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ORIGINAL ARTICLE

Synthesis, and Fluorescence Properties of Coumarin and Benzocoumarin Derivatives Conjugated Pyrimidine Scaffolds for Biological Imaging Applications

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Abstract Series of coumarin and 5,6-benzomcomarin substituted pyrimidine derivatives **11**–**15** and **22**–**25** were synthesized, aiming to develop new imaging fluorescent agents. Analogously, treatment of 4-chloropyrimidine analog **16** with coumarin 3-carbohyrazide **5** under MWI condition followed by boiling with NH_4OAc in HOAc furnished coumarin-1,2,4-triazolo-pyrimidine analog **18**. The fluorescence property was investigated spectrophotometrically in MeOH with Rhodamine 6G as standard dye. All the compounds showed emission in the region between 331 and 495 nm. The quantum yield of all the compounds were found to be weak, except methyl benzocoumarin 3-carboxylate **22** which showed (Φ_F=0.98) in comparison to Rhodamine 6G as standard (Φ_F=0.95).

Keywords Coumarins · Fluorescence · Pyrimidines · UV-visible absorption

Introduction

Coumarin and its derivatives are one of the important classes of heterocyclic compounds which occur in many natural products with pharmacological activity [1–6]. Coumarin compounds are known to possess a wide range of biological activities such as antibacterial [7], anticancer [8, 9], anticoagulants [10], anti-HIV protease inhibitors [11], anti-HIV integrases [12, 13], serine protease inhibitors [14], inhibitors of steroid 5α-reductase [15], and

NO synthase inhibitors [16]. Geiparvarin **1** (Fig. 1), a naturally occurring product bearing the coumarin residue, has been shown to possess a significant inhibitory activity against a variety of cancer cell lines [17]. Due to efficient light emission properties, the coumarin derivatives are of the most importance as significant organic fluorescent materials [18] for biochemical and biological imaging applications [19]. Lee et al. [20] have reported novel coumarin-based fluorogenic probe bearing the 2-picolyl as a fluorescent chemosensor with high selectivity and suitable affinity in biological systems toward Cu^{2+}, meanwhile Rajisha et al. [21] have synthesized new benzocoumarin-oxadiazolyls as strong blue-green fluorescent brighteners research with good bathochromic shifts. Other coumarin derivatives are used as fluorescent imaging agents such as 7-amino-4-methyl coumarin-3-acetic acid (AMCA) **2** [22] in fluorohistochemical examination of human kidney glomeruli and 3-(4-aminophenyl)-2H-chromen-2-one (CMC) **3** (Fig. 1) can be used for in situ fluorescent imaging of myelin in the vertebrate nervous system [23].

Fluorescence Imaging (FI) is one of the most popular imaging modes in biomedical sciences for the visualisation of cells and tissues both in vitro and in vivo [24]. Despite that a number of fluorescent imaging agents have been reported, we synthesized here new coumarin and benzocoumarin derivatives conjugated pyrimidines scaffolds with study of their fluorescence properties, aiming to develop new imaging agents can be used in living cells.

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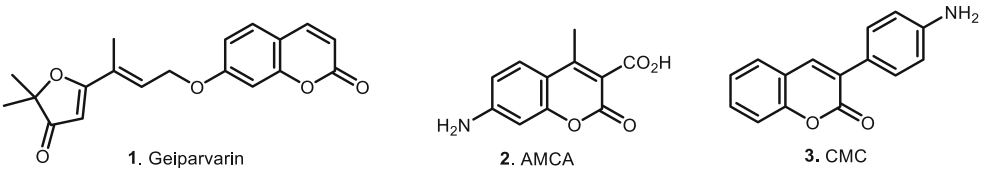
Fig. 1 Some potential coumarin derivatives



Experimental Details

Physical Measurements

Melting points are uncorrected and were measured with a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). NMR data were obtained on 400 and 600 MHz (^{1}H) and 100 and 150.91 MHz (^{13}C) spectrometers (Avance III, Bruker, Germany), respectively, with TMS as internal standard and on the δ scale in ppm. Heteronuclear assignments were verified by ^{1}H, ^{13}C HMBC and ^{1}H, ^{13}C HSQC NMR experiments. Microanalytical data were obtained with a Vario EL (Shimadzu, Japan). Analytical silica gel TLC plates 60 F_{254} were purchased from Merck. Microwave-assisted reactions were carried out in a CEM Focused Microwave Synthesis System (100–450 W). All reagents were obtained from commercial suppliers and were used without further purification.

*General Procedure for the Preparation of Coumarinyl-Pyrimidine Analogues (***11**–**15***)*

To a stirred solution of coumarin-3-carbohysrazide (**5**) (100 mg, 0.50 mmol) in DMF (10 mL) and substituted arylazopyrimidines **6**–**10** (0.50 mmol) were heated in an oil bath at 90–100 °C for 4–5 h. The mixture was evaporated to dryness and the residue was co-evaporated with silica gel (1.0 g) in MeOH then poured on a short SiO_2 column (5.0 g). Elution, in gradient, with MeOH (0–10 % *v:v*) and $CHCl_3$ as eluent afforded the desired products.

*N'-(2,6-Diamino-5-((4-chlorophenyl)diazenyl) pyrimidin-4-yl)-2-oxo-2H-chromene-3-carbohydrazide (***11***)*

From 2,6-diamino-4-chloro-5-*p*-chlorophenylazopyrimidine (**6**) (141 mg). Yield: 120 mg (53 %), m.p. 203–210 °C R_f= 0.65. ^{1}H NMR (DMSO-d_6): δ 11.14 (br s., 1H, NH), 9.26 (br s., 1H, NH), 9.01 (s, 1H, $H_{coum.}$-4), 8.18 (br s., 2H, NH_2), 7.82 (dd, 1H, $J_{5,7}$=1.4 Hz, $J_{5,6}$=7.7 Hz, H-5), 7.70 (dt, 1H, $J_{6,7}$=$J_{7,8}$=7.8 Hz, H-7), 7.57 (d, 2H, $J_{2',3'}$=8.0 Hz, $H_{arom.}$-2′+$H_{arom.}$-6′), 7.41 (d, 2H, $J_{5',6'}$=8.0 Hz, $H_{arom.}$-3′+$H_{arom.}$-5′), 7.00 (m, 2H, $H_{coum.}$-6+$H_{coum.}$-8), 6.81 (d, 2H, *J*=4.5 Hz, NH_2). ^{13}C NMR (DMSO-d_6): δ 165.1 (C_9=O), 163.2 ($C_{pyrimid.}$-4), 161.7 ($C_{pyrimid.}$-2), 159.1 ($C_{coum.}$-2), 157.0 ($C_{pyrimid.}$-6), 151.1 ($C_{coum.}$-8a), 133.9 (C-Cl), 131.1 ($C_{arom.}$-2′+$C_{arom.}$-6′), 129.8 ($C_{arom.}$-3′+$C_{arom.}$-5′), 129.0 ($C_{coum.}$-5+$C_{coum.}$-7), 128.6 ($C_{arom.}$-1′), 123.5 ($C_{coum.}$-6), 119.2 ($C_{coum.}$-4), 118.7 ($C_{coum.}$-4a), 117.0 ($C_{coum.}$-8), 113.1 ($C_{coum.}$-3), 102.4 ($C_{pyrimid.}$-5). Anal. Calcd for $C_{20}H_{15}ClN_8O_3$ (450.84): C, 53.28; H, 3.35; N, 24.85. Found: C, 53.01; H, 3.28; N, 24.77 %.

*N'-(2,6-Diamino-5-((4-bromophenyl)diazenyl) pyrimidin-4-yl)-2-oxo-2H-chromene-3-carbohydrazide (***12***)*

From 2,6-diamino-4-chloro-5-*p*-bromophenylazopyrimidine (**7**) (164 mg). Yield: 153 mg (62 %), m. p. 222–223 °C (dec.). R_f=0.72. ^{1}H NMR (DMSO-d_6): δ 11.11 (br s., 1H, NH), 9.40 (br s., 1H, NH), 9.00 (s, 1H, $H_{coum.}$-4), 8.35 (m, 3H, NH_2+H-5), 7.98 (dt, 1H, $J_{5,7}$=1.5 Hz, $J_{7,8}$=$J_{6,7}$=7.7 Hz, $H_{coum.}$-7), 7.68 (d, 2H, $J_{2',3'}$=7.9 Hz, $H_{arom.}$-2′+$H_{arom.}$-6′), 7.41 (m, 3H, $H_{arom.}$-3′+ $H_{arom.}$-5′+$H_{coum.}$-6), 6.97 (m, 3H, NH_2+$H_{coum.}$-8). ^{13}C NMR (DMSO-d_6): δ 165.1 (C_9=O), 163.2 ($C_{pyrimid.}$-4), 161.7 ($C_{pyrimid.}$-2), 159.1 ($C_{coum.}$-2), 156.4 ($C_{pyrimid.}$-6), 151.8 ($C_{coum.}$-8a), 133.7 (C-Br), 132.7 ($C_{arom.}$-2′+$C_{arom.}$-6′), 131.3 ($C_{coum.}$-5+$C_{coum.}$-7), 123.8 ($C_{coum.}$-6), 120.1 ($C_{coum.}$-4), 119.1 ($C_{coum.}$-4a), 118.7 ($C_{coum.}$-8), 112.5 ($C_{coum.}$-3), 102.7 ($C_{pyrimid.}$-5′). Anal. Calcd for $C_{20}H_{15}BrN_8O_3$ (495.29): C, 48.50; H, 3.05; N, 22.62. Found: C, 48.31; H, 2.98; N, 22.40 %.

*N'-(2,6-Diamino-5-((4-nitrophenyl)diazenyl)pyrimidin-4-yl) -2-oxo-2H-chromene-3-carbohydrazide (***13***)*

From 2,6-diamino-4-chloro-5-*p*-nitrophenylazopyrimidine (**8**) (164 mg). Yield: 138 mg (60 %), m.p. 198–201 °C (dec.). R_f=0.79. ^{1}H NMR (DMSO-d_6): δ 11.11 (br s., 1H, NH), 9.40 (br s., 1H, NH), 9.00 (s, 1H, $H_{coum.}$-4), 8.48 (br s., 2H, NH_2), 8.36 (d, 2H, $J_{5',6'}$=8.9 Hz, $H_{arom.}$-3′+$H_{arom.}$-5′), 7.98 (m, 2H, $H_{coum.}$-5+ $H_{coum.}$-7), 7.69 (d, 2H, $J_{2',3'}$=8.9 Hz, $H_{arom.}$-2′+ $H_{arom.}$-6′), 7.40 (m, 2H, $H_{coum.}$-6+$H_{coum.}$-8), 6.98 (d, 2H, *J*= 5.9 Hz, NH_2). ^{13}C NMR (DMSO-d_6): δ 163.6 (C_9=O), 161.3 ($C_{pyrimid.}$-4′), 159.4 ($C_{pyrimid.}$-2′), 158.5 ($C_{coum.}$-2), 156.2 ($C_{pyrimid.}$-6), 155.7 ($C_{coum.}$-8a), 146.5 (C-NO_2), 133.3 ($C_{arom.}$-1′), 130.7 ($C_{arom.}$-2′+$C_{arom.}$-6′), 126.9 ($C_{coum.}$-5+$C_{coum.}$-7), 124.9 ($C_{coum.}$-6), 122.0 ($C_{arom.}$-3′+$C_{arom.}$-5′), 119.5 ($C_{coum.}$-4), 118.1 ($C_{coum.}$-4a), 116.4 ($C_{coum.}$-8), 112.5 ($C_{coum.}$-3), 103.0 ($C_{pyrimid.}$-5). Anal. Calcd for $C_{20}H_{15}BrN_9O_5$ (461.39): C, 52.06; H, 3.28; N, 27.32. Found: C, 51.89; H, 3.17; N, 27.08 %.

*Methyl 4-((2,6-diamino-6-(2-oxo-2H-chromene-3-carbonyl) hydrazinyl)pyrimidin-5-yl) diazenyl)benzoate (***14***)*

From methyl 4-((2,6-diamino-4-chloropyrimidin-5-yl)diazenyl)benzoate (**9**) (153 mg). Yield: 52 mg (22 %),

m.p. 212–216 °C. R_f=0.60. ^{1}H NMR (DMSO-d_6): δ 11.12 (br s., 1H, NH), 9.00 (s, 2H, NH+H-4), 8.00 (d, 1H, J=8.7 Hz, $H_{arom.}$-2′+$H_{arom.}$-6′), 7.69 (m, 2H, $H_{coum.}$-5+ $H_{coum.}$-7), 7.43 (d, 2H, J=8.7 Hz, $H_{arom.}$-3′+$H_{arom.}$-5′), 7.00-6.95 (m, 4H, NH_2+ $H_{coum.}$-6+$H_{coum.}$-8), 3.90 (s, 3H, CO_2Me). ^{13}C NMR (DMSO-d_6): δ 164.1 (CO_2Me), 163.3 (C_9=O), 162.6 ($C_{pyrimid.}$-4), 159.6 ($C_{pyrimid.}$-2), 158.5 ($C_{coum.}$-2), 155.5 ($C_{pyrimid.}$-6), 152.8 ($C_{coum.}$-8a), 133.1 ($C_{arom.}$-1′), 130.7 ($C_{arom.}$-4′), 129.4 ($C_{arom.}$-3′+$C_{arom.}$-5′), 127.8 ($C_{coum.}$-5+$C_{coum.}$-7+ $C_{arom.}$-2′+$C_{arom.}$-6′), 124.2 ($C_{coum.}$-6), 119.5 ($C_{coum.}$-4), 118.1 ($C_{coum.}$-4a), 116.4 ($C_{coum.}$-8), 113.1 ($C_{coum.}$-3), 99.9 ($C_{pyrimid.}$-5), 51.6 (CO_2*Me*). Anal. Calcd for $C_{22}H_{18}N_8O_5$ (474.43): C, 55.70; H, 3.82; N, 23.62. Found: C, 55.49; H, 3.70; N, 23.40 %.

*N-(4-((2,4-Diamino-6-(2-oxo-2H-chromene-3-carbonyl) hydrazinyl)pyrimidin-5-yl)diazenyl)phenyl)acetamide (***15***)*

From 2,6-diamino-4-chloro-5-*p*-acetamidophenylazopyrimidine (**8**)**10** (153 mg). Yield: 88 mg (37 %), m.p. 288 °C (dec.). R_f= 0.62. ^{1}H NMR (DMSO-d_6): δ 11.11 (br s., 1H, NH), 9.00 (s, 1H, $H_{coum.}$-4), 7.70 (m, 6H, NH_2+$H_{arom.}$-2′+$H_{arom.}$-6′+$H_{coum.}$-5+ $H_{coum.}$-7), 7.42 (d, 2H, $J_{3',4'}$=8.5 Hz, $H_{arom.}$-3′+$H_{arom.}$-5′), 7.39 (m, 2H, $H_{coum.}$-6+$H_{coum.}$-8), 7.11 (s, 1H, *NH*Me), 6.97 (d, 2H, J=6.0 Hz, NH_2), 2.32 (s, 3H, NH*Me*). ^{13}C NMR (DMSO-d_6): δ 169.0 (CONH), 165.0 (C_9=O), 162.6 ($C_{pyrimid.}$-4), 160.1 ($C_{pyrimid.}$-2), 158.5 ($C_{coum.}$-2), 156.5 ($C_{pyrimid.}$-6), 153.1 ($C_{coum.}$-8a), 137.8 ($C_{arom.}$4′), 133.1 ($C_{arom.}$-2′+$C_{arom.}$-6′), 130.7 ($C_{arom.}$-1′), 127.7 ($C_{coum.}$-5+$C_{coum.}$-7), 123.6 ($C_{coum.}$-6), 119.5 ($C_{coum.}$-4+$C_{arom.}$-3′+$C_{arom.}$-5′), 118.1 ($C_{coum.}$-4a), 116.4 ($C_{coum.}$-8), 113.9 ($C_{coum.}$-3), 99.4 ($C_{pyrimid.}$-5), 25.0 (CONH*Me*). Anal. Calcd for ($C_{22}H_{19}N_9O_4$ (473.44): C, 55.81, H, 4.05; N, 26.63. Found: C, 54.61; H, 3.93; N, 26.4 %.

*3-(5,7-Diamino[1,2,4]triazolo[4,3-c]pyrimidin-3-yl) -2H-chromene-2-one (***18***)*

A mixture of 2,6-diamino-4-chloropyrimidine (**16**) (200 mg, 1.39 mmol) and **5** (283 mg, 1.39 mmol) was irradiated under microwave at 180 °C for 25 min. The crude mixture was heated under reflux in glacial HOAc (10 ml) for 4 h. After cooling, water was added the precipitate was filtered, washed with cold EtOH and dried. Recrystallization from EtOH afforded **18** (176 mg, 43 %), m.p. 210 °C (dec.), R_f=0.17. ^{1}H NMR (DMSO-d_6): δ 9.72 (br s., 2H, NH_2), 8.04 (s, 1H, $H_{coum.}$-4), 7.73 (dd, 1H, $J_{5,6}$=8.3 Hz, $J_{5,7}$=2.3 Hz, $H_{coum.}$-5), 7.59-7.32 (m, 3H, $H_{coum.}$-6+$H_{coum.}$-7+$H_{coum.}$-8), 6.32 (br s., 2H, NH_2), 5.71 (s, 1H, $H_{pyrimid.}$-5). ^{13}C NMR (DMSO-d_6): δ 168.2 ($C_{pyrimid.}$-2′), 164.9 ($C_{pyrimid.}$-6′), 159.3 ($C_{coum.}$-2), 152.8 ($C_{coum.}$-8a), 149.3 $C_{pyrimid.}$-4′), 146.1 ($C_{triazol}$-3), 144.7 ($C_{coum.}$-4), 130.4 ($C_{coum.}$-3), 128.3 ($C_{coum.}$-7), 126.5 ($C_{coum.}$-5), 124.7 ($C_{coum.}$-6), 120.4 ($C_{coum.}$-4a), 115.8 ($C_{coum.}$-8), 93.7 ($C_{pyrimid.}$-5). Anal. Calcd for $C_{14}H_{10}N_6O_2$ (294.27): C, 57.14; H, 3.33; N, 28.56. Found: C, 56.83; H, 3.33; N, 28.29 %.

*Methyl 3-oxo-3H-benzochromene-2-carboxylate (***22***)*

A mixture of 2-hydroxynaphthaldehyde (**19**) (500 mg, 2.91 mmol) and dimethylmalonate (**20**) (385 mg, 2.91 mmol) in the presence of piperidine (2.0 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was acidified with HCl and cooled. The precipitate was filtered, washed with small amount of cold water, the product was dried and recrystallized from MeOH to give **22** (593 mg, 85 %), m.p. 148 °C, R_f=0.69. ^{1}H NMR (DMSO-d_6): δ 9.45 (s, 1H, H-4), 8.63 (dd, 1H, $J_{5,6}$= 7.6 Hz, $J_{5,7}$=1.5 Hz, H-5), 8.36 (dd, 1H, $J_{7,8}$=7.6 Hz, $J_{6,8}$=1.5 Hz, H-8), 8.11 (m, 1H, H-6), 7.82 (m, 1H, H-7), 7.70 (d, 1H, $J_{9,10}$=8.0 Hz, H-9), 7.64 (d, 1H, $J_{9,10}$=8.0 Hz, H-10), 3.90 (s, 3H, CO_2Me). ^{13}C NMR (DMSO-d_6): δ 163.9 (CO_2Me), 155.8 (C-2), 145.2 (C-10a) 136.7 (C-4), 129.6 (C-9+C-5a), 129.5 (C-8), 127.0 (C-6), 122.8 (C-7+C-8a), 120.3 (C-5), 117.0 (C-4a), 115.3 (C-10), 115.3 (C-4a), 100.0 (C-3), 53.0 (OMe). Anal. Calcd for $C_{15}H_{10}O_4$ (254.24): C, 70.86; H 3.96. Found: C, 70.66; H, 3.89 %.

*Ethyl 3-imino-3H-benzochromene-2-carboxylate (***23***)*

To a stirred solution of **19** (361 mg, 2.01 mmol) in EtOH (10 ml) were added ethyl cyanoacetate (**21**) (227 mg, 2.01 mmol), NH_4OAc (200 mg) and glacial HOAc (1.0 ml). The mixture was heated under reflux for 1 h, and then poured onto crushed ice. The precipitate was filtered, dried and recrystallized from MeOH to give **23** (456 mg, 81 %), m.p. 202–205 °C (dec.), R_f=0.32. ^{1}H NMR (DMSO-d_6): δ 9.29 (s, 1H, H-4), 9.07 (s, 1H, NH), 8.66 (d, 1H, J=8.5 Hz, H-5), 8.34 (d, 1H, J= 9.1 Hz, H-8), 8.12 (d, 1H, $J_{6,7}$=8.1 Hz, H-6), 7.82 (m, 1H, H-7), 7.81 (d, 1H, $J_{9,10}$=8.0 Hz, H-9), 7.68 (m, 2H, H-9+H-10), 4.23 (q, 2H, J=7.8 Hz, CH_2CH_3), 1.27 (t, 3H, CH_2CH_3). ^{13}C NMR (DMSO-d_6): δ 167.1 (CO_2Et), 165.0 (C=NH), 154.3 (C-10a) 135.6 (C-8), 130.6 (C-5a), 129.5, 129.4 (C-4+C-6), 127.1 (C-9), 123.1 (C-5), 122.1 (C-7+C-8a), 118.8 (C-4a), 117.0 (C-10), 112.6 (C-3), 60.5 (CH_2), 14.9 (CH_3). Anal. Calcd for $C_{16}H_{13}NO_3$ (267.28): C, 71.90; H, 4.91; N, 5.24. Found: C, 71.74; H, 7.80; N, 5.07 %.

*3-Oxo-3H-benzochromene-2-carbohydrazide (***24***)*

A solution of **22** (100 mg, 0.37 mmol) in EtOH (7 mL) and hydrazine hydrate (2 mL) was heated under reflux for 3 h. After cooling, the solution was poured into crushed ice with stirring. The separated solid was filtered, washed with water, dried and recrystallised from EtOH to give **24** (75 mg, 80 %), as a yellow solid, m. p. 260–263 °C (Lit. [25], m.p. 260–262 °C),

N'-(2,6-Diamino-5-((4-bromophenyl)diazenyl) pyrimidin-4-yl)-3-oxo-3H-benzochromene-2-carbohydrazide (**25**)

To a stirred solution of **24** (67 mg, 0.30 mmol) in DMF (5 mL) was added the azopyrimidine **7** (98 mg, 0.30 mmol) and the mixture was heated at 100 °C for 3 h. After cooling, the residue was evaporated to dryness and the residue was purified on a short SiO_2 column (5.0 g). Elution, in gradient, with MeOH (0–10 %) and $CHCl_3$ as eluent afforded **25** (95 mg, 58 %), m.p. 279–282 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 10.02 (br s., 2H, NH_2), 9.26 (br s., 1H, NH), 8.78 (d, 1H, $J_{9,10}$= 8.8 Hz, $H_{coum.}$-10), 8.00 (d, 1H, $J_{7,8}$=8.0 Hz, $H_{coum.}$-8), 7.89 (d, 1H, $J_{5,6}$=8.0 Hz, H-5), 7.82 (d, 1H, $J_{9,10}$=8.8 Hz, $H_{coum.}$-9), 7.55 (d, 2H, $J_{2',3'}$=8.0 Hz, $H_{arom.}$-2′+$H_{arom.}$-6′), 7.50 (m, 2H, $H_{coum.}$-6+$H_{coum.}$-7), 7.38 (d, 2H, $J_{5',6'}$=8.0 Hz, $H_{arom.}$-3′+$H_{arom.}$-5′), 7.25 (s, 1H, $H_{coum.}$-4), 6.97 (d, 2H, J=5.0 Hz, NH_2). ^{13}C NMR (DMSO-d_6): δ 164.9 ($CONH_.$), 162.1 ($C_{pyrimid.}$-4), 161.2 ($C_{pyrimid.}$-2), 157.8 ($C_{coum.2}$=O), 156.1 ($C_{pyrimid.}$-6), 137.2 ($C_{coum.}$-3), 134.5 (C-8a), 132.8 (C-4a), 132.3, 131.0, 130.3, 129.4, 128.4, 128.0, 127.3, 127.0, 125.2, 124.3, 123.1 ($C_{coum.}$+$C_{arom.}$), 120.1 ($C_{coum.}$-4), 102.1 ($C_{pyrimid.}$-5). Anal. Calcd for $C_{24}H_{17}BrN_8O_3$ (545.35): C, 52.86; H, 3.14; N, 20.55. Found: C, 52.66; H, 3.03; N, 20.36 %.

Results and Discussion

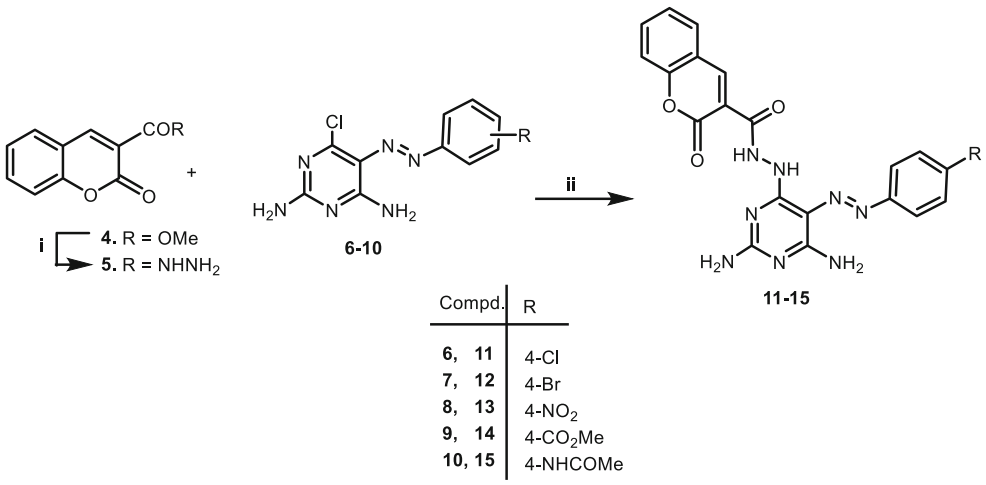
Chemistry

Treatment of methyl coumarin-3-carboxylate **4** with hydrazine hydrate afforded the carbohydrazide analog **5**. 4-Chloro-azopyrimidine derivatives **6**–**10** have been selected as starting materials for the synthesis of the target compounds **11**–**15**, since the electron withdrawing group, azo residue would facilitated replacement of the chloro group at C-4 by the carbohydrazide scaffolds. Thus, treatment of **6**–**10** with **5** in hot DMF for 4–5 h furnished, after chromatographic purification, **11**–**15** in 22–62 % yield (Scheme 1).

The structures of **11**–**15** were determined by ^{1}H, ^{13}C and 2D NMR spectroscopy. In the ^{1}H NMR spectra, H-4 of the coumarin ring were resonated as singlets at δ=9.01–9.00 ppm, meanwhile H-5 - H-8 together with the aromatic protons conjugated pyrimidine ring and other substituents were fully analysed. In the ^{13}C NMR spectra of **11**–**15**, the carbonyl carbon atom (C_9=O) appeared at the regions δ=165.2–163.3 ppm, while C-4 of the pyrimidine ring resonated at δ=163.3–161.3 ppm. The resonances at the regions δ= 161.7–159.4, 157.0–155.7 and 103–99.4 ppm were assigned to carbon atoms 2, 6 and 5 of the pyrimidine backbone, respectively, whereas the signals at the regions δ=159.1–158.5 and 155.7–151 ppm were attributed to carbon atoms 2, and 8a of the coumarin scaffold, respectively. Other coumarin and aromatic carbon atoms as well as the substituents were fully analysed (*c.f.* Experimental section).

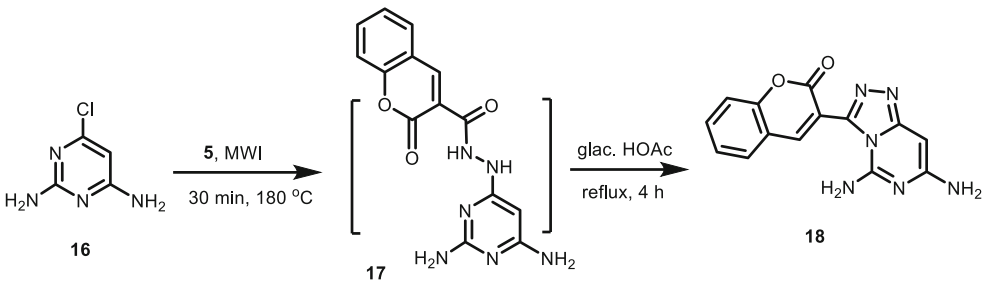
Next, treatment of **16** with carbohydrazide **5** under microwave irradiation (400 Watt, 180 °C) for 20 min afforded the crud product, 4-coumarinyl-pyrimidine carbohydrazide derivative **17** which has been used directly, without purification. Boiling of the crude **17** with glacial HOAc for 4 h furnished , after chromatographic purification, the cyclized triazolo analog **18** (43 %) (Scheme 2).

The structure of **18** was determined from its ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectrum, H-4 of the coumarin ring appeared as a singlet at δ=8.04 ppm, while H-5 resonated as a doublet of doublets at δ=7.73 ppm ($J_{5,6}$=8.3 Hz, $J_{5,7}$= 2.3 Hz). H-5 of the pyrimidine backbone appeared as a singlet δ=7.71 ppm, whereas the other coumarin protons were analysed. The ^{13}C NMR spectrum showed four signals at the lower fields, δ=168.2, 164.9, 159.3 and 152.8 ppm, were assigned to C-2′, C-6′ of the pyrimidine ring and C-2, 8a of coumarin moiety, respectively. The resonances at δ=149.3, 146.1 and 144.7 ppm were attributed C-4′ of the pyrimidine



Scheme 1 *Conditions and reagents*: (i) $NH_2NH_2.H_2O$, EtOH, reflux, 6 h; (ii) DMF, 90–100 °C, 4–5 h

Scheme 2 Synthesis of 3-(5,7-diamino[1,2,4-]triazolo[4,3-*c*]pyrimidin-3-yl)-2*H*-chromene-2-one (**18**)



ring, C-3 of the triazole and C-4 of coumarin moiety, respectively. C-5 of the pyrimidine ring resonated at δ=93.7 ppm.

Our work was modified by selecting **19** as a precursor for the synthesis of new benzocoumarinyl-pyrimidine, aiming to examine its fluorescence property in comparison to the coumarin analogs **11**–**15**. Thus, treatment of **19** with dimethylmalonate **20** in the presence of piperidine afforded, after purification, the benzocoumarin 3-methyl ester **22** (85 %). Alternatively, treatment of **19** with ethyl cyanoacetate **21** in the presence of NH_4OAc and HOAc in boiling EtOH furnished the 3-iminoethyl benzocoumarin ester **23** (81 %) which has been structurally confirmd by the ^1H and ^{13}C NMR spectra. Treatment of **22** with hydrazine hydrate afforded the carbohydrazide derivative **24** in 30 % yield. Compound **25** was obtained in 58 % yield by condensation of **24** with the 4-bromo-azopyrimidine derivative **7** in hot DMF (Scheme 3).

The structures of **22**–**25** were assigned by their ^1H and ^{13}C NMR spectra, where the protons and carbon atoms of the coumarin moiety showed almost similar pattern. The singlets at δ=9.24, 9.29, 8.87 and 7.25 ppm were assigned for H-4 of the coumarin ring, respectively. The other aromatic, NH and NH_2 protons were fully analysed (*c.f.* Experimental section). In the ^{13}C NMR spectrum of **23**, CO_2Et and C=NH carbon atoms resonated at δ=167.1 and 165.0 ppm, respectively. Further, the resonances at δ=164.9 and 162.1 ppm were assigned for CONH of coumarin and C-4′ of pyrimidine backbone, respectively. The rest of benzocoumarin and aromatic carbon atoms were fully assigned (*cf.* Experimental section).

All the synthesized compounds were further analysed by the HSQC [26] and HMBC [27] NMR spectroscopy.

UV–Vis Absorption and Fluorescence Spectra

The UV-visible absorption and fluorescence spectra of the synthesized coumarin analogs **4**, **5**, **11**–**15**, **18** and **22**–**25** in MeOH were obtained and are presented in Table 1. The absorption and fluorescence maxima of some of the synthesized analogs showed good bathochromic shifts with wavelength of maximum absorption in the UV or visible region ($\lambda_{ex.}$) (315–405 nm). Wavelengths of maximum emission for the compounds ($\lambda_{em.}$) (322–483 nm) were observed in MeOH at room temperature. Although, compounds **4**, **12**, **22** and **23** showed low quantum yield values (Φ_F) of 0.02, 0.01 and 0.15 nm, respectively, but exhibited fluorescence spectral properties with Stoke's shift of 89, 87, 72 and 85 nm, respectively (Fig. 2). However, **22**

Scheme 3 *Conditions and reagents*: (i) **20**, piperidine, EtOH, reflux, 1 h; (ii) **21**, NH_4OAc, HOAc, EtOH, reflux, 1 h; (iii) $NH_2NH_2.H_2O$, EtOH, reflux, 3 h; (iv) **7**, DMF, 100 °C, 3 h

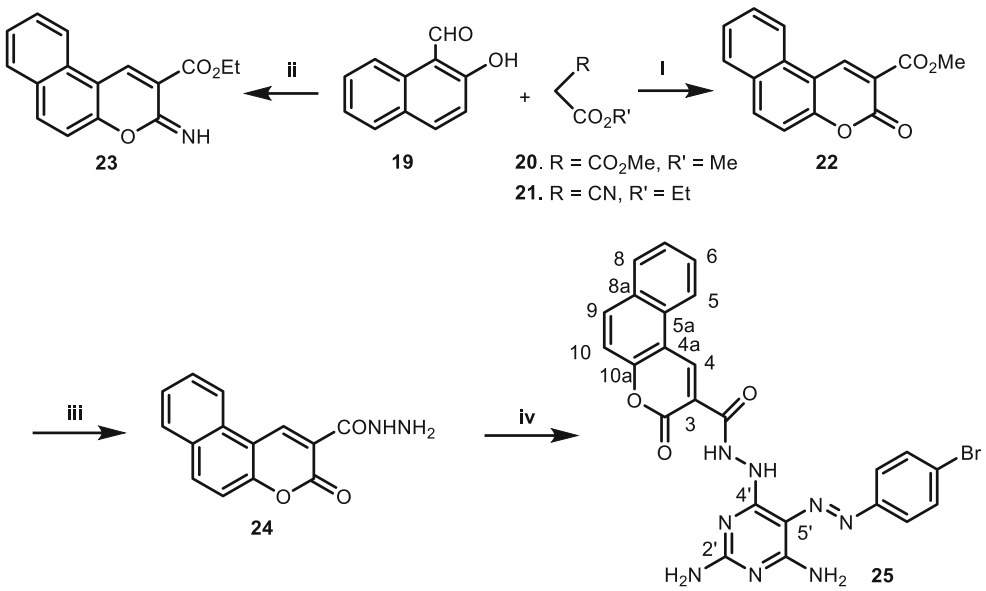


Table 1 UV–vis, fluorescence data, and quantum yields (Φ_F) of compounds some coumarin and benzocoumarin analogs

Compd.	λ_{ex}[nm]	log ε [mM]$^{-1}$.[cm]$^{-1}$	λ_{em}[nm]	Φ_F	Stoke's shift
4	331	4.04	331	0.02	89
5	315	4.03	322	<0.01	7
11	365	4.41	385	-<0.01	20
12	365	4.78	483	0.01	87
13	405	4.43	405	<0.01-	10
14	[355]	[4.61]	355	<0.01-	8
15	[355]	[4.42]	360	<0.01-	5
18	293	4.56	300	<0.01-	7
22	371	3.90	443	0.98	72
23	363	3.81	448	0.15	85
24	360	4.09	373	0.01	13
25	379	4.27	391	0.12	12

Annotations: Ref.: Rh6G in EtOH (Φ_F: 0.95) at room temperature; Equipment and software: Perkin Elmer LS 50, Cary UV/vis 50; SpekWin 1.71.3, FL WinLab. [ ]: shoulder

showed a very exciting fluorescence result in MeOH with a quantum yield (Φ_F=0.98) with stoke's shift of 72 nm (Fig. 2), which characterized by a brightly intense blue emission light at λ_{em}=443 nm, in comparison for those of Rh6G (Φ_F=0.95). These result encouraged us to proceed further in our current fluorescence imaging (FI) for the visualisation of cells and tissues both in vitro and in vivo as well as in coronary angiographic applications, with full biochemical study of **22**.

In conclusion, the replacement of 3-ester group in the coumarin of benzocoumarin backbone by hydrazide group (e.g.,: **5** and **24**) or hydrazide-pyrimidine moiety (e.g.,: **11**–**15** and **25**) would reduced the fluorescence property unless there is an electron donating group at the aromatic ring of the coumarin moiety. Such argument is in agreement with Mahadevan et al. results [28].

Biological Imaging Application

Organic fluorescent dyes are routinely used as contrast agents in immunohistochemical staining protocols. Owing to their low molecular weight, high quantum yield, relatively low cost and the existence of well-developed conjugation protocols, they represent an ideal optical label for molecular imaging probes [29]. Additionally, fluorescent imaging is a relatively recent imaging method and thus still developing in many ways, and typically performed by exogenous delivery of a fluorescent probe (e g, an organic dye) that interacts with the target or by direct imaging of an endogenously expressed fluorescent protein. Signal generation is achieved by exciting the fluorescent probe or protein at a given light wavelength (λ) and detecting light emission at another λ with a charge-coupled device camera built into either a planar (fluorescence reflectance imaging) or a tomographic (fluorescence molecular tomography) imaging system [30, 31]. Most of the above imaging fluorescence agents exhibited several side effects in vivo (especially by human treatment), including the allergy, toxicity, solubility and radioactivity. Update ^{125}I and sodium diatrizoate (sodium 3-(acetylamino)-2,4,6-triiodobenzoate) are used in the coronary arterials angiography [32].

Our small molecule, the benzocoumarin ester **22** has been selected for a priminary flourescence imaging application by using an experimental animal model (rabbit, weight~3.2 kg) induced inrtravenasouly by **22** with a concentration of 10^{-3} M in a mixture of MeOH-H_2O (1:9 v:v) solution, following Johnston et al. method [33]. The X-ray contrast media for coronary angiography showed some encouraging peripheral vascular angiographic imaging pictures. The application is under further investigation to overcome the solubility problem of **22** in vivo by introducing a more polar group such hydroxyl group.

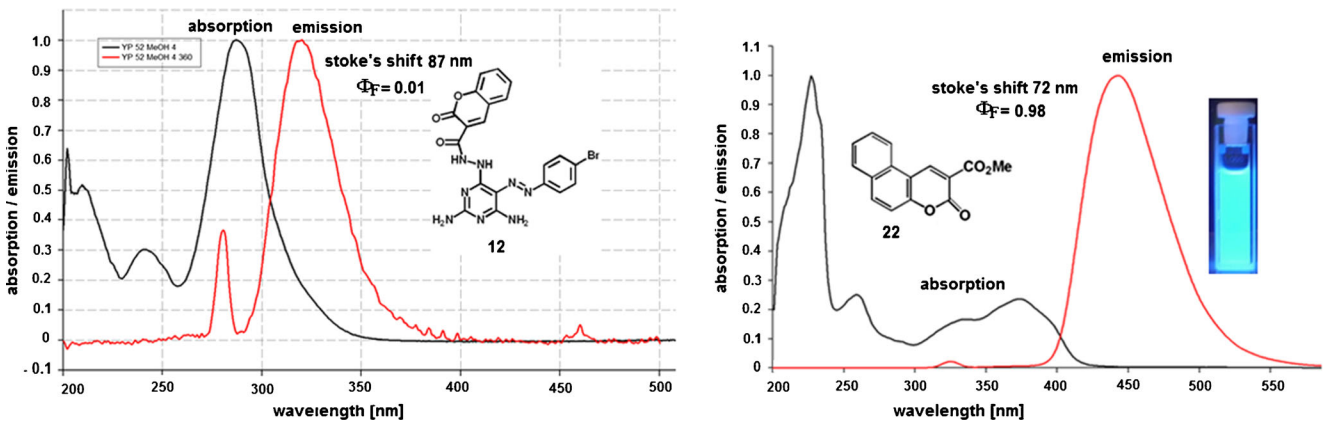


Fig. 2 UV-visible and fluorescence spectra of compounds **12** and **22** in MeOH

Conclusion

A new series of coumarin and 5,6-benzomcomarin substituted pyrimidine derivatives **11**–**15** and **22**–**25** were synthesized from treatment of coumarin and benzocoumarin 3-carbohydrazide with 4-chloro-5-azoarylpyrimidines analogs, Further, the coumarin-1,2,4-triazolo-pyrimidine analog **18** was prepared from 4-chloropyrimidine analog **16** with coumarin 3-carbohyrazide **5** under MWI condition, aiming to develop new imaging fluorescence agents. The new analogs were investigated spectrophotometrically in MeOH for their fluorescence properties, which showed emission in the region between 331 and 495 nm. Interestingly, methyl benzocoumarin 3-carboxylate (**22**) showed a higher quantum yield (Φ_F=0.98) in comparison for those of Rhodamine 6G as standard (Φ_F=0.95). The priminary biological fluorescence imaging aaplication of **22** in an experimental animal model showed some peripheral vascular angiographic imaging pictures by using a flourescence emission with some pharmakinetic studies.

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