Research Article

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ABSTRACT

The novel coumarin derivatives (2, 3, 4, 5, 6, 7) have been synthesized from the reaction of o-acetyloxy benzoic acid with thiophen 2-yl chloride yielding 2-acetoxy benzooy chloride, which on further treatment with ethylacetoacetate gave 4-hydroxycoumarin. Substituted pyrazolones and thiazoles reacted with 4-hydroxy coumarin to give pyrazolone and methyl thiazoles related coumarin derivatives. The newly synthesized products were characterized with IR, 'H and 13C NMR, mass spectroscopic techniques and elemental analysis. The synthesized compounds were screened for their antibacterial and antifungal activity. All the compounds were found to have significant activity against the tested microorganisms.

KEYWORDS

Coumarin, thiazole, pyrazolone, antibacterial activity, antifungal activity.

1. Introduction

In plants, coumarin derivatives are present in significant quantities and more than 1300 coumarins are identified from natural sources. This family of compounds serves as an important model for the advanced design and synthesis of more active analogous coumarins, since natural compounds possess potent antioxidative and radical-scavenging properties as reported in various experimental models. The synthesis of coumarin and its derivatives have attracted considerable attention from organic and medicinal chemists for many years, as large numbers of natural products contain this heterocyclic nucleus. They are widely used as optical brighteners and dispersed fluorescent and laser dyes, additives in food, perfumes, cosmetics and pharmaceuticals so the synthesis of this heterocyclic nucleus is of much interest. The coumarin derivatives possess a broad spectrum of biological activities such as, antifungal, antibacterial, anti-inflammatory, antiproliferative, antiviral, antioxidant, anticancer and anti-HIV activities. The reaction of o-acetyloxy benzoic acid and thiophen 2-yl chloride to give 2-acetoxy benzooy chloride, which then reacts with ethylacetoacetate to yield hydroxycoumarin. Coumarin heterocycles have been found to be very useful compounds for different types of activities. 4-Hydroxy coumarin derivatives are useful starting materials for the synthesis of new Coumarin derivatives. Coumarins have been synthesized by several routes including Pechmann, Perkin, Knoevenagel, Reformatsky and the Wittig reaction. Bucumolol, Chromonar, Folescuitol and 4-methyl umbelliferone are coumarin derivatives and are clinically used as antirheumatic, vasodilator, capillary and antispesitively agents. Warfarin and acenocoumarol, which are coumarin derivatives, exhibit anticoagulant activity. Coumarin derivatives are also used to synthesize fluorescent polymers and fluorescent chemosensors for Mg2+ ion. We report herein the design and synthesis of pyrazolone and methyl thiazole related coumarins derivatives with significant biological importance.

2. Experimental

2.1. Methods and Materials

All the chemicals were procured from Sigma-Aldrich, and used without further purification. The 4-hydroxy coumarin was prepared by Knoevenagel’s literature procedure. Initially, the purity of synthesized compounds was confirmed using aluminium-coated TLC plates (E. Merck). Melting points were determined using a Stuart SMP10 MP apparatus and are uncorrected. The IR spectra (ν, cm⁻¹) were recorded on a 8400 FT-IR-435 spectrometer using KBr pellets. Elemental analysis was performed on an ECS 4010 Elemental Combustion System. A Bruker-Avance 400 MHz spectrometer was used to record the 1H-NMR and 13C-NMR spectra using TMS as an internal standard. The chemical shifts were reported in parts per million (δ-LC, ppm). Mass spectra was carried out on Waters Micro mass Q-Tof Micro having range of 4000 amu in quadrupole and 20 000 amu in ToF.

2.1.1. Synthesis of Intermediates Chromen-2-one Derivatives (2, 3) (Scheme 1)

4-Hydroxy coumarin 1 (1.0 mole), DMF (4 vol) and potassium carbonate (1.1 mole) was heated to 85 °C. Dibromoalkane (2.3 mole) in DMF (1 vol) was added in to the reaction mixture. The reaction mass stirred for 5 h at 85 °C. The completion of the reaction was checked on TLC (hexane/ethyl acetate 7:3, RF = 0.85 for compound 2 and 0.57 for compound 1). After completion of the reaction, the reaction mass was filtered and washed with DMF. The collected filtrate was poured into water and stirred for 30 min. The obtained solid was filtered, washed with water and purified by recrystallization from acetone to give compound 2 and compound 3.

During the reaction, Impurity-A and Impurity-B formed, which are insoluble in DMF so they can easily be removed through filtration. The method of preparation for Impurity-A and Impurity-B is incorporated in the supplementary information. The purification of compounds 2 and 3 were carried out via recrystallization in acetone.
3-(2-Bromoethyl)-4-hydroxy-2H-chromen-2-one (2)

Off-white solid. Yield 73 %, m.p. 155–158 °C, 1H NMR (400 MHz, CDCl3) (Fig. S3) 7.25–7.83 (m, 4H, Ar-H), 5.79 (s, 1H, OH), 4.32–4.37 (m, 2H, CH2), 4.10–4.15 (m, 2H, CH2), 2.76 (s, 3H, CH3), 2.74 (m, 2H, CH2), 2.02–2.16 (m, 4H, CH2), 1.40 (t, 3H, CH3); 13C NMR (100 MHz, CDCl3) (Fig. S10) 13.99, 17.10, 28.63, 60.06, 68.75, 90.56, 114.95, 115.21, 116.04, 120.94, 122.64, 123.57, 125.0, 125.06, 127.92, 152.70, 160.85, 160.93, 161.7 164.94, 168.8; C26H25NO6S, (297.03); Found (C-52.50; H-4.32 %) requires (C-52.56, H-4.38, Br-26.90, O-16.16 %)

4-Hydroxy coumarin (1.0 mole) and potassium carbonate (1.0 mole) were heated at 75 °C and solution of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1.5 mole) in DMF (3 vol) as a solvent media was heated up to 75 °C and solution of 3-methyl-1-phenyl-1H-pyrazol–5(4H)–one (1.0 mole) in place of 3-(4-bromobutyl)-4-hydroxy-2H-chromen-3-yl was synthesized by following the similar synthetic procedure of compound (4) by changing 3-(4-bromobutyl)-4-hydroxy-2H-chromen-2-one (1.0 mole) in place of 3-(2-bromoethyl)-4-hydroxy-2H-chromen-2-one. (TLC monitoring: hexane-ethyl acetate 7:3, Rf = 0.52).

Off-white crystals. Yield 77 %, m.p. 170–175 °C, IR (KBr, cm−1) (Fig. S14): 1710 cm−1 (C=O), 3084–3176 cm−1 (OH), 1375–1390 cm−1 (CH), 759 cm, 775 cm−1 (substituted benzene); 1H NMR (400 MHz, DMSO) (Fig. S7): 7.11–8.03 (m, 9H, Ar-H), 7.11–8.03 (m, 9H, Ar-H), 2.78 (s, 3H, CH3), 2.76 (m, 2H, CH2), 2.18–2.19 (s, 3H, CH3); C13H13BrO3 (297.03); Found (C-52.50; H-4.32 %) requires (C-52.56, H-4.38, Br-26.90, O-16.16 %)

2.1.3. Synthesis of Compound Ethyl-2,4-(3-(butyl)-4-hydroxy-2H-chromen-2-one)phenyloxy-4,5-dihydro-4-methylthiazole-5-carboxylate (4) (Scheme 2)

Ethyl-4,5-dihydro-2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylate (1.0 mole) in DMF (2 vol) at 75 °C was added. The reaction mass stirred for 5 h at 75 °C and solution of 3-(2-bromoethyl)-4-hydroxy-2H-chromen-2-one (1.0 mole) solution in DMF (2 vol) at 75 °C. The reaction mass stirred for 6 h at 75 °C (TLC monitoring: hexane/ethyl acetate 5:5, Rf = 0.52). After the completion of reaction, the product was filtered, dried and recrystallized from ethyl acetate to give the ethyl-2,4-(3-(butyl)-4-hydroxy-2H-chromen-2-one)phenyloxy-4,5-dihydro-4-methylthiazole-5-carboxylate (4).

Off-white crystals. Yield 72 %, m.p. 183–187 °C, IR (KBr, cm−1) (Fig. S13): 29810–3053 cm−1 (OH), 1710 cm−1 (C=O), 3084–3176 cm−1 (OH), 1375–1390 cm−1 (CH), 759 cm, 775 cm−1 (substituted benzene); 1H NMR (400 MHz, CDCl3) (Fig. S5): 7.04–7.94 (m, 8H, Ar-H), 5.88 (s, 1H, OH), 4.32–4.37 (m, 2H, CH2), 4.10–4.15 (m, 2H, CH2), 2.76 (s, 3H, CH3), 2.74 (m, 2H, CH2), 2.02–2.16 (m, 4H, CH2), 1.40 (t, 3H, CH3); 13C NMR (100 MHz, CDCl3) (Fig. S19): 1631 cm–1 (C=O), 3084–3176 cm–1 (OH), 1375–1390 cm–1 (CH), 759 cm, 775 cm–1 (substituted benzene); 1H NMR (400 MHz, CDCl3) (Fig. S6): 6.93–7.92 (m, 8H, Ar-H), 5.69 (s, 1H, OH), 4.32–4.37 (m, 2H, CH2), 4.10–4.15 (m, 2H, CH2), 2.76 (s, 3H, CH3), 2.74 (m, 2H, CH2), 2.02–2.16 (m, 4H, CH2), 1.40 (t, 3H, CH3); 13C NMR (100 MHz, CDCl3) (Fig. S10) 13.99, 17.10, 28.63, 60.06, 68.75, 90.56, 114.95, 115.21, 116.04, 120.94, 122.64, 123.57, 125.0, 125.06, 127.92, 152.70, 160.85, 160.93, 161.7 164.94, 168.8; C13H13BrO3 (297.03); Found (C-49.16; H-3.30 %) requires (C-49.10, H-3.35, Br-29.70, O-17.84 %)

2.1.4. Preparation of 4-(4-Hydroxy-2-oxo-2H-chromen-3-yl)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5) (Scheme 3)

4-Hydroxy coumarin (1.0 mole) and potassium carbonate (1.5 mole) in DMF (3 vol) as a solvent media was heated up to 75 °C and solution of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1.0 mole) in DMF (2 vol) at 75 °C was added. The reaction mass stirred for 5 h at 75 °C (TLC monitoring: hexane/ethyl acetate 5:5, Rf = 0.47). After the completion of reaction, the product was obtained by adjusting the pH at 4-5 by HCl. It was filtered, dried and recrystallized from ethyl acetate and hexane to give the 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (5).

Light cream crystal. Yield 73 %, m.p. 153–157 °C, IR (KBr, cm−1) (Fig. S15): 29810–3053 cm−1 (OH), 1710 cm−1 (C=O), 1440–1415 cm−1 (CH), 806–833 cm–1 (substituted benzene); 1H NMR (400 MHz, DMSO) (Fig. S7): 3.91–4.60 (m, 1H, CH), 2.96 (m, 2H, CH), 2.13–2.18 (m, 4H, CH2); C16H16BrO4 (378.10); Found (C-63.92, H-4.65; N-2.98 %) requires (C-63.85, H-4.69, N-2.98 %)MS: m/z: 452.2 (M+H) (Fig. S17).

2.1.5. Preparation of 4-(4-Hydroxy-2-oxo-2H-chromen-3-yl)-3-methyl-1H-pyrazol-5(4H)-one (7)

The compound 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-3-methyl-1H-pyrazol-5(4H)-one (7) was synthesized by changing 3-(4-bromobutyl)-4-hydroxy-2H-chromen-2-one (1.0 mole) in place of 3-(2-bromoethyl)-4-hydroxy-2H-chromen-2-one. (TLC monitoring: hexane-ethyl acetate 7:3, Rf = 0.52).
The paper impregnated with the test compounds (100 µg disc–1) the microorganism (matched to McFarland barium sulphate pounds) was tested against nutrient agar medium. The antifungal activity of the compounds was formed in triplicate. The sterilized medium was inoculated Sabouraud dextrose agar medium. All of the tests were performed by Agar diffusion method.40 The standard microbial strains used for antimicrobial activity were procured from the Institute of Microbial Technology, Chandigarh. The antibacterial activity of the synthesized compounds was tested against two Gram-positive and two Gram-negative bacteria [Escherichia coli (MTCC 96), Pseudomonas aeruginosa (MTCC 1688), and Bacillus subtilis (MTCC 443)] using Sabouraud dextrose agar medium. All of the tests were performed in triplicate. The sterilized medium was inoculated (1 mL 100 mL–1 of medium) with the suspension (10⁵ cfu mL–1) of the microbial strains (matched to McFarland barium sulphate standard) and poured into a Petri dish to give a depth of 3–4 mm. The paper impregnated with the test compounds (100 µg disc–1) was placed on the solidified medium. The plates were pre-incubated for 1 hour at room temperature and incubated at 37 °C for 24 h for antibacterial and 48 h for antifungal activities. Ampicillin43 (100 µg disc–1) and Fluconazole41 (10 µg disc–1) were used as standard for anti-bacterial and anti-fungal activities respectively. The MIC values for standards are obtained under the identical experimental condition as the test compounds. The results are presented in Table 1.

2.2. Antimicrobials and Antifungal Activities (MIC)
The antimicrobial and antifungal activities were performed by the similar synthetic procedure of compound (6) by changing 3-methyl-1H-pyrazol-5(4H)-one to 3-methyl-1H-pyrazol-5(4H)-one (1.0 mole) in place of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one. After the completion of reaction, the isolation of product carried out at pH 7–8. (TLC monitoring: hexane/ethyl acetate 5:5, Rf = 0.43) Light lemon-yellow crystal. Yield 68 %, m.p. 181.3–185.3 °C, IR (KBr, cm–1) (Fig. S16): 1631 cm –1 (C=O), 3084–3176 cm –1 (OH), 1375–1390 cm–1 (CH2), 759 cm–1, 775 cm–1 (substituted benzene); 1H NMR (400 MHz, DMSO) (Fig. S8) 11.01 (s, 1H, OH), 8.50 (s, 1H, NH), 7.29–7.91 (m, 4H, Ar-H), 2.60 (m, 1H, CH), 1.96 (s, 3H, CH3); MS: m/z (C-60.47, H-3.89, O-24.78, N-10.85 %) MS: m/z: 259.1 (M+H).

3. Results and Discussion
Ethyl-2-4-(3-(ethyl)-4-hydroxy-2H-chromen-2-one)phenyloxycarbonyl-4,5-dihydro-4-methylthiazole-5-carboxylate (4) was prepared from 3-(2-bromoethyl)-4-hydroxy-2H-chromen-2-one (2) in the presence of base. 3-(2-Bromoalkyl)-4-hydroxy-2H-chromen-2-one (2) was prepared from 4-hydroxy coumarin (1) and dibromo ethane in the presence of base. The synthesis of ethyl-2-4-(3-(alkyl)-4-hydroxy-2H-chromen-2-one)phenyloxycarbonyl-4,5-dihydro-4-methylthiazole-5-carboxylate (4, 5) was carried out from 3-(2-bromoalkyl)-4-hydroxy-2H-chromen-2-one (2) and Ethyl-4,5-dihydro-2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylate in presence of acetone at 40–45 °C, to obtain 90 % yield. Compounds 4 and 5 were purified by crystallization in methanol at 45–50 °C with 70 % yield. Compound 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (6) was prepared from 4-hydroxy coumarin (1) and 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one in presence of potassium carbonate as base and DMF as solvent. The synthesis of 4-(4-hydroxy-2-oxo-2H-chromen-3-yl)-3-methyl-1H-pyrazol-5(4H)-one (7) was carried out following same procedure as for compound (6) using 3-methyl-1H-pyrazol-5(4H)-one. The crystallization of compounds (6) and (7) was carried out in ethyl acetate and hexane at RT, to obtain 65–75 % yield. All the synthesized compounds were characterized by IR, 1H NMR, and MS.

Table 1 MIC’s of the test compounds (2–7) against bacterial and fungal species.

| Tested compounds (300 µg/mL)* | Minimum inhibitory concentration (MIC’s)* in µg mL–1 |
|-------------------------------|-----------------------------------------------|
|                               | Antibacterial activity | Antifungal activity |
|                               | E. coli | P. aeruginosa | B. subtilis | S. aureus | C. albicans (40 µg mL–1)* |
| 2                             | 10     | 14             | 11          | 10         | 10                        |
| 3                             | 12     | 11             | 12          | 13         | 10                        |
| 4                             | 16     | 16             | 12          | 12         | 10                        |
| 5                             | 14     | 15             | 14          | 10         | 10                        |
| 6                             | 11     | 10             | 14          | 11         | 10                        |
| 7                             | 11     | 10             | 12          | 11         | 10                        |
| Ampicillin*                   | 18     | 18             | 21          | 19         | 19                        |
| Fluconazoleb                  | –      | –              | –           | –          |                           |

* Values are mean of three replicates. (Standard error ± 1 µg mL–1).

* Concentration of solutions for respective activities.

* Ampicillin was used as a standard against bacterial species (100 µg mL–1).

* Fluconazole was used as a standard against fungi species (10 µg mL–1).
and $^{13}$C NMR spectroscopic techniques, mass spectra and elemental analysis. The IR spectra of compounds (4, 5) showed a band in the region 2993–3076 cm$^{-1}$ due to the -OH groups. The C-H bending vibrations are observed as a sharp medium to strong band at 1329 cm$^{-1}$ in all compounds. The C-H linkage of the six-member ring caused a weak and sharp absorption band at 800–850 cm$^{-1}$. The C=O group is observed as a strong and sharp band at 1650–1710 cm$^{-1}$ in these compounds. Further, H NMR spectra exhibited multiplet in the region at $\delta$ 7.54–8.03 ppm for eight aromatic protons (four aromatic protons of benzene) (4). Four protons present in –CH$_2$ of compounds (4) are found to resonate as multiplet at $\delta$-LC 4.54–4.34 ppm (alkene). One proton present in –OH of compound (4) is found to resonate as singlet at $\delta$-LC 5.88 ppm. Six protons present in –CH$_3$ of compounds (4) are found to resonate as triplets at $\delta$-LC 1.35–1.39 ppm (thiazole) and singlet at $\delta$-LC 2.71 ppm (thiazole). Two protons of the –CH$_2$ group are observed at $\delta$-LC 2.71 ppm as a multiplet compound (4). The IR spectra of compounds (6–7) showed a broad band in the region 3084–3176 cm$^{-1}$ due to the -OH groups. The C=O group is observed as a strong and sharp band at 1631 cm$^{-1}$ in these compounds. The C-H linkage of the mono substituted six-member ring caused a weak and sharp absorption band at 730–780 cm$^{-1}$ in all the compounds. The H NMR spectra of compounds 6 and 7 show identical peaks for coumarin moiety but show significant differences for pyrazolone moiety. The mono substituted aromatic rings proton found in between $\delta$-LC 7.54–8.03 ppm for compounds 6 and –NH proton observed as broad peak at $\delta$-LC 8.50 ppm for compound 7.

The anti-bacterial activity of the synthesized compounds was tested against two Gram-negative bacteria (Staphylococcus aureus, Bacillus subtilis) and two Gram-positive bacteria (Escherichia coli and Pseudomonas aeruginosa) using nutrient agar medium. The antifungal activity of the compounds was tested against fungi species Candida albicans. The Ampicillin used as standard antibacterial agent and Fluconazole used as standard antifungal agent. The MIC values for standards were obtained under the identical experimental condition as the test compounds. The MIC values for the other reported drugs are listed in Table 2.

### 4. Conclusion

In summary, a series of novel bioactive coumarin derivatives have been synthesized, purified and characterized. The result of antibacterial activity with the Ampicillin as standard at MIC > 100 µg mL$^{-1}$ against Pseudomonas aeruginosa and Escherichia coli at 300 µg mL$^{-1}$. All compounds exhibited slightly reduced activities (than the positive controls), and the best activity was for compounds 4 and 5. Against Staphylococcus aureus, Bacillus subtilis 300 µg all compound showed slightly reduces activities, and the best performance was shown by compounds 4 and 7. The result of antifungal activities with the comparison of Fluconazole at MIC 10 µg against Candida albicans at 40 µg all compound activity showed less activity. Given the above result, these kind of compounds could be further studied and explored as good antibacterial agents.

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### Supplementary Material

Supplementary information is provided in the online supplement.

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Supplementary Information

Novel coumarin derivatives: Synthesis, characterization and antimicrobial activity

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Process for the impurity-A and impurity-B preparation

4-Hydroxy coumarin 1 (1.0 mole), DMF (4 vol) and potassium carbonate (3.1 mole) was heated up to 85 °C. Dibromoalkane (0.55 mole) in DMF (1 vol) was added into the reaction mixture. The reaction mass stirred for 5 hours at 85 °C (TLC monitoring: Hexane/Ethyl acetate 7:3, Rf = 0.69). After completion of reaction, the reaction mass was filtered and washed with DMF. Dry the obtained product in oven at 50 °C. The Impurity-A (Figure-S1) and Impurity-B (Figure-S2) were characterized by 1H-NMR spectroscopy.

![Figure-S1: 1H NMR spectra of Impurity-A](image-url)

**Figure-S1**: $^1$H NMR spectra of Impurity-A
Figure-S2: $^1$H NMR spectra of Impurity-B
Figure-S3: $^1$H NMR spectra of Compound 2
Figure-S4: $^1$H NMR spectra of Compound 3
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Figure-S17: Mass spectra of Compound 4

Figure-S18: Mass spectra of Compound 5