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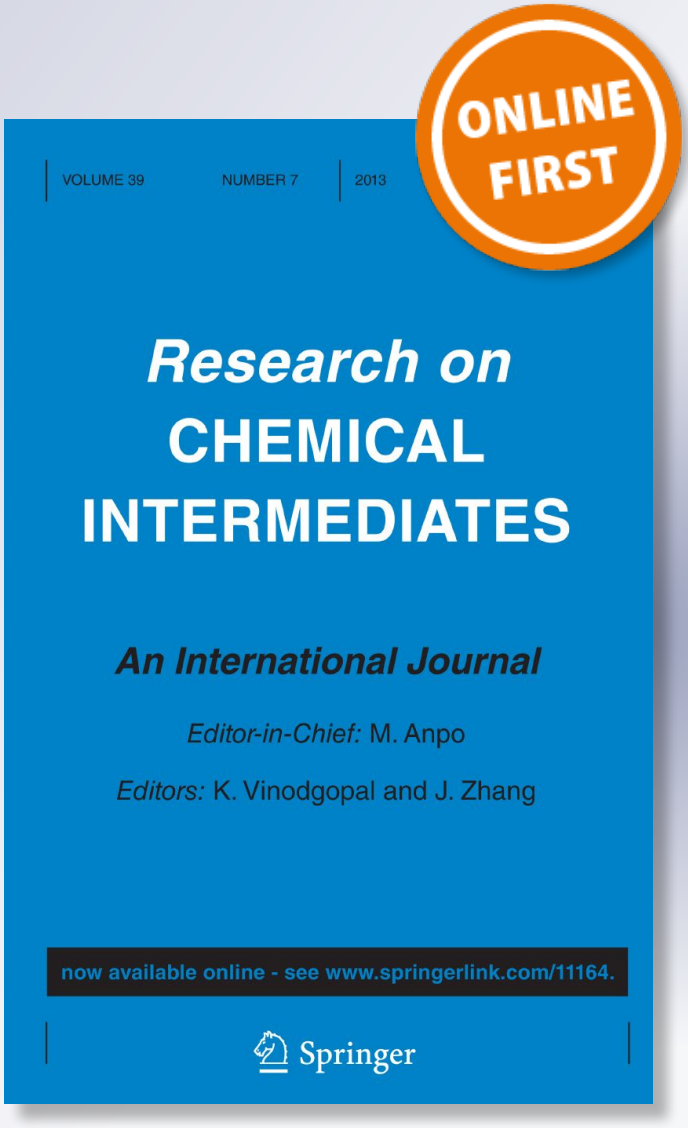
Research on Chemical Intermediates

ISSN 0922-6168

Res Chem Intermed
DOI 10.1007/s11164-017-3184-0



Springer

Res Chem Intermed
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Synthesis, antibacterial activity, and fluorescence properties of a novel series from [2,4-dioxochromen-3(4*H*)methyl]amino acid

Rasha A. M. Faty[1] · Asmaa K. Mourad[2] · Ramadan M. Abd Elmotaleb[2] · Rasha M. Radewan[2]

Received: 23 September 2016 / Accepted: 24 October 2017

Abstract A new solvent-free method for synthesis of starting compounds 2,4-dioxochromen-3(4*H*)methyl amino acetic acid derivatives **1a–e** via a green approach is reported. Also, the behavior of compound **1a** towards various nitrogen nucleophiles such as primary amines, hydrazine hydrate, and hydroxylamine hydrochloride to give corresponding compounds **2–4** was studied. Furthermore, chlorination of compound **1a** using a mixture of $PCl_5/POCl_3$ to yield acid chloride derivative **5** and the reaction of the latter compound **5** with various amino acids to obtain dipeptide compounds **6a–e** are described. Moreover, cyclization of compound **1a** in alkaline medium to afford dihydrochromeno[3,4-*c*]pyrrole-1-carboxylic acid **7** and cyclization of **6b** in acidic medium, namely Ac_2O, to yield piperazine derivative **8** are reported. Also, reaction of compound **1a** with maleic anhydride in dioxane to afford Diels–Alder adduct **9**, which posteriorly reacted with hydrazine hydrate to give **10**, was investigated. Most of the newly synthesized compounds were screened against Gram-positive and Gram-negative bacteria, with compound **5** exhibiting the maximum inhibition zone towards all four types of bacteria. In addition, the absorption and fluorescence emission of some of the substituted coumarins were studied in dioxane, revealing that the substituents altered both the absorption and fluorescence emission maxima.

Keywords Coumarin acetic acid · Dipeptide · Antibacterial activity · Fluorescence

✉ Rasha A. M. Faty
rashafaty@yahoo.com

[1] Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt

[2] Department of Chemistry, Faculty of Science, Fayoum University, Fayoum, Egypt

Published online: 13 November 2017

Introduction

Fluorescent heterocyclic compounds are of interest in many fields as molecular probes for biochemical research [1–4], emitters for electroluminescence devices [5–7], and in traditional textile and polymer fields [8, 9]. We present herein the continuation of our previous work directed at synthesis of different heterocyclic compounds for evaluation of their biological properties [10–12]. Among all naturally occurring heterocyclic compounds, coumarins occupy an important position in view of their wide-ranging biological and fluorescent properties [13–15]. Coumarins are used as fluorescent dyes for synthetic fibers and daylight fluorescent pigments. Moreover, coumarin derivatives have been used as fluorescent pH probes as well as for detection of nitric oxide, nitroxide, and hydrogen peroxide [16, 17].

The aim of the work presented herein is to prepare a novel series of coumarins with biological and fluorescent properties.

Experimental

Melting points were determined using Electrothermal melting point apparatus and are uncorrected. Reaction time was determined using thin-layer chromatography (TLC) on fluorescent silica gel plates HF_{245} (Merck) viewed under ultraviolet (UV) light at 245 and 265 nm. Silica gel (230–400 mesh) was used for flash chromatography separation. Elemental analysis was carried out at the Micro-analytical Unit, Faculty of Science, Cairo University.

Infrared (IR) (KBr) spectra were recorded on a Pye-Unicam infrared spectrophotometer SP2000 (Faculty of Science, Fayoum University). Mass spectra were run on a Shimadzu-GC-MS-GP EX using the direct inlet system (Faculty of Science, Cairo University). Nuclear magnetic resonance spectra were recorded on Varian Mercury 300 MHz spectrometer using tetramethylsilane (TMS) as internal standard; chemical shifts were recorded in δ units (National Research Center). Ultraviolet–visible absorption spectra were measured on a PerkinElmer Lambda 35 spectrophotometer at room temperature. Microwave experiments were conducted utilizing a CEM Discover LabMate microwave apparatus (300 W/Chem. Driver Software). Steady-state fluorescence spectra were measured on a PerkinElmer LS 55 spectrophotometer. The instrument provides corrected excitation spectra directly. Fluorescence spectra were corrected for the characteristics of the emission monochromator and for the photomultiplier response. For fluorescence measurements, very weakly absorbing solutions (optical density $\sim$0.05 at excitation wavelength in 1-cm cell) were used.

Fluorescence spectra were recorded by excitation at the wavelength of the most intense absorption vibronic band; the optical density at this wavelength was about 0.05 in a 1-cm cell (Pardubice University).

[2,4-Dioxochromen-3(4*H*)-ylidenemethylamino] acetic acid derivatives (1a–e)

Method A Known compounds **1a–e** were prepared according to literature procedures [18].

Compound **1a** Crystallized from dioxane as yellow crystals in 95% yield; m.p. 233 °C; IR (KBr, ν/cm^{-1}): 3404 (OH), 3304 (NH), 2964, 2893 (aliph.), 1721, 1680, 1664 (3CO); ^{1}H NMR (DMSO-d_6): δ 4.43 ppm (d, 2H, CH_2), 7.22–8.00 (m, 4H, ArH), 8.52 (dd, 1H, $J = 7.5$, 1.5 Hz, CH, *Z* and *E*), 10.35 and 11.65 (dd, 1H, $J = 9.5$, 3.8 Hz, NH, *Z* and *E*); MS *m*/*z* (%) = 247 (M^+, 1), 201 (1), 188 (3.5), 73 (94), 41 (73), 30 (100); Anal. Calcd. for $C_{12}H_9NO_5$ (247.20): C 58.30, H 3.64, N 5.66%. Found: C 58.06, H 3.67, N 5.70%.

Compound **1b** Crystallized from dioxane as orange crystals in 50% yield; m.p. 245 °C; IR (KBr, ν/cm^{-1}): 3432 (OH), 3244 (NH), 2957, 2900 (aliph.), 1733, 1689, 1633 (3CO); ^{1}H NMR (DMSO-d_6): δ 1.55 ppm (d, 3H, CH_3), 4.68 (m, 1H, CH), 7.18–7.97 (m, 4H, ArH), 8.58 (dd, 1H, $J = 7.3$, 1.1 Hz, CH, *Z* and *E*), 10.42 and 11.94 (dd, 1H, $J = 9.8$, 3.5 Hz, NH, *Z* and *E*); MS *m*/*z* (%) = 261 (M^+, 11), 216 (6), 188 (5), 73 (100), 41 (27), 30 (29); Anal. Calcd. for $C_{13}H_{11}NO_5$ (261.06): C 59.77, H 4.21, N 5.36%. Found: C 59.98, H 4.26, N 5.25%.

Compound **1c** Crystallized from ethanol as brown crystals in 55% yield; m.p. 226 °C; IR (KBr, ν/cm^{-1}): 3451 (OH), 3237 (NH), 2920 (aliph.), 1745, 1701, 1644 (3CO); ^{1}H NMR (DMSO-d_6): δ 3.32 ppm (m, 2H, CH_2), 4.95 (m, 1H, CH), 7.20–7.95 (m, 9H, ArH), 8.37 (dd, $J = 7.8$, 1.3 Hz 1H, CH, *Z* and *E*), 10.35 and 11.53 (dd, 1H, NH, $J = 9.5$, 3.9 Hz, *Z* and *E*); MS *m*/*z* (%) = 337 (M^+, 61), 246 (100), 191 (24), 91 (82), 77 (19), 65 (18); Anal. Calcd. for $C_{19}H_{15}NO_5$ (337.33): C 67.65, H 4.45, N 4.15%. Found: C 67.54, H 4.51, N 4.13%.

Compound **1d** Crystallized from dioxane as yellow crystals in 80% yield; m.p. 166 °C; IR (KBr, ν/cm^{-1}): 3461 (OH), 3197 (NH), 2954, 2878 (aliph.), 1712, 1667, 1645 (3CO); ^{1}H NMR (DMSO-d_6): δ 0.92 ppm (d, 6H, $J = 6.1$ Hz, $2CH_3$), 1.72 (m, 1H, CH),1.76 (m, 2H, CH_2), 4.65 (m, 1H, CH), 7.22–8.00 (m, 4H, ArH), 8.62 (dd, 1H, $J = 7.5$, 1.5 Hz, CH, *Z* and *E*), 10.39 and 11.88 (dd, $J = 9.3$, 3.9 Hz, 1H, NH, *Z* and *E*); MS *m*/*z* (%) = 303 (M^+, 47), 258 (57), 188 (17), 175 (23), 121 (46), 77 (25), 69 (40), 43 (100); Anal. Calcd. for $C_{16}H_{17}NO_5$ (303.31): C 63.36, H 5.65, N 4.62%. Found: C 63.78, H 5.29, N 4.91%.

Compound **1e** Crystallized from dioxane as yellow crystals in 70% yield; m.p. 145 °C; IR (KBr, ν/cm^{-1}): 3422 (OH), 3185 (NH), 2855, 2820 (aliph.), 1726, 1695, 1654 (3CO); ^{1}H NMR (DMSO-d_6): δ 3.26 ppm (d, 2H, CH_2), 4.91 (m, 1H, CH), 7.20–7.95 (m, 4H, ArH), 8.60 (dd, $J = 7.8$, 1.5 Hz, 1H, CH, *Z* and *E*), 10.50 and 11.90 (dd, $J = 9.8$, 4.0 Hz 1H, NH, *Z* and *E*); MS *m*/*z* (%) = 293 (M^+, 0.1), 248 (0.3), 188 (3), 86 (100), 59 (45), 44 (76); Anal. Calcd. for $C_{13}H_{11}NO_5S$ (293.30): C 53.24, H 3.75, N 4.7%. Found: C 53.60, H 4.02, N 4.75%.

Method B using microwaves A mixture of 4-hydroxycoumarin (16.2 g, 0.1 mol) and glycine (7.5 g, 0.1 mol) wetted by triethyl orthoformate (0.5 mol) in a closed vessel was irradiated in a focused microwave reactor for 8 min at 90 °C (250 W) (controlled by TLC). The product was obtained as yellow crystals in 54% yield.

General procedure for synthesis of 2-oxo-4-iminochromen-3(4*H*)-ylidene-methyl-amino]acetic acid derivatives (2a–e)

A mixture of compound **1a** (2.47 g, 0.01 mol) and appropriate amine, namely aniline, *p*-toluidine, *p*-aminoacetophenone, benzylamine, and *n*-butylamine (0.01 mol), was refluxed in 15 mL boiling pyridine for 12 h. The reaction mixture was cooled and poured into ice and hydrochloric acid. The solid product was filtered off, washed with water, and crystallized from suitable solvent.

Compound **2a** Crystallized from dioxane as yellow crystals in 70% yield; m.p. 225 °C; IR (KBr, ν/cm^{-1}): 3472–3225 (OH, NH), 3055 (Ar), 2947 (aliph.), 1720, 1683 (2CO); ^{1}H NMR (DMSO-d_6): δ 2.11 ppm (s, 1H, NH, D_2O exchangeable), 3.58 (s, 2H, –CH_2CO), 7.10–7.69 (m, 10H, 2ArH, ethylene), 11.02 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 45.3 ppm (CH_2), 94.2 (C=C, chromen-C3), 120.3, 121.1, 123.3, 126.1, 128.2, 131.4, 133.1, 135.2, 148.3, 150.0 (Ar–C), 158.6 (=CN), 163.5 (CO), 166.4 (C=N), 170.6 (CO); MS *m*/*z* (%) = 322 (M^+, 9.6), 305 (21), 262 (100); Anal. Calcd. for $C_{18}H_{14}N_2O_4$ (322.31): C, 67.07; H, 4.38; N, 8.69%. Found: C, 67.29; H, 4.11; N, 8.90%.

Compound **2b** Crystallized from dioxane as brown crystals in 76% yield; m.p. 218 °C; IR (KBr, ν/cm^{-1}): 3439–3304 (OH, NH), 3083 (Ar), 2915 (aliph.), 1746, 1707 (2CO); ^{1}H NMR (DMSO-d_6): δ 2.11 ppm (s, 3H, CH_3), 3.78 (s, 2H, –CH_2CO), 4.61 (s, 1H, NH, D_2O exchangeable), 7.10–7.69 (m, 9H, 8 ArH, ethylene-H), 11.45 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 18.5 ppm (CH_3), 45.1 (CH_2), 95.2 (C=C, chromen-C3), 120.3, 121.0, 122.4, 126.2, 128.1, 131.2, 133.1, 134.6, 148.3, 149.2 (Ar–C), 158.6 (=CN), 164.2 (CO), 166.4 (C=N), 171.5 (CO); MS *m*/*z* (%) = 336 (M^+, 12.4), 319 (24), 276 (100); Anal. Calcd. for $C_{19}H_{16}N_2O_4$ (336.34): C, 67.85; H, 4.79; N, 8.33%. Found: C, 67.74; H, 4.83; N, 8.01%.

Compound **2c** Crystallized from dioxane as brown crystals in 65% yield; m.p. 190 °C; IR (KBr, ν/cm^{-1}): 3410–3284 (OH, NH), 3026 (Ar), 2820 (aliph.), 1734, 1710 (2CO); ^{1}H NMR (DMSO-d_6): δ 3.44 ppm (s, 3H, CH_3), 3.76 (s, 2H, –CH_2CO), 4.57 (s, 1H, NH, D_2O exchangeable), 7.10–7.69 (m, 9H, 8 ArH, ethylene-H), 11.50 (s, 1H, OH, D_2O exchangeable); MS *m*/*z* (%) = 364 (M^+, 22.5), 347 (16), 304 (100); Anal. Calcd. for $C_{20}H_{16}N_2O_5$ (364.35): C, 65.93; H, 4.43; N, 7.69%. Found: C, 65.53; H, 4.31; N, 7.43%.

Compound **2d** Crystallized from dioxane as yellow crystals in 63% yield; m.p. 257 °C; IR (KBr, ν/cm^{-1}): 3455–3266 (OH, NH), 3050 (Ar), 2898 (aliph.), 1765, 1669 (2CO); ^{1}H NMR (DMSO-d_6): δ 3.94 ppm (s, 2H, –CH_2CO), 4.57 (s, 2H, CH_2-Ph), 7.10–7.61 (m, 9H, ArH), 7.93 (s, 1H, ethylene-H), 9.43 (s, 1H, NH, D_2O

exchangeable), 12.31 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 45.1 ppm (CH_2), 49.3 (CH_2–Ph), 93.2 (C=C, chromen-C3), 120.3, 124.0, 126.2, 128.1, 129.3, 131.2, 136.2, 148.3 (Ar–C), 157.5 (=CN), 160.2 (CO), 165.6 (C=N), 169.5 (CO); MS *m*/*z* (%) = 336 (M^+, 15.6), 319 (22), 276 (70), 91 (100); Anal. Calcd. for $C_{19}H_{16}N_2O_4$ (336.34): C, 67.85; H, 4.79; N, 8.33%. Found: C, 67.68; H, 4.47; N, 8.09%.

Compound **2e** Crystallized from dioxane as red crystals in 51% yield; m.p. 107 °C; IR (KBr, ν/cm^{-1}): 3410–3193 (OH, NH), 3015 (Ar), 2945, 2876 (aliph.), 1712, 1669 (2CO); ^1H NMR (DMSO-d_6): δ 0.92 ppm (t, 3H, J = 5.31 Hz, CH_3), 1.41 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 3.65 (t, 2H, J = 5.32 Hz, CH_2), 4.31 (s,2H, CH_2), 7.13–7.76 (m, 5H, Ar + ethylene-H), 7.91 (s, 1H, NH, D_2O exchangeable), 11.9 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 13.2 ppm (CH_3), 16.3, 21.2, 45.2, 52.7 (4 CH_2), 93.2 (C=C, chromen-C3), 120.3, 123.0, 127.2, 129.1, 131.2, 149.5 (Ar–C), 157.5 (=CN), 160.2 (CO), 164.4 (C=N), 170.2 (CO); MS *m*/*z* (%) = 302 (M^+, 13.6), 285 (20), 245 (77), 185 (100); Anal. Calcd. for $C_{16}H_{18}N_2O_4$ (302.33): C, 63.56; H, 6.00; N, 9.27%. Found: C, 63.38; H, 6.12; N, 9.38%.

Synthesis of (4-oxo-2,3,3*a*,4-tetrahydrochromeno[4,3-*c*]pyrazol-3-ylamino)acetic acid derivatives (3a, b)

A mixture of compound **1a** (2.47 g, 0.01 mol) and excess of hydrazine hydrate (98%) or phenyl hydrazine (0.01 mol) was heated under reflux in 15 mL boiling pyridine for 10 h. The reaction mixture was poured into ice and hydrochloric acid. The mixture was filtered off and crystallized from proper solvent.

Compound **3a** Crystallized from dioxane as brown crystals in 65% yield; m.p. 232 °C; IR (KBr, ν/cm^{-1}): 3442–3208 (OH, NH), 3090 (Ar), 2978 (aliph.), 1720, 1669 (2CO); ^1H NMR (DMSO-d_6): δ 2.62 ppm (d, 1H, J = 6.82 Hz, CH, chromen H-3), 3.52 (s, 2H, CH_2), 4.61 (d, 1H, J = 6.81 Hz, CH, pyrazol H-3), 5.21 (s, 1H, NH, D_2O exchangeable), 6.98–7.67 (m, 4H, Ar), 7.91 (s, 1H, NH, D_2O exchangeable), 12.10 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 45.2 ppm (CH_2), 55.2 (CH, chromen-C3), 63.2 (CH, pyrazole C-3), 120.3, 123.0, 124.1, 129.4, 131.2 (Ar–C), 146.7 (C=N, chromen C-4), 159.3, 167.2 (2CO); MS *m*/*z* (%) = 261 (M^+, 14.46), 244 (22), 201 (100); Anal. Calcd. for $C_{12}H_{11}N_3O_4$ (261.23): C, 55.17; H, 4.24; N, 16.09%. Found: C, 55.38; H, 4.41; N, 16.2%.

Compound **3b** Crystallized from dioxane as pale-brown crystals in 62% yield; m.p. 321 °C; IR (KBr, ν/cm^{-1}): 3412–3217 (OH, NH), 3012 (Ar), 2881 (aliph.), 1716, 1687 (2CO); ^1H NMR (DMSO-d_6): δ 2.62 ppm (d, 1H, J = 6.84 Hz, CH, chromen H-3), 3.52 (s, 2H, CH_2), 4.61 (d, 1H, J = 6.84 Hz, CH, pyrazol H-3), 5.21 (s, 1H, NH, D_2O exchangeable), 6.74–7.72 (m, 9H, Ar), 12.41 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 45.4 ppm (CH_2), 56.2 (CH, chromen-C3), 68.2 (CH, pyrazole C-3), 118.2, 120.3, 123.0, 124.1, 128.4, 130.2, 139.3 (Ar–C), 148.6 (C=N, chromen C-4), 161.2, 166.2 (2CO); MS *m*/*z* (%) = 337 (M^+, 21), 277

(31), 199 (100); Anal. Calcd. for $C_{18}H_{15}N_3O_4$ (337.33): C, 64.09; H, 4.48; N, 12.46%. Found: C, 63.85; H, 4.21; N, 12.33%.

Synthesis of (4-oxo-3*a*,4-dihydro-3*H*-chromeno[4,3-*c*]isoxazol-3-yl)amino)acetic acid (4)

A mixture of **1a** (2.47 g, 0.01 mol) and hydroxylamine hydrochloride (0.7 g, 0.01 mol) was heated under reflux in 15 mL boiling pyridine for 10 h. On cooling, the reaction mixture was poured into ice and hydrochloric acid; the precipitated solid was filtered off, washed several times with water, and crystallized from methanol as pale-brown crystals in 86% yield; m.p. 335 °C; IR (KBr, ν/cm^{-1}): 3438–3390 (OH, NH), 3077 (Ar), 2912 (aliph.), 1716, 1680 (2CO); ^{1}H NMR (DMSO-d_6): δ 2.64 ppm (d, 1H, $J = 6.82$ Hz, CH, chromen H-3), 3.52 (s, 2H, CH_2), 4.46 (d, 1H, $J = 6.81$ Hz, CH, pyrazol H-3), 5.11 (s, 1H, NH, D_2O exchangeable), 7.10–7.66 (m, 4H, Ar), 12.33 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 46.3 ppm (CH_2), 55.4 (CH, chromen-C3), 64.2 (CH, pyrazole C-3), 120.3, 123.0, 124.5, 129.6, 132.4 (Ar–C), 146.6 (C=N, chromen C-4), 160.3, 165.5 (2CO); MS *m*/*z* (%) = 262 (M^+, 18), 202 (31), 188 (100); Anal. Calcd. for $C_{12}H_{10}N_2O_5$ (262.22): C, 54.97; H, 3.84; N, 10.68%. Found: C, 54.89; H, 3.84; N, 10.71%.

Synthesis of [2,4–dioxochromen-3(4*H*)-ylidene methylamino]acetyl chloride (5)

A mixture of compound **1a** (2.47 g, 0.01 mol), phosphorus pentachloride (1 g), and phosphorus oxychloride (15 mL) was heated under reflux on water bath for 7 h. The solid product was precipitated by diethyl ether, filtered off, and crystallized from dry dioxane as brown crystals in 75% yield; m.p. 225 °C; IR (KBr, ν/cm^{-1}): 3225–2990 (NH), 2891 (aliph.), 1745, 1712, 1675 (3CO); ^{1}H NMR (DMSO-d_6): δ 4.65 ppm (s, 2H, CH_2), 5.43 (s, 1H, NH, D_2O exchangeable), 7.32–7.81 (m, 5H, Ar + ethylene-H); ^{13}C NMR (DMSO-d_6): δ 49.3 ppm (CH_2), 65.4 (CH, chromen-C3), 118.1, 120.3, 124.5, 129.6, 132.4 (Ar–C), 162.3, 167.4, 169.2 (3CO); MS *m*/*z* (%) = 265/267 (M^+/M^{+2}, 60/17), 187 (100); Anal. Calcd. for $C_{12}H_8ClNO_4$ (265.65): C, 54.26; H, 3.04; Cl, 13.35; N, 5.27%. Found: C, 54.06; H, 3.32; Cl, 13.36; N 5.30%.

General procedure for synthesis of [2,4-dioxo-2*H*-chromen-3(4*H*)-ylidene methylamino] acetylaminoacetic acid derivatives (6a–e)

Solution of (0.01 mol) amino acid (glycine, L-alanine, phenyl alanine, leucine, and L-cysteine) in 5 mL 2% NaOH was added with 0.6 mL triethylamine on a solution of compound **5** (2.81 g, 0.01 mol) in 20 mL dioxane. The reaction mixture was left for 18 h at room temperature. Then, the reaction mixture was acidified, filtered off, and crystallized from proper solvent.

Compound **6a** Crystallized from dioxane as yellow crystals in 72% yield; m.p. 262 °C; IR (KBr, ν/cm^{-1}): 3482–3200 (OH, NH), 2987 (aliph.), 1726, 1701, 1686,

1659 (4CO); ^{1}H NMR (DMSO-d_6): δ 2.17 ppm (s, 1H, NH, D_2O exchangeable), 3.50 (s, 2H, CH_2), 4.10 (s, 2H, CH_2COOH), 7.22–7.84 (m, 5H, Ar–H + ethylene-H), 8.20 (s, 1H, NH sec.imide, D_2O exchangeable), 11.16 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 44.2, 47.1 ppm (2 CH_2), 85.4 (CH, chromen-C3), 118.3, 122.3, 126.4, 129.6, 132.4 (Ar–C), 162.3 (=CN), 163.4, 164.2,168.5, 170.2 (4CO); MS *m/z* (%) = 304 (M^+, 6.4), 244 (13.4), 185 (100); Anal. Calcd. for $C_{14}H_{12}N_2O_8$ (304.08): C, 55.27; H, 3.98; N, 9.21%. Found: C, 55.19; H, 4.22; N, 9.17%.

Compound **6b** Crystallized from dioxane as brown crystals in 63% yield; m.p. 289 °C; IR (KBr, *v*/cm^{-1}): 3482–3200 (OH, NH), 2987 (aliph.), 1730, 1714, 1686, 1659 (4CO); ^{1}H NMR (DMSO-d_6): δ 1.82 ppm (d, 3H, J = 6.34 Hz, CH_3), 3.90 (q, 1H, J = 6.31 Hz, CH), 4.23 (s, 2H, CH_2), 5.53 (s, 1H, NH, D_2O exchangeable), 7.30–7.74 (m, 4H, Ar), 8.21 (s, 1H, ethylene-H), 8.65 (s, 1H, NH, D_2O exchangeable), 12.34 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 18.29 ppm (CH_3), 47.84 (CH_2), 52.47 (CH), 85.38 (CH, chromen-C3), 118.22, 121.98, 126.23, 128.76, 135.03 (Ar–C), 151.54 (C-O), 161.76 (=CN), 164.21, 166.33, 168.16, 170.02 (4CO). MS *m/z* (%) = 319 (M^++1, 5.7), 318 (M^+, 4.4), 301 (7.7), 244 (100); Anal. Calcd. for $C_{15}H_{14}N_2O_6$ (318.28): C, 56.60; H, 4.43; N, 8.80%. Found: C, 56.34, H, 4.58; N, 9.01%.

Compound **6c** Crystallized from methanol as orange crystals in 69% yield; m.p. 300 °C; IR (KBr, *v*/cm^{-1}): 3408–3289 (OH, NH), 3099 (Ar), 2867 (aliph.), 1730, 1714, 1686, 1659 (4CO); ^{1}H NMR (DMSO-d_6): δ 2.85 ppm (d, 2H, J = 6.65 Hz, CH_2), 3.90 (s, 2H, CH_2), 4.23 (t, 1H, J = 6.65 Hz, CH), 5.53 (s, 1H, NH, D_2O exchangeable), 6.85–7.73 (m, 9H, Ar), 8.21 (s, 1H, ethylene-H), 8.65 (s, 1H, NH, D_2O exchangeable), 12.34 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 44.2, 47.1 ppm ($2CH_2$), 53.3 (CH), 85.4 (CH, chromen-C3), 118.3, 124.3, 126.4, 129.6, 132.4, 134.6 (Ar–C), 162.3(=CN), 163.5, 165.1, 168.5, 170.3 (4CO); MS *m/z* (%) = 394 (M^+, 3.2), 349 (9.2), 189 (44.2), 91 (100); Anal. Calcd. for $C_{21}H_{18}N_2O_6$ (394.38): C, 63.96; H, 4.60; N, 7.10%. Found: C, 63.60; H, 4.71; N, 7.05%.

Compound **6d** Crystallized from methanol as orange crystals in 54% yield; m.p. 312 °C; IR (KBr, *v*/cm^{-1}): 3442–3265 (OH, NH), 3049 (Ar), 2876 (aliph.), 1728, 1713, 1689, 1659 (4CO); ^{1}H NMR (DMSO-d_6): δ 1.12 ppm (d, 6H, $2CH_3$), 1.39 (m, 1H, CH), 1.84 (m, 2H, CH_2), 4.21 (s, 2H, CH_2), 4.55 (t, 1H, CH), 5.53 (s, 1H, NH, D_2O exchangeable), 7.12–7.74 (m, 4H, Ar), 8.21 (s, 1H, ethylene-H), 8.65 (s, 1H, NH, D_2O exchangeable), 12.34 (s,1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 22.54 ppm ($2CH_3$), 24.89 (CH), 40.24, 47.99 ($2CH_2$), 52.73 (CH), 85.98 (CH, chromen-C3), 118.03, 124.18, 126.33, 128.76, 132.11, 135.59 (Ar–C), 161.76 (=CN), 164.11, 165.28, 168.16, 170.36 (4CO); MS *m/z* (%) = 360 (M^+, 3.2), 325 (9.2), 222 (44.2), 167 (74), 91 (100); Anal. Calcd. for $C_{18}H_{20}N_2O_6$ (360.35): C, 59.99; H, 5.59; N, 7.77%. Found: C, 59.51; H, 5.40; N, 7.53%.

Compound **6e** Crystallized from ethanol as brown crystals in 75% yield; m.p. 225 °C; IR (KBr, *v*/cm^{-1}): 3419–3225 (OH, NH), 3077 (Ar), 2881 (aliph.), 1728,

1714, 1686, 1660 (4CO); ^{1}H NMR (DMSO-d_6): δ 1.45 ppm (s, 1H, SH), 2.71–2.97 (m, 2H, CH_2), 4.12 (s, 2H, CH_2), 4.75 (t, 1H, *J* 6.62 Hz, CH), 5.10 (s, 1H, NH, D_2O exchangeable), 7.19–7.82 (m, 5H, Ar–H + ethylene-H), 8.55 (s, 1H, NH, D_2O exchangeable), 12.23 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6): δ 28.15 ppm (CH_2), 47.99 (CH_2), 54.28 (CH), 87.93 (CH, chromen-C3), 118.10, 121.98, 124.53, 129.86, 135.32 (Ar–C), 153.64 (C–O), 161.12 (=CN), 164.11, 165.48, 169.16, 171.32 (4CO). MS *m*/*z* (%) = 350 (M^+, 5.6), 305 (11.2), 243 (45.0), 167 (100); Anal. Calcd. for $C_{15}H_{14}N_2O_6S$ (350.38): C, 51.42; H, 4.03; N, 8.00; S, 9.14%. Found: C, 51.11; H, 3.71; N, 7.70; S, 8.63%.

Synthesis of 4-oxo-2,4-dihydro-chromeno[3,4-*c*]pyrrole-1-carboxylic acid (7)

Compound **1a** (2.47 g, 0.01 mol) was refluxed in 15 mL boiling pyridine for 10 h. The reaction mixture was acidified with hydrochloric acid. The obtained solid was filtered off and crystallized from dioxane as yellow crystals in 85% yield; m.p. 319 °C; IR (KBr, ν/cm^{-1}): 3478–3207 (OH, NH), 3098 (Ar), 2923 (aliph.), 1720, 1713 (2CO); ^{1}H NMR (DMSO-d_6): δ 5.00 ppm (s, 1H, NH, D_2O exchangeable), 7.12–7.51 (m, 5H, 4Ar + pyrrole H-2), 11.09 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 103.2 ppm (CH, chromen-C3), 113.0 (CH, pyrrole C-5), 118, 122.3, 124.1, 129.4, 131.2, 136.5 (Ar–C), 162.3, 168.5 (2CO); MS *m*/*z* (%) = 229 (M^+, 1.58), 184 (100); Anal. Calcd. for $C_{12}H_7NO_4$ (229.04): C, 62.89; H, 3.08; N, 6.11%. Found: C, 62.66, H, 2.92, N, 6.14%.

Synthesis of 1-(2,4-dioxo-2*H*-chromen-3(4*H*)-ylidene methyl)-3-methyl piperazine-2,5-dione (8)

A mixture of compound **6b** (3.18 g, 0.01 mol) and excess of acetic anhydride (7 mL, 99%) was heated in 25 mL water bath for 4 h. The reaction mixture was cooled and precipitated by diethyl ether. The obtained solid was filtered off, and crystallized from dioxane as dark-brown crystals in 61% yield; m.p. 253 °C; IR (KBr, ν/cm^{-1}): 3243 (NH), 3084 (Ar), 2956 (aliph.), 1720, 1690, 1683 (3CO); ^{1}H NMR (DMSO-d_6): δ 1.50 ppm (d, 3H, $J = 6.82$ Hz, CH_3), 4.12 (s, 2H, CH_2), 4.80 (q, 1H, $J = 6.81$, CH), 7.25–7.78 (m, 4H, 4Ar), 8.00 (s, 1H, NH, D_2O exchangeable), 9.15 (s,1H, CHN); ^{13}C NMR (DMSO-d_6): δ 19.3, 49.2, 52.6 ppm (CH_3, CH, CH_2), 116.7, 123.3, 125.1, 131.2, 136.5 (Ar–C), 146 (ethylene–C), 160.3, 165.5, 169.3 (3CO); MS *m*/*z* (%) = 300 (M^+, 45), 299 ($M^+ - 1$, 3.38), 173 (100), Anal. Calcd. for $C_{15}H_{12}N_2O_5$ (300.27): C, 60.00; H, 4.03; N, 9.33%. Found: C, 59.89; H, 4.21; N, 9.39%.

Synthesis of (6,8,10-trioxo-7*a*,8,10,10*a*-tetrahydro-6*H*,7*H*-furo[3′,4′:5,6]pyrano[3,2-*c*]chromen-7-yl)glycine (9)

A solution of maleic anhydride (0.98 g, 0.01 mol) in 10 mL dioxane was added on solution of **1a** (2.47 g, 0.01 mol) in 15 mL dioxane and heated under reflux for 15 h. The reaction mixture was filtered off and crystallized from dioxane as yellow

crystals in 82% yield; m.p. 320 °C; IR (KBr, ν/cm^{-1}): 3410–3227 (OH, NH), 3083 (Ar), 2916 (aliph.), 1731, 1709 and 1679 (3CO); ^{1}H NMR (DMSO-d$_6$): δ 2.89 ppm (m, 1H, CH, furane–H3), 3.81 (s, 2H, CH$_2$), 4.46 (d, 1H, J = 5.74 Hz, CH, pyrane–H4), 5.21 (d, 1H, J = 5.68 Hz, CH, furane–H4), 5.77 (s, 1H, NH, D$_2$O exchangeable), 6.98–7.45 (m, 4H, Ar), 12.10 (s, 1H, OH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d$_6$): δ 42,3, 44.2, 48.2 ppm (2CH + CH$_2$), 72.0 (CH, chromen-C3), 89.5 (CH, chromen H-3), 118.3, 123.3, 125.1, 128.3, 141.2 (Ar–C), 158.4 (chromen H-4), 163.3, 167.2, 170.2 (3CO); MS m/z (%) = 345 (M$^+$, 22), 272 (100); Anal. Calcd. for $C_{16}H_{11}NO_8$ (345.28): C, 55.65; H, 3.18; N, 4.05%. Found: C, 55.20; H, 2.97; N, 3.79%.

Synthesis of (6,8,11-trioxo-6*a*,7*a*,8,9,10,11,11*a*,12*a*-octahydro-6*H*,7*H*-chromeno [3′,4′:5,6]pyrano[2,3-*d*]pyridazin-7-yl)glycine (10)

Compound **9** (3.47 g, 0.01 mol) was boiled with hydrazine hydrate (2.5 mL, 0.05 mol) in 15 mL pyridine for 10 h. The reaction mixture was poured into ice and hydrochloric acid. The obtained solid was filtered off and crystallized from dimethylformamide (DMF) as brown crystals; m.p. 335 °C; IR (KBr, ν/cm^{-1}): 3400–3200 (OH, NH), 2873 (aliph.), 1740,1717 and 1680 (3CO); ^{1}H NMR (DMSO-d$_6$): δ 2.89 ppm (m, 1H, CH, pyrane H-4), 3.12 (m, 1H, CH), 3.83 (s, 2H, CH$_2$), 3.96 (m, 1H, CH), 4.82 (d, 1H, J = 7.32 Hz, CH, pyrane H-2), 5.21 (d, 1H, J = 7.30 Hz, CH, chromen H-3), 5.77 (s, 1H, NH, D$_2$O exchangeable), 7.14–7.62 (m, 4H, Ar), 9.68 (s, 1H, NH, D$_2$O exchangeable), 12.21 (s, 1H, OH, D$_2$O exchangeable); ^{13}C NMR (DMSO-d$_6$): δ 34.5, 42.3, 45.3, 51.1 ppm (3CH + CH$_2$), 72.0, 76.4 (2CH), 120.3, 123.3, 125.2, 128.3, 133.6 (Ar–C), 164.3, 167.4, 170.2 (3CO); MS m/z (%) = 359 (M$^+$, 4.79), 357 (100); Anal. Calcd. for $C_{16}H_{15}N_3O_7$ (361.29): C, 53.49; H, 3.65; N, 11.70%. Found: C, 53.66; H, 3.48; N, 11.32%.

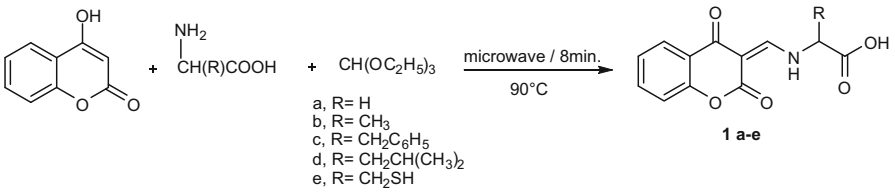
Results and discussion

2,4-Dioxo-chromen-3(4*H*)methyl amino acetic acid derivatives **1a–e** were reported for the first time by Spéziale and coworkers [18]. The synthesis involved reflux of 4-hydroxycoumarin, triethyl orthoformate, and various amino acids in 2-propanol for 2 h.

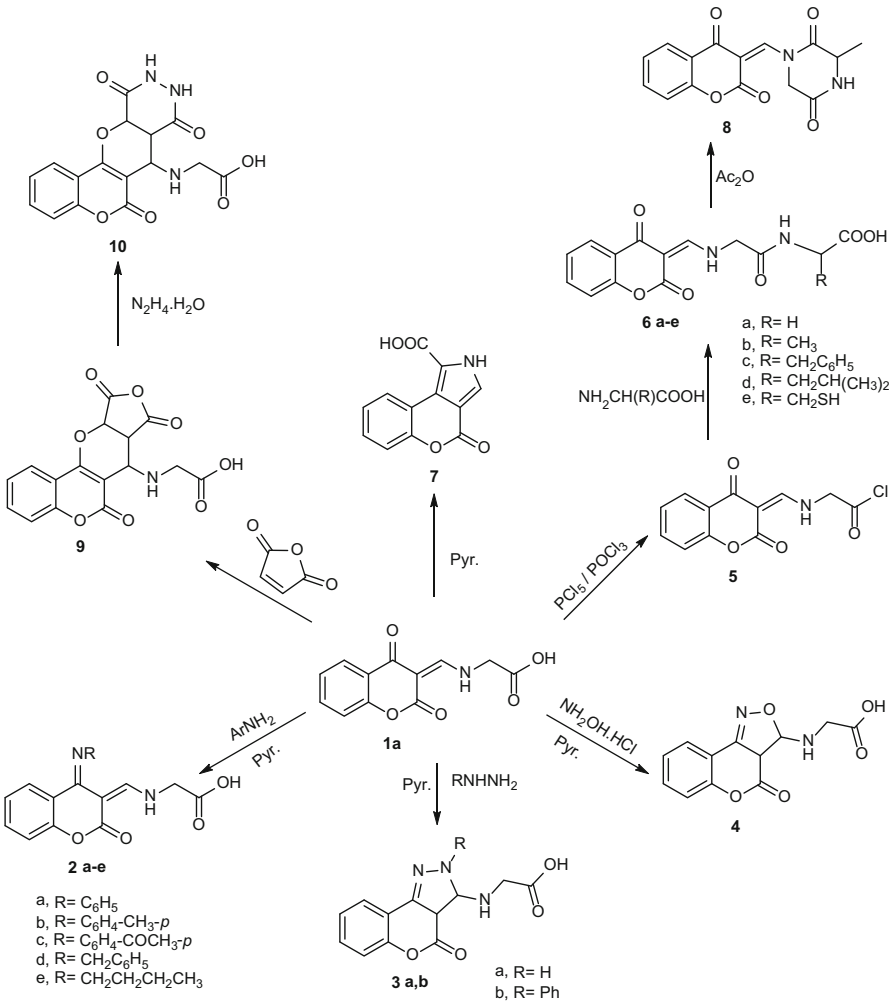
In this work, we developed a new approach for synthesis of 2,4-dioxo-chromen-3(4*H*)methyl amino acetic acid derivatives **1a–e** using microwaves as a result of the interaction between 4-hydroxycoumarin with triethyl orthoformate and amino acids [13]. Microwave dielectric heating provides a rapid and expedient route for synthesis of organic compounds without solvent [19] (Scheme 1).

Subsequently, compound **1a** was used as starting material for synthesis of a novel series of coumarin acetic acid derivatives (Scheme 2).

The behavior of compound **1a** towards primary amines such as aniline, *p*-toluidine, *p*-aminoacetophenone, benzylamine, and *n*-butylamine was investigated, resulting in preparation of 2-oxo-4-iminochromen-3(4*H*)ylidene)methyl amino acetic acid derivatives **2a–e** (Scheme 2). When compound **1a** was allowed to



Scheme 1 Synthesis of compounds **1a–e** using microwaves



Scheme 2 Reactions of compound **1a**

condense with amines in boiling pyridine, it afforded corresponding Schiff base compounds **2a–e** in good yield. The structure of compounds **2a–e** was confirmed by elemental analysis and spectral data. The IR spectra exhibited strong absorption bands at 3472 to 3410 cm^{-1} due to ν_{OH}, at 3304 to 3193 cm^{-1} due to ν_{NH}, and at 1765 to 1669 cm^{-1} due to ν_{CO} of δ-lactone and carboxylic group. The ^{1}H NMR (DMSO-d_6) spectrum of **2a** showed signals at δ 2.11 ppm (s, 1H, NH), 3.58 ppm (s, 2H, $-CH_2CO$), 7.10–7.69 ppm, (m, 10H, 2ArH, ethylene), and 11.02 ppm (s, 1H, OH). The mass spectrum of compound **2a** revealed an ion peak at $m/z = (M^+)$ 322 (9.6%), equivalent to molecular formula $C_{18}H_{14}N_2O_4$.

Action of hydrazine derivatives on compound **1a** yielded chromeno[4,3-*c*]pyrazol-3-amino acetic acid derivatives **3a**, **b** (Scheme 2). The structure of compounds **3a**, **b** was confirmed by elemental analysis and spectral data. The IR spectra showed strong absorption bands at 3442 to 3417 cm^{-1} due to ν_{OH}, 3273 to 3208 cm^{-1} due to ν_{NH} group, strong absorption bands at 1720 to 1716 cm^{-1} due to ν_{CO} of δ-lactone, and absorption bands at 1687 to 1669 cm^{-1} due to ν_{CO} of carboxylic groups. The ^{1}H NMR (DMSO-d_6) spectrum of **3a** showed signals at δ 2.62 ppm (d, 1H, $J = 6.82$ Hz, CH, chromen H-3), 3.52 (s, 2H, CH_2), 4.61 (d, 1H, $J = 6.81$ Hz, CH, pyrazol H-3), 5.21 (s, 1H, NH, D_2O exchangeable), 6.98–7.67 (m, 4H, Ar), 7.91 (s, 1H, NH, D_2O exchangeable), and 12.10 (s, 1H, OH, D_2O exchangeable); The mass spectrum of **3a** revealed an ion peak at $m/z = 261$ (14.46%) equivalent to molecular formula $C_{12}H_{11}N_3O_4$.

In addition, compound **1a** reacted with hydroxylamine hydrochloride in boiling pyridine under reflux for 10 h to afford corresponding 2-(4-oxo-3a,4-dihydro-3*H*-chromeno[4,3-*c*]isoxazol-3-yl)aminoacetic acid **4** (Scheme 2). The structure of this compound was confirmed by elemental analysis and spectral data. The IR spectrum showed strong absorption band at 3438 cm^{-1} due to ν_{OH} group in carboxylic acid group, absorption band at 3390 cm^{-1} due to ν_{NH} group, strong absorption band at 1716 cm^{-1} due to ν_{CO} of δ-lactone, and absorption bands at 1680 cm^{-1} due to ν_{CO} of carboxylic group (Experimental).

Chlorination of compound **1a** using a mixture of phosphorus pentachloride and phosphorus oxychloride under reflux in water bath afforded acid chloride derivative **5** (Scheme 2). The structure of compound **5** was proved by elemental analysis and spectral data. The IR spectrum showed disappearance of the absorption band of ν_{OH} of carboxylic acid group and showed a strong absorption band at 3225 cm^{-1} due to ν_{NH} and a broad band centered at 1712 cm^{-1} due to ν_{CO} of δ-lactone and acid chloride. The ^{1}H NMR (DMSO-d_6) spectrum of compound **5** showed signals at δ 4.65 ppm (s, 2H, CH_2), 5.43 (s, 1H, NH, D_2O exchangeable), and 7.32–7.81 (m, 5H, Ar + ethylene-H) (Experimental).

Acid chloride **5** was allowed to react with various amino acid such as glycine, L-alanine, L-phenylalanine, L-leucine, and L-cysteine in dioxane with a few drops of triethylamine at room temperature to afford dipeptide derivatives **6a–e**, respectively. The structure of compounds **6a–e** was confirmed by elemental analysis and spectral data. The IR spectra showed strong absorption bands at 3482 to 3408 cm^{-1} due to ν_{OH} group, at 3370 to 3200 cm^{-1} due to $2\nu_{NH}$, a broad band centered at 1714 cm^{-1} due to ν_{CO} of δ-lactone, and at 1659 cm^{-1} due to ν_{CO} of amide. The ^{1}H NMR (DMSO-d_6) spectrum of compound **6a** showed signals at δ 2.17 ppm (s, 1H, NH,

D_2O exchangeable), 3.50 (s, 2H, CH_2), 4.10 (s, 2H, CH_2COOH), 7.22–7.84 (m, 5H, Ar–H + ethylene-H), 8.20 (s, 1H, NH sec.imide, D_2O exchangeable), and 11.16 (s, 1H, OH, D_2O exchangeable); The mass spectrum of compound **6b** revealed an ion peak at $m/z = 318$ (4.4%) equivalent to molecular formula $C_{15}H_{14}N_2O_6$.

Attempts were made to cyclize the side-chain of compound **1a** in refluxing pyridine for long time (10 h), which afforded 4-oxo-2,4-dihydrochromeno[3,4-*c*]pyrrole-1-carboxylic acid **7** (Scheme 2) via elimination of water molecule. The structure of compound **7** was confirmed via elemental analysis and spectral data. The IR spectrum showed strong absorption band at 3478 cm^{-1} due to ν_{OH} of the carboxylic function group, band at 3207 cm^{-1} due to ν_{NH} and at 1730 cm^{-1} due to ν_{CO} of carboxylic acid group, 1714 cm^{-1} due to ν_{CO} of δ-lactone, 1686 cm^{-1} due to ν_{CO} of conjugated ketone, and 1659 cm^{-1} due to ν_{CO} of amidic carbonyl. The ^1H NMR (DMSO-d_6) spectrum of **7** showed signals at δ 5.00 ppm (s, 1H, NH), 7.12–7.51 ppm (m, 5H, Ar–H, CH pyrrole), and 11.09 ppm (s, 1H, OH) . The mass spectrum of compound **7** revealed an ion peak at $m/z = 229$ (1.58%) equivalent to molecular formula $C_{12}H_7NO_4$.

Cyclization of side-chain in peptide **6b** via elimination of water took place on refluxing compound **6b** with acetic anhydride for 4 h to yield 1-[2,4-dioxo-chromen-3(4*H*)-yieldene methyl]-3-methyl piperazine-2,5-dione **8**. The structure of compound **8** was confirmed by elemental analysis and spectral data. The IR spectrum of this compound showed strong absorption band at 3243 cm^{-1} due to ν_{NH}, and broad band at 1720 and 1683 cm^{-1} due to ν_{CO} of δ-lactone, ketone, and amide. The ^1H NMR (DMSO-d_6) spectrum of **8** showed signals at δ 1.5 ppm (d, 3H, CH_3), 4.12 ppm (s, 2H, $-CH_2CO$), 4.80 ppm (q, 1H, $CHCH_3$), 7.25–7.78 ppm (m, 4H, Ar–H), 8.00 ppm (s, 1H, NH), and δ 9.15 ppm (s, 1H, CHN). The mass spectrum of compound **8** revealed an ion peak at $m/z = 300$ (45%) equivalent to molecular formula $C_{15}H_{12}N_2O_5$.

Compound **1a** reacted with maleic anhydride in dioxane under reflux for 15 h to afford Diels–Alder adduct **9** (Scheme 2). The structure of compound **9** was confirmed by elemental analysis and spectral data. The IR spectrum of this compound showed strong absorption band at 3410 cm^{-1} due to ν_{OH} in carboxylic acid group, band at 3227 cm^{-1} due to ν_{NH}, strong absorption bands at 1731, 1709, and 1679 cm^{-1} due to ν_{CO} of anhydride ring, δ-lactone, and carboxylic group.

Action of hydrazine hydrate on compound **9** in boiling pyridine afforded phthalizenedione **10**. The structure of compound **10** was confirmed by elemental analysis and spectral data. The IR spectrum of **10** showed strong absorption band at 3400 cm^{-1} due to ν_{OH} in carboxylic acid group, 3340, 3250, and 3200 cm^{-1} due to $3\nu_{NH}$, and broad band at 1740, 1717, and 1680 cm^{-1} due to ν_{CO} of δ-lactone, carboxylic group, and amide. The ^1H NMR (DMSO-d_6) spectrum of compound **10** showed signals at δ 2.89 ppm (m, 1H, CH, pyrane H-4), 3.12 (m, 1H, CH), 3.83 (s, 2H, CH_2), 3.96 (m, 1H, CH), 4.82 (d, 1H, $J = 7.32$ Hz, CH, pyrane H-2), 5.21 (d, 1H, $J = 7.30$ Hz, CH, chromen H-3), 5.77 (s, 1H, NH, D_2O exchangeable), 7.14–7.62 (m, 4H, Ar), 9.68 (s, 1H, NH, D_2O exchangeable), and 12.21 (s, 1H, OH, D_2O exchangeable). The mass spectrum of compound **10** revealed an ion peak at $m/z = M^+$ 359 (4.79%) equivalent to molecular formula $C_{16}H_{13}N_3O_7$.

Fluorescence and absorption spectra

The UV–Vis absorption spectra of all compounds as well as the fluorescence spectra of the compounds exhibiting fluorescence in solution were measured in 1,4-dioxane. The absorption and fluorescence emission maxima are listed in Table 1. The fluorescence properties of the compounds depend on the presence of electron-donating and electron-withdrawing substituents on the acceptor part. The acceptor part of (6,8,11-trioxo-6*a*,7*a*,8,9,10,11,11*a*,12*a*-octahydro-6*H*,7*H*-5,12-dioxa-9,10-diaza-benzo[*a*]antheracen-7-ylamino) acetic acid (compound **10**) contains a long-chain carboxyl group when compared with other compounds. Hence, due to the less positive inductive effect of **10**, the donating tendency becomes less and compound **10** exhibits high quantum yield of 0.80, much higher than other compounds. Compounds **2b**, **6b** exhibited intense fluorescence in solid phase, while compounds **7**, **10** exhibited high quantum yield ϕ_F of 0.73 and 0.80, respectively, which may be due to the presence of one additional aromatic nucleus in acceptor part, enabling extended conjugation (Table 1).

No fluorescence was detected in solution for all studied compounds except **5** and **10**. However, compounds **1a**, **2b**, **6b** exhibited intense fluorescence in solid phase (Table 1; Figs. 1–4). Simultaneously, it was observed that only compound **5** showed fluorescence in both solution and solid phase, and the fluorescence maximum in solid phase was shifted bathochromically by about 50 nm compared with the maximum in solution. Conversely, compound **10** exhibited fluorescence only in solution.

Figure 4 demonstrates that only compound **2b** showed excellent fluorescence, while acid chloride derivative **5** showed weak fluorescence.

Antimicrobial evaluation

The antimicrobial properties were tested against Gram-positive bacteria (*Bacillus subtilis* and *Streptococcus*) and Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*). The culture medium used was Müller–Hinton agar (g/L)

Table 1 Absorption (λ_A) and fluorescence (λ_F) maxima (nm) of prepared compounds

Compound	Yield (%)	M.p. (°C)	λ_A	λ_F	Quantum yield ϕ_F
1a	74	305	321	373	0.26
2b	76	218	299	309	0.34
2c	65	190	309/373	–	–
2d	63	257	325	–	–
2e	51	107	–	–	–
5	75	225	311/283	537	0.70
6b	63	289	313/282	–	–
6d	54	312	326	–	–
7	85	319	320/270	530/373	0.73
10	82	320	321		0.80

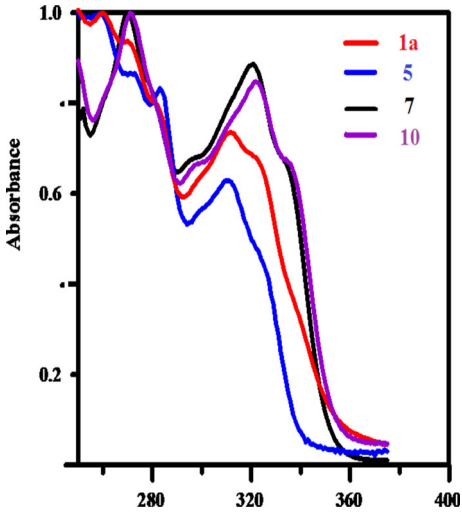


Fig. 1 Absorption spectra of compounds **1a**, **5**, **7**, **10**

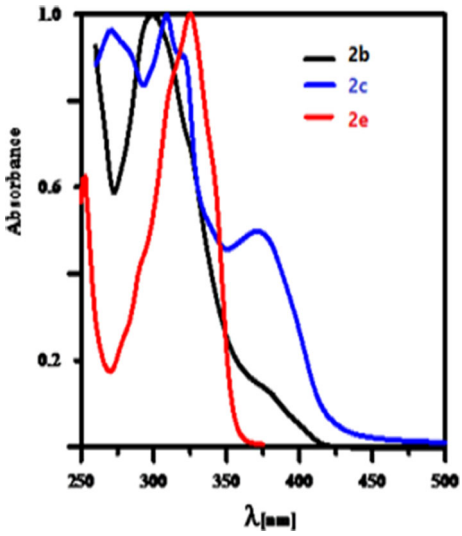


Fig. 2 Absorption spectra of compounds **2b**, **c**, **e**

comprising beef extract powder (3.0), casein hydrolase (17.5), starch (1.5), and agar (17.0).

Preparation of agar Müller–Hinton agar (38 g) was suspended in one liter of distilled water, heated to dissolve the medium completely, then sterilized by autoclaving at 121 °C for 15 min.

The tested compounds showed variation in their antibacterial activity and corresponding minimum inhibitory concentration (MIC) values (Table 2).

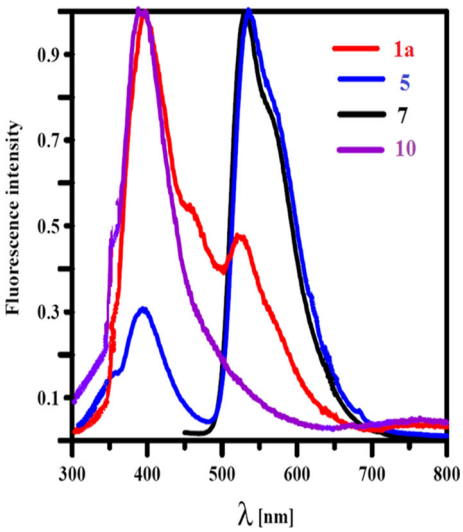


Fig. 3 Fluorescence spectra of compounds **1a**, **5**, **7**, **10** in dioxane

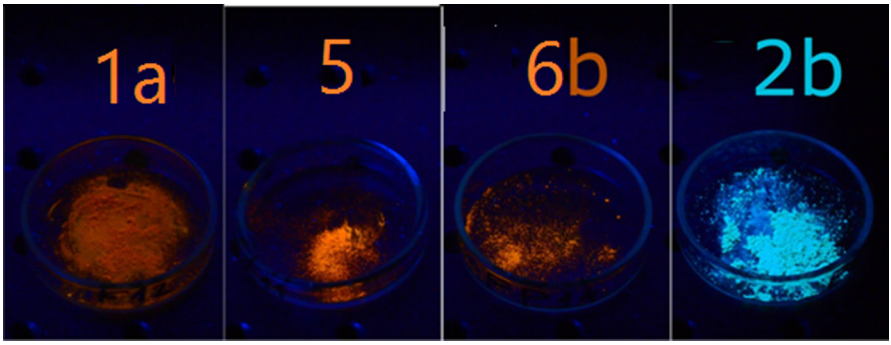


Fig. 4 Compounds **1a**, **5**, **6b**, **2b** in solid phase under UV light irradiation

Ciprofloxacin was used as reference standard. All compounds demonstrated moderate to excellent activity, except a few. Starting compound **1a** was biologically inactive against Gram-positive and Gram-negative bacteria. Gram-negative *K. pneumoniae* was not inhibited by **6a–e** but was most efficiently inhibited by **5** and **7** with the best activity observed for the latter with MIC of 8.87 ± 1.20 μg/mL compared with 8.00 ± 2.50 μg/mL for the reference. Compounds **6b**, **d** were inactive against *E. coli*, whereas moderate to good activity was exhibited by the effective compounds **5**, **6a**, **6c**, **6e**, **7**. The most active compound was **6a** with MIC of 7.23 ± 1.81 μg/mL relative to 7.96 ± 1.14 μg/mL. *B. subtilis* was moderately inhibited by all compounds except **6b**, **7**, and efficiently by **5** with MIC of 11.15 ± 1.87 μg/mL in comparison with 9.72 ± 1.00 μg/mL. Compound **5** was the most efficient against *Streptococcus* with MIC of 8.32 ± 2.87 μg/mL relative to 7.43 ± 0.45 μg/mL. Compounds **6a–c**, **e** were completely inactive against this

Table 2 MIC values for antibacterial activity of newly synthesized compounds against different types of bacteria

Compound	MIC (μg/mL)			
	Gram positive		Gram negative	
	K. pneumoniae	*E. coli*	*B. subtilis*	*Streptococcus*
1	–	–	–	–
5	14.46 ± 1.10	8.91 ± 1.94	11.15 ± 1.87	8.32 ± 2.87
6a	–	7.23 ± 1.81	16.51 ± 1.98	–
6b	–	–	–	–
6c	–	14.24 ± 3.14	16.64 ± 3.85	–
6d	–	–	17.71 ± 2.71	18.75 ± 0.65
6e	–	9.46 ± 0.50	15.18 ± 4.71	–
7	8.87 ± 1.20	10.41 ± 1.44	–	11.14 ± 2.25
Ciprofloxacin	8.00 ± 2.54	7.96 ± 1.14	9.72 ± 1.00	7.43 ± 0.45

MIC measured using suitable dilutions (5–30 μg/well)

strain. Overall, Gram-negative strains were more efficiently inhibited by the synthesized molecules relative to Gram-positive ones. Acid chloride **5** and pyrrole derivative **7** exhibited excellent activity against Gram-positive and Gram-negative bacteria.

Conclusions

The synthesis, antibacterial activity, and fluorescence properties of novel coumarin derivatives were studied. Compounds **1a–e** were synthesized using microwaves as heat source, resulting in enhanced yield in shorter time compared with traditional method. Compound **1a** reacted with primary amines, hydrazine hydrate, and hydroxylamine hydrochloride to give corresponding compounds **2a–e** to **4**. Acid chloride **5** was obtained by reaction of compound **1a** with $PCl_5/POCl_3$ and reacted with various amino acid to obtain dipeptide compounds **6a–e**. Cyclization of the side-chain of compounds **1a**, **6b** was carried out under various conditions. Also, compound **1a** reacted with maleic anhydride to give compound **9**, which reacted with hydrazine hydrate to give **10**. Most of the newly compounds were screened against two types of Gram-positive (*Bacillus subtilis* and *Streptococcus*) and two types of Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*). Compound **5** exhibited the maximum inhibition zone against all types of bacteria. The absorption and fluorescence emission properties of some of the substituted coumarins were studied in dioxane.

Acknowledgements The authors would like to thank the Chemistry Department, Faculty of Science, Fayoum University for their valuable support of this work.

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