Synthesis, Characterization and Testing of Biological Activity of Some Novel Chalones Derivatives of Coumarin

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Abstract: In the present communication, we herein report the synthesis of some novel derivatives of coumarin i.e. 3-((2E)-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl)acryloyl)-2H-chromen-2-one (2a-2g). Various aromatic amines and salicylaldehyde in the presence of sodium nitrite and conc. HCl yielded 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (1a-1g). A series of 3-((2E)-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl)acryloyl)-2H-chromen-2-one were synthesized by the reaction of 2-hydroxy-5-((aryl)diazenyl) benzaldehyde and coumarin in presence of ethanol and piperidine. Different analytical techniques such as elemental analysis, IR spectra, 1H NMR spectra and mass spectra were used to elucidate the structures of all newly synthesized compounds (1a-1g and 2a-2g). The anti-bacterial and anti-fungal activity of newly synthesized chalcone derivatives (2a-2g) were evaluated against different bacterial and fungal stains. Some of these compounds showed promising activity against different stains.

Keywords: Chalcone derivatives, Coumarins, Anti-bacterial activity

Introduction

Coumarin nucleus is widely distributed in nature in plant kingdom and forms an important class of oxygen heterocycle1. Over the years, coumarins have been established as well-known naturally occurring oxygen-heterocyclic compounds isolated from various plants2. They are the family of lactones containing benzopyrone skeletal framework that have enjoyed isolation from plant as well as total synthesis in the laboratory. The plant extracts containing coumarin-related heterocycles are employed as herbal remedies in traditional systems of medicine. The synthesis of coumarin (2-oxo-2H-chromene) derivatives has attracted considerable attention of organic and medicinal chemists due to its wide usage in food additives, fragrances, pharmaceuticals and agrochemicals. In view of this, coumarins
have attracted intense interest in recent years because of their diverse pharmacological properties. Hence, many researchers have reported different biological activities of coumarins derivatives such as anticoagulant, antitubercular, antileucemic, antimicrobial, anti-inflammatory, anti-HIV, analgesic, anticancer, antitumoral, anticonvulsant, antiplatelet, antifungal, antiviral, antibacterial, antimalarial activities and other pharmacological activities.

Chalcones are α,β-unsaturated ketones which constitute an important group of natural products that serve as precursors for the synthesis of various heterocyclic compounds like pyrimidines, imidazoles, pyrazoles, 2-pyrazoline and flavonoids. Cyclization of chalcones, leading to thiazines, pyrimidines, pyrazolines has been a developing field within the realm of heterocyclic chemistry for the past several years because of their ready accessibility and the broad spectrum of biological activity of the products as antibacterial, antifungal, antiprotozoal, anti-inflammatory substances. With this background it has been thought worth to synthesize some novel heterocyclic compounds comprising of chalcone having azo-linkage and coumarin in a single moiety. Also, to evaluate these new compounds for their potency as an anti-bacterial and anti-fungal agent.

**Experimental**

All the chemicals and solvents were obtained from E-Merck, India (AR grade) and were used without further purification. Melting points were taken in an open capillary tube. IR spectra were recorded on a Shimadzu DR-8031 instrument. Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400. ^1^H NMR spectra of the synthesized compounds were recorded on a Bruker-Advance (300 MHz) and Varian-Gemini (200 MHz) spectrophotometer using CDCl_3 solvent and TMS as an internal standard. EI-MS spectra were determined on a LCQ ion trap mass spectrometer (Thermo Fisher, San Jose, CA, USA), equipped with an EI source.

**General procedure for the synthesis of 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (1a-1g)**

Aromatic amines (0.01 mol) was added in conc. HCl (5 mL) boiled for 10 minutes. This solution was then cooled to 0-5 °C in ice water bath. Aqueous sodium nitrite (0.01 mol, 10 mL) solution in cold condition was then added to this solution dropwise with vigorous stirring. The temperature of the reaction mixture was kept to 0-5 °C for 1 h, to give diazonium chloride solution. Then the resulting diazonium solution was poured drop wise with vigorous stirring to alkaline suspension of salicyladehyde in water (10 mL, 0.01 mol) at 0-5 °C. The pH of the reaction mixture was maintained at 8 to 9 by simultaneous addition of 10% aqueous sodium hydroxide (NaOH) solution. The precipitate was collected as a crystalline solid compound after keeping the reaction mixture overnight in the reaction flask. The precipitates were filtered by using Whatmann filter paper (no. 40). The compounds were recrystallized in EtOH.

**General procedure for the synthesis of 3-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl)propanoyl)-2H-chromen-2-one (2a-2g)**

A mixture of 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (1a-1g) and 3-acetyl-coumarin (0.01 mols, 1:1 ratio) in ethanol was stirred in presence of few drops of piperidine under reflux condition for 10-12 h. Mixture was cooled and poured into ice cold water and stirred vigorously. The compounds were precipitated as a crystalline product. These crude compounds were recrystallized with methanol.
Evaluation of antimicrobial activity (agar diffusion method)

Antimicrobial activity of all synthesized compounds was determined by agar diffusion method. All human pathogenic bacteria viz. *Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* were obtained from the Department of Biotechnology, S. F. S. College, Nagpur. Stock solutions of compounds were diluted in dimethyl sulfoxide (DMSO) to give a final concentration for determining the Minimum inhibitory concentration (MIC) value. MIC was defined as the lowest concentration of compound is required for a complete inhibition of the bacterial growth after incubation time. For antibacterial activity Muller Hinton agar was used. The wells of 6 mm diameter were filled with 0.1 mL of each compound is diluted separately for each test of bacterial strain. The antibiotic Ampicillin used as reference antibacterial agent, for comparison. Inoculated plates were then incubated at 37 °C for antibacterial activity for 24 h. After incubation the antimicrobial activity was measured in terms of the zone of inhibition in mm.

Results and Discussion

General

The synthetic route for the preparation of some novel derivatives of coumarin *i.e.* 3-((2E)- (3-(2-hydroxy-5-((aryl)diazenyl)phenyl)acryloyl)-2H-chromen-2-one (2a-2g) have been depicted in Scheme 1. The key intermediate 2-hydroxy-5-((aryl)diazenyl)benzaldehyde (1a-1g) was synthesized by the reaction of various aromatic amines and salicylaldehyde in appropriate quantity of conc. HCl and sodium nitrite mixture. The spectral data of newly synthesized compounds have been collected here in the following part.

![Scheme 1. Synthetic route to obtain 3-((2E)-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl) acryloyl)-2H-chromen-2-one](image)

Where: Ar = (a) 4-NO₂-C₆H₄, (b) 3-NO₂-C₆H₄, (c) 2-NO₂-C₆H₄, (d) o-CH₃-C₆H₄, (e) p-CH₃-C₆H₄, (f) m-CH₃-C₆H₄, (g) p-Cl-C₆H₄

Scheme 1. Synthetic route to obtain 3-((2E)-(3-(2-hydroxy-5-((aryl)diazenyl)phenyl) acryloyl)-2H-chromen-2-one

Characterization data of 2-hydroxy-5-((4-nitrophenyl)diazenyl)benzaldehyde (1a)

Yield: 75; m.p.: 112 °C, IR (KBr): 1530 (C=C), 1570 (N=N); 1710 (aldehydic C=O); 2920 (Ar-CH), 2990 cm⁻¹ (Ar-OH); ¹H NMR: δ = 7.40 (d, 1H, Ar-CH); 7.90 (d, 2H, (Ar-CH); 8.10
Characterization data of 2-hydroxy-5-((3-nitrophenyl)diazenyl)benzaldehyde (1b)
Yield: 68; m.p.: 105 °C, IR (KBr): 1550 (C=C), 1580 (N=N); 2930 (Ar-CH), 3110 cm\(^{-1}\) (Ar-OH); \(^1\)H NMR: δ = 7.10 (d, 1H, Ar-CH); 7.60 (d, 2H, Ar-CH); 8.30 (d, 2H, Ar-CH); 8.60 (d, 2H, Ar-CH); 10.60 (s, 1H, Ar-CHO); 11.70 (s, 1H, Ar-OH); Anal. Caled. For C\(_{13}H_9N_3O_4\) : C, 57.57; H, 3.34; N, 15.49; Found: C, 57.40; H, 3.20; N, 15.20; Mass spectra, \(m/z = 271\) (100%).

Characterization data of 2-hydroxy-5-((2-nitrophenyl)diazenyl)benzaldehyde (1c)
Yield: 70; m.p.: 110 °C, IR (KBr): 1510 (C=C), 1570 (N=N); 2960 (Ar-CH), 3140 cm\(^{-1}\) (Ar-OH); \(^1\)H NMR: δ = 7.20 (d, 1H, Ar-CH); 7.70 (d, 2H, Ar-CH); 8.50 (d, 2H, Ar-CH); 8.80 (d, 2H, Ar-CH); 10.80 (s, 1H, Ar-CHO); 11.90 (s, 1H, Ar-OH); Anal. Caled. For C\(_{13}H_9N_3O_4\) : C, 57.57; H, 3.34; N, 15.49; Found: C, 57.20; H, 3.30; N, 15.10; Mass spectra, \(m/z = 271\) (100%).

Characterization data of 2-hydroxy-5-((o-tolyldiazenyl)benzaldehyde (1d)
Yield: 73; m.p.: 172 °C, IR (KBr): 1540 (C=C), 1560 (N=N); 2990 (Ar-CH), 3180 cm\(^{-1}\) (Ar-OH); \(^1\)H NMR: δ = 2.40 (s, 3H, Ar-CH\(_3\)); 7.10-7.40 (m, 4H, Ar-CH); 7.90 (d, 1H, Ar-CH); 8.10 (d, 2H, Ar-CH); 10.40 (s, 1H, Ar-CHO); 11.70 (s, 1H, Ar-OH); Anal. Caled. For C\(_{14}H_{12}N_2O_2\) : C, 69.99; H, 5.03; N, 11.66; Found: C, 69.50; H, 5.00; N, 11.40; Mass spectra, \(m/z = 240\) (100%).

Characterization data of 2-hydroxy-5-((p-tolyldiazenyl)benzaldehyde (1e)
Yield: 65; m.p.: 140 °C, IR (KBr): 1520 (C=C), 1570 (N=N); 2980 (Ar-CH), 3210 cm\(^{-1}\) (Ar-OH); \(^1\)H NMR: δ = 2.20 (s, 3H, Ar-CH\(_3\)); 7.20-7.50 (m, 4H, Ar-CH); 7.70 (d, 1H, Ar-CH); 8.30 (d, 2H, Ar-CH); 10.70 (s, 1H, Ar-CHO); 11.60 (s, 1H, Ar-OH); Anal. Caled. For C\(_{14}H_{12}N_2O_2\) : C, 69.99; H, 5.03; N, 11.66; Found: C, 69.70; H, 5.10; N, 11.50; Mass spectra, \(m/z = 240\) (100%).

Characterization data of 2-hydroxy-5-((m-tolyldiazenyl)benzaldehyde (1f)
Yield: 65; m.p.: 140 °C, IR (KBr): 1520 (C=C), 1570 (N=N); 2980 (Ar-CH), 3210 cm\(^{-1}\) (Ar-OH); \(^1\)H NMR: δ = 2.30 (s, 3H, Ar-CH\(_3\)); 7.30-7.50 (m, 4H, Ar-CH); 7.60 (d, 1H, Ar-CH); 8.30 (d, 2H, Ar-CH); 10.70 (s, 1H, Ar-CHO); 11.60 (s, 1H, Ar-OH); Anal. Caled. For C\(_{14}H_{12}N_2O_2\) : C, 69.99; H, 5.03; N, 11.66; Found: C, 69.40; H, 5.00; N, 11.30; Mass spectra, \(m/z = 240\) (100%).

Characterization data of 5-((4-chlorophenyl)diazenyl)-2-hydroxybenzaldehyde (1g)
Yield: 70; m.p.: 130 °C, IR (KBr): 1510 (C=C), 1560 (N=N); 2960 (Ar-CH), 3260 cm\(^{-1}\) (Ar-OH); \(^1\)H NMR: δ = 7.40 (d, 1H, Ar-CH); 7.70 (d, 2H, Ar-CH); 7.90-8.10 (m, 4H, Ar-CH); 10.40 (s, 1H, Ar-CHO); 11.90 (s, 1H, Ar-OH); Anal. Caled. For C\(_{13}H_{9}N_2O_2Cl\) : C, 59.90; H, 3.48; N, 10.75; Found: C, 59.50; H, 3.20; N, 10.60; Mass spectra, \(m/z = 260\) (100%).

Characterization data of 3-(2E)-(3-(2-hydroxy-5-((4-nitrophenyl)diazenyl)-phenyl)acyrloyl)-2H-chromen-2-one (2a)
Yield: 69; m.p.: 145 °C, IR (KBr): 1490 (C=C), 1530 (N=N); 1680 cm\(^{-1}\) (ketonic C=O); 2960 (Ar-CH), 3210 cm\(^{-1}\) (Ar-OH); \(^1\)H NMR: δ = 6.80 (d, 1H, Ar-CH); 7.10 (d, 1H, Ar-CH);
7.40-7.60 (m, 5H, (Ar-CH); 7.80 (t, 3H, Ar-CH); 8.10 (d, 1H, Ar-CH); 8.40 (d, 2H, Ar-CH); 8.60 (s, 1H, Ar-CH); 11.90 (s, 1H, Ar-OH); Anal. Calcd. For C_{24}H_{15}N_{3}O_{6}: C, 65.31; H, 3.43; N, 9.52; Found: C, 65.20; H, 3.30; N, 9.40; Mass spectra, m/z = 441.00 (100%).

**Characterization data of 3-((2E)-(3-(2-hydroxy-5-((3-nitrophenyl)diazenyl)phenyl)acryloyl)-2H-chromen-2-one (2b)**
Yield: 74; m.p.: 151 °C, IR (KBr): 1470 (C=C), 1540 (N=N); 1690 cm^{-1} (ketonic C=O); 2940 (Ar-CH), 3270 cm^{-1} (Ar-OH); ^1H NMR: δ = 6.50 (d, 1H, Ar-CH); 7.20 (d, 1H, Ar-CH); 7.50-7.60 (m, 5H, (Ar-CH)); 7.90 (t, 3H, Ar-CH); 8.30 (d, 1H, Ar-CH); 8.50 (d, 2H, Ar-CH); 8.70 (s, 1H, Ar-CH); 11.60 (s, 1H, Ar-OH); Anal. Calcd. For C_{24}H_{15}N_{3}O_{6}: C, 65.31; H, 3.43; N, 9.52; Found: C, 65.10; H, 3.20; N, 9.30; Mass spectra, m/z = 441.00 (100%).

**Characterization data of 3-((2E)-(3-(2-hydroxy-5-((2-nitrophenyl)diazenyl)phenyl)acryloyl)-2H-chromen-2-one (2c)**
Yield: 72; m.p.: 141 °C, IR (KBr): 1480 (C=C), 1580 (N=N); 1710 cm^{-1} (ketonic C=O); 2960 (Ar-CH), 3290 cm^{-1} (Ar-OH); ^1H NMR: δ = 6.30 (d, 1H, Ar-CH); 7.30 (d, 1H, Ar-CH); 7.50-7.70 (m, 5H, (Ar-CH)); 7.90 (t, 3H, Ar-CH); 8.20 (d, 1H, Ar-CH); 8.60 (d, 2H, Ar-CH); 8.90 (s, 1H, Ar-CH); 11.30 (s, 1H, Ar-OH); Anal. Calcd. For C_{24}H_{15}N_{3}O_{6}: C, 65.31; H, 3.43; N, 9.52; Found: C, 65.20; H, 3.10; N, 9.40; Mass spectra, m/z = 441.00 (100%).

**Characterization data of 3-((2E)-(3-(2-hydroxy-5-((o-tolyldiazenyl)phenyl)acryloyl)-2H-chromen-2-one (2d)**
Yield: 68; m.p.: 170 °C, IR (KBr): 1460 (C=C), 1590 (N=N); 1730 cm^{-1} (ketonic C=O); 2980 (Ar-CH), 3260 cm^{-1} (Ar-OH); ^1H NMR: δ = 2.40 (s, 3H, Ar-CH_{3}); 6.70 (d, 1H, Ar-CH); 7.10 (d, 1H, Ar-CH); 7.40-7.70 (m, 7H, (Ar-CH)); 7.90 (d, 2H, Ar-CH); 8.10 (d, 1H, Ar-CH); 8.60 (s, 1H, Ar-CH); 11.90 (s, 1H, Ar-OH); Anal. Calcd. For C_{25}H_{18}N_{2}O_{4}: C, 73.16; H, 4.42; N, 6.83; Found: C, 73.10; H, 4.20; N, 6.50; Mass spectra, m/z = 410.10 (100%).

**Characterization data of 3-((2E)-(3-(2-hydroxy-5-((p-tolyldiazenyl)phenyl)acryloyl)-2H-chromen-2-one (2e)**
Yield: 74; m.p.: 160 °C, IR (KBr): 1480 (C=C), 1560 (N=N); 1750 cm^{-1} (ketonic C=O); 2970 (Ar-CH), 3280 cm^{-1} (Ar-OH); ^1H NMR: δ = 2.20 (s, 3H, Ar-CH_{3}); 6.50 (d, 1H, Ar-CH); 6.90 (d, 1H, Ar-CH); 7.20 (d, 1H, Ar-CH); 7.40-7.70 (m, 7H, (Ar-CH)); 7.80 (d, 2H, Ar-CH); 8.30 (d, 1H, Ar-CH); 8.70 (s, 1H, Ar-CH); 11.50 (s, 1H, Ar-OH); Anal. Calcd. For C_{25}H_{18}N_{2}O_{4}: C, 73.16; H, 4.42; N, 6.83; Found: C, 72.90; H, 4.30; N, 6.70; Mass spectra, m/z = 410.00 (100%).

**Characterization data of 3-((2E)-(3-(2-hydroxy-5-((m-tolyldiazenyl)phenyl)acryloyl)-2H-chromen-2-one (2f)**
Yield: 69; m.p.: 152 °C, IR (KBr): 1450 (C=C), 1550 (N=N); 1760 cm^{-1} (ketonic C=O); 2960 (Ar-CH), 3250 cm^{-1} (Ar-OH); ^1H NMR: δ = 2.30 (s, 3H, Ar-CH_{3}); 6.20 (d, 1H, Ar-CH); 6.90 (d, 1H, Ar-CH); 7.20-7.50 (m, 7H, (Ar-CH)); 7.70 (d, 2H, Ar-CH); 8.50 (d, 1H, Ar-CH); 8.90 (s, 1H, Ar-CH); 11.70 (s, 1H, Ar-OH); Anal. Calcd. For C_{25}H_{18}N_{2}O_{4}: C, 73.16; H, 4.42; N, 6.83; Found: C, 73.00; H, 4.20; N, 6.40; Mass spectra, m/z = 410.00 (100%).
Characterization data of 3-((2E)-3-(5-(2-(4-chlorophenyl)diazenyl)-2-hydroxyphenyl)acryloyl)-2H-chromen-2-one (2g)

Yield: 78; m.p.: 145 °C, IR (KBr): 1460 (C=C), 1580 (N=N); 1790 cm⁻¹ (ketonic C=O); 2980 (Ar-CH), 3190 cm⁻¹ (Ar-OH); ¹H NMR: δ = 6.80 (d, 1H, Ar-CH); 7.10-7.20 (m, 1H, Ar-CH); 7.40 (d, 2H, Ar-CH); 7.60 (d, 1H, Ar-CH); 7.80-7.90 (m, 4H, Ar-CH); 8.40 (s, 1H, Ar-CH); 11.80 (s, 1H, Ar-OH); Anal. Calcd. For C₂₄H₁₅N₂O₄Cl: C, 66.91; H, 3.51; N, 6.50; Found: C, 66.50; H, 3.30; N, 6.20; Mass spectra, m/z = 430.00 (100%).

Biological activity

From the investigation of antibacterial screening data depicted in Table 1, it is revealed that most of the tested compounds showed moderate to good bacterial inhibition.

Table 1. Antibacterial activity of newly synthesized chalcone derivatives (2a-2g) (zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram (+ve) bacteria</th>
<th>Gram (-ve) Bacteria</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>2a</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>2b</td>
<td>+</td>
<td>++</td>
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<td>2c</td>
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<tr>
<td>2d</td>
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<td>++</td>
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<tr>
<td>2e</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>2f</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2g</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Standard Ampicillin: +++ +++ +++ +++ +++

Gram +ve Bacteria: (A) Bacillus subtilis; (B) Proteus vulgaris; (C) Staphylococcus aureus. Gram -ve Bacteria: (D) Escherichia coli; (E) Klebsiella pneumonia. Zone of Inhibitions: Inactive = - (inhibition zone < 5 mm); Slightly active = + (inhibition zone 5-12 mm); Moderately active = ++ (inhibition zone 13-17 mm); Highly active = +++ (inhibition zone > 17 mm)

Compound 2a have shown moderate activity against Bacillus subtilis, Escherichia coli and Klebsiella pneumonia bacteria. Compound 2b were found to be moderately active against Proteus vulgaris and Staphylococcus aureus. Compound 2c was highly active against Staphylococcus aureus whereas moderately active against Bacillus subtilis. Compound 2d was found to be moderately active against Proteus vulgaris and Klebsiella pneumonia. Compound 2e showed promising good activity against Bacillus subtilis and moderate activity against Staphylococcus aureus and Escherichia coli. Compound 2f have shown good activity against Klebsiella pneumonia. Among all the newly synthesized compounds, compound 2g have shown good activity against gram positive bacteria i.e. Bacillus subtilis, Proteus vulgaris and Staphylococcus aureus whereas this compound shown slight activity against both gram negative bacteria i.e. Escherichia coli and Klebsiella pneumonia. Thus, it can be concluded that compound 2g can be used a promising antibacterial agent.
References