An efficient cyclocondensation reactions, antimicrobial activity and molecular orbital calculations of α-benzopyrone derivatives

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ABSTRACT
An efficient synthesis of 4,6,8-trimethyl-2-oxo-2H-chromene-3-carbonitrile (2) via Claisen condensation of 3,5-dimethyl-2-hydroxyacetophenone with ethyl cyanoacetate in the presence of sodium metal is reported. Cyclocondensation reactions of compound (2) with ethyl acetate or with ethyl cyanoacetate in the presence of ethoxide gave sodium salt of 7-amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromen-6-one derivatives (3) and (4), respectively, which upon neutralization with 10% hydrochloric acid gave 7-amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromen-6-one derivatives (5). Hydrolysis of compound (2) with ethanolic sodium hydroxide solution gave 4,6,8-trimethyl-2-oxo-2H-chromene-3-carboxylic acid (6). Treatment of compound (2) with Vilsmier reagent using excess POCl3 gave 4-(chloroformyl)ethyl-6,8-dimethyl-2-oxo-2H-chromene-3-carbonitrile (7). Also, condensation of compound (2) with DMF-DMA in xylene or with POCl3/DMF in pyridine gave the same product 4-((E)-2-(dimethylamino)vinyl)-6,8-dimethyl-2-oxo-2H-chromene-3-carbonitrile (8). The cyclocondensation reactions of compound (8) with hydroxylamine hydrochloride, urea, and with hydrazinecarbothioic acid gave 3,4-dihydro-3-hydroxy-4-imino-7,9-dimethylchromeno[3,4-c]pyridin-5-one (9), 4-amino-7,9-dimethyl-5-oxo-4H-chromeno[3,4-c]pyridine-3(5H)-carboxamide (10) and 3-amino-3,4-dihydro-4-imino-7,9-dimethylchromeno[3,4-c]pyridin-5-one (11), respectively. Also, acid hydrolysis of compound (8) gave 7,9-dimethyl-5H-chromeno[3,4-c]pyridine-4,5-dione (12). Structures of the products were established on the basis of elemental analysis, IR, 1H and 13C NMR, mass spectra and semi-empirical AM1-MO calculations. The antimicrobial activities of the synthesized products were also studied.

1. Introduction
α-Benzopyrone derivatives constitute an important class of oxygenated heterocycles [1]. Many compounds containing the benzopyrone nucleus, both naturally occurring and synthetic, are known to exhibit pharmacological activity such as antifungal [2-4], antibacterial [5], anti-mycobacterial [6,7], anticoagulants [8,9], inhibitors of some enzymes [10,11], and antitumor [12-14]. With the expectations to find biological activity, we decided to investigate the synthesis of some novel systems of α-benzopyrones derivatives bearing fused and isolated moiety. Recently, the synthesis [15], photochemical [16] and theoretical [1,2,17] properties of α-benzopyrone derivatives were investigated.

The aim of the present paper is to investigate an efficient synthesis of coumarin derivatives containing active methyl and cyano groups and study their cyclocondensation reactions with ethyl acetate, ethyl cyanoacetate, hydroxymaline hydrochloride, urea, and with hydrazine carbothioic acid. The condensations of 4-methyl group of coumarin derivatives with Vilsmier reagents at different conditions and with DMF-DMA were also studied.

The antimicrobial activities for the prepared compounds were investigated. Also, semi-empirical AM1 and Ab Initio (STO-3G) molecular orbital calculations for the new compounds were performed and compared with their experimental data.

2. Experimental
2.1. Instrumentation
The uncorrected melting point was determined in an open capillary tube on a digital Stuart SMP-3 apparatus. 1H NMR/13C NMR spectra were obtained on a 300 MHz/75.46 MHz Varian Mercury VX-300 NMR spectrometer in DMSO-d6 with tetramethylsilane as an internal standard. Elemental analyses were performed on Vario El Elementar apparatus. IR spectra were recorded on FTIR Nicolet iS10 spectrophotometer (cm⁻¹), using KBr disks. Mass spectra were recorded on a Gas Chromatographic GCMSqp 1000 ex Shimadzu instrument at 70 eV.

The chemicals were purchased from the suppliers as the highest purity grade. The theoretical data which were obtained from molecular mechanical calculations on the basis of the semi-empirical AM1 and Ab Initio (STO-3G) methods of HyperChem 8.03 computer program.
2.2. Synthesis

2.2.1. 4,6,8-Trimethyl-2-oxo-2H-chromene-3-carbonitrile (2)

Sodium metal (2 g, 8.6 mmol) was added in small portion to a solution of 3,5-dimethyl-2-hydroxyacetophenone (1) (4 g, 24 mmol) in ethyl cyanoacetate (30 cm³). The reaction mixture was heated on water-bath for 3 h, and then cooled to room temperature, treated with ethanol (10 cm³) and refluxed for 1 h. The solid obtained was filtered, and crystallized from ethanol to give (2) as pale green crystals (Scheme 1). Yield: 62%. M.p.: 200-202 °C. FT-IR (KBr, cm⁻¹): 3072 (CHarom.), 2921, 2953 (CHaleph.), 7.72 (s, 1H, CH₃). 7.49 (s, 1H, H-5). 1H NMR (300 MHz, DMSO-δ, δ ppm): 2.25 (6 s, 3H, CH₃), 2.31 (6 s, 3H, CH₃), 7.42 (s, 1H, H-7). 7.49 (s, 1H, H-5). 13C NMR (75 MHz, DMSO-δ, δ ppm): 24.2, 27.7, 29.6, 109.9, 123.5, 126.7, 133.6, 134.9, 143.6, 146.7, 158.3, 165.9, 172.9. MS (EI, m/z (%)): 212.4 (M⁻, 86.9), 212.8 (M⁺, 100), 213.8 (M⁻+1, 14.5). Anal. calcd. for C₃₂H₂₂NO₃: C, 73.23; H, 5.20; N, 6.57. Found: C, 73.10; H, 5.00; N, 6.60%.

2.2.2. Sodium salt of 7-amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromene-6-one derivatives (3) and 7-amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromene-6-one (5)

A solution of compound (2) (2 g, 0.93 mmol) in ethoxide (1.0 g sodium, 40 cm³ absolute ethanol), and then ethyl cyanoacetate (9 cm³) was added and the mixture was refluxed on water-bath for 3 h, cooled to room temperature. The solid obtained was filtered, and crystallized from ethanol to give (3) as pale-yellow crystals (Scheme 1 and 2). Yield: 89%. M.p.: 168-170 °C. The compound (3) (0.5 g, 0.18 mmol) was acidified with 10% HCl and the solid obtained was filtered, and crystallized from water to give (5) as pale-yellow crystals (Scheme 1 and 2). Yield: 89%. M.p.: 166-169 °C.

7-Amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromene-6-one derivatives (3): FT-IR (KBr, cm⁻¹): 3309, 3489 (br., NH₃), 2921 (CHaleph.), 1721 (w, C=Oamide), 1655 (s, C=Ocylic ketone). 1H NMR (300 MHz, DMSO-δ, δ ppm): 1.63 (6 s, 2H, NH₂, exchangeable with D₂O), 2.22 (6 s, 3H, CH₃), 2.28 (6 s, 3H, CH₃), 6.23 (6 s, 1H, H-8), 7.09 (6 s, 1H, H-3), 7.19 (6 s, 1H, H-1), 7.31 (6 s, 1H, H-10). 7-Amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromene-6-one (5): FT-IR (KBr, cm⁻¹): 2922, 3455 (br., OH, NH₃), 1725 (m, C=Ocoumarin). 1H NMR (300 MHz, DMSO-δ, δ ppm): 2.29 (6 s, 3H, CH₃), 2.33 (6 s, 3H, CH₃), 3.83 (6 s, 2H, NH₂, exchangeable with D₂O), 6.22 (6 s, 1H, OH, exchangeable with D₂O), 7.21 (6 s, 1H, H-7), 7.26 (6 s, 1H, H-2), 7.28 (6 s, 1H, H-1), 7.37 (6 s, 1H, H-10). 13C NMR (75 MHz, DMSO-δ, δ ppm): 24.5, 25.3, 27.7, 29.8, 52.5, 53.8, 123.4, 127.8, 132.1, 132.7, 142.7, 143.3, 143.9, 157.9, 166.8, 175.5. MS (EI, m/z (%)): 255.7 (M⁺, 6.5). Anal. calcd. for C₃₂H₂₂NO₃: C, 70.58; H, 4.90; N, 5.49. Found: C, 70.30; H, 4.90; N, 5.40%.

2.2.3. Sodium salt of 7-amino-9-hydroxy-2,4-dimethyl-6-oxo-6H-benzo[c]chromene-8-carbonitrile (4)

A solution of compound (2) (2 g, 0.93 mmol) in ethoxide (0.8 g sodium, 40 cm³ absolute ethanol), and then ethyl cyanoacetate (9 cm³) was added and the mixture was refluxed on water-bath for 3 h, cooled to room temperature. The solid obtained was filtered, and crystallized from ethanol to give (4) as white crystal (Scheme 1 and 2). Yield: 31%. M.p.: 183-185 °C. FT-IR (KBr, cm⁻¹): 3397 (br., NH₃), 2976 (CHaleph.), 2263 (CN), 1623 (br., C=O), MS (EI, m/z (%)): 301.0 (M⁻-1, 16.7). Anal. calcd. for C₃₂H₂₂N₂NaO₂: C, 63.58; H, 3.67; N, 9.27. Found: C, 63.10; H, 3.20; N, 9.10%.

2.2.4. 4,6,8-Trimethyl-2-oxo-2H-chromene-3-carboxylic acid (6)

A solution of compound (2) (0.5 g, 0.23 mmol) in ethanol (2 cm³), sodium hydroxide solution (0.09 g sodium hydroxide, 2 cm³ water) was added and the mixture was refluxed for 2 h, cooled to room temperature. The solid obtained was filtered, and crystallized from ethanol to give (6) as dark brown crystals (Scheme 3). M.p. 206-207 °C. Yield: 87%. FT-IR (KBr, cm⁻¹): 3081 (br., OH), 2920, 2959 (CHaleph.), 1742 (s, C=Oacid). 1660 (br., C=Oamide).
Phosphorus oxychloride (2.4 cm³) was added dropwise to DMF (6 cm³) with stirring at 30-35 °C, after the addition was completed, the solution was stirred at 50-60 °C for 30 min. A solution of compound (2) (1 g, 0.46 mmol) in dry pyridine (6 cm³) was added to the above mixture dropwise at 30-35 °C, and after the addition was completed, the mixture was stirred at 50-60 °C for 3 h, cooled to room temperature. The mixture was poured over cold water. The solid obtained was filtered, and crystallized from DMF to give (7) as yellow crystals (Scheme 3). M.p.: 268-270 °C. Yield: 85%. FT-IR (KBr, cm⁻¹): 3424 (enolic OH), 2930, 2958 (CH₃), 2722 (CH₇), 2244 (CN), 1735 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 2.28 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 2.19 (s, 3H, CH₃), 2.25 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 6.17 (s, 1H, OH; exchangeable with D₂O), 7.18 (s, 1H, H-7), 7.28 (s, 1H, H-5). 13C NMR (75 MHz, DMSO-d₆, δ ppm): 24.3, 25.3, 29.8, 127.5, 130.8, 132.7, 134.3, 142.7, 144.1, 157.8, 158.3, 166.6, 175.3. MS (EI, m/z (%)): 243.0 (M⁻, 100%), 244.0 (M⁺, 21.2), 245.0 (M⁺+, 5.7). Anal. calcd. for C₁₁H₁₀ClNO₃: C, 60.99; H, 3.66; N, 5.08. Found: C, 61.00; H, 3.60; N, 5.10%.

2.2.5. 4-(Chloroformyl)methyl-6,8-dimethyl-2-oxo-2H-chromene-3-carbonitrile (7)

Phosphorusoxy chloride (2.4 cm³) was added dropwise to DMF (6 cm³) with stirring at 30-35 °C, after the addition was completed, the solution was stirred at 50-60 °C for 30 min. A solution of compound (2) (1 g, 0.46 mmol) in dry pyridine (6 cm³) was added to the above mixture dropwise at 30-35 °C, and after the addition was completed, the mixture was stirred at 50-60 °C for 3 h, cooled to room temperature. The mixture was poured over cold water. The solid obtained was filtered, and crystallized from DMF to give (7) as yellow crystals (Scheme 3). M.p.: 268-270 °C. Yield: 85%. FT-IR (KBr, cm⁻¹): 3424 (enolic OH), 2930, 2958 (CH₃), 2722 (CH₇), 2244 (CN), 1735 (C=O). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 2.28 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 7.14 (s, 1H, H-Cl), 7.44 (s, 1H, H-7), 7.69 (s, 1H, H-5), 9.01 (s, 1H, H=O). 13C NMR (75 MHz, DMSO-d₆, δ ppm): 24.3, 25.3, 29.8, 127.5, 130.8, 132.7, 134.3, 142.7, 144.1, 157.8, 158.3, 166.6, 175.3. MS (EI, m/z (%)): 243.0 (M⁻, 100%), 244.0 (M⁺, 21.2), 245.0 (M⁺+, 5.7). Anal. calcd. for C₁₁H₁₀ClNO₃: C, 60.99; H, 3.66; N, 5.08. Found: C, 61.00; H, 3.60; N, 5.10%.

2.2.6. 4-((E)-2-(Dimethylamino)vinyl)-6,8-dimethyl-2-oxo-2H-chromene-3-carbonitrile (8)

Method A: A solution of compound (2) (0.5 g, 0.23 mmol) in dry xylene (10 cm³), and then DMF-DMA (0.35 cm³), 0.29 mmol was added and the mixture was refluxed for 0.5 h, cooled to room temperature. The solid obtained was filtered, and crystallized from DMF to give (8) as green crystals (Scheme 4). M.p.: 271-272 °C. Yield: 63%. FT-IR (KBr, cm⁻¹): 3438 (br., OH), 2935, 2999 (CH₂), 2202 (CN), 1767 (C=O), 1618 (C=C). 1H NMR (300 MHz, DMSO-d₆, δ ppm): 2.28 (s, 6H, CH₃), 2.35 (s, 3H, CH₃), 2.49 (s, 3H, CH₃), 5.71 (d, J = 12.6 Hz, 1H, H-9), 7.31 (s, 1H, H-7), 7.73 (s, 1H, H-5), 8.45 (d, J = 12.3 Hz, 1H, H-10). 13C NMR (75 MHz, DMSO-d₆, δ ppm): 18.4, 24.8, 29.8, 46.7, 55.3, 97.6, 108.8, 126.4, 129.3, 132.3, 135.0, 142.4, 144.8, 164.3, 238.5.
2.2.7. 4-Dihydro-3-hydroxy-4-imino-7,9-dimethylchromeno [3,4-c]pyridin-5-one (9)

A mixture of compound (8) (0.5 g, 0.19 mmol), hydroxylamine hydrochloride (0.13 g, 0.19 mmol), absolute ethanol (15 cm³), and drops of acetic acid was refluxed for 3 h, cooled to room temperature. The solid obtained was filtered, and crystallized from DMF to give (9) as yellow crystals (Scheme 5). M.p.: 315-316 °C. Yield: 85%. FT-IR (KBr, cm⁻¹): 3440 (br., NH), 3223 (br., NH₂), 2920 (CHαleph.), 1702 (C=O), 1669 (C=Oamide). MS (El, m/z (%)): 280.0 (M-3, 18.8), 281.0 (M-2, 2.6). Anal. calcd. for C₁₉H₁₅N₃O₂: C, 69.26; H, 4.63; N, 14.83. Found: C, 69.02; H, 4.50; N, 14.62.

2.2.8. 4-Imino-7,9-dimethyl-5-oxo-4H-chromeno[3,4-c]pyridine-3(5H)-carboxamide (10)

A mixture of compound (8) (0.5 g, 0.19 mmol), urea (0.11 g, 0.19 mmol), and glacial acetic acid (10 cm³) was refluxed for 3 h, cooled to room temperature. The solid obtained was filtered, and crystallized from DMF to give (10) as yellow crystals (Scheme 6). M.p.: >300 °C. Yield: 73%. FT-IR (KBr, cm⁻¹): 3359, 3095 (br., NH, NH₂), 2970, 2919 (CHαleph.), 1728 (C=Oamide). MS (El, m/z (%)): 254.0 (M⁺, 4.5), 256.0 (M⁺+1, 2.7). Anal. calcd. for C₁₉H₁₆N₃O₂: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.30; H, 4.60; N, 11.11.

2.2.9. 3-Amino-3,4-dihydro-4-imino-7,9-dimethylchromeno [3,4-c]pyridin-5-one (11)

A mixture of compound (8) (0.5 g, 0.19 mmol), hydrazinecarbodithioic acid (0.2 g, 0.19 mmol), and glacial acetic acid (10 cm³) was refluxed for 3 h, cooled to room temperature. The solid obtained was filtered, and crystallized from DMF to give (11) as yellow crystals (Scheme 6). M.p.: >300 °C. Yield: 73%. FT-IR (KBr, cm⁻¹): 3359, 3095 (br., NH, NH₂), 2970, 2919 (CHαleph.), 1728 (C=Oamide). MS (El, m/z (%)): 254.0 (M⁺, 4.5), 256.0 (M⁺+1, 2.7). Anal. calcd. for C₁₈H₁₅N₄O₂: C, 65.62; H, 4.72; N, 11.11. Found: C, 65.30; H, 4.60; N, 11.11.
2.2.10. 7,9-Dimethyl-3H-chromeno[3,4-c]pyridine-4,5-dione (12)

A mixture of compound (8) (0.4 g, 0.15 mmol) and conc. hydrochloric acid (3 cm³), was stirred at 60-70 °C for 0.5 h, cooled to room temperature and diluted with water (5 cm³). The solid obtained was filtered, and crystallized from DMF to give (12) as yellow crystals (Scheme 6). M.p.: 303-304 °C. Yield: 82%. FT-IR (KBr, cm⁻¹): 3166 (br., NH), 1732 (s, C=O coumarin), 1667 (w, C=Oamide), 1620 (C=C). 1H NMR (300 MHz, DMSO-d₆, cooled to room temperature and diluted with water (5 cm³), δ, ppm): 2.29 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 7.10-7.20 (m, 2H, H-1, H-2), 7.80 (s, 1H, H-10), 9.40 (S, 1H, H-2). MS (EI, m/z (%)): 241.0 (M⁺, 100), 242.0 (M⁺+1, 21.7). Anal. calc. for C₁₂H₁₀N₂O₂: C, 69.50; H, 4.50; N, 5.60%.

3. Results and discussion

3.1. Chemistry

The Claisen condensation of 2-hydroxycacetophenone derivatives with ethyl acetate in the presence of sodium metal gave β-dicarbonyl compound derivatives which were cyclized under the effect of concentrated sulfuric acid to give 2-methylchromone derivatives [17].

In our recent work [2], we described the Claisen condensation of 3,5-dichloro-2-hydroxycacetophenone and unsubstituted 2-hydroxycacetophenone with ethyl cyanoacetate in the presence of sodium metal gave 7-amino-2,4-dichloro-9-hydroxy-6-oxo-6H-benzo[c]chromene-8-carbonitrile and a mixture of 4-methyl-2-oxo-2H-chromene-3-carbonitrile and 7-amino-9-hydroxy-6-oxo-6H-benzo[c]chromene-8-carbonitrile, respectively.

The present work describes the Claisen condensation of 3,5-dimethyl-2-hydroxycacetophenone with ethyl cyanoacetate in the presence of sodium metal to give 4,6,8-trimethyl-2-oxo-2H-chromene-3-carbonitrile (2) (Scheme 1). The presence of cyano group in a position 3 activates the methyl group in a position 4 of compound (2) which facilitates the condensation reactions with ester derivatives containing active methylene group, so the reaction of compound (2) with ethyl acetate or with ethyl cyanoacetate in the presence of ethoxide gave sodium salt of 7-amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromen-6-one derivatives (3) and (4) respectively (Scheme 1). When the sodium salt of compound (3) was neutralized with 10% hydrochloric acid gave sodium-7-amino-9-hydroxy-2,4-dimethyl-6H-benzo[c]chromen-6-one (5) (Scheme 1).

The formation of compounds 3a, 4a and 5a proceeded via Claisen condensation on active methyl group of compound (2) followed by nucleophilic cyclization (Scheme 2).

The hydrolysis of cyano group of 4,6,8-trimethyl-2-oxo-2H-chromene-3-carbonitrile (2) in ethanolic sodium hydroxide solution gave 4,6,8-trimethyl-2-oxo-2H-chromene-3-carboxylic acid (6) (Scheme 3). Also, the Vilsmeier reaction of compound (2) in excess POCl₃ gave 4-(chloro(formyl)methyl)-6,8-dimethyl-2-oxo-2H-chromene-3-carbonitrile (7) (Scheme 3).

The presence of carbonyl group in a position 2 and cyano group in a position 3 of coumarin moiety facilitate the condensation reactions of 4-methyl group of coumarin, so the reaction of DMF-DMA with compound (2) in dry xylene gave 4-{(E)-2-(dimethylamino)vinyl}-6,8-dimethyl-2-oxo-2H-chromene-3-carbonitrile (8). Also, compound (8) was formed by condensation with dimethylformamide in the presence of POCl₃ (Scheme 4). The compound (8) was formed in the form of E-isomer from 1H NMR spectra which showed JCH = 12.3 Hz at δ = 5.71 and 8.45 ppm.

The cyclocondensation reactions of 4-{(E)-2-(dimethyl amino)vinyl}-6,8-dimethyl-2-oxo-2H-chromene-3-carbonitrile (8) with primary amines in ethanol gave 4-imino-7,9-dimethylchromeno[3,4-c]pyridine derivatives. The cyclo condensation reactions of compound (8) with hydroxylamine hydrochloride, urea, and with hydrazinecarbodithioic acid gave 3,4-dihydro-3-hydroxy-4-imino-7,9-dimethylchromen [3,4-c]pyridin-5-one (9), 4-imino-7,9-dimethyl-5-oxo-4H-chromeno [3,4-c]pyridine-3(SH)–carboxamide (10) (Scheme 5) and 3-amino-3,4-dihydro-4-imino-7,9-dimethylchromeno[3,4-c] pyridin-5-one (11) (Scheme 6) respectively. Also, the cyclocondensation reaction of compound (8) by acid hydrolysis of cyano group, followed by cyclization to give 7,9-dimethyl-3H-chromeno[3,4-c]pyridine-4,5-dione (12) (Scheme 6).

The formation of cyclocondensation products (9-11) proceeded via the nucleophilic replacement of NH₂ group of amine derivatives to -N(CH₃)₂; group of compound 8 to give the intermediate products 13, which can be cyclized by nucleophilic addition of NH group to C=N group (Scheme 7).

3.2. Molecular orbital calculations

The experimental IR frequencies of C=O groups of coumarin derivatives were compared with theoretical bond lengths of C=O groups which were obtained from molecular mechanical calculations on the basis of the semi-empirical AM1 and Ab Initio (STO-3G) methods of HyperChem 8.03 computer program after geometrical optimization of the structures for compounds [2-12] (Table 1).
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Table 1. Calculated bond lengths of C=O of coumarin derivatives by Semi-empirical AM1 and Ab initio (STO-3G) methods and their experimental IR \( \nu_{C=O} \) values for compounds (2-12).

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<th>Bond lengths of C=O (Å) by Semi-empirical AM1</th>
<th>Bond lengths of C=O (Å) by Ab Initio (STO-3G)</th>
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\( ^{a} \text{C=O} \text{coumarin} \)

\( ^{b} \text{C=O} \text{cyclic ketone} \)

\( ^{c} \text{C=O} \text{acid} \)

\( ^{d} \text{C=O} \text{aldehyde} \)

\( ^{e} \text{C=O} \text{amide} \)

The calculated bond lengths of C=O groups (Å) on the basis of semi-empirical AM1 method are linearly related to the measured IR \( \nu_{C=O} \) groups (cm\(^{-1}\)) for compounds (2-12) and from the linear relation bond length (C=O) = 1.699-2.743 \( \nu_{C=O} \), \( r = 0.913 \) except 4a, 4b (br. band of \( \nu_{C=O} \)), where \( r \) is regression coefficient. The negative slope reveals indirect proportionality of the calculated bond lengths with measured \( \nu_{C=O} \) values, which agreement with Hooke’s law and these support the proposed structures for the prepared compounds. On the other hand, when Ab initio (STO-3G) method was used, the linear relation between bond length (C=O) and \( \nu_{C=O} \) not agreement which is less efficient method than the semi-empirical AM1 method.

3.3. Antimicrobial activity

The newly synthesized compounds were screened against Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635), Gram-negative bacteria: *Salmonella typhimurium* (ATCC 14028) and *Escherichia coli* (ATCC 25922), Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*. The standardized disc-agar diffusion method [18] was followed to determine the activity of the synthesized compounds against the tested microorganisms.

The tested compounds were dissolved in dimethyl formamide (DMF) solvent and prepared in two concentrations 2 and 1 mg/mL. The antibiotic chloramphencol was used as standard reference in the case of Gram-negative bacteria, Cephalothin was used as standard reference in the case of Gram-positive bacteria and cycloheximide was used as standard reference in the case of yeasts and fungi. Compound 9 showed high activities against *Aspergillus fumigatus* at concentration of 2 mg and 1 mg, while it showed intermediate activities against *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* (Table 2).

Acknowledgements

I am particularly gratitude to Dr. Ibrahim Hassan, Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, for his kind cooperation in carrying out the antimicrobial screening throughout this work.
### Table 2. Antimicrobial activities data of compounds (2-12).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
<th>Yeasts&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus (ATCC 29213)</td>
<td>Bacillus subtilis (ATCC 6633)</td>
<td>Salmonella typhimurium (ATCC 14020)</td>
</tr>
<tr>
<td></td>
<td>Concentration&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Concentration</td>
<td>Concentration</td>
</tr>
<tr>
<td></td>
<td>Mean of zone diameters, mm</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>2</td>
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<tr>
<td>Control&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup> As chloramphenicol in the case of Gram-positive bacteria, Cephalothin in the case of Gram-negative bacteria and cycloheximide in the case of fungi.

<sup>b</sup> Identify the basis of routine cultural, morphological and microscopic characteristics.

<sup>c</sup> Low activity = Mean of zone diameter ≤ 1/3 of mean zone diameter of control.

<sup>d</sup> Intermediate activity = Mean of zone diameter ≤ 2/3 of mean zone diameter of control.

<sup>e</sup> High activity = Mean of zone diameter > 2/3 of mean zone diameter of control.

<sup>f</sup> Concentration, mg/mL.

**References**