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Original article

Synthesis, characterization and antibacterial activity of novel heterocycle, coumacine, and two of its derivatives

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ABSTRACT

Heterocyclic nucleus plays a fundamental role in the medicinal chemistry and serves as a key template for the development of various therapeutic agents including broad spectrum antibacterial drugs. In an effort to develop new antibacterial agents, a bicyclic twelve-membered heterocyclic nucleus derived from coumarin was prepared by an uncomplicated method. The rate of ring closure for this nucleus, which was given the name coumacine, in addition to two of its derivatives was monitored spectroscopically and this rate followed zero order kinetics. The chemical structures of the synthesized products were established by detecting their physicochemical properties and analyzing their IR, ^{1}H NMR and ^{13}C NMR spectra. The *in vitro* antibacterial activity of coumacines was evaluated via agar dilution method against different standard aerobic and anaerobic bacterial strains using ciprofloxacin and metronidazole as positive controls, respectively; the results indicated that coumacine I has an excellent broad spectrum antibacterial activity against the tested bacterial strains with percentage of growth inhibition approximating to those of positive controls.

1. Introduction

There is a growing interest in the development of new antibacterial agents as a consequence to the gradual growth of bacterial resistance toward a variety of agents; besides, with the alarming rise of multi-drug resistance bacterial species (York, 2017); nowadays, physicians are forced to prescribe the second or even third option of antibiotic to fight these resistant bacteria (Perron et al., 2012).

With their origin firmly established in organic and medicinal chemistry, heterocyclic compounds introduce themselves as an essential sort of organic chemicals; they are defined by IUPAC as "cyclic compounds having, as ring members, atoms of at least two different elements" (IUPAC, 2017). In accordance with heteroatom(s) present in the ring system, heterocycles can be grouped as oxygen, nitrogen or sulfur-based orderliness; within each group, they are also organized according to the size of ring structure that is determined by the total number of atoms (St. Jean, and Fotsch, 2012). The type of heteroatom and the ring size along with the substituent groups on the lead structure are the principal determinants of heterocycle's physicochemical properties (Martins et al., 2015). Although there is a massive number of pharmacologically active heterocyclic compounds acting as antitumor (Chen et al., 2014; Sadhasivam et al., 2015; Abdel-Aziem 2017), anti-fungal (Cao et al., 2014; Chitra et al., 2017; Zhao et al., 2017), anti-viral (Zhang et al., 2014; Da Costa et al., 2017; Asif, 2017), anti-inflammatory (Khan et al., 2012; Malik, 2016; Gomha et al. 2017) and anticonvulsant (Zayed, 2014; Shakya et al., 2016; Saravanan et al., 2017), an increasing number of heterocycles have shown a potent antibacterial effect (Azab et al., 2013; Hafez et al., 2015; Chand et al., 2017).

Among the different biologically active oxygenated heterocyclic compounds, coumarin (Fig. 1) and its derivatives have become a fascinating subject of research due to their broad distribution in nature and their well-defined synthetic reactions (Al-Majedy et al., 2017; Detsi et al., 2017; El-Naggar et al., 2017). Also, they serve as important precursors for advanced design and synthesis of more pharmacologically active compounds (Tasior et al., 2015; Han et al., 2015; Valadbeigi and Ghodsi, 2017).

The aim of this work is the synthesis of heterocyclic compounds containing a novel chemical nucleus herein called coumacine that has not been synthesized before with the testing of their spectrum of antibacterial activity through achieving the following objectives:

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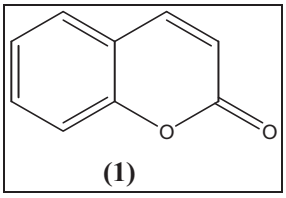

Fig. 1. Chemical structure of coumarin.

synthesis of these compounds from coumarins, detecting the kinetics of their ring closure, and testing there *in vitro* antibacterial activity against different standard aerobic and anaerobic bacterial strains using ciprofloxacin and metronidazole as positive controls, respectively.

2. Materials and methods

The chemicals and solvents used in this work were purchased from documented international sources and utilized without further purification. The microbiological cultures and anaerobe indicator test were supplied from Sigma-Aldrich. The melting points of the synthesized products were determined on an electrochemical CIA 9300 melting point apparatus using an open capillary method and they were uncorrected. The purity of compounds and the follow up of reactions were checked by ascending TLC on precoated silica gel plates (GF_{254} type 60, Merck); the spots on chromatograms were eluted by $CHCl_3$: acetone (4:1) as a mobile phase.

Bruker-Alpha ATR was used to scan IR spectra while the instrument used to identify UV spectra of the synthesized products and to follow up the kinetic study was Varian UV/Visible spectrophotometer. Among other UV absorption bands, the wavelength of maximum absorption (λ_{max}) was utilized in this work. Proton-nuclear magnetic resonance (^{1}H NMR) and carbon-nuclear magnetic resonance (^{13}C NMR) spectra of the synthesized products were scanned on Bruker Avance 300 and 400 MHz. the chemical shifts (δ) of these spectra were expressed in part per million (ppm) downfield to tetramethylsilane as an internal standard. In ^{1}H NMR, spin-spin coupling was identified by the following terms: singlet (s), doublet (d), triplet (t) and multiplet (m).

2.1. Synthesis

2.1.1. Synthesis of 4,6-Dimethylcoumarine (2)

A solution of *p*-cresol (1.05 ml, 10 mmol) and ethyl acetoacetate (1.4 ml, 11 mmol) was added dropwise over 30 min with a constant stirring to concentrated H_2SO_4 (25 ml) placed in an ice bath. The reaction mixture was stirred for 1 h in the ice bath, kept at room temperature overnight, heated to 50 °C and then directly poured into a mixture of crushed ice and H_2O with vigorous stirring. The precipitate was collected by filtration and washed with H_2O. The crude product was purified by dissolving it in 50 ml of 10% NaOH solution and the filtrate was then acidified with 1.026 N HCl. The titled product was gathered after 30 min, washed with H_2O and recrystallized from benzene. This compound was synthesized via a modified method to that reported by (Ahluwalia et al., 2005).

4,6-Dimethylcoumarine **(2)** off white powder (1.36 g, 78.12% yield), m.p 154–157 °C, λ_{max} (EtOH) 279 nm, R_f 0.526, IR (ν, cm^{-1}): 3035 (=C—H str.), 2950 and 2883 (C—H str., alkyl), 1700 (C=O str., ester), 1666 (C=C str.), 1270 (C—O str., ester); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.8 (s, 1H, Ar **H**), 7.5 (d, 1H, Ar **H**), 7.3 (d, 1H, Ar **H**), 6.4 (s, 1H, =C**H**), 2.35 (s, 3H, Ar—C**H**$_3$), 1.9 (s, 3H, =C—C**H**$_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ ppm: 160 (**C**=O), 154 (CH_3—**C**=C), 143 (Ar **C**—O), 136 (Ar **C**—CH_3), 131, 129.9, 129.7 (Ar **C**), 124 (Ar **C**—C—O), 111 (=**C**H), 26 (=C—**C**H_3), 20 (Ar—**C**H_3).

2.1.2. Synthesis of 6-Methyl-4-phenylcoumarin (3)

Through a solution of *p*-cresol (1.05 ml, 10 mmol) and ethyl benzoylacetate (1.9 ml, 11 mmol) in 30 ml absolute EtOH placed in a two-necked round-bottomed flask wrapped with aluminum foil, dry HCl gas was passed for 3 h under anhydrous conditions. The reaction mixture was kept for 48 h at room temperature and then in refrigerator for 24 h to complete the precipitation. The titled product was filtered, washed with cold EtOH and recrystallized from a mixture of CH_3OH: ether (1:3). This compound was synthesized via a modified method to that reported by (Ibrahim et al., 2014).

6-Methyl-4-phenylcoumarin (**3**) white crystals (1.58 g, 66.79% yield), m.p 132–134 °C, λ_{max} (EtOH) 316 nm, R_f 0.704, IR (ν, cm^{-1}): 3063 (=C—H str.), 2838(C—H str., alkyl), 1700 (C=O str., ester), 1641 (C=C str.), 1310 (C—O str., ester); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.7 (s, 1H, Ar **H**), 7.57 (dd, 2H, Ar **H**), 7.42 (m, 3H, Ar′ **H**), 6.6 (s, 1H, =C**H**), 2.1 (s, 3H, Ar—C**H**$_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ ppm: 160 (**C**=O), 156 (Ar′—**C**=), 143 (Ar **C**—O), 139 (Ar′ **C**—C=CH), 138 (Ar **C**—CH_3), 130.8, 130.1, 129.7, 124 (Ar **C**), 130.2, 129.9, 127 (Ar′ **C**), 107 (=**C**H), 20 (Ar—**C**H_3).

2.1.3. General procedure for the reduction of coumarins

A solution of pure $LiAlH_4$ (0.76 g, 20 mmol) in 20 ml dry ether was added dropwise to a solution of coumarin derivative (10 mmol) in dry ether placed in an ice bath. After stirring for 15 min at 0 °C, 3 M HCl (8 ml) was gradually added and the pH of the reaction mixture was adjusted to 5 with HCl (1.032 N). The ether layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was dissolved in aqueous EtOH, filtered and evaporated (Dehaen et al., 2011).

Z-2-(3-hydroxypropenyl)phenol **(1a)** white crystals from EtOH (0.60 g, 40.23% yield), m.p 148–151 °C, λ_{max} (EtOH) 286 nm, R_f 0.337, IR (ν, cm^{-1}): 3301 (phenolic OH, str.), 3276 (alcoholic OH, str.), 3057 (=C—H str.), 2894(C—H str., alkyl), 1640 (C=C str.); ^{1}H NMR (CD_3OD, 300 MHz) δ ppm: 10 (s, 1H, Ar—O**H**), 7.05 (d, 1H, Ar **H**), 6.6–6.7 (dd, 2H, Ar **H**), 6.4 (d, 1H, Ar **H**), 6.3 (d, 1H, Ar—C**H**=), 5.75 (m, 1H, =C**H**—CH_2), 4.25 (d, 2H, C**H**$_2$—OH), 3.5 (s, 1H, CH_2—O**H**); ^{13}C NMR (CD_3OD, 100 MHz) δ ppm: 157 (Ar **C**—OH), 130, 128, 123, 121, 117 (Ar **C**), 129 (Ar—**C**H=), 127 (=**C**—CH_2), 62 (**C**H_2—OH).

Z-2-(3-Hydroxy-1-methyl-propenyl)-4-methylphenol **(2a)** white crystals from EtOH (0.70 g, 39.19% yield), m.p 178–180 °C, λ_{max} (EtOH) 289 nm, R_f 0.358, IR (ν, cm^{-1}): 3312 (phenolic OH, str.), 3257 (alcoholic OH, str.), 3077 (=C—H str.), 2890 (C—H str., alkyl), 1644 (C=C str.); ^{1}H NMR (CD_3OD, 300 MHz) δ ppm: 9.9 (s, 1H, Ar—O**H**), 6.8 (s, 1H, Ar **H**), 6.5 (d, 1H, Ar **H**), 6.2 (d, 1H, Ar **H**), 6.0 (t, 1H, =C**H**), 4.2 (d, 2H, —C**H**$_2$), 3.5 (s, 1H, CH_2—O**H**), 2.5 (s, 3H, Ar—C**H**$_3$), 2 (s, 3H, =C—C**H**$_3$); ^{13}C NMR (CD_3OD, 75.47 MHz) δ ppm: 155 (Ar **C**—OH), 137 (CH_3—**C**=C), 133 (Ar **C**—CH_3), 130, 129, 124, 117 (Ar **C**), 121 (=**C**H), 62 (**C**H_2—OH), 26 (=C—**C**H_3), 20 (Ar—**C**H_3).

Z-2-(3-Hydroxy-1-phenyl-propenyl)-4-methylphenol **(3a)** off-white powder from EtOH (0.79 g, 32.89% yield), m.p 140–143 °C, λ_{max} (EtOH) 336 nm, R_f 0.567, IR (ν, cm^{-1}): 3308 (phenolic OH, str.), 3259 (alcoholic OH, str.), 3063 (=C—H str.), 2928, 2883 (C—H str., alkyl), 1642 (C=C str.); ^{1}H NMR (CD_3OD, 300 MHz) δ ppm: 9.6 (s, 1H, Ar—O**H**), 7.3–7.4 (m, 3H, Ar′ **H**), 6.8 (s, 1H, Ar **H**), 6.5–6.6 (dd, 2H, Ar **H**), 6.1 (t, 1H, =C**H**), 4.2 (d, 2H, C**H**$_2$—OH), 4