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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

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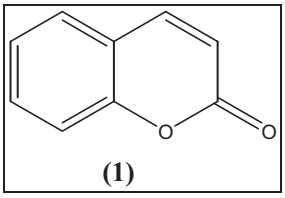


Fig. 1. Chemical structure of coumarin.

objectives: 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 (s, 1H, CH_2—O**H**), 2.1 (s, 3H, Ar—C**H**$_3$); ^{13}C NMR (CD_3OD, 100 MHz) δ ppm: 155 (Ar **C**—OH), 145 (Ar′—**C**=), 142 (Ar′ **C**—C=CH), 133 (Ar **C**—CH_3), 130, 129.3, 124, 117 (Ar **C**), 129.7, 128, 127 (Ar′ **C**), 116 (=**C**H), 62 (**C**H_2—OH), 20 (Ar—**C**H_3).

2.1.4. General procedure for the preparation of disodium salt of diol derivative

A mixture of diol derivative (10 mmol) in 19 ml NaOH (1.029 N) was heated at 60 °C in a water bath with constant stirring for 1 h. The reaction mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was re-dissolved in an adequate amount of DMF, filtered and the filtrate was concentrated to dryness under reduced pressure to afford a disodium salt of diol derivative. This procedure can be considered as a modified one to that reported by (Sethuraman et al., 2014).

2.1.5. General procedure for the synthesis of coumacines

To a conical flask enveloped with aluminum foil and lodged in an ice bath, a suspension of disodium salt of diol derivative (10 mmol) in 45 ml dry ethyl acetate was added. As the suspension temperature falls to 0 °C, a solution of methylene iodide (0.4 ml, 5 mmol) in dry ethyl acetate (4.6 ml) was added in portions over 15 min. The reaction mixture was stirred for 6 h at 80 °C and subsequently the solvent was evaporated under reduced pressure. H_2O (20 ml) was added to the residue and the aqueous layer was extracted with $CHCl_3$ (3 × 20 ml). The combined organic layer was dried over $CaCl_2$ and evaporated to afford the target product. This procedure can be considered as a modified one to that reported by (Razali and Ahmad, 2017).

4H-Benzo[d][1,3]dioxocine **(coumacine)** white powder from a mixture of ether: $CHCl_3$ (1:2), (0.90 g, 55.46% yield), m.p 128–130 °C, λ_{max} (EtOH) 268 nm, R_f 0.577, IR (ν, cm^{-1}): 3065 (=C—H str.), 2900, 2866 (C—H str., alkyl), 1648 (C=C str.), 1258 (asymmetrical str. aryl alkyl ether), 1152 (asymmetrical str. aliphatic ether), 1050 (symmetrical str. aryl alkyl ether); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.0–7.2 (m, 4H, Ar **H**), 6.8 (d, 1H, Ar—C**H**=), 6.1 (m, 1H, =C**H**—CH_2), 5.7 (s, 2H, O—C**H**$_2$—O), 3.8 (d, 2H, =CH—C**H**$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 158 (Ar **C**—O—), 128, 127.3, 120.6, 120.4, 111 (Ar **C**), 127.5 (Ar—**C**H=), 125 (=**C**—CH_2), 99 (O—**C**H_2—O), 63 (=CH—**C**H_2—O).

6,8-Dimethyl-4H-benzo[d][1,3]dioxocine **(coumacine I)** white powder from a mixture of ether: $CHCl_3$ (1:1.5), (1.18 g, 62.17% yield), m.p 176–178 °C, λ_{max} (EtOH) 279 nm, R_f 0.589, IR (ν, cm^{-1}): 3078 (=C—H str.), 2933, 2888 (C—H str., alkyl), 1650 (C=C str.), 1255 (asymmetrical str. aryl alkyl ether), 1157 (asymmetrical str. aliphatic ether), 1067 (symmetrical str. aryl alkyl ether); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.0 (s, 1H, Ar **H**), 6.7–6.8 (dd, 2H, Ar **H**), 6.1 (t, 1H, =C**H**—CH_2), 5.8 (s, 2H, O—C**H**$_2$—O), 3.9 (d, 2H, =CH—C**H**$_2$), 2.5 (s, 3H, Ar—C**H**$_3$), 2.0 (s, 3H, =C—C**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 159 (Ar **C**—O), 140 (CH_3—**C**=C), 130 (Ar **C**—CH_3), 129.5, 129.0, 122, 114 (Ar **C**), 119 (=**C**H—CH_2), 95 (O—**C**H_2—O), 66 (=CH—**C**H_2—O), 25 (=C—**C**H_3), 20 (Ar—**C**H_3).

8-Methyl-6-phenyl-4H-benzo[d][1,3]dioxocine **(coumacine II)** white powder from a mixture of ether: $CHCl_3$ (1:2), (1.99 g, 79.04% yield), m.p 156–159 °C, λ_{max} (EtOH) 288 nm, R_f 0.788, IR (ν, cm^{-1}): 3066 (=C—H str.), 2931, 2837 (C—H str., alkyl), 1652 (C=C str.), 1254 (asymmetrical str. aryl alkyl ether), 1144 (asymmetrical str. aliphatic ether), 1045 (symmetrical str. aryl alkyl ether); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm: 7.1–7.3 (m, 3H, Ar′ **H**), 7 (s, 1H, Ar **H**), 6.7–6.9 (dd, 2H, Ar **H**), 6.2 (t, 1H, =C**H**—CH_2), 5.7 (s, 2H, O—C**H**$_2$—O), 3.9 (d, 2H, =CH—C**H**$_2$), 2.2 (s, 3H, Ar—C**H**$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 159 (Ar **C**—O—), 140 (Ar′—**C**=CH), 136 (Ar′ **C**—C=CH), 130 (Ar **C**—CH_3), 129.5, 127.9, 117, 109 (Ar **C**), 129.0, 127.3, 127.1 (Ar′ **C**), 112 (=**C**H—CH_2), 98 (O—**C**H_2—O), 60 (=CH—**C**H_2—O), 23 (Ar—**C**H_3).

2.2. Kinetic study

The rate of ring closure in the formation of coumacine and its two derivatives was followed up spectrophotometrically and monitored for the increase in the product concentration with time by applying Beer-Lambert law (Abood et al., 2015). At selected time intervals, a drop was taken from a reaction mixture; diluted to 2 ml with EtOH and subsequently estimated at defined λ_{max} to determine the concentration of the formed product.

2.3. Activity against aerobic bacteria

The standard aerobic bacterial strains involved in the antibacterial study using ciprofloxacin as a positive control were: *Escherichia coli* ATCC 25922, *Haemophilus influenzae* ATCC 49247, *Klebsiella pneumonia* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853. The susceptibility of these bacteria was verified via agar dilution technique using cation-adjusted Mueller-Hinton agar (NCCLS, 2015a). Coumacine or one of its derivatives (1 mg) was dissolved in DMSO (1 ml) and then diluted with sterile distilled H_2O to achieve the following concentrations: 2.5, 5, 10, 25, 50, 100 and 200 μg/ml.

The inoculum of 10^5 CFU/spot was employed on the agar plates utilizing multiple inoculator (Steers replicator). The inoculated agar plates and that free from the tested compound were incubated at 37 °C for 24 h under aerobic conditions. Afterwards, the minimal inhibitory concentration (MIC) and the percentage of bacterial growth inhibition comparing with negative control of each tested compound were pointed.

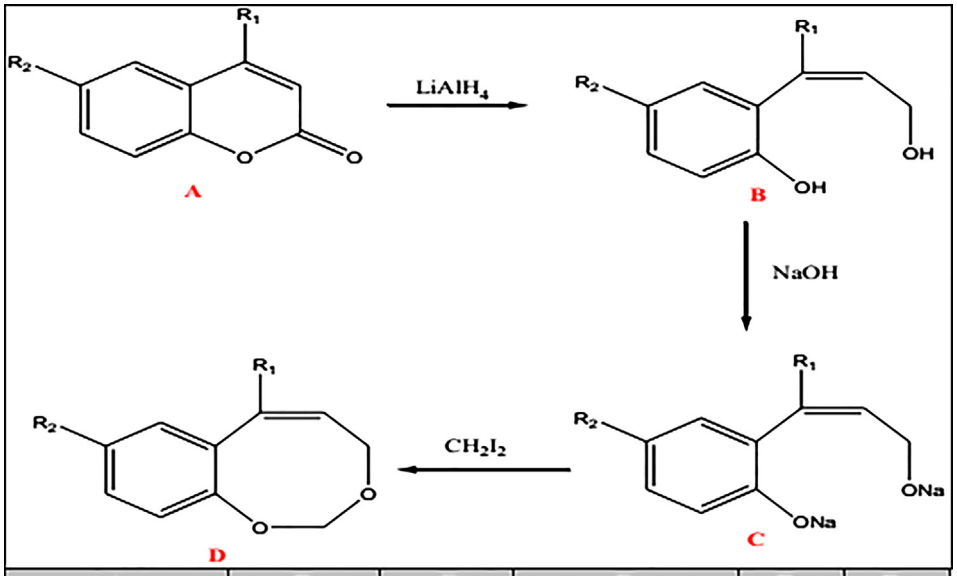
2.4. Activity against anaerobic bacteria

The investigation of anti-anaerobic activity of coumacine and its two derivatives using metronidazole as a positive control was conducted against the following standard bacterial strains: *Clostridium perfringens* ATCC 13124, *Bacteroides fragilis* ATCC 25285, *Prevotella melaninogenica* ATCC 25845 and *Fusobacterium necrophorum* ATCC 25286. This activity was tested via a plate dilution technique using Brucella agar supported with 5% sheep blood (NCCLS, 2015b). Coumacine or one of its derivatives (1 mg) was dissolved in DMSO (1 ml) and then diluted with sterile distilled H_2O to achieve the following concentrations: 2.5, 5, 10, 25, 50, 100 and 200 μg/ml. The inoculum of 10^5 CFU/spot was employed on the agar plates utilizing multiple inoculator.

The inoculated agar plates and that free from the tested compound were incubated in an anaerobic jar for 48 h at 37 °C under anaerobic environment which consists of aerophobic atmosphere (10% CO_2, 10% H_2 and 80% N_2), palladium catalyst and anaerobe indicator test. Subsequently, MIC and the percentage of bacterial growth inhibition comparing with negative control of each tested compound were measured.

3. Results and discussion

Since the gap between antibacterial agents and their efficacy against different pathogenic bacteria has been continuously expanded due to prevalent and haphazard use (Sampaio et al., 2014), the discovery of new compounds with proven antimicrobial activity becomes an urgent need especially those with unusual heterocyclic structure.



A	B	C	D	R_1	R_2
Coumarin (1)	1a	1b	Coumacine	H	H
2	2a	2b	Coumacine I	CH_3	CH_3
3	3a	3b	Coumacine II	Phenyl	CH_3

Scheme 1. General synthetic pathway of coumacines.

3.1. Synthesis

The novelty of this work comes from the synthesis of a bicyclic twelve-membered heterocyclic nucleus derived from coumarin by a convenient method as displayed in Scheme 1; the aforementioned nucleus, herein called coumacine, has not been synthesized before.

Coumarins (2) and (3) were prepared via Pechmann condensation though modified methods to those reported by (Ahluwalia et al., 2005) and (Ibrahim et al., 2014), respectively. $LiAlH_4$, a powerful and non-selective reducing agent, was utilized to reduce coumarins 1, 2 and 3 into corresponding open ring diols 1a, 2a and 3a respectively under restricted reaction conditions of solvent, duration and temperature (Dehaen et al., 2011).

The resulting diols were transformed to their disodium salts by NaOH to enhance the nucleophilicity of alcoholic and phenolic hydroxyl groups that is required to efficiently attack methylene iodide under anhydrous condition affording coumacine and its two derivatives in acceptable percentage yields.

3.2. Kinetic study

Based on the parameters obtained from triple trials as shown in Table 1, the rate of ring closure in the formation of synthesized coumacines was found to follow zero order kinetics.

The kinetic data used in the application of Beer-Lambert law are listed in Table 2 and they indicated that coumacine II was formed

Table 1
Parameters obtained from the kinetic study.

Product name	λ_{max} (nm, EtOH)	ε (M^{-1} cm^{-1})	k_{obs}(M s^{-1})	$t_{1/2}$ (s)
Coumacine	268	416.2	0.19×10^{-6}	184.21
Coumacine I	279	401.8	0.28×10^{-6}	175.00
Coumacine II	288	379.6	0.41×10^{-6}	166.46

Table 2
The kinetic data used in the application of Beer-Lambert law.

Absorbance	Product name	Time (min.)	A ($M \times 10^6$)
0.0025	Coumacine	30	6.0067
0.0030	Coumacine I		7.4664
0.0038	Coumacine II		10.0105
0.0055	Coumacine	60	13.2148
0.0066	Coumacine I		16.4261
0.0088	Coumacine II		23.1823
0.0077	Coumacine	90	18.5007
0.0102	Coumacine I		25.3858
0.0136	Coumacine II		35.8272
0.0092	Coumacine	120	22.1048
0.0130	Coumacine I		32.3544
0.0181	Coumacine II		47.6818
0.0122	Coumacine	150	29.3128
0.0165	Coumacine I		41.0652
0.0213	Coumacine II		56.1117
0.0146	Coumacine	180	35.0793
0.0197	Coumacine I		49.0294
0.0260	Coumacine II		68.4932
0.0167	Coumacine	210	40.1249
0.0236	Coumacine I		58.7357
0.0315	Coumacine II		82.9821
0.0197	Coumacine	240	47.3330
0.0253	Coumacine I		62.9667
0.0349	Coumacine II		91.9389
0.0216	Coumacine	270	51.8981
0.0299	Coumacine I		74.4151
0.0400	Coumacine II		105.3741
0.0242	Coumacine	300	58.1451
0.0328	Coumacine I		81.6327
0.0430	Coumacine II		113.2771
0.0267	Coumacine	330	64.1519
0.0363	Coumacine I		90.3435
0.0481	Coumacine II		126.7123
0.0291	Coumacine	360	69.9183
0.0394	Coumacine I		98.0587
0.0522	Coumacine II		137.5132

Table 3
The MIC (μg/ml) and percentage of bacterial growth inhibition for the synthesized products and positive controls.

Aerobic bacteria	Ciprofloxacin		Coumacine		Coumacine I		Coumacine II	
	MIC	%	MIC	%	MIC	%	MIC	%
Escherichia coli	0.80	87	100	54.3	5	80.5	50	52.2
Haemophilus influenzae	0.65	82	N	–	25	56.9	N	–
Klebsiella pneumonia	0.5	88	50	48.5	5	79.3	200	49.4
Pseudomonas aeruginosa	0.76	92	200	35.9	5	77.9	N	–
Anaerobic bacteria	Metronidazole		Coumacine		Coumacine I		Coumacine II	
	MIC	%	MIC	%	MIC	%	MIC	%
Clostridium perfringens	0.78	85	N	–	10	65.3	N	–
Bacteroides fragilis	3.5	94	25	39.9	5	82.7	100	37.8
Prevotella melaninogenica	0.65	92	N	–	5	82.3	5	33.1
Fusobacterium necrophorum	1.6	90	50	33.8	5	78.8	50	36.7

N = no antibacterial activity at 200 μg/ml.

in a rate faster than coumacine I, which is formed faster than coumacine. This is possibly due to the bulkiness of electron-donating group substituted on position 4 of the starting materials. It is proposed that as the repulsion between this group and the aromatic constituent of compounds 1b, 2b and 3b increases, the rate of ring closure to form coumacines will increase (Liao et al., 2000).

3.3. Antibacterial activity

Novel coumacines were screened for their antibacterial activity against four standard aerobic bacterial strains (*Escherichia coli* ATCC 25922, *Haemophilus influenzae* ATCC 49247, *Klebsiella pneumonia* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853) and against four standard anaerobic bacterial strains (*Clostridium perfringens* ATCC 13124, *Bacteroides fragilis* ATCC 25285, *Prevotella melaninogenica* ATCC 25845 and *Fusobacterium necrophorum* ATCC 25286) via agar dilution method using a solution of ciprofloxacin or of metronidazole as a positive control, respectively.

The results of this preliminary screening study as shown in Table 3 indicated that coumacine I has an excellent antibacterial activity against the tested aerobic and anaerobic bacteria with percentages of growth inhibition approximating to those of ciprofloxacin and metronidazole; so, this novel compound can be considered as an encouraging broad spectrum antibacterial agent. Although coumacine and coumacine II have a narrow spectrum and less potent antibacterial activity compared with coumacine I, they can be also regarded as heartening candidates for further microbiological screening tests.

4. Conclusion

Novel oxygenated heterocyclic nucleus termed coumacine in addition to two of its derivatives were prepared with good yields via a facile synthetic pathway, and their chemical structures were characterized by detecting their physicochemical properties and analyzing their IR, ^{1}H NMR and ^{13}C NMR spectra. The rate of their ring closure was studied via UV/Vis spectroscopy and followed zero order kinetics. The synthesized products were screened for their antibacterial activity against different aerobic and anaerobic standard bacterial strains. Although the three final products showed encouraging antibacterial activity, coumacine I has a broader and more potent activity compared with the other two using agar dilution method.

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