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Reactions of coumarin-3-carboxylate, its crystallographic study and antimicrobial activity

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ABSTRACT

Synthesis of various phenyl hydrazide derivatives have been carried out by reaction of various coumarin-3-carboxylate derivatives with phenyl hydrazine. The reaction of coumarin-3-carboxylate with hydrazine hydrate gave novel Schiff base products. The structures of these compounds have been confirmed by X-ray Single Crystal analysis. Mechanism for formation of Schiff base is proposed. All compounds were screened for antimicrobial activity against two Gram positive bacteria S. aureus and B. subtilis, two Gram negative bacteria E. coli and P. aeruginosa and one fungus C. albicans.

Keywords: coumarin-3-carboxylate, hydrazide, Schiff bases, single crystal

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INTRODUCTION

Coumarin-3-carboxylate derivatives possess wide range of activities [1-5]. Various amide derivatives of coumarin-3-caboxylate have showed anti-helicobacter pylori activity [1, 6] and various 3-carbonyl, acyl and hydrazide derivatives of coumarin-3-caboxylate have been reported to show human monoamine oxidase inhibition [2]. The ester and amide derivatives of 6-chloromethyl coumarin-3-carboxylic acid have been showed α-chymotrypsin inhibitory activity [3]. Various amide and ester derivatives of coumarin-3-carboxylate have been reported to show anticancer activity [5] or inhibition of enzyme gGAPDH [4].

Coumarin-3-carbohydrazide [7, 8] is an important class of compound, from which synthesis of variety of heterocyclic rings have been reported to get variety of amide derivatives of coumarin-3-carboxylate having antimicrobial [7, 9-11] anticonvulsant activity [8] and analgesic activity [12]. Oxazadiazole derivatives from naphthocoumarin-3-carboxylate [13] were reported as strong fluorescent brighteners [13].

In continuation of our work on 3-substituted coumarins [14, 15] and their activity, we have synthesized various coumarin-3-carboxylate **2a-d** by reported procedure. In our attempt to synthesize various heterocycles from hydrazide derivatives of coumarin-3-carboxylate using reported procedures [8, 9, 12]. We report herein formation of novel products, the structure of which was proved by IR, ^{1}H NMR, ^{13}C NMR, elemental analyses and X-ray single crystal analyses.

MATERIALS AND METHODS

Chemistry

Reagent grade chemicals and solvents were purchased from commercial supplier and used without purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (400 MHz) with $CDCl_3$ or

DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI/MS, using a Shimadzu LCMS 2020 apparatus. Elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA. X-ray diffraction data were collected using Mo X-ray source (λ= 0.71073 A°) and Cu X-ray source (λ= 1.54180 A°) radiation on Xcalibur, Eos, Gemini diffractometer equipped with a CCD area detector. Data collection, data reduction, structure solution/refinement were carried out using the software package of OLEX 1.2.2. Graphics were generated using MERCURY 2.2.

General procedure for synthesis of 2a-d

Aldehydes **1a-d** (5 g, 1 eq.) dissolved in mixture of pyridine (10 ml) and piperidine (0.5 ml) and diethylmalonate (1.1 eq.) added in the reaction flask and reaction mixture heated at 60-70ºC for 6 hours in water bath. Reaction mass poured into crushed ice containing concentrated HCl (25 ml) which gave solid product. It was filtered, dried and crystallized using ethanol afforded pure products **2a-d**.

Spectral Data:

Ethyl 8-methoxy-2-oxo-2H-chromene-3-carboxylate (2a)

This compound obtained as white solid. Yield 47%; mp 90-95°C; IR (KBr, cm^{-1}): 3053, 2996, 2978, 2949, 2847, 1755, 1731; ^{1}H NMR ($CDCl_3$, δ ppm): 1.40-1.44 (3H, t, CH_3), 3.98 (3H, s, OCH_3), 4.40-4.45 (2H, q, OCH_2), 7.18-7.20 (2H, m, *J*= 1.2, 7.6 Hz, ArH), 7.27-7.30 (1H, m, ArH), 8.52 (1H, s, C-4 H); ^{13}C NMR ($CDCl_3$, δ ppm): 14.2, 56.3, 62.0, 115.8, 118.4, 118.5, 120.6, 124.7, 144.9, 147.1, 148.9, 156.2, 163.1; Mol. Formula: $C_{13}H_{12}O_4$.

Ethyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate (2b)

This compound obtained as brown solid, Yield 42%; mp 162-166°C (Lit. 166-167°C [16]); IR (KBr, cm^{-1}): 3542, 3471, 1728, 1621; ^{1}H NMR (DMSO, d_6, δ ppm): 1.27-1.30 (3H, t, CH_3), 4.22-4.28 (2H, q, OCH_2), 6.72-6.73 (1H, d, *J*= 2.0 Hz, ArH), 6.82-6.85 (1H, dd, *J*= 2.4, 8.8 Hz, ArH), 7.74-7.76 (1H, d, *J*=8.8 Hz, ArH), 8.67 (1H, s, C-4 H), 11.12 (1H, s, OH); ^{13}C NMR (DMSO, d_6, δ ppm): 19.3, 66.1, 107.0, 115.6, 117.2, 119.2, 137.3, 154.7, 161.7, 162.3, 168.2, 169.3; ESI/MS m/z 235.2 $[M+1]^+$, base peak at m/z 189.1 for M-OCH_2CH_3 and 233.3 $[M-1]^+$ calculated for $C_{12}H_{10}O_5$.

Ethyl 2-oxo-2H-chromene-3-carboxylate (2c)

This compound obtained as white solid. mp 91-93°C (Lit. 93°C [16]); Yield 45 % (4.07 g); IR (KBr, cm^{-1}): 3061, 2977, 2916, 1767, 1609; ^{1}H NMR ($CDCl_3$, δ ppm): 1.41-1.45 (3H, t, CH_3), 4.41-4.46 (2H, q, OCH_2) 7.34-7.39 (2H, m, ArH), 7.63-7.69 (2H, m, ArH), 8.56 (1H, s, C-4 H); ^{13}C NMR ($CDCl_3$, δ ppm): 14.2, 62.0, 116.8, 117.9, 118.3, 124.9, 129.5, 134.4, 148.7, 155.2, 156.8, 163.1; ESI/MS m/z 219.1 $[M+1]^+$ and base peak at m/z 173.2 for M-OCH_2CH_3 calculated for $C_{12}H_{10}O_4$.

Ethyl 3-oxo-3H-benzo[f]chromene-2-carboxylate (2d)

This compound obtained as light yellow solid. Yield 46%; mp 115-117°C (Lit. 117 °C [13]); IR (KBr, cm^{-1}): 3128, 1631; ^{1}H NMR ($CDCl_3$, δ ppm): 1.46-1.50 (3H, t, CH_3), 4.48-4.53 (2H, q, OCH_2), 7.49-7.51 (1H, d, *J*= 9.2 Hz, ArH), 7.62-7.66 (1H, m, ArH), 7.76-7.80 (1H, m, ArH), 7.95-7.97 (1H, d, *J*= 8.0 Hz, ArH), 8.12-8.14 (1H, d, *J*= 9.2 Hz, ArH), 8.35-8.37 (1H, d, *J*= 8.0 Hz, ArH) 9.35 (1H, s, C-3 H); ^{13}C NMR ($CDCl_3$, δ ppm): 14.3, 62.1, 112.3, 116.5, 116.7, 121.5, 126.6, 129.1, 129.3, 129.4, 130.2, 136.2, 144.6, 156.0, 156.9, 163.6; ESI/MS m/z 268.9 $[M+1]^+$ and base peak at m/z 222.9 for M-OCH_2CH_3 calculated for $C_{16}H_{12}O_4$.

General procedure for synthesis of 3a-d

A solution of **2a-d** (1 g, 1 eq.) in ethanol (50 ml) and phenyl hydrazine (1.1 eq.) refluxed for 8 hours in water bath. Solvent distilled under reduced pressure and reaction mass poured into ice. Crude product extracted in ethyl acetate, dried over sodium sulphate and concentrated under reduced pressure. Crude product was purified by column chromatography using petroleum ether (60-80°C): ethyl acetate (8:2) as eluent.

Spectral Data:

8-Methoxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide (3a)

This compound obtained as light brown solid. Yield 45%; mp 236-240°C; IR (KBr, cm^{-1}): 3324, 3214, 1722, 1654, 1608; ^{1}H NMR ($CDCl_3$, δ ppm): 4.03 (3H, s, OCH_3), 6.93-6.96 (3H, m, ArH), 7.24-7.37 (6H, m, ArH), 8.91 (1H, s, NH), 10.41 (1H, s, NH, D_2O exch.); ^{1}H NMR ($CDCl_3$, δ ppm): 56.4, 113.9, 115.9, 119.1, 121.0, 121.5, 125.4, 129.2, 147.1, 149.5, 160.6; Elemental Analysis for $C_{17}H_{14}N_2O_4$; Calculated, %: C, 65.80; H, 4.55; N, 9.03; Found, %: C, 66.03; H, 4.29; N, 9.33.

7-Hydroxy-2-oxo-N'-phenyl-2H-chromene-3-carbohydrazide (3b)

This compound obtained as brown solid. Yield 49%; mp 158-160°C; IR (KBr, cm^{-1}): 3484, 3402, 3313, 3054, 3032, 2971, 2891, 2816, 1670, 1629; ^{1}H NMR (DMSO, d_6, δ ppm): 6.30-6.33 (2H, m, ArH), 6.73 (1H, s, ArH), 6.88-6.90

(2H, m, *J*= 0.8, 7.6 Hz, ArH), 7.20-7.29 (3H, m, ArH), 8.04 (1H, s, C-4 H), 9.74 (1H, s, NH, D_2O exch.), 10.14 (1H, s, OH, D_2O exch.), 10.75 (1H, s, NH, D_2O exch.); ^{13}C NMR (DMSO, d_6, δ ppm): 102.9, 107.9, 111.9, 112.4, 118.9, 129.5, 129,7, 139.6, 145.5, 157.9, 159.4; Elemental Analysis for $C_{16}H_{21}N_2O_4$; Calculated, %: C, 64.86; H, 4.08; N, 9.46; Found, %: C, 64.62; H, 3.81; N, 9.73.

2-Oxo-N'-phenyl-2H-chromene-3-carbohydrazide (3c)

This compound obtained as light brown solid. mp 142-145°C; Yield 49 % (0.63 g); IR (KBr, cm^{-1}): 3440, 3202, 3052, 1701, 1621; ^1H NMR ($CDCl_3$, δ ppm): 6.91-6.97 (2H, m, ArH), 7.00-7.04 (3H, m, ArH), 7.16-7.19 (1H, dd, *J*= 1.6, 7.6 Hz, ArH), 7.24-7.29 (1H, m, ArH), 7.31-7.35 (2H, m, ArH), 7.52 (1H, s, NH), 7.88 (1H, s, C-4 H), 10.89 (1H, s, NH); ^{13}C NMR ($CDCl_3$, δ ppm): 98.6, 115.0, 118.9, 120.8, 121.9, 123.3, 131.8, 132.0, 132.4, 143.5, 145.7, 159.2; Elemental Analysis for $C_{16}H_{12}N_2O_3$; Calculated, %: C, 68.56; H, 4.32; N, 9.99; Found, %: C, 68.37; H, 4.60; N, 10.27.

3-Oxo-N'-phenyl-3H-benzo[f]chromene-2-carbohydrazide (3d)

This compound obtained as light brown solid. Yield 52%; mp 242-244°C; IR (KBr, cm^{-1}): 3334, 3294, 3051, 1708, 1680; ^1H NMR ($CDCl_3$, δ ppm): 6.94-7.00 (3H, m, ArH), 7.29-7.31 (3H, d, *J*= 8.4 Hz, ArH), 7.56-7.58 (1H, d, *J*= 8.8 Hz, ArH), 7.65-7.69 (1H, t, *J*= 7.2, 7.6 Hz, ArH), 7.78-7.82 (1H, t, *J*= 7.2, 7.6 Hz, ArH), 7.98-8.00 (1H, d, *J*= 8 Hz, ArH), 8.17-8.20 (1H, d, *J*= 8.8 Hz, ArH), 8.43-8.45 (1H, d, *J*= 8.4 Hz, ArH), 9.71 (1H, s, NH, D_2O Exch.), 10.48 (1H, s, NH); ^{13}C NMR ($CDCl_3$, δ ppm): 113.3, 114.0, 116.4, 121.6, 122.0, 127.0, 129.2, 129.3, 129.4, 129.5, 130.4, 136.5, 144.8, 155.3; ESI/MS m/z 332.1 $[M+2]^+$ calculated for $C_{20}H_{14}N_2O_3$; Elemental Analysis for $C_{20}H_{14}N_2O_3$; Calculated, %: C, 72.72; H, 4.27; N, 8.48; Found, %: C, 72.86; H, 4.11; N, 8.68.

General procedure for synthesis of 4a-c and 5d

A solution of **2a-d** (5 g, 1 eq.) in ethanol (50 ml) and hydrazine hydrate (1.1 eq.) refluxed for 8 hours in water bath. Solvent removed under reduced pressure and reaction mass poured into crushed ice to obtain solid. Crude product filtered, dried and crystallized using ethanol to give products.

Spectral Data:

6,6'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxy phenol) (4a)

This compound obtained as yellow solid. Yield 43%; mp 152-155°C; IR (KBr, cm^{-1}): 3390, 3295, 3213, 3076, 3005, 2963, 2939, 2839, 1627, 1578; ^1H NMR ($CDCl_3$, δ ppm): 3.96 (3H, s, OCH_3), 6.92-6.96 (1H, t, *J*= 7.6, 8.0 Hz, ArH), 7.02-7.04 (2H, m, ArH), 8.73 (1H, s, CH=N), 11.61 (1H, s, OH, D_2O exch.); ^{13}C NMR ($CDCl_3$, δ ppm): 56.2, 115.1, 117.3, 119.4, 124.1, 148.3, 149.7, 164.9; ESI/MS m/z 301.0 $[M+1]^+$ calculated for $C_{16}H_{16}N_2O_4$.

4,4'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)dibenzene-1,3-diol (4b)

This compound obtained as yellow solid. Yield 54%; mp 220-240°C (dec.); IR (KBr, cm^{-1}): 3467, 3223, 1634, 1615; ^1H NMR (DMSO, d_6, δ ppm): 6.32-6.33 (1H, d, *J*= 2.0 Hz, ArH), 6.38-6.41 (1H, dd, *J*= 2.0, 8.4 Hz, ArH), 7.41-7.43 (1H, d, *J*= 8.8 Hz, ArH), 8.77 (1H, s, CH=N), 10.29 (1H, s, OH, D_2O exch.), 11.42 (1H, s, OH, D_2O exch.); ^{13}C NMR (DMSO, d_6, δ ppm): 102.9, 108.7, 110.7, 133.4, 161.1, 162.3, 162.5; ESI/MS m/z 273.2 $[M+1]^+$ and 271.1 $[M-1]^+$ calculated for $C_{14}H_{12}N_2O_4$.

2,2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol (4c)

This compound obtained as yellow solid. mp 210-215°C; Yield 40 % (0.97 g); IR (KBr, cm^{-1}): 3436, 3290, 1622, 1603; ^1H NMR ($CDCl_3$, δ ppm): 6.96-7.00 (2H, m, ArH), 7.39-7.43 (1H, m, ArH), 7.69-7.72 (1H, dd, *J*= 1.6, 7.6 Hz ArH), 9.02 (1H, s, CH=N), 11.15 (1H, s, OH); ^{13}C NMR ($CDCl_3$, δ ppm): 117.0, 118.6, 120.1, 125.8, 131.3, 133.7, 159.1, 163.3; ESI/MS *m/z* 241.1 $[M+1]^+$ and 239.0 $[M-1]^+$ calculated for $C_{14}H_{12}N_2O_2$; Elemental Analysis for $C_{14}H_{12}N_2O_2$; Calculated, %: C, 69.99; H, 5.03; N, 11.66; Found, %: C, 69.96; H, 4.55; N, 11.69.

3-Oxo-3H-benzo[f]chromene-2-carbohydrazide (5d)

This compound obtained as yellow solid. Yield 16%; mp 280-282°C; IR (KBr, cm^{-1}): 3323, 3282, 3019, 1708, 1622, 1580; ^1H NMR (DMSO, d_6, δ ppm): 4.79 (2H, s, NH_2), 7.66-7.70 (2H, t, *J*= 7.6, 8.8 Hz, ArH), 7.79-7.82 (1H, m, *J*= 7.6 Hz, ArH), 8.11-8.13 (1H, d, *J*= 8.0 Hz, ArH), 8.33-8.35 (1H, d, *J*= 8.8 Hz, ArH), 8.62-8.64 (1H, d, *J*= 8.4 Hz, ArH), 9.43 (1H, s, C-4 H), 9.70 (1H, s, NH); ^{13}C NMR (DMSO, d_6, δ ppm): 113.4, 116.9, 118.4, 122.8, 127.1, 129.5, 130.4, 136.0, 142.3, 154.4, 161.0; ESI/MS m/z 254.9 $[M+1]^+$ calculated for $C_{14}H_{10}N_2O_3$.

Single Crystal X-Ray Diffraction

X-ray quality single crystals of 6,6'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxy phenol) **4a**, 2,2'-(1E,1'E)-hydrazine-1,2-diylidenebis(methan-1-yl-1-ylidene)diphenol **4c** and 3-oxo-3H-benzo[f]chromene-2-carbohydrazide **5d** were grown by slow evaporation condition at room temperature. Crystals were obtained from a mixture of methanol: acetone (1:1). The structure was solved by direct methods and refined in

routine manner. All hydrogen atoms were geometrically fixed and refined.

Crystal Data for **4a** (CCDC 968041): $C_{16}H_{16}N_2O_4$, Monoclinic, Space group P21/n, a (Å)= 5.98503(15), b (Å)= 18.6664(5), c (Å)= 6.86260(17), a (°)= 90.00, b (°)= 106.399(3), g (°)= 90.00, Volume (Å3)= 735.49(3), Z= 3, Density (mg/m^3)= 1.3560, Wavelength (Å)= 0.71073, F(000)= 316.2, R1= 0.0378, wR2= 0.0826, Largest diff. peak and hole (eA^{-3})= 0.15/-0.11.

Crystal Data for **4c** (CCDC 968039): $C_{14}H_{12}N_2O_2$, Monoclinic, Space group P21/n, a (Å)= 8.5070(3), b (Å)= 6.2955(2), c (Å)= 11.7908(4), a (°)= 90.00, b (°)= 107.941(2), g (°)= 90.00, Volume (Å^3)= 600.76(4), Z= 2, Density (mg/m3)= 1.328, Wavelength (Å)= 0.71073, F(000)= 252.0, R1= 0.0455, wR2= 0.0942, Largest diff. peak and hole (eA^{-3})= 0.13/-0.14.

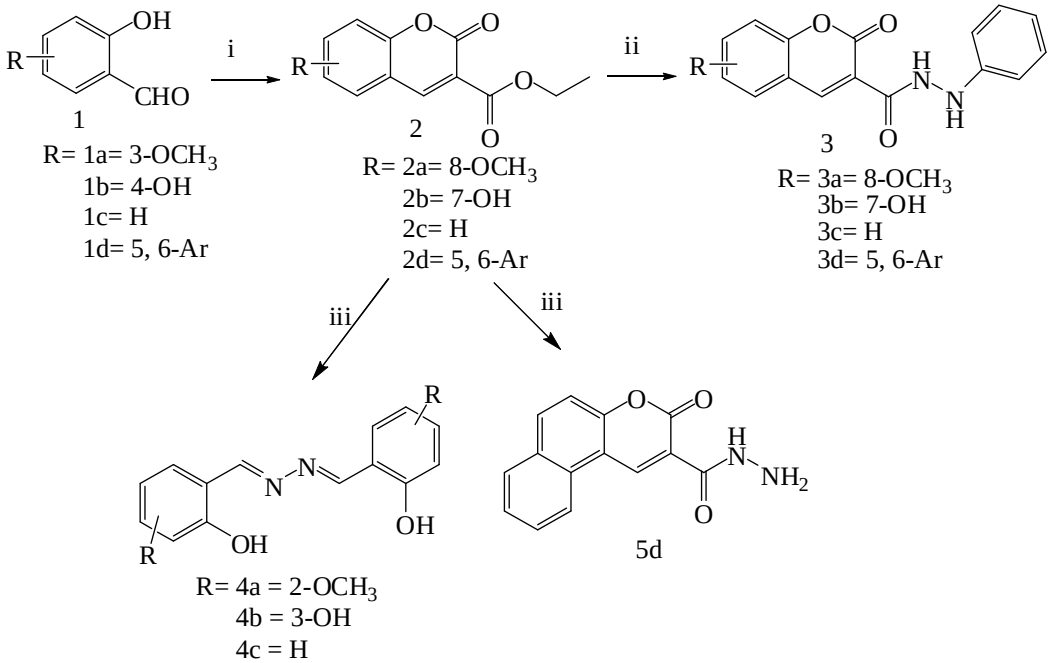
Crystal Data for **5d** (CCDC 968040): $C_{17}H_{14}N_2O_3$.H2O, Triclinic, Space group P-1, a (Å)= 7.9556(3), b (Å)= 10.0729(4), c (Å)= 10.6217(5), a (°)= 79.607(4), b (°)= 73.525(4), g (°)= 67.657(4), Volume (Å^3)= 752.42(6), Z= 2, Density (mg/m3)= 1.3785, Wavelength (Å)= 1.54180, F(000)= 329.1, R1= 0.0455, wR2= 0.1317, Largest diff. peak and hole (eA^{-3})= 0.21/-0.15.

RESULTS AND DISCUSSION

Chemistry

Knovenagel reaction of various substituted salicylaldehyde derivatives **1a-d** with diethylmalonate using pyridine and catalytic amount of piperidine gave corresponding substituted coumarin-3-carboxylate derivatives [9] **2a-d** as shown in Scheme 1. IR spectrum of **2a** exhibited broad band at 1755 and 1731 cm^{-1} for carbonyl group of lactone as well as ester. In ^{1}H NMR, triplet at δ 1.40 to 1.44 for three protons and quartet at δ 4.40 to 4.45 for two protons indicated presence of –OCH_2CH_3 group, singlet at δ 3.98 for three protons indicated presence of –OCH_3 group at C-8 position, multiplet between δ 7.18 to 7.30 for three protons indicated aromatic ring protons, while singlet at δ 8.52 for one proton indicated proton at C-4 position, thus confirmed the formation of **2a**. In ^{13}C NMR of this compound, peaks at δ 156.2 and 163.1 indicated ester and lactone carbonyl group carbon.

Various coumarin-3-carboxylate derivatives **2a-d** when refluxed with phenyl hydrazine in ethanol gave corresponding phenyl hydrazide derivatives [2, 6] **3a-d**. IR spectrum of **3a** exhibited band at 3324 cm^{-1} for –NH stretching vibrations. In ^{1}H NMR of **3a**, now the absence of triplet and quartet at δ 1.40 and 4.40 for –OCH_2CH_3 group clearly indicated formation of phenyl hydrazide. In ^{1}H NMR of **3a**, singlet at δ 4.03 for three protons indicated –OCH_3 group at C-8 position, multiplet at δ 6.93 to 7.37 for nine protons indicated nine aromatic protons, while singlet at δ 8.91 and 10.41 for one proton each indicated two –NH protons confirmed the formation of **3a**. In ^{13}C NMR of **3a**, two peaks at δ 149.5 and 160.0 for one carbon each indicated -CONH and lactone carbonyl carbons.



R= 1a= 3-OCH_3
1b= 4-OH
1c= H
1d= 5, 6-Ar

R= 2a= 8-OCH_3
2b= 7-OH
2c= H
2d= 5, 6-Ar

R= 3a= 8-OCH_3
3b= 7-OH
3c= H
3d= 5, 6-Ar

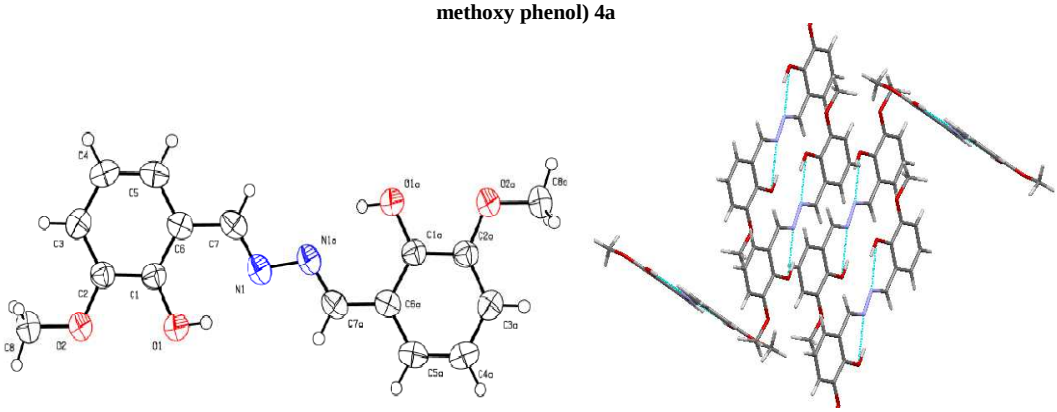
R= 4a = 2-OCH_3
4b = 3-OH
4c = H

Scheme 1: Reagents and Condition: (i) Diethylmalonate, Pyridine, Piperidine, 6 h (ii) Ethanol, Phenyl hydrazine, 8 h (iii) Ethanol, Hydrazine hydrate, 8 h

Various coumarin-3-carboxylate **2a-c** when refluxed with hydrazine hydrate in ethanol by reported procedures [7-12], were expected to give corresponding hydrazide derivatives. The spectral data of hydrazides was not matching with the corresponding structure. The attempt of further reaction of hydrazide, with carbon disulfide was also failed which indicated that it has not been formed. Hence single crystal of it was developed in methanol: acetone (1:1) and X-ray analysis of single crystal indicated formation of unexpected Schiff base **4a** (CCDC No. 968041). The ORTEP diagram is as shown in figure 1. Now the IR, ^{1}H NMR and ^{13}C NMR spectra were consistent with the structures **4a-c**.

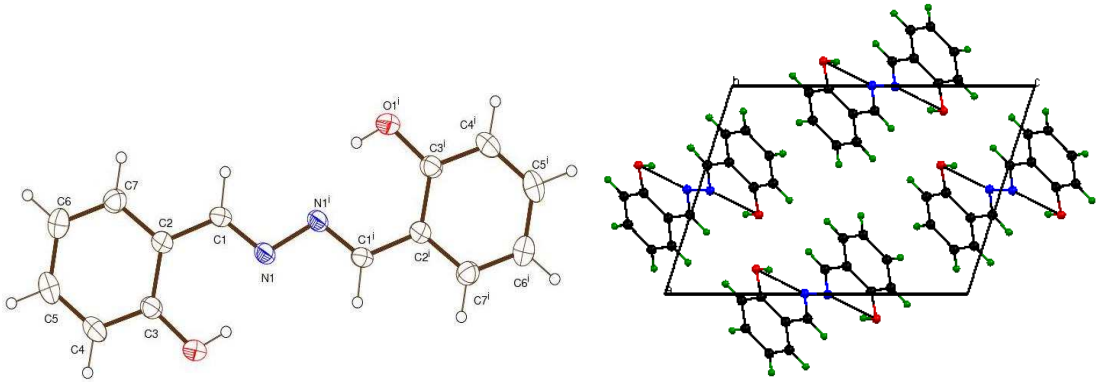
The IR spectrum of **4a** showed band at 1627 cm^{-1} for C=N. The ^{1}H NMR spectrum of **4a** showed singlet at δ 3.96 for three protons indicated –OCH_3 group. The peaks at δ 6.92 to 7.04 for three protons indicated aromatic protons. Singlet at δ 8.73 for one proton indicated –CH=N proton while singlet at δ 11.61 for one proton indicated –OH proton which disappeared on D_2O exchange. The ESI/MS spectrum of it showed $[M+1]^+$ peak at m/z 301.0 confirmed the structure of **4a**. Structure of **4a** was confirmed by its X-ray single crystal analysis which is as shown in figure 1 with CCDC No. 968041. Compound **4a** crystallizes in centrosymmetric monoclinic space group P21/n. Figure 1 shows ORTEP diagram with atom numbering scheme and the crystal packing diagram of **4a** generated in Mercury software.

Figure 1: ORTEP diagram and Crystal packing diagram of 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxy phenol) 4a



Similar Schiff base formations were observed for **4b-c**. Structure of **4c** was confirmed by its X-ray single crystal analysis which is as shown in figure 2 with CCDC No. 968039. Compound **4c** crystallizes in centrosymmetric monoclinic space group P21/n. Figure 2 shows ORTEP diagram with atom numbering scheme and the crystal packing diagram of **4c** generated in Mercury software.

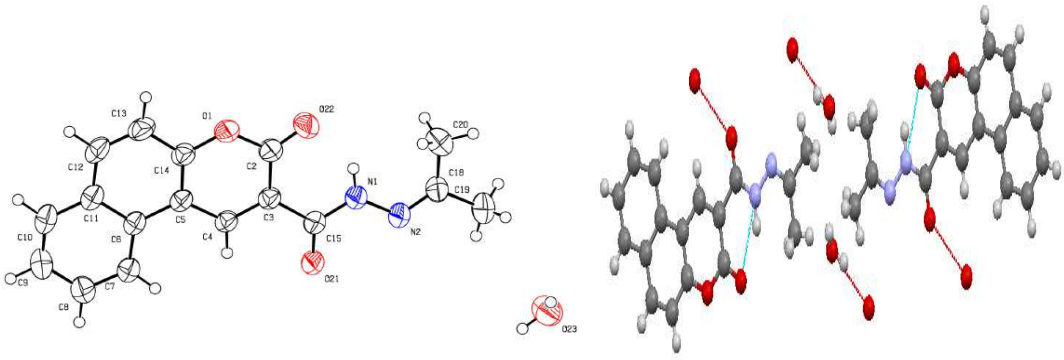
Figure 2: ORTEP diagram and Crystal packing diagram of 6, 6'-(1E, 1'E)-hydrazine-1, 2-diylidenebis(methan-1-yl-1-ylidene)bis(2-methoxy phenol) 4c



In case of reaction of naphthocoumarin-3-carboxylate **2d** with hydrazine hydrate, the product **5d** obtained was found to be hydrazide. The IR spectrum of **5d** showed band at 1704 and 1688 cm^{-1} indicated lactone carbonyl and amide carbonyl group. The ^{1}H NMR spectrum for **5d** in DMSO-d_6 showed broad peak at δ 4.8 for two protons indicated –NH_2 protons. Multiplet from δ 7.66 to 8.64 for six protons indicated all six aromatic protons of naphthalene ring. Singlet at δ 9.4 for one proton indicated proton at C-4 position. Another singlet at δ 9.7 for one proton indicted –NH proton. The ^{13}C NMR of **5d** showed two peaks for one carbon each at δ 154.4 and 161.0 indicated amide and lactone carbonyl carbon. The ESI/MS spectrum of **5d** showed $[M+1]^+$ peak at m/z 254.9 confirmed hydrazide structure. To prove the formation of hydrazide in case of **5d**, the single crystal was developed in methanol: acetone (1:1) system

and its X-ray single crystal analysis was carried out. In single crystal analysis of **5d** (CCDC No. is CCDC 968040, ORTEP diagram and crystal packing diagram as shown in figure 3), the formation of hydrazide was observed and the solvent acetone molecule was also observed in it and found to form Schiff base of acetone with free $–NH_2$ group of **5d**. The free $–NH_2$ group of hydrazide formed Schiff base with acetone during single crystal development. Compound **5d** crystallizes in triclinic space group P-1. Figure 3 shows ORTEP diagram with atom numbering scheme and the crystal packing diagram of **5d** generated in Mercury software.

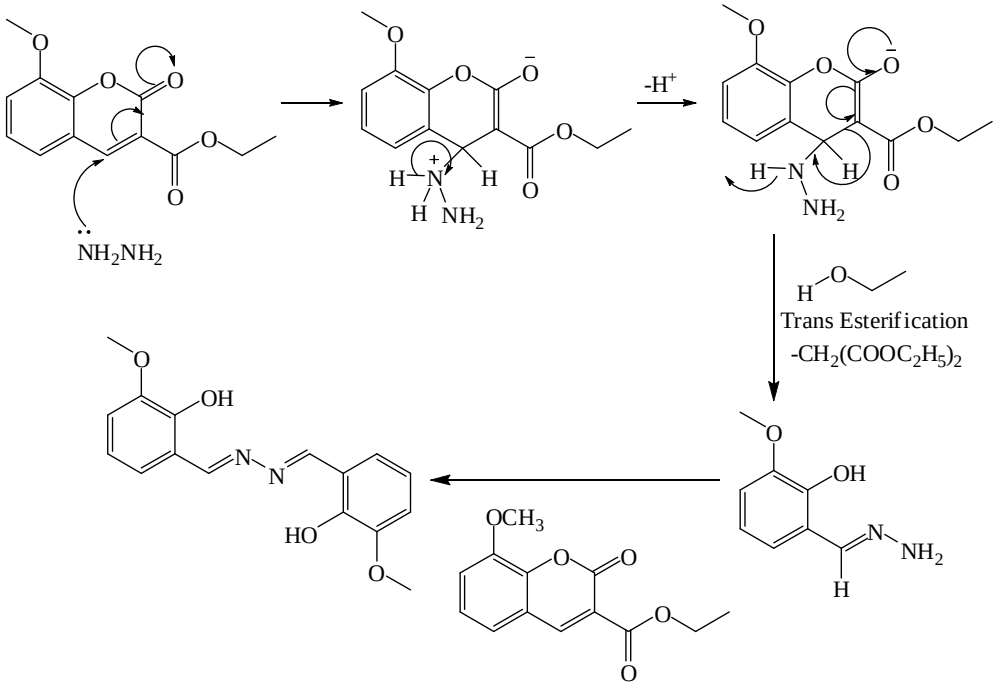
Figure 3: ORTEP diagram and Crystal packing diagram of adduct of 3-oxo-N'-(propan-2-ylidene)-3H-benzo[f]chromene-2-carbohydrazide 5d with acetone



Crystallographic data (excluding structural factors) for the structure in this article have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 968041 (**4a**), CCDC 968039 (**4c**) and CCDC 968040 (**5d**).

The formation of novel Schiff bases of **2a-c** can be possible only if retero Knovenagel reaction or reverse Michael reaction occurs. So the possible mechanism for it is suggested in Scheme 2. In case of **2a-c**, hydrazine hydrate attacks on β carbon of carbonyl instead of ester carbonyl, then lactone ring opens up and retero Knovenagel reaction or reverse Michael reaction occurs.

Scheme 2: Plausible Mechanism



While in case of **2d**, due to presence of naphthalene ring the attack on β carbon to carbonyl is sterically hindered, hence hydrazine hydrate attacks on ester carbonyl, thus gives hydrazide formation **5d**. The formations of phenyl hydrazides **3a-d** by reaction of **2a-d** with phenyl hydrazine also support the mechanism. Because of presence of phenyl ring (steric hindrance and less basicity) in phenyl hydrazine, it attacks only on ester carbonyl and not on β carbon of carbonyl, hence give phenyl hydrazides. The yields of reactions are less than 50% which also support the dimeric formation of products **4a-c**.

Biological activity

All the synthesized compounds were screened by Broth dilution method [17] for their antibacterial activity against two Gram positive bacteria *S. Aureus* and *B. Subtilis*, two Gram negative bacteria *E. Coli* and *P. Aeruginosa*. They were also evaluated for their *in vitro* antifungal activity against *C. Albicans*. Concentration of compounds was ranging from 20 µg to 600 µg. The lowest concentrations of the compounds that prevented visible growth are given in Table-1. It was determined that the solvent had no antibacterial or antifungal activities against any of the test microorganisms. Ciprofloxacin and Flucanazole were used as standard drugs also tested under the similar conditions for comparison. The minimum inhibitory concentration (MIC) of the synthesized compounds against highly inhibited organisms is reported in Table 1.

Table 1: MIC determination of antibacterial and antifungal agent (µg)

Sr. No.	MIC (µg)				
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
	(gm +ve bacteria)		(gm –ve bacteria)		fungi
3a	200	100	400	≥ 300	300
3b	100	100	≥ 600	≥ 300	≥ 300
3c	80	80	200	300	≥ 300
3d	200	200	600	300	≥ 300
4a	200	20	80	300	200
4b	100	20	80	300	200
4c	80	20	160	200	200
5d	80	40	60	200	200
Ciprofloxacin	5	2	15	7.5	-
Flucanazole	-	-	-	-	5

Compounds **3c**, **4c** and **5d** showed moderate effects against Gram positive bacteria *S. aureus* while compound **4a**, **4b**, **4c** and **5d** showed moderate activity against Gram positive bacteria *B. subtilis.* Compound **4a, 4b** and **5d** showed moderate activity against Gram negative bacteria *E. coli* while all these compounds found less active against *P. aeruginosa* (MIC more than 200 µg/ml). It was observed that phenyl hydrazide derivatives were inactive in all Gram +ve and Gram –ve bacteria while some of the Schiff bases were active against all Gram +ve and Gram –ve bacteria. Methoxy group at 2nd (**4a**) position and hydroxyl group (**4b**) at 3rd position of Schiff bases showed moderate activity while compound **5d** also showed moderate activity against some Gram +ve and Gram –ve bacteria. All compounds showed high MIC values (more than 200 µg/ml) against fungus *C. albicans.*

CONCLUSION

We have observed formation of novel Schiff bases **4a-c** during reaction of **2a-c** with hydrazine hydrate. The formation of hydrazide **5d** was observed from **2d** due to steric hindrance of naphthalene ring which prevent attack of hydrazine on β carbon of carbonyl group and hence attack occur on ester carbonyl to give hydrazide. The plausible mechanism for retero Knovenagel reaction or reverse Michael reaction is proposed. The formation of phenyl hydrazides also supports the mechanism. Steric hindrance by naphthalene ring or phenyl ring, gives corresponding hydrazides only. Compounds **4a**, **4b** and **4c** showed moderate activity against one of the bacteria *B. subtilis.*

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