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Synthesis and bio-evaluation of novel 7-hydroxy coumarin derivatives via Knoevenagel reaction

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Abstract Coumarin and 7-hydroxy coumarins have great significance as natural fragrances, having a characteristic odour like vanilla beans and their hydroxy position at 7 has importance in biosynthesis. Treatment of 8-formyl-7-hydroxy coumarin with *N,N*-disubstituted cyano acetamides in the presence of piperidine afforded novel 8-substituted-7-hydroxy coumarin derivatives. Their structures were characterized by IR, ^{1}H, ^{13}C NMR, mass spectral and elemental analysis data. Two out of these 12 compounds, i.e. 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide and 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acryl amide showed enhanced in vitro antifungal activity against *Candida albicans* and *Aspergillus niger* vis-à-vis standard, i.e. fluconazole, and enhanced in vitro antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* vis-à-vis standard, i.e. norfloxacin.

Keywords Cyano acetamide derivatives · Knoevenagel reaction · Fluconazole · *Aspergillus niger* · *Candida albicans* · *Escherichia coli* · *Staphylococcus aureus* · *Pseudomonas aeruginosa* · Norfloxacin

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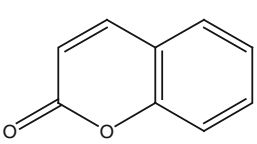
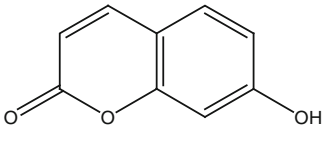
Introduction



coumarin



7-Hydroxy coumarin

The parent molecule of coumarin derivatives is the simplest compound of a large class of naturally occurring phenolic substances made of fused benzene and α-pyrone rings [1]. Coumarin itself and 7-hydroxycoumarin have been reported to inhibit the proliferation of a number of human malignant cell lines in vitro [2–5], and have demonstrated activity against several types of animal tumors [6–10]. Coumarin is a natural substance that has shown anti-tumor activity in vivo, with the effect believed to be due to its metabolites (e.g., 7-hydroxycoumarin). These compounds have also been reported in clinical trials to demonstrate activity against prostate cancer, malignant melanoma, and metastatic renal cell carcinoma [11–13].

7-hydroxy coumarin was condensed with N,N-dialkyl substituted cyano acetamide derivatives to get the novel knoevenagel products, which are expected to be biologically active.

Experimental procedure

General

All chemicals, pyrrolidine, piperidine, cyclohexylamine, benzylamine, 1-phenyl-ethylamine, 1-(4-methoxy-phenyl)-ethylamine, ortho nitro aniline, 3,5-di methoxy benzylamine, 2-(2-methoxy-phenoxy)-ethylamine, diphenyl methylamine, 5-meth-oxyl-pyridin-3-ylamine, and 2,5-dihydro-thiazol-2-ylamine were purchased from Merck, India. Melting points were determined on a Buchi-540 melting point apparatus and are uncorrected. TLC was performed on pre-coated silica gel, which were visualized by UV light and ninhydrin spray. FT-IR spectra were recorded as KBr pellet on a Nicolet-380 FT-IR instrument (Spectrum one; Thermo Electron) and gas chromatographic (GC) analysis wass performed on a Thermo Fisher GC-1000 model. ^{1}H and ^{13}C NMR (proton decoupled) spectra were recorded on a Varian 400 MHz spectrometer using DMSO-d_6 and $CDCl_3$ as solvent. Mass spectra was recorded on an Agilent triple quadrapole mass spectrometer equipped with a turbo ion spray interface at 360 °C.

Determination of zone of inhibition by Kirby Baur's method [14]

The antibacterial susceptibility test was done by determining the zone of inhibition by Kirby–Baur's Method. The 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(2) and ethyl-2-cyano acetate(3) and coumerin derivatives (4a–4l) were dissolved in

acetone to make a solution of 120 μM/ml. From this stock solution, serial dilutions have been done to 20, 10, 5, and 1.25 μM/ml with acetone in sterile test tubes. Sterilized filter discs were dipped in these solutions and subsequently dried to remove the acetone. Nutrient agar medium plates were prepared using Muller–Hinton agar and were allowed to solidify. Three different bacteria were selected, i.e. *E. coli*, *S. aureus*, *P. aeruginosa*, and 1 ml of each bacteria and culture broth were added to the plate and spread with the help of a sterile spreader. The filter paper discs soaked in solution of coumerin derivatives were placed aseptically over the inoculated plates using sterile forceps. The plates were incubated at 37 °C for 18 h in an upright position. The zone of inhibition was measured using the scale.

Determination of MIC by the micro dilution broth susceptibility test

Different concentrations 20, 10, 5, and 2.5 μM/ml, of all the compounds were prepared in sterile test tubes to determine minimum inhibitory concentrations (MIC). Nutrient broth was prepared using Muller–Hinton Broth (M391) and 4.9 ml of it was taken in each test tube which were sterilized after plugging. After cooling, 0.1 ml of each dilution was added to the test tube and the final volume was made up to 5.0 ml. The test tubes were shaken to uniformly mix the inoculums with the broth. The test tubes were incubated at 37 °C for 18 h. The appearance of any turbidity shows that the compound is not able to inhibit the growth of bacteria, while no turbidity indicates the inhibition of microorganisms by the sample.

In vitro antifungal test

For antifungal testing, the 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(**2**), ethyl-2-cyano acetate(3), and coumerin derivatives (4a–4l) were prepared in acetone at an initial concentration of 120 μM/ml and serially diluted to make effective concentrations of 20, 10, and 5 μM/ml. A dermatophyte was isolated from nail chippings from a suspected case of onychomycosis referred from a skin clinic as the most prevalent causative organism, i.e. *C. albicans*, in the nails of patient diagnosed with onychomycosis. For testing, isolates were subcultured on a subourad dextrose agar slant. *A. niger* was isolated from soil, diagnosed, and incubated at 30 °C for 10 days with and without antibiotics. Sterile discs with 150 mm diameter were further sterilized and charged with compounds (7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(2), ethyl-2-cyano acetate(3), and compounds 4a–4l) and after drying these discs were stored at 4 °C.

A standardized inoculum was prepared by counting microconidia. Cultures were grown on soluble dextrose agar for 10 days at 37 °C, sterile saline solution (0.85 %) was added to the slant, and cultures were gently swabbed with a cotton-tipped applicator to dislodge conidia from the hyphal mat. The suspension was transferred to a sterile tube and the volume was adjusted to 5 ml with sterile saline solution. The resulting suspension was counted with a homocytometer and the dilution in sabourad broth to 5 ml sabourad dextrose agar was poured to a depth of 5 mm in 90-mm Petri dishes and stored at 4 °C. The plates were dried, and the standardized

suspension was poured and uniformly spread by means of swab discs. The excess inoculum was drained off.

Procedure for the preparation of 7-hydroxy coumarin (1) [15]

Resorcinol (1.5 g, 0.1 mol) and malic acid (20.1 g, 0.15 mol) were added to a stirred solution of concentrated sulfuric acid (200 mL) in a reaction flask equipped with a thermometer and addition funnel. The reaction mass was maintained and poured into crushed ice under vigorous stirring. The off-white solid obtained was filtered, and the crude product was further purified by re-crystallization using solvent ethanol. 82 % of yield; m.p: 180–182 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.49 (s, 3H, C4–CH_3), 6.31 (s, 1H, C3–H), 6.92 (d, 1H, C6–H, $J = 9.0$ Hz), 6.94 (s, 1H, C8–H), 7.57 (d, 1H, C5–H, $J = 9.0$ Hz); IR (KBr,ν max): 3,423 (–OH), 1,733 (–CO), 1,555 (–C=C–) cm^{-1}. MS: m/z(M^++1); 163.15

Procedure for the preparation of 7-hydroxy-8-formyl coumarin (2) [16]

7-hydroxy coumarin (16.20 g, 0.1 mol) was dissolved in glacial acetic acid (200 ml), and hexamethylene tetramine (42.0 g, 0.3 mol) was added in one portion. The reaction mixture was maintained for an additional 1 h, followed by extraction with diethylether, and the combined organic layer was collected, dried over sodium sulfate, and concentrated in vacuo. The crude product was quenched into a pool of cold ethanol followed by crystallization, filtration, and drying to obtain pale yellow needles of 7-hydroxy-8-formyl coumarin (2). 8.40 g, 44 % of yield; m.p. 176–178 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.22 (s, 1H, C3-H), 6.90–6.93 (d, 1H, C6-H, $J = 9$ Hz), 7.73–7.76 (d, 1H, C5-H, $J = 9$ Hz), 10.63 (s, 1H, HCO), 12.28 (s, 1H, OH); IR (KBr,ν max):3 442 (–OH), 1 742 (–CO), 1 644 (–CHO), 1 594 (–C=C–) cm^{-1}. MS: m/z(M^++1); 191.15

Preparation of 3-oxo-3-pyrrolidin-1-yl-propionitrile(3a)[17–20]

To a stirred solution of pyrrolidine (0.71 g, 0.01 mol) in 50 ml ethanol was added ethyl cyano acetate (1.36 g, 0.012 mol). The reaction mixture was maintained at reflux for 5 h. The reaction mass was cooled to crystallize, filtration and drying to obtain 3-oxo-3-pyrrolidin-1-yl-propionitrile(3a), 0.903 g, 65 % of yield.

Spectral data of cyanoacetamide derivatives

3-oxo-3-(pyrrolidin-1-yl)propanenitrile(3a) ^{1}H NMR (DMSO-d_6): δ 1.85 (4H, m, pyrrolidine), 3.10 (4H,m, pyrrolidine),3.38 (2H,s, $C\underline{H_2}$–CN); MS: m/z (M^++1); 139.17

3-oxo-3-(piperidin-1-yl)propanenitrile(3b) ^{1}H NMR (DMSO-d_6): δ 1.53 (4H, m, piperidine),1.59 (2H, q, piperidine), 3.37 (2H,s, –$C\underline{H_2}$–CN) 3.42 (4H,m, piperidine); MS: m/z (M^++1); 153.19

2-cyano-N-cyclohexylacetamide(3c) ^{1}H NMR (DMSO-d_6): δ 1.1–1.9 (10H, m, cyclohexane), 3.30 (2H,s, –C$\underline{H_2}$–CN) 3.54 (H,quintet,); MS: m/z (M^++1); 167.22

N-benzyl-2-cyanoacetamide (3d) ^{1}H NMR (DMSO-d_6): δ 3.33 (2H,s, –C$\underline{H_2}$–CN), 4.30 (2H,s,–C$\underline{H_2}$Ar),7.2–7.4 (5H,m, Ar),8.8 (1H,s,–NH); MS: m/z (M^++1); 175.20

2-cyano-N-(1-phenylethyl)acetamide(3e) ^{1}H NMR (DMSO-d_6): δ 1.48 (3H,d,–CH_3), 3.31 (2H,s, –C$\underline{H_2}$–CN), 5.0 (1H,q), 7.21–7.44 (5H,m, Ar), 8.28 (1H,s,–NH); MS: m/z (M^++1); 189.23

2-cyano-N-(1-(4-methoxyphenyl)ethyl)acetamide(3f) ^{1}H NMR (DMSO-d_6): δ 1.45 (3H,d,-CH_3), 3.30 (2H,s, –C$\underline{H_2}$–CN),3.80 (3H,s,–OCH_3), 5.10 (1H,q), 6.9–7.24 (4H,m, Ar), 8.34 (1H,s,–NH); MS: m/z (M^++1); 219.25

2-cyano-N-(2-nitrophenyl)acetamide(3 g) ^{1}H NMR (DMSO-d_6): δ 3.31 (2H,s,-–$\underline{H_2}$CN), 7.28 (Ar–H), 7.80 (ArH), 7.9 (ArH), 10.0 (H,s,NH); MS: m/z (M^++1); 206.17

2-cyano-N-(3,5-dimethoxybenzyl)acetamide(3 h) ^{1}H NMR (DMSO-d_6): δ 3.30 (2H,s, –C$\underline{H_2}$–CN),3.83 (6H,s,–$(OCH_3)_2$),4.20(2H,s,C$\underline{H_2}$–Ar), 6.18(1H,s,ArH),6.6 (H,s,ArH),8.74(1H,s,–NH); MS: m/z (M^++1); 235.25

2-cyano-N-(2-(2-methoxyphenoxy)ethyl)acetamide(3i) ^{1}H NMR (DMSO-d_6):δ 3.30 (2H,s, –C$\underline{H_2}$–CN), 3.53 (2H,t,–C$\underline{H_2}$–NH), 3.80 (3H,s,–OCH_3), 4.10 (2H,t,–C$\underline{H_2}$–O), 6.8–6.9 (4H,m,Ar), 8.04 (1H,s,–NH); MS: m/z (M^++1); 235.25

N-benzhydryl-2-cyanoacetamide(3j) ^{1}H NMR (DMSO-d_6): δ 3.33(2H,s, –C$\underline{H_2}$–CN),6.16(H,s,Ar–C$\underline{H}$–Ar),7.3–7.5(10H,m,Ar), 8.34(1H,s,–NH); MS: m/z (M^++1); 251.30

2-cyano-N-(5-methylpyridin-3-yl)acetamide(3 k) ^{1}HNMR (DMSO-d_6): δ 2.3 (3H,s,–CH_3), 3.34 (2H,s, –C$\underline{H_2}$–CN), 8.19 (1H,s), 9.30 (1H,s), 10.10 (H,s,–NH); MS: m/z (M^++1); 176.19

3-(2,5-dihydrothiazol-2-yl)-3-oxopropanenitrile(3 l) ^{1}HNMR (DMSO-d_6): δ2.3 (2H,m), 3.32 (2H,s, –C$\underline{H_2}$–CN), 4.6 (1H,s,S-C$\underline{H}$-), 8.10 (H,s,-NH); MS: m/z (M^++1); 170.20

Preparation of 3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)-acrylonitrile(4a) by Knoevenagel condensation [21]

7-Hydroxy-8-formyl coumarin (**2**, 1.90 g, 0.01 mol) and a catalytic amount of piperidine were dissolved in ethanol in a 100-mL reaction flask equipped with reflux condenser, thermometer, stirrer and addition funnel followed by addition of 3-oxo-3-pyrrolidin-1-yl-propionitrile (3a, 1.39 g, 0.01 mol). The reaction mass was heated

to reflux temperature and maintained for 2 h. The reaction mass was then cooled to 0–5 °C followed by crystallization, filtration, and drying. Crude product obtained was further purified by recrystallization using solvent dioxane:ethanol to get (E)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)acrylonitrile (4a). 1.63 g, 52 % of yield. IR (KBr, cm^{-1}): 3,442 (–OH), 2,246 (–CN), 1,742 (–CO), 1,644 (cyclic –CO), 1,594 (–C=C); ^{1}H NMR (DMSO-d_6): δ 11.8 (s, –OH), 8.35 (s, H, exocyclic CH=C), 6.23–7.5 (3H, coumarin), 3.25 (t, 4H, pyrrolidine), 1.72 (t, 4H, pyrrolidine); ^{13}C NMR(DMSO-d_6): δ170.7 (–CO), 160, (C-2), 155 (C-7), 153 (C, expcyclic ethylene), 146 (C-10), 125.2 (C-5), 118.2 (C-8), 115 (–CN), 114.0 (C-6), 112.5 (C-3), 112.0 (C-9), 106 (C, ethylene), 45.7 (2C, of pyrrolidine), 25.0 (2C, of pyrrolidine); MS: m/z (M^++1) 311.3; M.P: 225–227 °C. Anal. Calcd for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03; O, 20.62. Found: C, 65.76; H, 4.42; N, 9.04; O, 19.90.

Similar procedure was used for the synthesis of compounds (4b-l).

*3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-2-(piperidine-1-carbonyl)-acrylonitrile (**4b**)*

IR (KBr, cm^{-1}): 3,442 (–OH), 2,240.3 (–CN), 1,742 (-CO), 1,644 (cyclic –CO), 1,594 (–C=C); ^{1}H NMR (DMSO-d_6): δ 11.79 (s, –OH),8.32 (s, H, exocyclic CH=C), 6.2–7.55 (m, 3H, coumarinring), 3.7 (m, 4H, piperidine), 1.6 (m, 2H, piperidine),1.53 (m, 4H, piperidine); ^{13}CNMR (DMSO-d_6) : δ170.5 (CO), 160 (C-2), 155 (C-7),153 (C,ethylene), 152.7 (C-4),146 (C-10), 125.2 (C-5), 118.2 (C-8), 115 (CN), 114.0 (C-6), 112.5 (C-3), 112.0 (C-9), 106 (C, ethylene), 47.0 (2C, piperidine), 25.0 (2C, piperidine), 24.1 (C, piperidine); MS: m/z (M^++1) 325.3; M.P: 215–217 °C. Anal. Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64; O, 19.73. Found: C, 66.40; H, 5.03; N, 8.71; O,19.20

*2-cyano-N-cyclohexyl-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (**4c**)*

IR (KBr, cm^{-1}): 3,442 (–OH), 2,240.3 (–CN), 1,742 (–CO), 1,644 (cyclic –CO), 1,594 (–C=C); ^{1}H NMR (DMSO-d_6): δ 11.8 (s, –OH), 8.43 (s, H, exocyclic C<u>H</u>=C), 8.2 (s,H,–NH), 6.0 (d,H,C-3), 6.5 (d, H, C-6), 7.5 (d, H, C-5), 7.8 (d, H, C-4), 3.57 (m, H, C-1′), 1.11–1.3 ((m, 4H, C-3′, C-5′), 1.45–1.53 (m, 2H, C-4′), 1.6–1.8 (m, 4H, C-2′, C-6′); ^{13}CNMR (DMSO-d_6) : δ162.5 (CO), 160 (C-2), 155 (C-7), 153 (C,ethylene), 152.7 (C-4), 146 (C-10), 125.2 (C-5), 118.2 (C-8), 115 (CN), 114.0 (C-6), 112.5 (C-3), 112.0 (C-9), 106 (C,<u>C</u>–CN), 47.0 (C, C-1′), 33.0 (2C, C-2′, C-6′), 26.1 (C, C-4′), 24.5 (2C, C-3′, C-5′); MS: m/z (M^++1) 339.3; M.P: 219–221 °C. Anal. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28; O, 18.91. Found: C, 67.40; H, 5.23; N, 8.31; O,19.20

*N-benzyl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (**4d**)*

IR (KBr, cm^{-1}): 3,430 (–OH), 3,275 (–NH), 2,230 (–CN), 1,730.4 (–CO), 1,674 (cyclic –CO), 1,546 (–C=C) cm^{-1}; ^{1}H NMR(DMSO-d_6): δ, 11.7 (s, H, –OH), 8.9 (s, H, –NH)), 8.5 (s, H exocyclic CH=C), 7.5 (d, H, coumarin ring), 6.8 (d, H, coumarin ring), 7.2–7.4 (m, 5H, phenyl), 6.0 (s, H, endocyclic), 4.5 (s, 2H, $–CH_2$): ^{13}C NMR

(DMSO-d_6): δ162.7 (C-2),160, (-CO), 155 (C-7), 153.5 (C, ethylene),152.5 (C-4), 146 (C-10), 134 (C-4′), 128.5 (C-2′,C-6′), 126.5 (C-3′,C-5′), 126 (C-1′), 125.2 (C-5), 118.2 (C-7), 115 (C, –CN), 114.5 (C-6),,112.5 (C-3), 112 (C-9), 103 (C–CN), 43.0 (C, $–CH_2$); MS: m/z (M^++1) 347.5; M.P: 225–227 °C. Anal. Calcd for $C_{20}H_{14}N_2O_4$: C, 69.36; H, 4.07; N, 8.09; O, 18.48. Found: C, 69.59; H, 4.04; N, 7.96; O, 18.46.

*2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-N-(1-phenyl–ethyl)-acrylamide (**4e**)*

IR (KBr, cm^{-1}): 3,442 (–OH), 2,257 (–CN), 3,275 (–NH), 1,742 (–CO), 1,644 (cyclic –CO), 1,594 (–C=C); ^{1}H NMR (DMSO-d_6): δ 11.7 (s, H, –OH), 8.9 (s, H, –NH), 8.5 (s, H, exocyclic CH=C), 6.5–7.4 (m, 5H, phenyl), 6.0–6.4 (m, 2H, coumarin ring), 6.23 (d, 1H, endocyclic), 5.0 (q, H, –CH–NH), 1.5 (d, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 164.5 (C-4′), 160.7 (C-2), 159 (–CO), 155 (C-7), 153.5 (C, exocyclic ethylene), 152.5 (C-4), 146.8 (C-10), 128.7 (C-2′, C-6′), 127 (C-3′, C-5′), 126 (C-1′), 125 (C-5), 118 (C-8), 116 (–CN), 114.5 (C-6), 112.5 (C-3), 112 (C-9), 103 (C-exocyclic), 49 (−CH-NH-), 21.5 ($–CH_3$); MS: m/z (M^++1) 361.4; M.P: 235–237 °C. Anal. Calcd for $C_{21}H_{16}N_2O_4$: C, 69.99; H, 4.48; N, 7.77; O, 17.76. Found: C, 70.55; H, 4.80; N, 7.50; O, 17.0.

*2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-N-[1-(4-methoxy-phenyl)-ethyl]-acrylamide (**4f**)*

IR (KBr cm^{-1}): 3,437 (–OH), 3,275 (–NH), 2,240 (–CN), 1,729 (–CO, cyclic), 1,624 (–CO), 1,557 (–C=C–): ^{1}H NMR (DMSO-d_6): δ 11.8 (s, –OH), 8.9 (s, H –NH), 8.5 (s, H, exocyclic CH=C), 6.9–7.2 (m, 4H, phenyl), 6.0–6.5 (m, 2H, coumarin ring), 5.0 (q, H,–CH–NH), 3.8 (s, 3H, $–OCH_3$ 1.5 (d, 3H, $–CH_3$); ^{13}C NMR (DMSO-d_6): δ 160.8 (C-2), 160 (C=O), 158.9 (C-1′), 155 (C-7), 153.4 (C-exocyclic), 153 (C-4), 146 (C-10), 133 (C-4′), 126.5 (C-3′, C-5′), 125.2 (C-5), 118.2 (C-8), 115 (–CN), 114.0 (C-2′, C-6′, C-6), 112.5 (C-3), 112.0 (C-9), 103 (C-exocyclic), 56.1 ($–OCH_3$), 49.0 (C, –CH–NH), 19.5 ($–CH_3$ of C-4); MS: m/z (M^++1) 391.4; M.P: 222–224 °C. Anal. Calcd for $C_{22}H_{18}N_2O_5$: C, 67.69; H, 4.65; N, 7.18; O, 20.49. Found: C, 67.29; H, 4.69; N, 6.98; O,20.8.

*2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-N-(2-nitro-phenyl)-acrylamide (**4g**)*

IR (KBr, cm^{-1}): 3,430 (-OH), 3,275 (-NH), 2,230 (-CN), 1,730.4 (-CO), 1,674 (cyclic –CO), 1,546 (–C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ, 11.87 (s, H, -OH), 10.2 (s, H, -NH), 8.57 (d, H, C-6′), 8.55 (s, H, exocyclic CH = C), 8.24 (d, H, C-3′), 7.8 (t, 2H, C-4′, C-5′),7.58 (d, H, C-4), 7.44 (d, H, C-5), 6.88 (d, H, C-6), 6.26 (s, H, C-3), ^{13}C NMR (DMSO-d_6): δ163.7 (C-2),161, (-CO), 154 (C-7), 153.1 (C, ethylene), 152.1 (C-4), 117 (C-6′),145.2 (C-10), 126.4 (C-4′), 142.8 (C-2′), 132.5 (C-3′), 130.2 (C-5′), 115.9 (C-5), 115.8 (C, –CN), 114.2 (C-6), 112.1 (C-3), 111.2 (C-9), 108 (C–CN); MS: m/z (M^++1) 378.3; M.P: 228–241 °C. Anal. Calcd for $C_{19}H_{11}N_3O_6$; C, 60.48; H, 2.94; N, 11.14; O, 25.44. Found: C, 59.20; H, 3.04; N, 11.18; O, 24.86.

*2-cyano-N-(3,5-dimethoxy-benzyl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (**4h**)*

IR (KBr, cm^{-1}): 3,442 (–OH), 2,254 (–CN), 1,742 (–CO), 1,644 (cyclic–CO), 1,594 (–C=C), 1,549 (–C=C); ^{1}H NMR (DMSO-d_6):11.8 (s, OH), 10.5 (s, –NH), 8.35 (s, H exocyclic ethylene), 8.1 (d, H of C-3′), 6.23–7.55 (m, 3H coumarin ring), 6.53–6.8 (2H, of C-2′,C-6′), 3.8 (s, 6H of $–OCH_3$); ^{13}C NMR(DMSO-d_6): δ 169 (C-1′), 64 (C, -CO), 160.5 (C-2),155 (C-7), 153.6 (C, exocyclic ethylene), 154 (C-4, C-5′), 146 (C-10), 125.2 (C-5), 123 (C-3′), 118.2 (C-8), 117 (C-4′), 115 (–CN), 114.6 (C-6), 112.5 (C-3), 112.0 (C-9), 106 (C-2′), 104 (C, ethylene), 100 (C-6′), 56 (2C, $–OCH_3$); MS: m/z (M^++1): 407.4; M.P: 184–187 °C. Anal. Calcd for $C_{22}H_{18}N_2O_6$:C,65.02;H,4.46; N,6.89;O,23.62. Found: C, 65.07; H, 4.52; N, 6.79; O, 23.90.

*2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-N-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide (**4i**)*

IR (KBr cm^{-1}): 3,437 (–OH), 3,275 (–NH), 2,240(–CN), 1,729 (–CO, cyclic), 1,624 (–CO), 1,557 (–C=C–):^{1}H NMR (DMSO-d_6): δ 11.8 (s, –OH), 8.9 (s, H –NH), 8.5 (s, H, exocyclic CH=C), 8.57 (d, H, C-6′), 6.90 (d2H, C-3′, C-6′), 7.18 (t, 2H, C-4′, C-5′), 7.58 (d, H, C-4), 7.44 (d, H, C-5), 6.88 (d, H, C-6), 6.26 (s, H, C-3), 4.20 (t, 2H, $–C\underline{H}_2–O–$), 3.7 (s, 3H, $–OCH_3$), 3.28 (q, 2H, $–NH–C\underline{H}_2$); ^{13}C NMR (DMSO-d_6): δ 160.8 (C-2), 160 (C=O), 150.9 (C-1′), 155 (C-7), 153.4 (C-exocyclic), 153 (C-4), 151 (C-1′), 146 (C-10), 123 (C-4′), 122.5 (C-3′), 111 (C-5′), 125.2 (C-5), 118.2 (C-8), 115 (-CN), 114.0 (C-6′), 112.5 (C-3), 112.0 (C-9), 103 (C-exocyclic), 70 (C,–$O–CH_2–$), 56.1 ($–OCH_3$), 39.0 (C, $–\underline{C}H–NH$); MS: m/z (M^++1): 407.4; M.P: 210–212 °C. Anal. Calcd for $C_{22}H_{18}N_2O_6$: C, 65.02; H, 4.46; N, 6.89; O, 23.62. Found: C, 65.29; H, 4.69; N, 6.98; O, 23.08.

*N-benzhydryl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (**4j**)*

IR (KBr, cm^{-1}): 3,442 (-OH), 2,254 (-CN), 1,742 (-CO), 1,644 (cyclic –CO), 1,594 (–C=C), 1549 (–C=C); ^{1}H NMR (DMSO-d_6): 11.8 (s, OH), 8.35 (s, H, exocyclic ethylene), 8.0 (s, H, –NH), 6.23–7.55 (m, 3H coumarin ring), 7.22–7.44 (m, 10H, benzhydril), 6.16 (s, H,–CH); ^{13}C NMR (DMSO-d_6): δ 161 (C-2), 160 (C, exocyclic ethylene), 159 (CO),155 (C-7), 153 (C-4), 146 (C-10), 141.2 (C-4′, C-4″), 128 (C-5′, C-5″, C-3′, C-3″), 129.2 (C-6′, C-6″, C-2′, C-2″), 126.2 (C-1′, C-1″), 125.2 (C-5), 118.2 (C-8), 115 (–CN), 114.6 (C-6), 112.5 (C-3), 112.0 (C-9), 104 (C, ethylene), 52.5 (C, –CH of benzhydril); MS: m/z (M^++1): 423.5; M.P: 258–260 °C. Anal. Calcd for $C_{26}H_{18}N_2O_4$: C, 73.92; H, 4.29; N, 6.63; O, 15.15. Found: C, 74.15; H, 4.46; N, 6.56; O, 14.96.

*2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-N-(5-methyl-pyridin-3-yl)-acrylamide (**4k**)*

IR (KBr, cm^{-1}): 3,430 (–OH), 3,275 (–NH), 2,230 (–CN), 1,730.4 (–CO), 1,674 (cyclic –CO), 1,546 (–C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ, 11.82 (s,H,-OH), 8.00

(d,H,C-6′), 6.66 (d,H,C-6′), 6.65 (d,H,C-5′), 8.45 (s,H,exocyclic,CH=C), 8.16 (s,H,–NH), 6.26 (s,H,C-3), 6.88 (d,H,C-6), 7.44 (d,H,C-5), 2.42 (s, 3H, $–CH_3$) ^{13}C NMR (DMSO-d_6): δ160.7 (C-2),163, (–CO), 153.9 (C-7), 153.0 (C,ethylene), 152.0 (C-4), 146.2 (C-10), 158.4 (C-4′), 163.0 (C-2′), 145 (C-6′), 115.5 (C-3′), 125.5 (C-5), 123.2 (C-5′), 118.3 (C-8), 115.8 (C, –CN), 114.2 (C-6), 112.1 (C-3), 111.2 (C-9), 108 (C–CN), 21.3 (C,-CH_3 of pyridine); MS: m/z (M^++1) 348.5; M.P: 238–240 °C. Anal. Calcd for $C_{19}H_{13}N_3O_4$; C, 65.70; H, 3.77; N, 12.10; O, 18.43. Found: C, 67.020; H, 4.04; N, 12.18; O, 17.56.

*2-cyano-N-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (**4l**)*

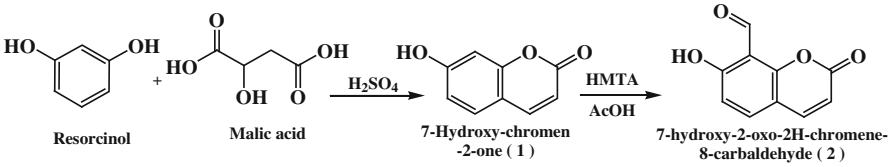
IR(KBr, cm^{-1}): 3,442 (-OH), 2,254 (-CN), 1,742 (-CO), 1,644 (cyclic–CO), 1,594 (–C=C), 1,549 (–C=C), 750 (C–S); ^{1}H NMR (DMSO-d_6): 11.8 (s, OH), 8.45 (s, H exocyclic ethylene), 8.0 (s, H, –NH), 6.23–7.55 (m, 3H coumarin ring), 3.8 (t, 2H, –N–CH_2–), 3.25 (t, 2H,–S–CH_2); ^{13}C NMR (DMSO-d_6) : δ 169 (CO), 163.5 (–N–C=N), 162 (C, exocyclic ethylene), 160.5 (C-2), 155 (C-7), 153 (C-4), 146 (C-10), 125.2 (C-5), 118.2 (C-8), 115 (–CN), 114.6 (C-6), 112.5 (C-3), 112.0 (C-9), 106 (C, ethylene), 54 (=N–CH_2), 24 (–S– CH_2); MS: m/z (M^++1): 342.37; M.P: 247–249 °C. Anal. Calcd for $C_{16}H_{11}N_3O_4S$: C, 56.30; H, 3.25; N, 12.31; O, 18.75; S, 9.39. Found: C, 57.02; H, 3.30; N, 11.98; O, 18.70, S, 9.40.

Results and discussion

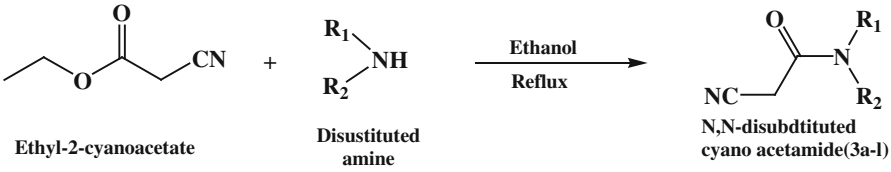
To develop a general method for the synthesis of 8-Formyl-7-hydroxy coumarin derivatives, experiments were conducted by reacting 8-Formyl-7-hydroxy coumarin with N,N-disubstituted cyano acetamides in the presence of catalytic amounts of piperidine at reflux temperature for 5 h, and N,N-disubstituted cyano acetamides were prepared by reacting corresponding amines with ethyl cyano acetate at reflux temperature for 5 h.

To explore the general method developed for the synthesis of 8-Formyl-7-hydroxy coumarin derivatives, the experiments were conducted with ethyl cyano acetate with corresponding secondary amines followed by reacting with 8-Formyl-7-hydroxy coumarin to give the corresponding 8-Formyl-7-hydroxy coumarin derivatives in substantial yields.

We found that this two-step reaction is general and is applicable to most secondary alkylamines, and that the reaction condition for the preparation of 7-hydroxy coumerin is a Peechman condensation with dl-Malic acid and 1,3-resorcinol in the presence of sulfuric acid, as summarized in Scheme 1. The reaction conditions for the preparation of *N*,*N*-disubstituted cyano acetamides starting from different secondary alkyl amines has been suggested in Scheme 2 and summarized in Table 1. A probable mechanism for the preparation of 8-Formyl-7-hydroxy coumarin derivatives from different N,N-disubstituted cyano acetamides has been suggested in Scheme 3 and summarized in Table 2. The structures of (4a-l) were



Scheme 1 Synthesis of 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde (2). Reagents and conditions: Resorcinol, malic acid, sulfuric acid, hexamine and acetic acid, 0–5 °C for 12 h and at 80–85 °C for 6 h



Scheme 2 Synthesis of pyrrolidine(3a), piperidine(3b), cyclohexylamine(3c), benzylamine(3d), 1-phenylethylamine(3e), 1-(4-methoxy-phenyl)-ethylamine(3f), ortho nitro aniline(3 g), 3,5-di methoxy benzylamine(3 h), 2-(2-methoxy-phenoxy)-ethylamine(3i), diphenyl methylamine(3j), 5-methoxyl-pyridin-3-ylamine(3 k), 2,5-dihydro-thiazol-2-ylamine(3 l) derivatives of ethyl-2-cyano acetate. Reagents and conditions: ethanol, 78–80 °C, 2 h

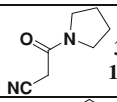
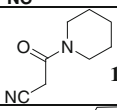
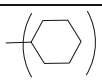
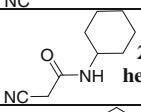
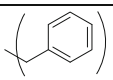
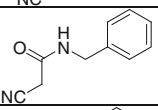
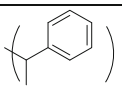
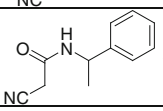
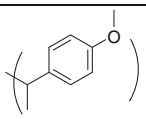
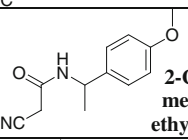
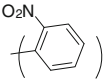
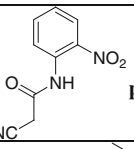
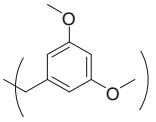
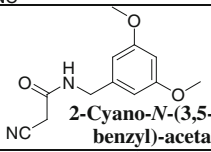
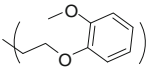
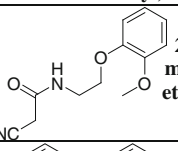
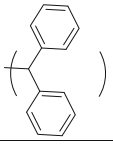
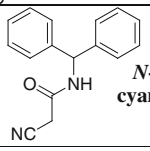
established on the basis of IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analysis.

These synthesized coumarin derivatives were subjected to tests for antifungal and antibacterial activities, as described below using different bacterial and fungal strains. The results are tabulated in Tables 3, 4, 5, and 6.

Antifungal activity

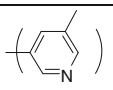
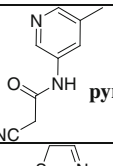
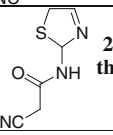
We have evaluated the antifungal activity of synthesized coumarin derivatives against specific fungi, i.e. *Candida albicans* and *Aspergillus niger,* using fluconazole as control fungicide. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with an antibiotic zone scale in mm and each test was performed in triplicate and the MICs reported the result of at least three repetitions. All 12 molecules 3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)-acrylonitrile(4a), 3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(piperidine-1-carbonyl)-acrylonitrile(4b), 2-cyano-*N*-cyclohexyl-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4c), *N*-benzyl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4d), 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(1-phenyl–ethyl)-acrylamide(4e), 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[1-(4-methoxyphenyl)-ethyl]-acrylamide(4f), 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(2-nitro-phenyl)-acrylamide(4g), 2-cyano-*N*-(3,5-dimethoxy-benzyl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4h), 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide(4i), *N*-benzhydryl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (4j), 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(5-methyl-pyridin-3-yl)-acrylamide (4k),

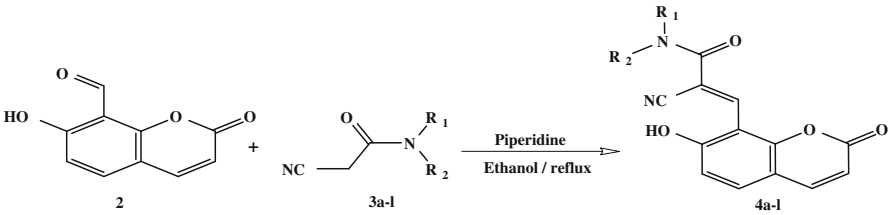
Table 1 Synthesis of *N,N*-disubstituted cyanoacetamide derivatives (3a-l)

Compound	R_1	R_2	structure
3a			 **3-Oxo-3-pyrrolidin-1-yl-propionitrile(3a)**
3b			 **3-Oxo-3-piperidin-1-yl-propionitrile(3b)**
3c	-H		 **2-Cyano-*N*-cyclo hexyl-acetamide(3c)**
3d	-H		 ***N*-Benzyl-2-cyano-acetamide(3d)**
3e	-H		 **2-Cyano-*N*-(1-phenyl-ethyl)-acetamide(3e)**
3f	-H		 **2-Cyano-*N*-[1-(4-methoxy-phenyl)-ethyl]-acetamide(3f)**
3g	-H		 **2-Cyano-*N*-(2-nitro-phenyl)-acetamide(3g)**
3h	-H		 **2-Cyano-*N*-(3,5-dimethoxy-benzyl)-acetamide(3h)**
3i	-H		 **2-Cyano-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acetamide(3i)**
3j	-H		 ***N*-Benzhydryl-2-cyano-acetamide(3j)**

2-cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4l) shows excellent anti fungal results against fluconazole, the best marketed antifungal drug. The results are shown in Table 3.

Table 1 continued

3k	-H		 **2-Cyano-*N*-(5-methyl-pyridin-3-yl)-acetamide(3k)**
3l	-H		 **2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-acetamide(3l)**



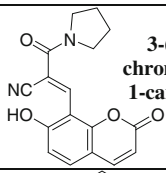
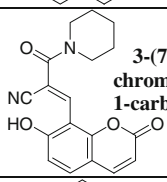
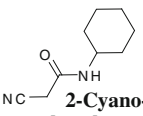
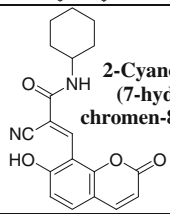
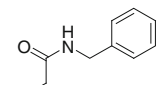
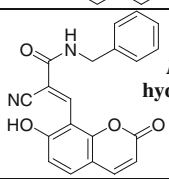
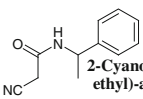
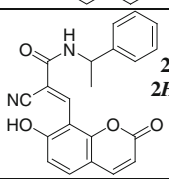
Scheme 3 Synthesis of 3-Oxo-3-pyrrolidin-1-yl-propionitrile(3a), 3-Oxo-3-piperidin-1-yl-propionitrile (3b), 2-Cyano-*N*-cyclohexyl-acetamide(3c), *N*-Benzyl-2-cyano-acetamide (3d),2-Cyano-*N*-(1-phenyl–ethyl)-acetamide(3e),2-Cyano-*N*-[1-(4-methoxy-phenyl)ethyl] -acetamide(3f), 2-Cyano-*N*-(2-nitro-phenyl)-acetamide(3 g), 2-Cyano-*N*-(3,5-dimethoxy-benzyl)-acetamide(3 h), 2-Cyano-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acetamide (3i), *N*-Benzhydryl-2-cyano-acetamide(3j), 2-Cyano-*N*-(5-methyl-pyridin-3-yl)-acetamide(3k), 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-acetamide(3l) derivatives of 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(2). Reagents and conditions: ethanol, piperidine, 0–5 °C, 2 h

The result of zone of inhibition is also encouraging as the disc containing fluconazole was purchased and the same amount of synthesized molecules were loaded on separate discs. The result are shown in Table 4. There is a wide inhibition zone around 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acryl-amide (4i), 2-cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (4l) were observed against fluconazole after 5 days of incubation has been shown in Fig. 1.

Twelve derivatives (4a–4l) show good positive results on multiresistant organism. The most encouraging results were obtained in case of 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide(4i), 2-cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4l) against *A. niger* and against *C. albicans,* Fluconazole, the best marketed antifungal drug as control, the MIC values of these compounds are 3 times more effective than fluconazole at the similar concentrations. From the results shown in the Table 3 we can infer that all synthesized molecules 4a–4l show almost double or equal activity against *A. niger* and *C. albicans*.

The result of zone of inhibition of fluconazole was 8–10 mm while the synthesized molecule 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-

Table 2 Synthesis of novel coumarin derivatives (4a-l)

Compound	Structure of 3a-l	Structure of 4a-l
4a	 **3-Oxo-3-pyrrolidin-1-yl-propionitrile(3a)**	 **3-(7-Hydroxy-2-oxo-2*H*-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)-acrylonitrile(4a)**
4b	 **3-Oxo-3-piperidin-1-yl-propionitrile(3b)**	 **3-(7-Hydroxy-2-oxo-2*H*-chromen-8-yl)-2-(piperidine-1-carbonyl)-acrylonitrile(4b)**
4c	 **2-Cyano-*N*-cyclo hexyl-acetamide(3c)**	 **2-Cyano-*N*-cyclohexyl-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-acrylamide(4c)**
4d	 ***N*-Benzyl-2-cyano-acetamide(3d)**	 ***N*-Benzyl-2-cyano-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-acrylamide(4d)**
4e	 **2-Cyano-*N*-(1-phenyl-ethyl)-acetamide(3e)**	 **2-Cyano-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-*N*-(1-phenyl-ethyl)-acrylamide (4e)**

methoxy-phenoxy)-ethyl]-acryl amide (4i) 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acryl amide (4l) shows 14–16 mm.

Antibacterial activity

A susceptibility test in vitro was done on multi-resistant bacteria especially those causing secondary infections in human beings, for example *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginos*. The results are summarized in Tables 5 and 6.

The antibacterial activity of coumarin derivatives (4a–4l) was compared with the known microdilution broth susceptibility test method.

Table 2 continued

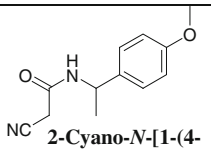
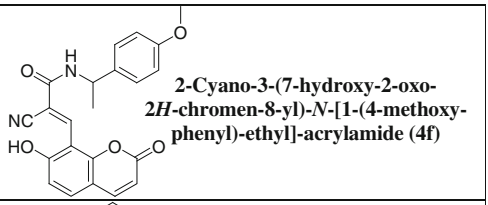
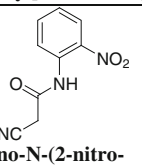
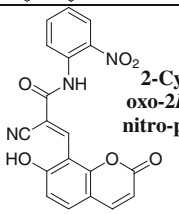
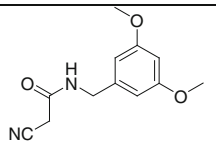
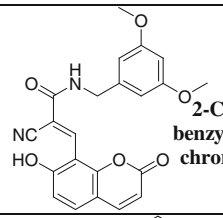
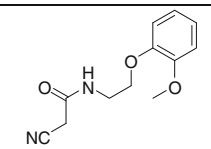
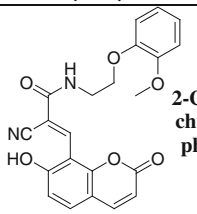
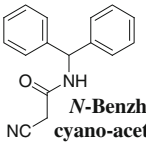
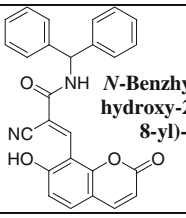
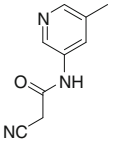
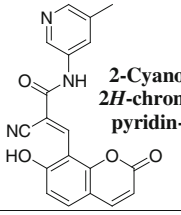
4f	 **2-Cyano-*N*-[1-(4-methoxy-phenyl)-ethyl]-acetamide(3f)**	 **2-Cyano-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-*N*-[1-(4-methoxy-phenyl)-ethyl]-acrylamide (4f)**
4g	 **2-Cyano-N-(2-nitro-phenyl)-acetamide(3g)**	 **2-Cyano-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-*N*-(2-nitro-phenyl)-acrylamide(4g)**
4h	 **2-Cyano-N-(3,5dimethoxy-benzyl)-acetamide(3h)**	 **2-Cyano-*N*-(3,5-dimethoxy-benzyl)-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-acrylamide(4h)**
4i	 **2-Cyano-N-[2-(2-methoxy-phenoxy)-ethyl] acetamide (3i)**	 **2-Cyano-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide(4i)**
4j	 ***N*-Benzhydryl-2-cyano-acetamide(3j)**	 ***N*-Benzhydryl-2-cyano-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-acrylamide(4j)**
4k	 **2-Cyano-N-(5-methyl-pyridin-3-yl)-acetamide (3k)**	 **2-Cyano-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-*N*-(5-methyl-pyridin-3-yl)-acrylamide(4k)**

Table 2 continued

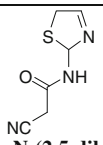
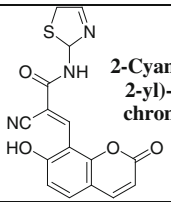
4l	 **2-Cyano-N-(2,5-dihydro-thiazol-2-yl)-acetamide(3l)**	 **2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2*H*-chromen-8-yl)-acrylamide(4l)**

Table 3 MIC (Minimum inhibitory concentration) Correlation Diagram (in μM/ml) against different fungal strains

Fungi	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	2	3	Control (Fluconazole)
Aspergillus niger	07	07	06	–	12	09	06	05	04	16	05	04	15	16	12
Candida albicans	06	06	06	–	10	08	06	06	04	12	05	05	14	14	14

(–) Resistant

3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)-acrylonitrile(4a), 3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(piperidine-1-carbonyl)-acrylonitrile(4b), 2-Cyano-*N*-cyclohexyl-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4c), *N*-Benzyl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4d), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(1-phenyl–ethyl)-acrylamide (4e), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[1-(4-methoxy-phenyl)-ethyl]-acrylamide(4f), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(2-nitro-phenyl)-acrylamide(4g),2-Cyano-*N*-(3,5-dimethoxy-benzyl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4h), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide(4i), *N*-Benzhydryl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4j), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(5-methyl-pyridin-3-yl)-acrylamide(4k), 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4l), 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(2), ethyl-2-cyano acetate(3)

The lowest concentration of synthesized molecules in μM/ml that prevented in vitro growth of microorganisms has been represented as correlated withthe zone of inhibition (Table 5) and MIC (minimum inhibitory concentration) (Table 6).

The result of the zone of inhibition is encouraging as the disc containing 20 μM/ml of norfloxacin was purchased and the same amount of synthesized molecules and 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(2), and ethyl-2-cyano acetate(3) were loaded on separate discs. From the results shown in the Table 5, the zone of inhibition of norfloxacin was 10 mm while the synthesized molecule 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide (4i), 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (4l) shows 12–16 mm against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginos*. Photographs showing the zone of inhibition in different bacterial strains by synthesized molecules are shown in Fig. 2.

Each test was performed in triplicate and the MICs reported represent the result of at least three repetitions. Twelve coumarin derivatives (4a–4l) show good

Table 4 Antifungal activity of coumarin derivatives (Zone of inhibition in mm) against different fungal stains

Fungi	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	2	3	Control (Fluconazole)
Aspergillus niger	08	10	08	04	04	10	10	08	16	–	10	14	2	4	08
Candida albicans	08	10	08	06	06	10	08	10	15	–	10	15	2	6	10

(–) resistant

3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)-acrylonitrile(4a), 3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(piperidine-1-carbonyl)-acrylonitrile(4b), 2-Cyano-*N*-cyclohexyl-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4c), *N*-Benzyl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide (4d), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(1-phenyl–ethyl)-acrylamide (4e), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[1-(4-methoxy-phenyl)-ethyl]-acrylamide(4f), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(2-nitro-phenyl)-acrylamide(4g),2-Cyano-*N*-(3,5-dimethoxy-benzyl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4h), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide(4i), *N*-Benzhydryl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4j), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(5-methyl-pyridin-3-yl)-acrylamide(4k), 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4l), 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(2), ethyl-2-cyano acetate(3)

Table 5 Antibacterial activity of coumarin derivatives (Zone of inhibition in mm) against different bacterial strains

Bacteria	4a	4b	4c	4d	4e	4f	4 g	4 h	4i	4j	4 k	4 l	2	3	Control (Norfloxacin)
Escherichia coli	10	–	–	06	05	–	08	06	14	–	–	12	–	2	10
Staphylococcus aureus	10	–	–	08	06	–	10	06	16	–	–	15	–	2	10
Pseudomonas aeruginosa		–		06	05	–	10	08	14	–	–	14	–	4	10

(–) resistant

3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)-acrylonitrile(4a), 3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(piperidine-1-carbonyl)-acrylonitrile(4b), 2-Cyano-*N*-cyclohexyl-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4c), *N*-Benzyl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4d), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(1-phenyl–ethyl)-acrylamide (4e), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[1-(4-methoxy-phenyl)-ethyl]-acrylamide(4f), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(2-nitro-phenyl)-acrylamide(4 g),2-Cyano-*N*-(3,5-dimethoxy-benzyl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4 h), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide(4i), *N*-Benzhydryl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4j), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-N-(5-methyl-pyridin-3-yl)-acrylamide(4 k), 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4 l)

positive results on multi-resistant organisms. The most encouraging results were obtained in the case of 2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acryl- amide (4i), 2-cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acryl amide (4l) having MIC value 4–6 μM/ml

Table 6 MIC (minimum inhibitory concentration) correlation diagram (in μM/ml) against different bacterial strains

Bacteria	4a	4b	4c	4d	4e	4f	4 g	4 h	4i	4j	4 k	4 l	2	3	Control (Norfloxacin)
Escherichia coli	12	–	–	14	15	–	10	12	03	–	–	04	–	–	10
Staphylococcus aureus	16	–	–	12	14	–	12	12	06	–	–	05	–	14	10
Pseudomonas aeruginosa	12	–	–	12	16	–	10	14	04	–	–	07	–	14	10

(–) resistant

3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(pyrrolidine-1-carbonyl)-acrylonitrile(4a), 3-(7-Hydroxy-2-oxo-2H-chromen-8-yl)-2-(piperidine-1-carbonyl)-acrylonitrile(4b), 2-Cyano-*N*-cyclohexyl-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4c), *N*-Benzyl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4d), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(1-phenyl–ethyl)-acrylamide (4e), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[1-(4-methoxy-phenyl)-ethyl]-acrylamide(4f), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(2-nitro-phenyl)-acrylamide(4 g),2-Cyano-*N*-(3,5-dimethoxy-benzyl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4 h), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-[2-(2-methoxy-phenoxy)-ethyl]-acrylamide(4i), *N*-Benzhydryl-2-cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4j), 2-Cyano-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-*N*-(5-methyl-pyridin-3-yl)-acrylamide(4 k), 2-Cyano-*N*-(2,5-dihydro-thiazol-2-yl)-3-(7-hydroxy-2-oxo-2H-chromen-8-yl)-acrylamide(4 l) 7-hydroxy-2-oxo-2H-chromene-8-carbaldehyde(2), ethyl-2-cyano acetate(3)

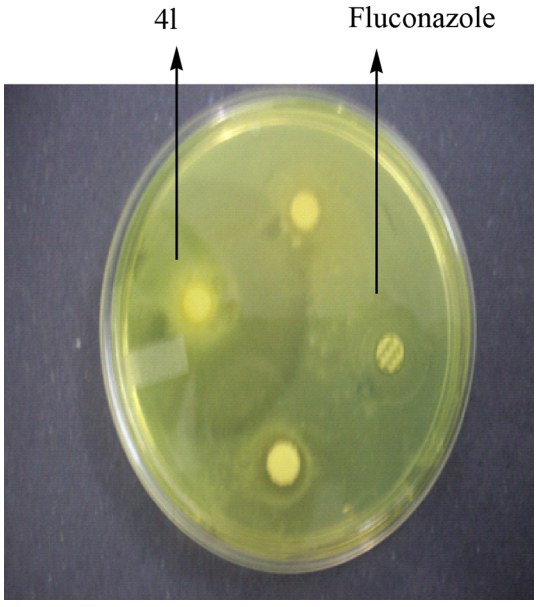


Fig. 1 The zone of inhibition in fungal strain (*A. niger*) by synthesized molecule fluconazole and 4l

against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginos*, while norfloxacin, the best marketed antibiotic, shows MIC of 10 μM/ml, thus these derivatives were 2 times more effective than norfloxacin at similar concentrations. The results are shown in Table 6.

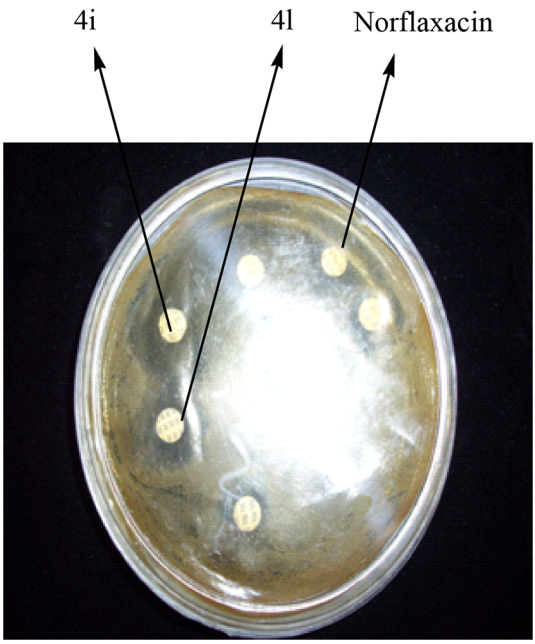


Fig. 2 The zones of inhibition by different bacterial strains by synthesized molecules 4i and 4l

Conclusion

The present method describes a simple efficient method for the synthesis of biologically active 8-Formyl-7-hydroxy coumarin derivatives from corresponding N,N-disubstituted cyano acetamides in the presence of catalytic amounts of piperidine. The notable feature of the method of preparation is that the process is economically viable, i.e. enhanced rate of reaction and negligible byproducts, and cleaner reaction profiles which makes it a useful and attractive process, specially for synthesis. The generality and simple experimental and product isolation procedures certainly play an important role in the development of a greener and much efficient commercial synthesis of coumarin derivatives. These 12 coumarin derivatives (4i–4l) show good positive results on multi-resistant organisms.

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