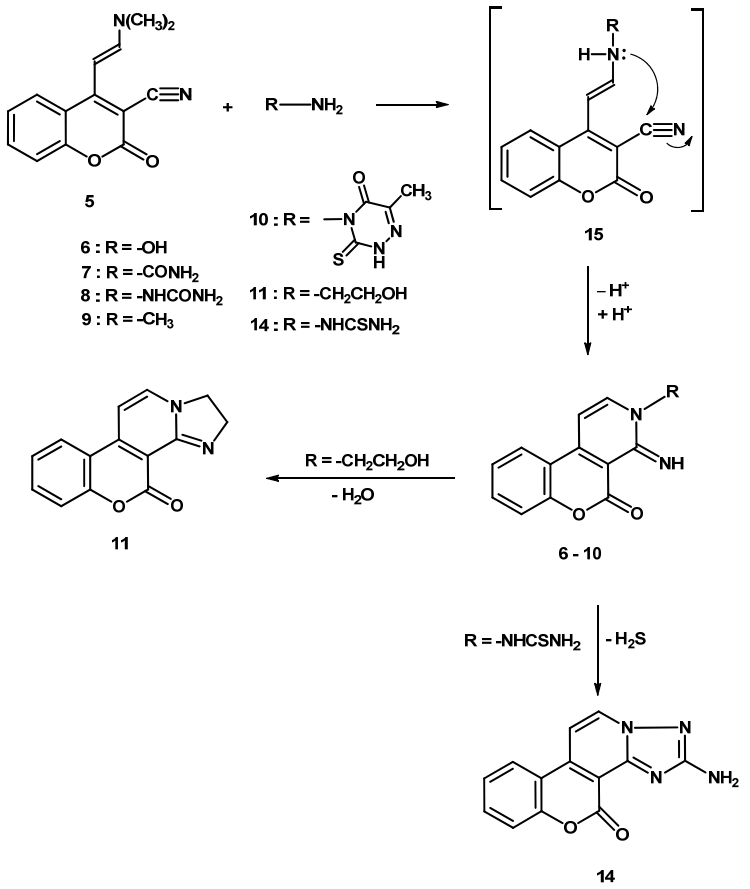
MS (EI, *m/z* (%)): 426.10 (M^+, 20.0), 427.10 (M^++1, 22.3), 428.10 (M^++2, 28.2). Anal. calcd. for $C_{24}H_{14}N_2O_6$: C, 67.61; H, 3.31; N, 6.57. Found: C, 67.34; H, 3.09; N, 6.25%.

2.3. Antimicrobial activity

The newly synthesized compounds were screened against Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635), Gram-negative bacteria: *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* (ATCC 25922), Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*. The standardized disc-agar diffusion method [20] was followed to determine the activity of the synthesized compounds against the tested microorganisms. The tested compounds were dissolved in dimethyl formamide solvent and prepared in two concentrations 1.0 and 0.5 mg/mL. Most of the prepared compounds showed a low to intermediate antimicrobial activity towards Gram-positive, Gram-negative bacteria and fungal strain (Table 1).

2.4. Antitumor activity in planta

An 18-gauge needle (no bezel) was used to produce holes on uniform fruits of summer squash (*Cucurbita pepo* cv. Eskandarany) at 12 sites distributed over 3 rows (4 sites / raw) per fruit. Into each hole, 2 µL of 10^8 CFU/mL *Agrobacterium tumefaciens* cell suspension was pipetted. After the liquid was absorbed by the plant tissue, the fruits were maintained for 24 hr in wetted plastic containers with transparent plastic covers. After 24 hr, sites were re-wounded and 2 µL from the concentration of 0.5 mg/mL of each chemical compounds suspension in dimethylformamide was pipetted into each site. After the suspension was absorbed, the fruits were again backed to the containers and kept wetted in growth chamber at 27 ± 2 °C until galls measuring were assessed. Number and size of formed galls were recorded after 10 days. Three replicates were carried out for each compound.



Scheme 8

3. Results and discussion

3.1. Chemistry

In our recent work, we described the condensation of 3,5-dichloro-2-hydroxyacetophenone [2], 3,5-dimethyl-2-hydroxy acetophenone [18] and unsubstituted 2-hydroxyacetophenone [2] with ethyl cyanoacetate in the presence of sodium metal gave 7-amino-2,4-dichloro(or 2,4-dimethyl)-9-hydroxy-6-oxo-6*H*-benzo[*c*]chromene-8-carbonitrile, and a mixture of 4-methyl-2-oxo-2*H*-chromene-3-carbonitrile and 7-amino-9-hydroxy-6-oxo-6*H*-benzo[*c*]chromene-8-carbonitrile, respectively.

The present work describes the hydrolysis of cyano group of 4-methyl-2-oxo-2*H*-chromene-3-carbonitrile (**1**) in ethanolic sodium hydroxide solution gave 4-methyl-2-oxo-2*H*-chromene-3-carboxylic acid (**2**), but when the reaction takes place in aqueous sodium hydroxide solution the formed amide group in one molecule was added to the cyano group of another molecule afforded *N*-[imino(4-methy-2-oxo-2*H*-chromen-3-yl)methyl]-4-methyl-2-oxo-2*H*-chromene-3-carboxamide (**3**) (Scheme 1).

On the other hand, the presence of carbonyl group at position 2 and cyano group at position 3 of 2-oxo-2*H*-chromene moiety facilitate the condensation reactions of 4-methyl group of compound **1**, so the Vilsmeier reaction of compound **1** in pyridine followed by reflux in absolute ethanol gave 4-(1-ethoxy-2-oxoethyl)-2-oxo-2*H*-chromene-3-carbonitrile (**4**), and condensation reaction of compound **1** with DMF-DMA in dry xylene gave 4-[(*E*)-2-(dimethylamino)ethenyl]-2-oxo-2*H*-chromene-3-carbonitrile (**5**) (Scheme 2). Compound **5** which was found to exist in *trans* form, as revealed by the ^{1}H NMR spectrum which displayed olefinic protons at δ 5.73 and 8.47 ppm with coupling constant 12.2 Hz, typical for *trans* protons.

Cyclocondensation reactions of compound **5** with primary amines afforded the corresponding 4-iminochromeno[3,4-*c*]pyridine derivatives. So, the reactions of compound **5** with hydroxylamine hydrochloride in ethanol, urea, semicarbazide, methylamine hydrochloride and 4-amino-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2*H*)-one in acetic acid afforded 3,4-dihydro-4-imino-5*H*-chromeno[3,4-c]pyridin-5-one derivatives, **6**-**10**, respectively (Scheme 3). The protons of NH_2 groups in compounds **7** and **8** in ^{1}H NMR spectra were displaced although their appearance in the IR spectra due to strong hydrogen bonding and keto-enol tautomerism (Scheme 4).

Also, the cyclocondensation reactions of compound **5** with 2-aminoethanol and with ethane-1,2-diamine in acetic acid produced 2,3-dihydro-imidazo[1,2-a]-12*H*-chromeno[3,4-c] pyridin-12-one (**11**) and 4,4′-{ethane-1,2-diyl-*bis*[imino(*z*) ethene-2,1-diyl]}*bis*(2-oxo-2*H*-chromene-3-carbonitrile) (**12**), respectively (Scheme 5).

On the other hand, condensation and/or cyclocondensation reactions of compound **5** with thiosemicarbazide in the presence of glacial acetic acid depend on reaction conditions. When the reaction was refluxed for 10 min afforded 2-[2-(3-cyano-2-oxo-2*H*-chromen-4-yl)ethenyl]hydrazinecarbothio amide (**13**), whereas refluxing for 4 hours gave 2-amino-12*H*-chromeno[3,4-c][1,2,4]triazolo[1,5-a]pyridin-12-one (**14**).

Also, compound **14** was formed by refluxing of compound **13** in glacial acetic acid for 3 hours (Scheme 6).

The formation of cyclocondensation products **6-10** proceeded via the nucleophilic replacement of NH_2 group of amine derivatives with -$N(CH_3)_2$ group of compound **5** to give the intermediate products **15**, which can be cyclized by nucleophilic addition of NH group to C≡N group. Also, the cyclocondensation products **11** and **14** were proceeded by elimination of H_2O or H_2S, respectively (Scheme 8).

On the other hand, cyclocondensation reaction of compound **5** in the presence of dilute acetic acid gave a mixture of 4-imino-4H,5H-pyrano[3,4-c]chromen-5-one (**16**) (22%) and (Z)-2-(3-cyano-2-oxo-2*H*-chromen-4-yl)vinyl-2-oxo-4-(2-oxoethyl)-2*H*-chromene-3-carbimidate (**17**) (34%) (Scheme 7). Structure **17** was assigned for this product based on ^{1}H NMR data, that revealed cis olefinic protons at δ 4.14 and 7.06 ppm with J = 6.9 Hz.

3.2. Antimicrobial activity

Compounds **2**, **4** and **5** showed intermediate activities against *Bacillus subtilis*, while compounds **9** and **10** showed intermediate activities against *Salmonella typhimurium*. Also, compound **4** showed intermediate activity against *Aspergillus fumigatus*.

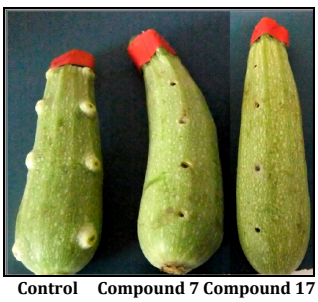
3.3. Antitumor activity in planta

Chemical compounds that yielded the greatest inhibition zones *in vitro* were selected to demonstrate its antitumor activity in *planta*. These compounds were examined for their capability to suppress gall formation by *Agrobacterium tumefaciens* on summer squash fruits. The strain of *Agrobacterium tumefaciens* used as tumrigenic agent in this study was the isolate designated 5A. This strain was originally isolated from rose plant with typical symptoms of crown gall disease. The obtained results revealed that compounds **7** and **17** yielded the greatest inhibition zones *in vitro* against *Agrobacterium tumefaciens* on summer squash fruits (Table 2 and Figure 1). The reason for the higher reactivity of compounds **7** and **17** as tumrigenic agents due to the presence of chromeno[3,4-c]pyridine carrying *N*-carboxamide group in compound **7** and two isolated bioactive coumarin moiety in compound **17**.

Table 2. Antitumor activity in *planta* data of some synthesized compounds

Compound	Gall incidence (%)	Gall size (mm) *
2	100	2
3	100	4
5	100	3
7	0	-
10	100	6
11	100	4
13	100	5
14	100	6
17	0	-
Control	100	7

* Mean, nearest whole, mm.



Control Compound 7 Compound 17

Figure 1. The inhibition zones of compounds **7** and **17** related to control.

4. Conclusion

An efficient synthesis of some new isolated and fused 2-oxo-2*H*-chromene derivatives starting from 2-hydroxyacetophenone with ethyl cyanoacetate followed by cyclocondensation reactions with nitrogen nucleophilic reagents is reported. The antitumor activities in *planta* for the prepared compounds indicated that compounds **7** and **17** yielded the greatest inhibition zones in vitro against *Agrobacterium tumefaciens* due to the presence of chromeno[3,4-c]pyridine carrying *N*-carboxamide group and two isolated bioactive coumarin moiety, respectively.

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