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REGULAR ARTICLE

Expeditious synthesis of coumarin-pyridone conjugates molecules and their anti-microbial evaluation

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Abstract. An expedient synthesis of coumarin-pyridone conjugate molecules **2a-o** is reported *via* one-pot reaction between (*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-ones **1a-o**, ethyl 2-nitroacetate and ammonium acetate. The structures of synthesized compounds have been unambiguously confirmed by spectroscopic analyses (NMR, IR and MS). All the compounds were screened for their anti-microbial activities against three Gram-positive bacterial strains, two Gram-negative bacterial strains and four fungal organisms. Compounds **2d**, **2i**, **2k**, **2o** exhibited mild anti-bacterial activity and **2d**, **2m** were found to be moderately active against all the tested fungal organisms. Compound **2k** showed good inhibitory potential against the tested yeasts organisms.

Keywords. (*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-ones; ethyl 2-nitroacetate; 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones; anti-bacterial; anti-fungal.

1. Introduction

Coumarin (2*H*-chromen-2-one) is a biologically active motif belonging to the flavonoid class of plant secondary metabolite and coumarin derivatives represent an elite class of C6–C3 plant metabolites, originating from the shikimate pathway.[1,2] This class of compounds has attracted significant attention in recent years owing to its widespread occurrence in natural products and exhibition of diverse pharmacological activities including anti-bacterial[3], anti-fungal[4], anti-coagulant,[5] anti-platelet,[5] anti-inflammatory,[6] anti-oxidant,[6] hypolipidemic[7] and anti-cancer.[8] They have also been evaluated for their *in vitro* inhibitory activity toward bovine α-chymotrypsin, human leukocyte elastase and cell proliferation of several human tumor cell lines (*viz.*, gastric carcinoma, colon-carcinoma, hepatoma-derived and lymphoblastic).[9] On the other hand, pyridin-2(1*H*)-one and its derivatives have also become important compounds because of their significance in medicinal chemistry and occurrence in many biologically active natural products, therapeutics and synthetic compounds.[10] Pyridin-2(1*H*)-ones display a variety of biological properties such as anti-bacterial,[11] cardiotonic,[12] cardiovascular,[13] anti-anaphylactic,[14] anti-inflammatory,[15] anti-oxidant[16] and anti-fungal.[17] Pyridin-2(1*H*)-ones have been used as lead compounds for the preparation of several drugs such as potent anti-hepatitis B,[18] pim-1 kinase inhibitors and inhibitors of amyloid-β peptide aggregation, an important character in amyloid formation in Alzheimer's disease.[19]

Further, in the design of new pharmaceutical agents, the development of conjugate molecules through the combination of different pharmacophores has resulted in the improvement of biological profiles. Adopting this approach, several research groups have reported the formation of conjugate molecules by coupling coumarin with different bioactive cores and new hybrids were found to exhibit remarkable anti-cancer,[20] anti-inflammatory[21] and anti-oxidant[22] activities.

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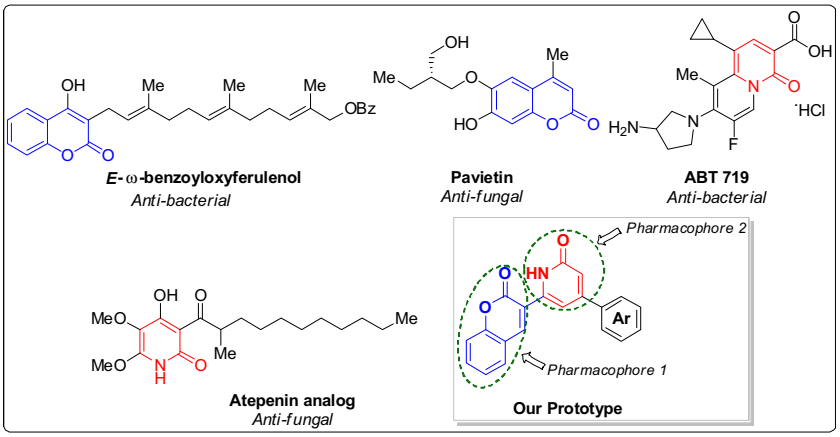


Figure 1. Representative examples of coumarin and pyridin-2(1*H*)-one core containing heterocyclic compounds possessing anti-microbial activities and our designed prototype.

These literature reports on conjugate molecules coupled with our successful one-pot preparation of 4, 6-diarylpyridin-2(1*H*)-ones[23] prompted us to synthesize 6-(2-oxo-2*H*-chromen-3-yl)-4-aryl derivatives **2a-o** of pyridin-2(1*H*)-one (a conjugate of coumarin and pyridin-2(1*H*)-one) for their *in vitro* anti-bacterial and anti-fungal screening. The structures of several significant anti-microbial coumarin and pyridin-2(1*H*)-one based compounds along with our designed prototype is shown in Figure 1.

2. Experimental

All experiments were performed in an oven dried glass apparatus. All the commercially available reagents were purchased from *Aldrich* and were used without further purification. Solvents used in purification were distilled prior to use. Melting points (°C) were measured in open glass capillaries using Perfit melting point apparatus and are uncorrected. (*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-one (**1a-o**, 80–91% yield) were prepared from 3-acetyl-2*H*-chromen-2-one and aryl/heteryl aldehydes using Literature method.[24] The progress of the reaction and the purity of the final products were monitored by thin layer chromatography (TLC) using silica gel pre-coated aluminium sheets (60 F254, Merck). Visualization of spots was effected by exposure to ultraviolet light (UV) at 365 nm and 254 nm, iodine vapours, 2% 2,4-dinitrophenylhydrazine in methanol containing few drops of H_2SO_4, draggendroff reagent and anisaldehyde reagent. IR spectra (υ_{max}, cm^{-1}) were recorded on Perkin-Elmer FTIR spectrophotometer using KBr discs. ^1H (at 400 MHz) and ^{13}C (at 100 MHz) NMR spectra were recorded on Bruker Avance III-400 MHz using $(CH_3)_4Si$ as an internal standard. Chemical shifts (δ) are expressed in parts per million. *J* values are given in hertz (Hz). The abbreviations s, br s, d, t, dd, dt and m in ^1H NMR spectra refer to singlet, broad singlet, doublet, triplet, double doublet, double triplet and multiplet respectively. Elemental analysis was performed on Leco CHNS-932 analyzer. MS (ESI) were recorded on Micro Mass VG-7070 H mass spectrometer at 70 eV.

Anti-bacterial and anti-fungal activities of 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones **2a-o**, 4-methyl-2*H*-chromeno[3,4-c]pyridine-2,5(3*H*)-dione **2p** were determined using broth microdilution method.[25–27] These compounds were screened for their *in vitro* anti-bacterial activity against five strains *viz*. three gram positive bacteria [*Bacillus subtilis* (MTCC 441), *Klebsiella pneumoniae* (ATCC 13883), *Staphylococcus aureus* (ATCC 29213] and two gram negative bacteria [*Escherichia coli* (MTC 119), *Pseudomonas aeruginosa* (MTCC 1934)]. In addition, these compounds were screened for anti-fungal activity against two yeast strains [*Candida albicans* (ATCC 22019), *Candida albicans* (V-01-27853)] and two filamentous fungi [*Aspergillus fumigatus* (LSI-II), *Aspergillus niger* (ATCC 16404)]. Anti-bacterial testing was performed in Muller Hinton Broth (Becton-Dickenson, Cockeysville, MD), and for anti-fungal testing RPMI 1640 with L-glutamine (Sigma Aldrich, St. Louis, MO) buffered to pH 7.0 supplemented with 0.165 M 3-(N-morpholino) propanesulfonic acid (Sigma Aldrich) was used. The stock solution of the compounds was prepared in dimethyl sulfoxide. The minimum inhibitory concentration (MIC) of the compounds was determined by serial twofold diluting the solution in the 100 µL volume of aforementioned media in a 96-well U bottom micro titre plate. Popular drugs such as ciprofloxacin and amphotetricin B (20–0.03 µg/mL) were used as standard anti-bacterial and anti-fungal agents respectively. The final concentrations of compounds ranged from 125–0.25 µg/mL. The bacterial and fungal suspension of the overnight grown bacterial and fungal sample was prepared in sterile normal saline, and the density was adjusted to 0.5 Mcfarland. The bacterial cultures were further diluted and added in 100 µL volume at final inoculums of 1×10^5 CFU/mL. For fungal cultures, 1×10^3 CFU/mL was used. The plates were incubated at 37°C for 24 h for

bacterial cultures and at 25°C for 48 h for fungal cultures. The plates were read visually, and the minimum concentration of the compound showing no turbidity was recorded as MIC.

2.1 *General procedure for the synthesis of 6-(2-oxo-2H-chromen-3-yl)-4-arylpyridin-2(1H)-ones (2a-O)*

In an oven-dried round-bottomed flask, a mixture of (*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-one **1a-o** (1.0 mmol), ethyl 2-nitroacetate (1.0 mmol) and ammonium acetate (6.0 mmol) in *n*-BuOH (10 mL) was refluxed for the appropriate time till the completion of reaction (Table 2). The reaction mixture was cooled to room temperature and the solid obtained was filtered, washed and recrystallized from EtOH to obtain the pure product (**2a-o**; 79–93%). All the products were characterized spectroscopically.

2.2 *Spectroscopic Characterization of All the Synthesized Compounds (2a–2p)*

2.2a *6-(2-Oxo-2H-chromen-3-yl)-4-phenylpyridin-2(1H)-one (2a)*: The title compound **2a** was obtained from 3-cinnamoyl-2*H*-chromen-2-one **1a** (1.0 mmol) as a pale yellow solid (0.272 g, 86% yield), M.p. 283–284°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.61 (br s, 1H, exchangeable with D_2O), 8.61 (s, 1H), 7.85–7.68 (m, 4H), 7.55–7.42 (m, 5H), 7.23 (s, 1H), 6.74 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.3, 159.3, 153.7, 152.0, 143.3, 137.5, 133.4, 130.0, 129.7, 129.6, 127.2, 125.5, 119.1, 116.5; IR (KBr) (υ_{max}, cm^{-1}): 3437.13, 1722.43, 1686.11; Anal. Calcd for $C_{20}H_{13}NO_3$: C, 76.18; H, 4.16; N, 4.44%. Found: C, 76.10; H, 4.12; N, 4.49%. MS (ESI): $m/z = 316$ $(M+H)^+$.

2.2b *6-(2-Oxo-2H-chromen-3-yl)-4-(p-tolyl)pyridin-2(1H)-one (2b)*: The title compound **2b** was obtained from (*E*)-3-(3-(*p*-tolyl)acryloyl)-2*H*-chromen-2-one **1b** (1.0 mmol) as a yellow solid (0.273 g, 83% yield), M.p. 299–300°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.49 (br s, 1H, exchangeable with D_2O), 8.45 (s, 1H), 7.66–7.48 (m, 5H), 7.34–7.15 (m, 4H), 6.65 (d, J = 10.9 Hz, 1H), 1.17 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6): δ 163.7, 159.5, 153.6, 152.5, 142.5, 139.6, 134.7, 133.1, 129.7, 129.1, 126.7, 125.1, 120.1, 118.7, 116.3, 106.7, 21.2; IR (KBr) (υ_{max}, cm^{-1}): 3397.91, 1719.56, 1642.18; Anal. Calcd for $C_{21}H_{15}NO_3$: C, 76.58; H, 4.59; N, 4.25%. Found: C, 76.62; H, 4.53; N, 4.20%. MS (ESI): $m/z = 330$ $(M+H)^+$.

2.2c *4-(3-Methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2(1H)-one (2c)*: The title compound **2c** was obtained from (*E*)-3-(3-(3-methoxyphenyl)acryloyl)-2*H*-chromen-2-one **1c** (1.0 mmol) as a light brown solid (0.276 g, 80% yield), M.p. 233°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.72 (br s, 1H, exchangeable with D_2O), 8.11 (s, 1H), 7.93 (d, J = 8.1 Hz, 2H), 7.86 (d, J = 10.9 Hz, 1H), 7.51 (br s, 3H), 7.06 (d, J = 7.8 Hz, 2H), 6.82 (s, 1H), 6.51 (s, 1H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 160.8, 158.9, 153.6, 151.6, 148.3, 136.1, 134.7, 134.4, 129.8, 129.1, 128.7, 124.8, 114.8, 111.4, 105.8, 55.7; IR (KBr) (υ_{max}, cm^{-1}): 3371.44, 1712.79, 1643.09; Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06%. Found: C, 73.10; H, 4.43; N, 4.10%. MS (ESI): $m/z = 346$ $(M+H)^+$.

2.2d *4-(4-Methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl)pyridin-2(1H)-one (2d)*: The title compound **2d** was obtained from (*E*)-3-(3-(4-methoxyphenyl)acryloyl)-2*H*-chromen-2-one **1d** (1.0 mmol) as a shiny brown solid (0.320 g, 93% yield), M.p. 245–247°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (br s, 1H, exchangeable with D_2O), 8.52 (s, IH), 7.95 (d, J = 8.1 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 7.56 (d, J = 7.8 Hz, 2H), 7.08–7.04 (m, 3H), 6.66 (s, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 160.8, 159.2, 151.6, 148.2, 143.2, 134.7, 134.4, 129.8, 129.2, 129.1, 128.7, 125.4, 114.8, 111.4, 105.5, 55.7; IR (KBr) (υ_{max}, cm^{-1}): 3396.04, 1722.23, 1657.99; Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06%. Found: C, 73.09; H, 4.45; N, 4.13%. MS (ESI): $m/z = 346$ $(M+H)^+$.

2.2e *4-(4-Hydroxyphenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2(1H)-one (2e)*: The title compound **2e** was obtained from (*E*)-3-(3-(4-hydroxyphenyl)acryloyl)-2*H*-chromen-2-one **1e** (1.0 mmol) as a yellow solid (0.265 g, 80% yield), M.p. 298°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.95 (br s, 1H, exchangeable with D_2O), 8.60 (s, 1H), 7.82 (d, J = 7.1 Hz, 1H), 7.67 (dd, J = 27.7, 7.9 Hz, 3H), 7.48 (dt, J = 15.0, 5.9 Hz, 3H), 7.17 (br s, exchangeable with D_2O, 1H), 6.89 (d, J = 8.6 Hz, 2H), 6.65 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 159.4, 159.3, 153.6, 151.6, 143.4, 143.1, 133.3, 129.7, 128.6, 127.9, 125.4, 120.3, 119.6, 119.1, 116.5, 116.3; IR (KBr) (υ_{max}, cm^{-1}): 3440.12, 3129.44, 1723.76, 1656.63; Anal. Calcd for $C_{20}H_{13}NO_4$: C, 72.50; H, 3.95; N, 4.23%. Found: C, 72.55; H, 3.89; N, 4.16%. MS (ESI): $m/z = 332$ $(M+H)^+$.

2.2f *4-(2-Chlorophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2(1H)-one (2f)*: The title compound **2f** was obtained from (*E*)-3-(3-(2-chlorophenyl)acryloyl)-2*H*-chromen-2-one **1f** (1.0 mmol) as a pale yellow solid (0.276 g, 79% yield), M.p. 267–268°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.85 (br s, 1H, exchangeable with D_2O), 8.31 (s, 1H), 7.86–7.77 (m, 4H), 7.56 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 7.9 Hz, 2H), 6.94 (s, 1H), 6.63 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 159.7, 158.0, 152.3, 149.0, 148.2, 141.0, 137.9, 133.5, 129.8, 129.4, 127.3, 121.8, 108.9, 107.7, 102.0; IR (KBr) (υ_{max}, cm^{-1}): 3439.32, 1749.02, 1667.88; Anal. Calcd for $C_{20}H_{12}ClNO_3$: C, 68.68; H, 3.46; N, 4.00%. Found: C, 68.73; H, 3.42; N, 4.07%. MS (ESI): $m/z = 350, 352$ $(M+H)^+$.

2.2g *4-(4-Chlorophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2(1H)-one (2g)*: The title compound **2g** was

obtained from (*E*)-3-(3-(4-chlorophenyl)acryloyl)-2*H*-chromen-2-one **1g** (1.0 mmol) as a light brown solid (0.293 g, 84% yield), M.p. 303–304°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.95 (br s, 1H, exchangeable with D_2O), 8.53 (s, 1H), 7.91–7.87 (m, 3H), 7.58–7.52 (m, 5H), 7.02 (s, 1H), 6.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.0, 159.8, 158.8, 153.6, 150.9, 144.9, 142.5, 136.7, 134.7, 133.1, 130.1, 128.1, 127.5, 119.7, 116.9, 113.0; IR (KBr) (υ_{max}, cm^{-1}): 3431.65, 1720.33, 1659.98; Anal. Calcd for $C_{20}H_{12}ClNO_3$: C, 68.68; H, 3.46; N, 4.00%. Found: C, 68.71; H, 3.49; N, 4.05%. MS (ESI): m/z = 350, 352 (M+H)$^+$.

2.2h *4-(4-Bromophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2(1H)-one (2h)*: The title compound **2h** was obtained from (*E*)-3-(3-(4-bromophenyl)acryloyl)-2*H*-chromen-2-one **1h** (1.0 mmol) as a brown solid (0.326 g, 83% yield), M.p. 258–260°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.78 (br s, 1H, exchangeable with D_2O), 8.50 (s, 1H), 7.91–7.86 (m, 3H), 7.57–7.49 (m, 5H), 7.02 (s, 1H), 6.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 160.3, 158.5, 154.3, 150.9, 146.2, 142.7, 136.6, 134.7, 130.1, 129.4, 129.3, 129.2, 127.5, 121.4, 113.1, 104.7; IR (KBr) (υ_{max}, cm^{-1}): 3429.54, 1717.69, 1649.02; Anal. Calcd for $C_{20}H_{12}BrNO_3$: C, 60.93; H, 3.07; N, 3.55%. Found: C, 60.97; H, 3.14; N, 3.49%. MS (ESI): m/z = 394, 396 (M+H)$^+$.

2.2i *4-(3-Nitrophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2(1H)-one (2i)*: The title compound **2i** was obtained from (*E*)-3-(3-(3-nitrophenyl)acryloyl)-2*H*-chromen-2-one **1i** (1.0 mmol) as a brown solid (0.284 g, 79% yield), M.p. 266 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (br s, 1H, exchangeable with D_2O), 8.52 (s, 1H), 8.30 (d, J = 8.2 Hz, 1H), 7.99–7.97 (m, 3H), 7.69–7.67 (m, 1H), 7.46–7.42 (m, 3H), 7.29 (d, J = 8.5 Hz, 1H), 7.03 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.2, 161.2, 156.6, 154.0, 150.4, 148.7, 140.1, 133.0, 129.6, 128.7, 126.9, 124.7, 123.8, 122.0, 121.2, 119.6, 115.9, 113.1; IR (KBr) (υ_{max}, cm^{-1}): 3445.91, 1734.43, 1676.19; Anal. Calcd for $C_{20}H_{12}N_2O_5$: C, 66.67; H, 3.36; N, 7.77%. Found: C, 66.72; H, 3.30; N, 7.73%. MS (ESI): m/z = 361 (M+H)$^+$.

2.2j *4-(4-Nitrophenyl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2(1H)-one (2j)*: The title compound **2j** was obtained from (*E*)-3-(3-(4-nitrophenyl)acryloyl)-2*H*-chromen-2-one **1j** (1.0 mmol) as a brown solid (0.303 g, 84% yield), M.p. 295–297°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.83 (br s, 1H, exchangeable with D_2O), 8.57 (s, 1H), 8.33–8.27 (m, 2H), 7.94 (d, J = 8.1 Hz, 2H), 7.80 (t, J = 7.0 Hz, 1H), 7.51 (d, J = 5.9 Hz, 3H), 7.13 (s, 1H), 6.79 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 160.1, 157.8, 154.0, 150.1, 148.8, 139.6, 134.9, 134.0, 131.0, 130.2, 129.1, 127.5, 124.4, 122.1, 113.6, 105.1; IR (KBr) (υ_{max}, cm^{-1}): 3437.28, 1729.24, 1680.00; Anal. Calcd for $C_{20}H_{12}N_2O_5$: C, 66.67; H, 3.36; N, 7.77%. Found: C, 66.74; H, 3.43; N, 7.70%. MS (ESI): m/z = 361 (M+H)$^+$.

2.2k *4-(3-Bromo-4-methoxyphenyl)-6-(2-oxo-2H-chromen-3-yl) Pyridin-2(1H)-one (2k)*: The title compound **2k** was obtained from (*E*)-3-(3-(3-bromo-4-methoxyphenyl) acryloyl)-2*H*-chromen-2-one **1k** (1.0 mmol) as a pale yellow solid (0.334 g, 79% yield), M.p. 306–308°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.72 (br s, 1H, exchangeable with D_2O), 8.51 (s, 1H), 7.94–7.85 (m, 3H), 7.51 (s, 3H), 7.23 (d, J = 8.5 Hz, 1H), 7.02 (s, 1H), 6.67 (s, 1H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 159.4, 156.8, 154.1, 148.7, 134.9, 131.7, 129.1, 128.2, 127.6, 126.5, 124.2, 122.7, 119.4, 113.3, 111.8, 104.5, 56.9; IR (KBr) (υ_{max}, cm^{-1}): 3421.09, 1718.94, 1653.89; Anal. Calcd for $C_{21}H_{14}BrNO_4$: C, 59.45; H, 3.33; N, 3.30%. Found: C, 59.51; H, 3.28; N, 3.37%. MS (ESI): m/z = 424, 426 (M+H)$^+$.

2.2l *6-(2-Oxo-2H-chromen-3-yl)-4-(3,4,5-trimethoxyphenyl)pyridin-2(1H)-one (2l)*: The title compound **2l** was obtained from (*E*)-3-(3-(3,4,5-trimethoxyphenyl) acryloyl)-2*H*-chromen-2-one **1l** (1.0 mmol) as a light brown solid (0.369 g, 91% yield), M.p. 312°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (br s, 1H, exchangeable with D_2O), 8.57 (d, J = 14.2 Hz, 1H), 7.73–7.68 (m, 2H), 7.52–7.42 (m, 3H), 7.01 (s, 2H), 6.79 (s, 1H), 3.88 (s, 6H), 3.72 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.5, 161.2, 153.6, 152.0, 150.7, 143.9, 143.4, 135.2, 133.3, 133.2, 133.1, 129.7, 125.7, 125.4, 125.3, 119.6, 119.1, 117.8, 113.4, 104.7, 60.5, 56.5; IR (KBr) (υ_{max}, cm^{-1}): 3428.39, 1733.40, 1644.19; Anal. Calcd for $C_{23}H_{19}NO_6$: C, 68.14; H, 4.72; N, 3.46%. Found: C, 68.19; H, 4.64; N, 3.41%. MS (ESI): m/z = 406 (M+H)$^+$.

2.2m *4-(Furan-2-yl)-6-(2-oxo-2H-chromen-3-yl) pyridin-2(1H)-one (2m)*: The title compound **2m** was obtained from (*E*)-3-(3-(furan-2-yl)acryloyl)-2*H*-chromen-2-one **1m** (1.0 mmol) as a grey solid (0.269 g, 88% yield), Mp 267–268°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.70 (br s, 1H, exchangeable with D_2O), 8.33 (s, 1H), 7.88 (d, J = 4.6 Hz, 3H), 7.76 (d, J = 5.0 Hz, 1H), 7.52 (d, J = 5.1 Hz, 3H), 7.24 (t, J = 4.2 Hz, 1H), 6.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.4, 159.1, 153.8, 150.1, 148.5, 141.2, 139.1, 137.9, 130.1, 129.2, 127.3, 124.9, 118.8, 116.3; IR (KBr) (υ_{max}, cm^{-1}): 3412.91, 1724.69, 1629.97; Anal. Calcd for $C_{18}H_{11}NO_4$: C, 70.82; H, 3.63; N, 4.59%. Found: C, 70.87; H, 3.59; N, 4.51%. MS (ESI): m/z = 306 (M+H)$^+$.

2.2n *6-(2-Oxo-2H-chromen-3-yl)-4-(thiophen-2-yl) pyridin-2(1H)-one (2n)*: The title compound **2n** was obtained from (*E*)-3-(3-(thiophen-2-yl)acryloyl)-2*H*-chromen-2-one **1n** (1.0 mmol) as a brown solid (0.280 g, 87% yield), M.p. 249°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.52 (br s, 1H, exchangeable with D_2O), 8.59 (s, 1H), 7.83 (d, J = 7.4 Hz, 1H), 7.77–7.69 (m, 2H), 7.54–7.39 (m, 3H), 7.28–7.19 (m, 2H), 6.71 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.2, 159.2, 153.7, 145.1, 143.3, 140.4, 133.4, 129.7, 129.3, 129.2, 127.6, 125.4, 119.1, 116.5; IR (KBr) (υ_{max}, cm^{-1}): 3415.89, 1729.34, 1673.00; Anal. Calcd for $C_{18}H_{11}NO_3S$: C,

67.28; H, 3.45; N, 4.36; S, 9.98%. Found: C, 67.33; H, 3.40; N, 4.41, S, 10.03%. MS (ESI): $m/z = 322$ $(M+H)^+$.

2.2o *6'-(2-Oxo-2H-chromen-3-yl)-[2,4'-bipyridin]-2'(1'H)-one (2o)*: The title compound **2o** was obtained from (*E*)-3-(3-(pyridin-2-yl)acryloyl)-2*H*-chromen-2-one **1o** (1.0 mmol) as a grey solid (0.275 g, 87% yield), M.p. 226–227°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.89 (br s, 1H, exchangeable with D_2O), 8.57 (s, 1H), 8.32 (d, $J = 6.9$ Hz, 1H), 7.95 (t, $J = 7.0$ Hz, 1H), 7.89 (d, $J = 7.0$ Hz, 2H), 7.59–7.47 (m, 4H), 7.10 (s, 1H), 6.93 (s, 1H);^{13}C NMR (100 MHz, DMSO-d_6): δ 164.4, 157.1, 153.8, 150.4, 150.1, 145.1, 139.1, 137.9, 130.1, 129.3, 129.2, 127.3, 124.9, 121.9, 119.5, 118.0, 116.3; IR (KBr) (υ_{max}, cm^{-1}): 3419.91, 1727.83, 1646.54; Anal. Calcd for $C_{19}H_{12}N_2O_3$: C, 72.15; H, 3.82; N, 8.86%. Found: C, 72.11; H, 3.89; N, 8.91%. MS (ESI): $m/z = 317$ $(M+H)^+$.

2.2p *Procedure for the synthesis of 4-methyl-2H-chromeno[3,4-c]pyridine-2,5(3H)-dione (2p)*: In an oven-dried round-bottomed flask, a mixture of 3-acetyl-2*H*-chromen-2-one (1.0 mmol), ethyl 2-nitroacetate (1.0 mmol) and ammonium acetate (6.0 mmol) in *n*-BuOH (10 mL) was refluxed for 3 h till the completion of reaction (TLC, Table 2). The reaction mixture was cooled to room temperature and the solid obtained was filtered, washed and recrystallized from EtOH to obtain the pure 4-methyl-2*H*-chromeno[3,4-c]pyridine-2,5(3*H*)-dione **2p** as a white solid in 79% yield.

2.2q *4-Methyl-2H-chromeno[3,4-c]pyridine-2,5(3H)-dione (2p)*: The title compound **2p** was obtained from 3-acetyl-2*H*-chromen-2-one (1.0 mmol) as a white solid (0.179 g, 79% yield), M.p. 271°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.39 (br s, 1H, exchangeable with D_2O), 8.20 (d, $J = 8.1$ Hz, 1H), 7.60 (t, $J = 7.9$ Hz, 1H), 7.34–7.27 (m, 2H), 7.00 (s, 1H), 2.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.6, 158.8, 151.9, 144.0, 133.3, 125.4, 125.0, 117.5, 116.3, 106.7, 21.0; IR (KBr) (υ_{max}, cm^{-1}): 3433.48, 1649.12, 1578.36; Anal. Calcd for $C_{13}H_9NO_3$: C, 68.72; H, 3.99; N, 6.16%. Found: C, 68.65; H, 4.08; N, 6.11%. MS (ESI): $m/z = 228$ $(M+H)^+$.

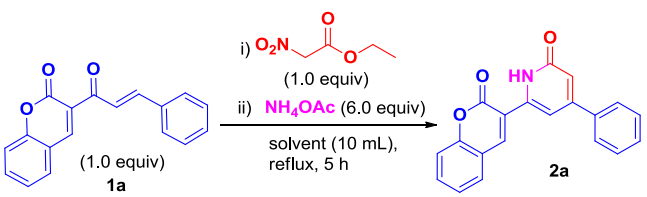
3. Results and Discussion

3.1 *Chemistry*

The required starting materials *viz.* (*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-ones (chalcones) **1a-o** were synthesized by an aldol/dehydration reaction of 3-acetyl-2*H*-chromen-2-one with aryl/heteryl aldehydes.[24] We had earlier reported the one pot synthesis of 4,6-diarylpyridin-2(1*H*)-ones.[23] With these previously optimized reaction conditions in hand, we attempted to synthesize 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones **2a-o** by replacing the aryl substitution at C-6 of 4,6-diarylpyridin-2(1*H*)-ones with 2-oxo-2*H*-chromen-3-yl motif. The model reaction was performed between 3-cinnamoyl-2*H*-chromen-2-one **1a** (1.0 equiv), ethyl 2-nitroacetate (1.0 equiv) and ammonium acetate (6.0 equiv) in refluxing EtOH for 5 h (entry 1; Table 1). Formation of desired product 6-(2-oxo-2*H*-chromen-3-yl)-4-phenylpyridin-2(1*H*)-one **2a** occurred *albeit* in a low yield (32%, entry 1; Table 1). This may be attributed to low solubility of **1a** in EtOH and examination of this reaction in various solvents such as *i*-PrOH, *n*-BuOH, DCE, $CHCl_3$, MeCN, dioxane, diglyme and 1:1 DMF:H_2O (entries 2–9, Table 1) led us to conclude that *n*-BuOH was the solvent of choice (entry 3; Table 1).

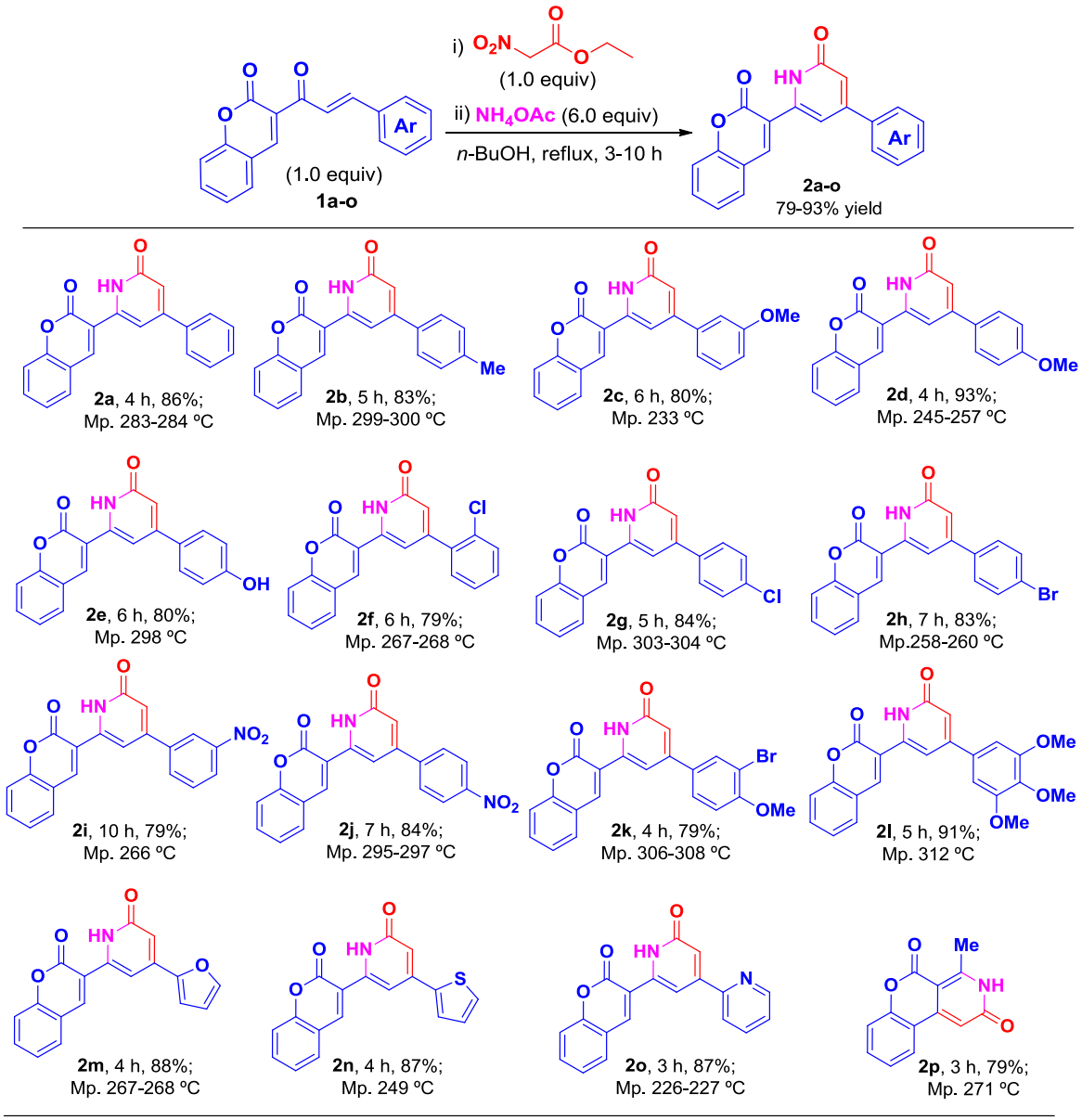
Under the optimized conditions, various electron-rich as well as electron-deficient (*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-ones **1b-o** were investigated. The corresponding products *viz.*, 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones **2b-o** were obtained in good to excellent yields (Table 2). The (*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-ones bearing electron donating (Me, OMe, OH) and electron withdrawing substitutions (Cl, Br, NO_2) on the phenyl ring (ortho, meta or para substitutions) were efficiently converted into their corresponding 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones **2b-j** (79–93%, Table 2). The (*E*)-3-(3-aryl acryloyl)-2*H*-chromen-2-one with di-substitution **1k**, and with tri-substitution **1l** on the phenyl ring gave the desired products **2k** (79%) and **2l** (91%) respectively. Further, to demonstrate the relevance of this method, (*E*)-3-(3-heteryl acryloyl)-2*H*-chromen-2-ones **1m-o** were also used as substrates to afford

Table 1. Screening of solvents for the one-pot preparation of 6-(2-oxo-2*H*-chromen-3-yl)-4-phenylpyridin-2(1*H*)-one **2a**.



Entry	Solvent	Yield (%)[b]
1	EtOH	32
2	*i*−PrOH	35
3	***n*-BuOH**	**86**
4	DCE	No reaction
5	$CHCl_3$	40
6	MeCN	40
7	1,4-dioxane	35
8	Diglyme	52
9	1:1 DMF:H_2O	62

Table 2. Substrate Scope for the synthesis of 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones **2b-o** and 4-methyl-2*H*-chromeno[3,4-c]pyridine-2,5(3*H*)-dione **2p**.

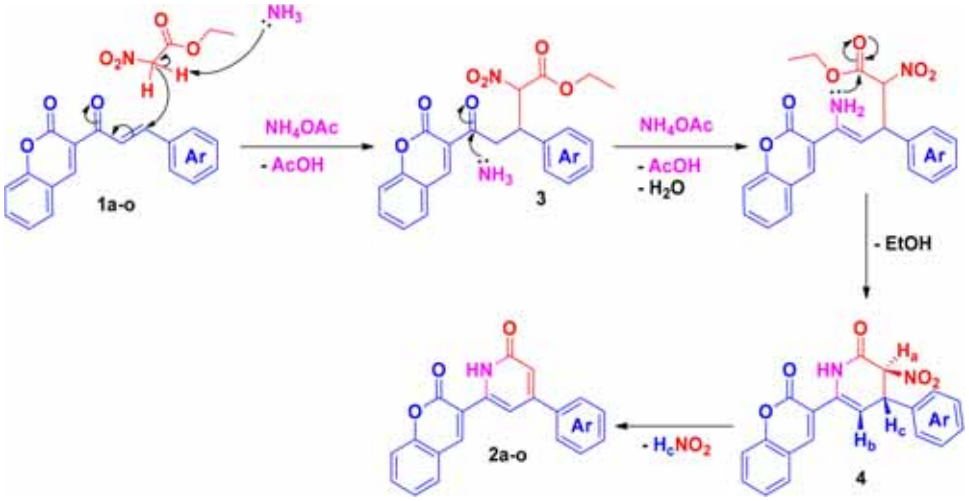


the corresponding products *viz*. 4-(furan-2-yl)-6-(2-oxo-2*H*-chromen-3-yl)pyridin-2(1*H*)-one (**2m**, 88%), 6-(2-oxo-2*H*-chromen-3-yl)-4-(thiophen-2-yl) pyridin-2(1*H*)-one (**2n**, 87%) and 6'-(2-oxo-2*H*-chromen-3-yl)-[2,4'-bipyridin]-2'(1'*H*)-one (**2o**, 87%) respectively. Pertinent to mention that the usage of 3-acetyl-2*H*-chromen-2-one afforded the 4-methyl-2*H*-chromeno [3,4-c]pyridine-2,5(3*H*)-dione (**2p**, 79%).

In the light of our earlier proposed mechanism for the preparation of 4,6-diarylpyridin-2(1*H*)-ones[23], the following mechanism is proposed for the current reaction (Scheme 1). (*E*)-3-(3-Arylacryloyl)-2*H*-chromen-2-one **1a-o** underwent NH_4OAc catalyzed Michael addition with ethyl 2-nitroacetate to give adduct **3**, which upon base-promoted cyclization resulted in 3-nitro-6-(2-oxo-2*H*-chromen-3-yl)-4-aryl-3,4-dihydropyridin-2(1*H*)-one **4** followed by the loss of one molecule of H_2O and EtOH each and subsequent aromatization of **4** (*syn* elimination of H_cNO_2) to yield the corresponding 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-one**2a-o**. The formation of 4-methyl-2*H*-chromeno[3,4-c]pyridine-2,5(3*H*)-dione **2p** from acetyl-2*H*-chromen-2-one also follows the same mechanism.

3.2 *Biology: anti-microbial evaluation*

The results of anti-microbial screening are summarized in Table 3. It was found that the test compounds **2d**, **2i**, **2k** and **2o** exhibited mild anti-bacterial activity whereas the rest of the compounds did not show any significant



Scheme 1. Plausible reaction mechanism for the one-pot formation of 6-(2-oxo-2*H*-chromen-3-yl)-4-phenylpyridin-2(1*H*)-one derivatives **2a-o**.

Table 3. Anti-microbial activity of 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones **2a-o** and 4-methyl-2*H*-chromeno[3,4-c]pyridine-2,5(3*H*)-dione **2p** (MICs, μg/mL).

Compd	*Gram +ve Bacteria*			*Gram –ve Bacteria*		*Yeasts*		*Filamentous Fungi*	
	B.s. (MTCC 441)	*K.p.* (ATCC 13883)	*S.a.* (ATCC 29213)	*E.c.* (MTC 119)	*P.a.* (MTCC 1934)	*C.a.* (ATCC 22019)	*C.al.* (V-01-27853)	*A.f.* (LSI-II)	*A.n.* (ATCC 16404)
SD-1	0.25	8.0	0.25	<0.03	0.5	–	–	–	–
SD-2	–	–	–	–	–	0.5	0.5	1.0	0.5
2a	>125	>125	>125	>100	>100	>125	>125	>125	>125
2b	>125	>125	>125	>125	>125	>125	>125	>125	>125
2c	>100	>125	>100	>125	>100	>110	>125	>100	>125
2d	>65	>68	>65	>75	>60	>75	>65	>60	>60
2e	>125	>125	>125	>125	>125	>110	>125	>110	>125
2f	>100	>125	>125	>110	>125	>125	>125	>110	>125
2g	>125	>125	>125	>125	>125	>125	>125	>125	>125
2h	>125	>110	>125	>100	>125	>125	>125	>125	>100
2i	>80	>60	>65	>80	>75	>120	>125	>110	>125
2j	>120	>125	>125	>110	>125	>110	>125	>125	>125
2k	>68	>60	>60	>65	>80	>65	>60	>125	>120
2l	>125	>125	>125	>125	>125	>125	>125	>125	>125
2m	>125	>110	>125	>125	>125	>75	>65	>75	>60
2n	>110	>125	>125	>125	>100	>125	>125	>100	>120
2o	>60	>70	>70	>60	>75	>125	>125	>125	>125
2p	>120	>125	>125	>110	>125	>125	>125	>110	>125

SD-1 = Ciprofloxacin; **SD-2** = Amphotetricin B;***B.s.*** *Bacillus subtilis*; ***K.p.****Klebsiella pneumoniae*; ***S.a.*** *Staphylococcus aureus*; ***E.c.*** *Escherichia coli*; ***P.a.****Pseudomonas aeruginosa*; ***C.a.*** *Candida albicans*; ***C.al.*** *Candida albicans*; ***A.f.*** *Aspergillus fumigatus*; ***A.n.*** *Aspergillus niger*.

anti-bacterial activity. The investigation of anti-fungal screening revealed that only two compounds *viz.*,**2d** and **2m** were moderately active against all the tested fungal organisms. Compound **2k** showed good anti-fungal potential against the both tested yeasts *Candida albicans* (ATCC 22019) and *Candida albicans* (V-01-27853) whereas the remaining compounds did not show any significant activity.

4. Conclusions

The utility of our previous successful endeavor for the one-pot synthesis of 4,6-diarylpyridin-2(1*H*)-ones led to the successful generation of coumarin-pyridone conjugate molecules *viz.* 6-(2-oxo-2*H*-chromen-3-yl)-4-arylpyridin-2(1*H*)-ones **2a-o** in good to excellent yields. Usage of 3-acetyl-2*H*-chromen-2-one in place of

(*E*)-3-(3-arylacryloyl)-2*H*-chromen-2-ones **1a-o** under the same reaction conditions afforded the corresponding 4-methyl-2*H*-chromeno[3,4-c]pyridine-2,5(3*H*)-dione **2p** (a conjugate tricyclic compound) in appreciable yield. The isolation and purification of all the synthesized compounds were done by crystallization, without the use of column chromatography technique. All the compounds were evaluated for anti-microbial activities against different bacterial and fungal strains. Only compounds **2d**, **2i**, **2k**, **2o** exhibited mild anti-bacterial activity whereas compounds **2d**, **2m** were found moderately active against all the tested fungal organisms. Compound **2k** showed good inhibitory potential against both tested yeasts. Rest of the compounds did not show any significant anti-bacterial and anti-fungal activity.

Supplementary Information (SI)

All additional information pertaining to characterization of the compounds using ^{1}H NMR and ^{13}C NMR spectra (Figures S1 to S32) are given in the supporting information available at www.ias.ac.in/chemsci.

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