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Development of Novel Imaging Fluorescent Agents Bearing Anti-Inflammatory Drugs: Synthesis, Structural Characterization and Evaluation of Biological Activity

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Abstract—A new series of 5,6-benzocoumarin derivatives carrying anti-inflammatory drugs were synthesized via alkylamide spacers, the target to develop novel imaging fluorescent agents as useful technique to cancer early discovery. Experimentally, treatment of *N*-Boc-1,3-propanediamine with ethyl 5,6-benzocoumarin-3-carboxlate (**III**) under reflux conditions followed by hydrolyzed with CF_3COOH to the corresponding primary amine (**V**), then treatment of derivative (**V**) with anti-inflammatory drugs have carboxylic group in presence of DCC (*N*,*N*'-dicyclohexylcarbodiimide) as catalyst and MeCN as solvent. The structure of the synthesized compounds was confirmed by FT-IR, NMR (^1H,^{13}C) spectral data as well as elemental analysis. The fluorescence properties study was investigated spectrophotometrically in methanol and maximum emission (λ_{em}) exhibited within range 411–436 nm, meanwhile, the quantum yields were calculated comparison to Rhodamine 6G as reference. Interestingly, the compound (**III**) gave a higher quantum yield (Φ_F = 0.96), meanwhile, compounds (**VII**, **VIII**, **IX** and **XI**) gave reasonable quantum yields (Φ_F) 0.60, 0.65, 0.78 and 0.84, respectively. All synthesized compounds were screened for their anti-AChE and antimicrobial activity. Based on the results, some of the compounds showed good activity in compared to the standard drugs.

Keywords: benzocoumarin, fluorescence, anti-inflammatory drugs (NSAIDs), acetylcholinesterase

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INTRODUCTION

Many natural and synthetic compounds containing coumarin nucleus, exhibit various biological and pharmacological activities, which led recently to appear an increasing interest in their synthesis [1–3]. Further, benzocoumarins and its derivatives were exhibited diverse biological activities such as anti-inflammatory [4], antimicrobial [5], antioxidant [6], antithrombotic [7], anti HIV [8], anti-tubercular [9], as well as lipid lowering properties [10]. Newly, benzocoumarin derivatives were showed significant antitumor activities against some cancer cell lines [11].

Fluorescence imaging (FI) is one of the importance techniques in biomedical field for monitoring the cells and tissues both in vitro and in vivo [12]. Due to the conjugated aromatic rings, coumarin and benzocomarin derivatives were showed good photophysical properties [13]. Al-Masoudi et al. [9] have synthesized several coumarin and benzocomarin analogs as well as their fluorescence properties study, they are found that the methyl 5,6-benzocoumarin-3-carboxylate have a higher quantum yield (Φ_F = 0.98) in comparison for those of Rhodamine 6G as reference (Φ_F = 0.95). The above results prompted us to synthesized new benzocoumarin derivatives conjugated anti-inflammatory drugs as well as study of their photophysical studies properties, in effort to develop novel imaging agents can be applied on living cells.

RESULTS AND DISCUSSION

Chemistry

The preparation methods of all compounds were described, as shown in Scheme 1, the physicochemical characteristic details are reported in Experimental section. The starting compound (**III**) was synthesized according to a literature method [15]. Treatment of ethyl benzocoumarin-3-carboxylate (**III**) with *N*-Boc-1,3-propanediamine afforded the carboxamide analog (**IV**), which subjected to deprotection of the Boc-group by TFA to give primary amine analog (**V**).

Next, treatment of the derivative **(V)** with anti-inflammatory drugs have carboxylic group in presence of DCC (*N*,*N*'-dicyclohexylcarbodiimide) as catalyst and MeCN as solvent to the synthesis of the target compounds.

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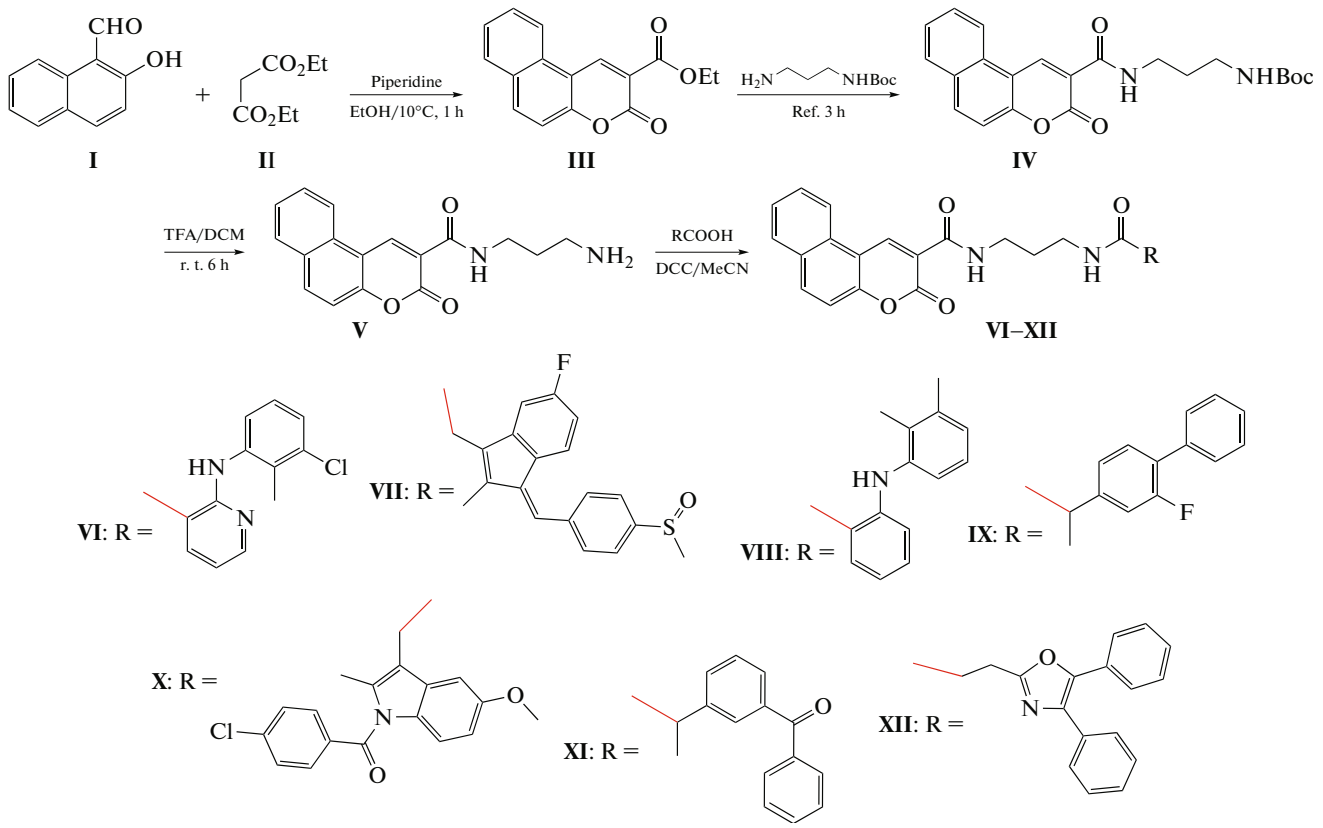

Scheme 1. The experimental steps for synthesis of benzocoumarin derivatives (**III–XII**).

Spectrally

The structures of (**III–XII**) were determined by FT-IR, NMR (^{1}H, ^{13}C) spectroscopy and by their elemental analysis. The FT-IR spectra of the prepared compounds exhibited characteristic absorption bands at 3332–3247 cm^{-1} attributable to (NH) stretching, and at 1751–1724 cm^{-1} due to ($C{=}O_{\text{lactone}}$), whereas at 1690–1650 cm^{-1} attributed to the ($C{=}O_{\text{amid}}$) stretching vibration. Other absorption bands attributed to substituents were fully analysed (c.f. Experimental section).

In the ^{1}H NMR spectra of the compounds (**IV–XII**), amid bonds protons (NH) were resonated as broad singlets at δ = 8.97–8.30 ppm. Further, the protons of the benzocoumarin moiety exhibited almost similar pattern, where H-4 of the benzocoumarin ring was resonated as singlets at δ = 8.96–8.64 ppm, meanwhile, the resonances at δ = 8.09–7.75 ppm due to for H-5, H-8 of the benzocoumarin compound. NCH_2 of propyl group at C-13, C-15 resonated as triplet at the regions 3.77–3.53, 3.69–3.40 ppm, respectively, whereas C-14 protons resonated as multiplets at the regions 2.75–1.25 ppm. In addition, the aromatic and other substituents protons were fully analysed (c.f. Experimental Section).

In the ^{13}C NMR spectra of the compounds (**III–XII**), the carbonyl carbon atom CO-17 showed at the regions δ = 176.8–173.9 ppm, while CO-11 and CO-2 were resonated at the regions δ = 161.4–161.0 ppm and δ = 160.9–160.3 ppm, respectively. The peaks at the regions δ = 154.8–147.9 ppm and δ = 148.6–140.1 ppm were assigned to carbon atoms C-10a and C-4 of the benzocoumarin backbone, respectively. In addition, the carbon atoms of propyl group C-13, C-15 and C-14 resonated at the regions δ = 43.3–42.5, 41.8–41.1 and 27.9–20.1 ppm, respectively. Other aromatic and benzocoumarin atoms as well as the atoms substituted were fully analysed (c.f. Experimental section).

Photophysical Studies

The UV–Vis and fluorescence spectra of the prepared benzocoumarin analogs (**III**, **V–XII**) in methanol were obtained and data are summarized in Table 1. The absorption and emission spectra of the compounds are presented in Fig. 1. Wavelengths of maximum absorption in the UV-visible region (λ_{ex}) were showed at (332–372 nm), meanwhile wavelengths of maximum emission (λ_{em}) (411–436 nm) were recorded in methanol at room temperature.

Although, compounds (**V**, **VI**, **X** and **XII**) gave low values of the quantum yield (Φ_F) 0.28, 0.08, 0.14 and

Table 1. UV–vis, fluorescence spectra data, and quantum yield values (Φ_F) of compounds (**III**, **V**–**XII**)

Compd.	λ_{ex}, nm	log ε, $mM^{-1} cm^{-1}$	λ_{em}, nm	Φ_F	Stoke's shift
III	361	4.459	435	0.96	74
V	352	4.414	417	0.28	65
VI	332	4.069	411	0.35	79
VII	368	4.230	420	0.60	52
VIII	345	4.160	436	0.84	91
IX	358	4.369	432	0.86	74
X	349	3.869	412	0.44	63
XI	372	4.512	422	0.91	50
XII	341	3.926	415	0.38	74

Table 2. The IC_{50} values of the benzocoumarin derivatives (**VI**–**XII**) against AChE

No. comp.	$IC_{50} \pm SD$, μM
VI	<100
VII	4.159 ± 0.245
VIII	<100
IX	1.828 ± 0.058
X	8.252 ± 0.642
XI	5.337 ± 0.461
XII	<100
Donepezil	0.014 ± 0.002

IC_{50}: (mean ± standard deviation)

0.18, respectively, but showed large Stoke's shifts of 65, 79, 63 and 74 nm, respectively, which may due to presence of a heavy atom (F and Cl) or a loose arm lead to decrease the radiative rate constant by spin-orbit coupling and "loose bolt effect" [44]. However, compound (**III**) exhibited a very exciting fluoresced in methanol with a quantum yield (Φ_F = 0.96) and Stoke's shift of 74 nm (Fig. 1), which characterized by a brightly intense blue emission light at λ_{em} = 435 nm, in comparison for those of Rh6G (Φ_F = 0.95). Meanwhile, compounds (**VII**, **VIII**, **IX** and **XI**) gave reasonable quantum yield values (Φ_F) 0.60, 0.65, 0.78 and 0.84, respectively, as well as showed Stoke's shifts 52, 91, 74 and 50 nm, respectively. All in all, the reported some benzocoumarin analogs exhibits reasonable fluorescence quantum yields can used for early detection applications of cancer by imaging of cells and also tissues both in vitro and in vivo as well as other applications such as coronary angiographic.

Biological Evaluation

Acetylcholinesterase inhibition assay. Inhibitory activity of the newly prepared compounds were screened in vitro for their anti-AChE activity, electric eel AChE was used as target enzyme, meanwhile donepezil hydrochloride was used as a reference compound. The obtained data were listed in Table 2 and expressed as IC_{50} values. The assay results of (**VII**, **IX**, **X** and **XI**) compounds were displayed good activity in the range of micromolar concentrations (μM), meanwhile, the other compounds inactive against AChE. Base on the results, it could be concluded that the flouro, chloro, and methoxy substituents increase the anti-AChE activity, this influence may be due to hydrophobic, bulky and electron withdrawing property. Therefore, IC_{50} values of unsubstituted benzocoumarins against AChE were significantly large than those of corresponding substituted benzocoumarins.

Biological assay of the synthesized compounds. Antibacterial and antifungal activity of the synthesized compounds were screened in vitro against both gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*) as well as fungi (*Candida albicans*, *Aspergillus niger*) using the broth dilution method [16]. Some of the compounds exhibited ability on inhibition both bacteria and fungi strains. The concentration of screened compounds is 5000 μg/mL. After 24h incubation at 37 ± 1°C, zone of inhibition resulted by each compound is measured in mm. Cephalexin and Fluconazole were used as standard antibiotics for antibacterial and antifungal activity respectively. The assay results were recorded as shown in the Fig.2.

EXPERIMENTAL

Melting points were measured with a Stuart SMP30 electrothermal apparatus and are uncorrected. Infrared (IR) spectra were recorded (KBr discs) on a Shimadzu FT-IR 8201 PC spectrophotometer. NMR data were obtained on Varian INOVA (^{1}H, 500 MHz; ^{13}C, 125.65 MHz) spectrometers with TMS as internal reference and on the δ scale in ppm (Shimadzu, Japan). Analytical silica gel TLC plates 60 F254 were supplied from Merck. Elemental analyses (C, H, N) were carried out by using Vario Elemental Anlayzer 3000 (Shimadzu). All drugs and reagents were obtained from commercial companies and were used without further purification.

Synthesis of ethyl 5,6-benzocoumarin-3-carboxylate (III). A mixture of 2-hydroxynaphthaldehyde (**I**) (2.68 g, 10 mmol) and diethylmalonate (**II**) (1.68 mL, 10 mmol) and few drops of piperidine in 20 mL of absolute EtOH was refluxed for 2h (TLC check). After cooling, the reaction mixture was acidified with HCl and cooled. The solid result was collected by filtration, washed with water, dried under vacuum and recrystallized from MeOH to give (**III**). White crystals, yield:

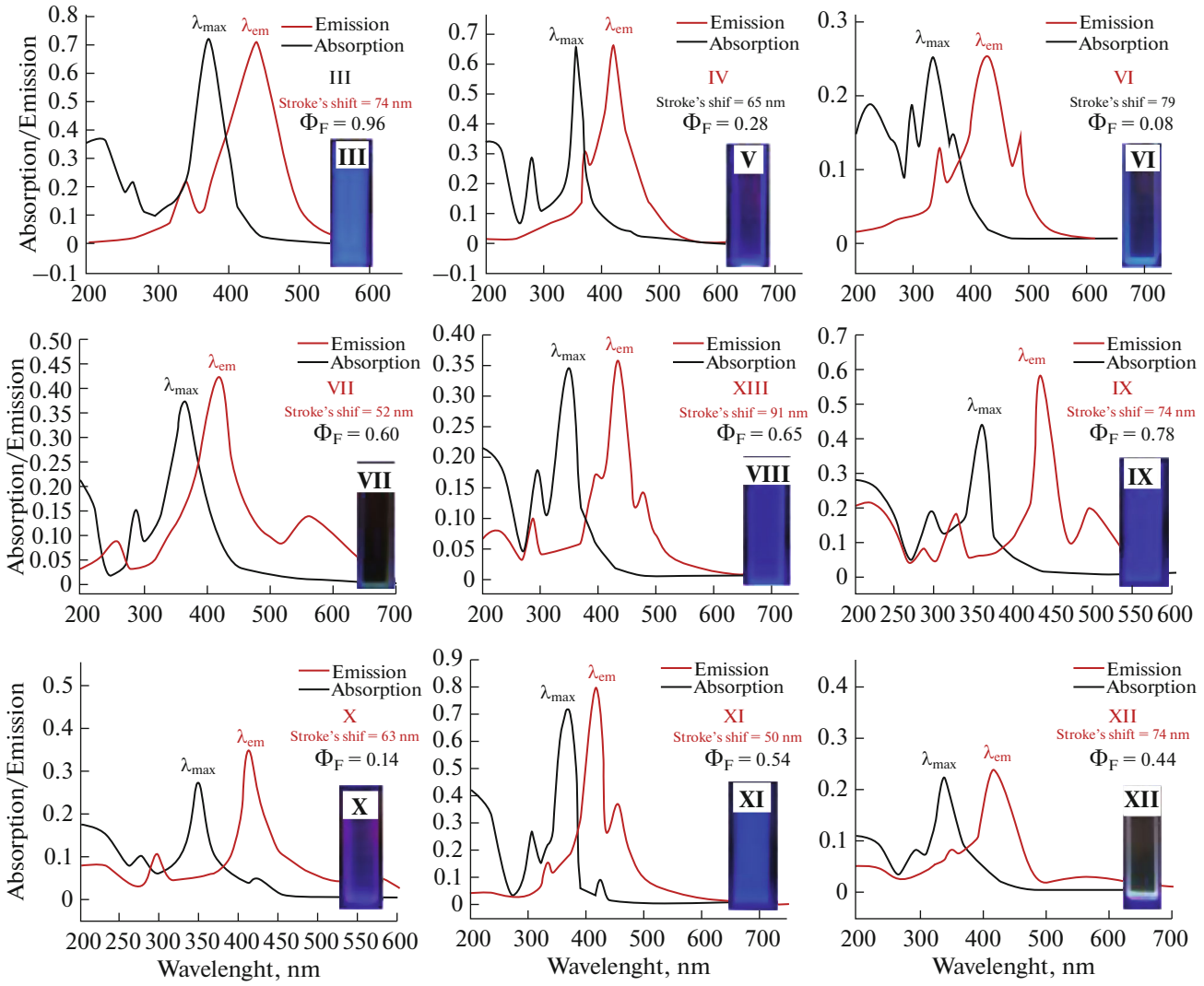

Fig. 1. UV-visible and fluorescence spectra of synthesised compounds (**III**, **V**–**XII**) in MeOH.

(3.76 g) 88%, mp: 116–119°C, R_f: 0.69. FT-IR (KBr, cm^{-1}): ν 3062, 2977(CH), 1748, 1732 (C=O), 1604, 1558, 1450 (C=C), 1203 (C–O). ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.25 (s, 1H, H_{coum}-4), 7.99 (d, 1H, H-5), 7.76 (d, 1H, H-8), 7.74-7.41 (4H, $H_{benzocoum}$), 3.76–3.75 (q, 2H, H-12), 1.22–1.20 (t, 3H, H-13). ^{13}C NMR (DMSO-d_6): δ 161.1 (CO-11), 160.2 (CO_{coum}-2), 153.7 (C_{coum}-10a), 147.9 ($C_{benzocoum}$-4), 134.3–116.4 ($C_{benzocoum}$), 57.7 (C-12), 15.7 (C-13). Anal. calculated for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51; Found: C, 70.86; H, 4.28.

Synthesis of N-(*N*-Boc-3-aminopropyl)-3-oxo-3*H*-benzo[*f*] chromene-3-carboxamide (IV). A mixture of (**III**) (1.34 g, 5 mmol), *N*-Boc-1,3-propanediamine (0.87 g, 5 mmol) in 20 mL of absolute EtOH was refluxed for 3 h (TLC check). After cooling, the reaction mixture was poured into ice water with stirring. The formed solid was filtered, washed with ethanol, dried and recrystallized from MeOH to give (**IV**). Yellow crystals; yield: (1.61 g) 73%; mp: 173–177°C; TLC system: hexane : ethyl acetate (8 : 2); R_f: 0.48. FT-IR (KBr, cm^{-1}): ν 3327 (NH), 3047, 2923 (CH), 1734, 1704, 1665 (C=O), 1612, 1566 (C=C), 1527 (NH bending), 1164 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.76 (br s, 1H, NH), 8.64 (s, 1H, H_{coum}-4), 7.99 (d, 1H, H-5), 7.98 (d, 1H, H-8), 7.76–7.42 (4H, $H_{benzocoum}$), 3.77–3.75 (t, 2H, H-13), 3.69–3.66 (t, 2H, H-15), 1.99–1.85 (m, 2H, H-14), 1.68 (s, 9H, $3CH_3$). ^{13}C NMR (DMSO-d_6): δ 161.3 (CONH-11), 160.3 (CO_{coum}-2), 156.1 (CONH-17), 153.9 (C_{coum}-10a), 147.9 (C_{coum}-4), 134.1–116.1 (C_{coum}), 85.0 (C-19), 43.2 (C-13), 41.3 (C-15), 32.5 (C-14), 28.6 ($3CH_3$). Anal. calculated for $C_{22}H_{24}N_2O_5$: C, 66.65; H, 6.10; N, 7,07; Found: C, 65.97; H, 5.89; N, 6.68.

General procedure for the deprotection of the Boc-group. Trifluoroacetic acid (TFA) 5 mL was slowly

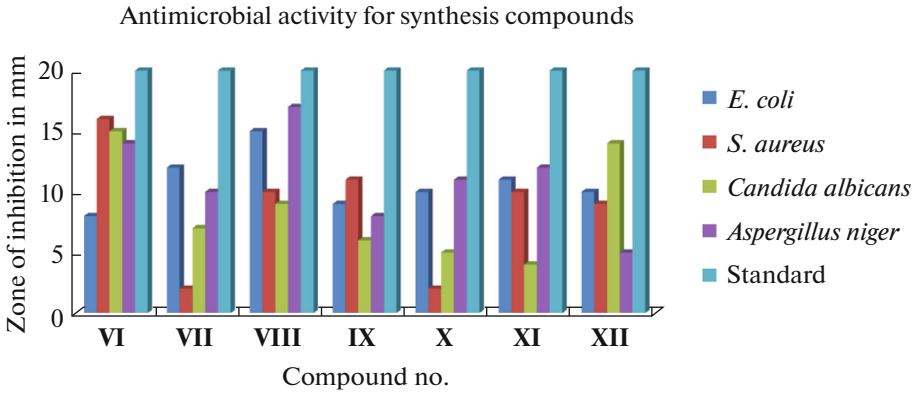

Fig. 2. Antimicrobial activity results of the benzocoumarin derivatives (**VI**–**XII**).

added to a cooled solution (0–5°C) of compound (**IV**) (25 mmol) dissolved in anhydrous CH_2Cl_2 (15 mL), the reaction mixture was stirred at room temperature for 6h (TLC check). After completion of the reaction, the solution was neutralized with saturated aqueous $NaHCO_3$ solution and then extracted with chloroform. The organic layer dried over Na_2SO_4, filtered, and solvent evaporated to give compound (**V**). white powder; yield: (2.05 g) 82%; mp: 158–160°C; TLC system: hexane : ethyl acetate (9:1); R_f : 0.68; FT-IR (KBr, cm^{-1}): ν 3394, 3332, 3262 (NH, NH_2), 3055, 2931 (CH), 1733, 1671 (C=O), 1604, 1566 (C=C), 1542 (NH bending), 1157 (C–O); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.76 (br s, 1H, NH), 8.64 (s, 1H, H_{coum}-4), 7.99 (d, 1H, H-5), 7.98 (d, 1H, H-8), 7.76–7.42 (4H, $H_{benzocoum}$), 3.77–3.75 (t, 2H, H-13), 3.69–3.66 (t, 2H, H-15), 2.16 (br s, 2H, NH_2), 1.89–1.86 (m, 2H, H-14). ^{13}C NMR (DMSO-d_6): δ 161.3 (CONH-11), 160.3 (CO_{coum}-2), 153.9 (C_{coum}-10a), 147.9 (C_{coum}-4), 134.2–116.1 (C_{coum}), 43.2 (C-13), 41.1 (C-15), 34.1 (C-14). Anal. calculated for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9,45. Found: C, 68.11; H, 5.25; N, 9.19.

General procedure for the preparation of compound (VI–XII). DCC (*N*,*N*'-dicyclohexylcarbodiimide) (2 mmol) was added to a stirred solution of anti-inflammatory drug have carboxylic group (Clonixic, Sulindac, Mefenamic acid, Fluribiprofen, Indomethacin, Ketoprofen, Oxaprozin) (2 mmol) dissolved in (20 mL) of anhydrous CH_3CN and stirred for 15 min. Next step, the compound (**V**) (2 mmol) was added to reaction mixture and stirred at 0–5°C for 8–10 h (TLC check). After completion of the reaction, the obtained precipitate of *N*,*N*-dicyclohexylurea (DCU) was filtered, the filtrate was kept overnight in freezer. The crystals of DCU were filtered off and the filtrate was evaporated under reduced pressure. The formed solids were washed with ether and recrystallized from appropriate solvents.

Physical and spectral data for new compounds. 2-((3-chloro-2-methylphenyl)amino)-N-(3-(3-oxo-3*H*-benzo[*f*]chromene-3-carboxamido)propyl) nicotinamide (VI). Brown powder; yield: (0.42 g) 65%; mp: 209–211°C; TLC system: hexane : ethyl acetate (7:3); R_f : 0.58. FT-IR (KBr, cm^{-1}): ν 3365, 3329, 3296 (NH), 3039, 2947(CH), 1720, 1675 (C=O), 1627 (C=N), 1604, 1576 (C=C), 1558 (NH bending), 1215 (C–O), 694 (C-Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.44 (br s, 1H, $NH_{cloin.}$), 8.96 (br s, 1H, NH-16), 8.89 (s, 1H, H_{coum}-4), 8.66 (br s, 1H, NH-12), 8.19 (d, 1H, 1H, 6'$_{arom}$A), 8.02 (d,1H, H-5), 7.99 (d, 1H, H-8), 7.92–7.09 (9H, H_{coum} + H_{arom}), 3.78–3.75 (t, 2H, H-13), 3.69–3.66 (t, 2H, H-15), 2.48 (s, 3H, Me), 1.87–1.85 (m, 2H, H-13). ^{13}C NMR (DMSO-d_6): δ 176.8 (CO-17), 161.3 (CO-11), 160.3 (CO_{coum}-2), 147.9 (C_{coum}-10a), 141.5 (C_{coum}-4), 141.2 (C-3'), 139.5 (C-2'), 134.2–116.1 (C_{coum} + C_{arom}), 43.2 (C-13), 41.1 (C-15), 20.7 (C-14), 13.2 (C–Me). Anal. calculated for $C_{32}H_{29}N_3O_4$: C, 73.97; H, 5.63; N, 8,09; Found: C, 73.21; H, 5.39; N, 7.81.

(*Z*)-*N*-(3-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)-benzylidene)-1*H*-indene-3-carboxamido) propyl)-3-oxo-3*H*-benzo[*f*]chromene-3-carboxamide (VII). Yellow powder; yield: (0.55 g) 62%; mp: 198–201°C; TLC system: hexane : ethyl acetate (9 : 1); R_f : 0.46. FT-IR (KBr, cm^{-1}): ν 3332, 3317 (NH), 3047, 2923 (CH), 1739, 1669 (C=O), 1604, 1542 (C=C), 1488 (NH bending), 1164 (C–O), 848 (C–F); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.95 (br s, 1H, NH-16), 8.89 (s, 1H, H_{coum}-4), 8.30 (br s, 1H, NH-12), 7.99 (d, 1H, H-5), 7.76 (d, 1H, H-8), 7.74–7.14 (12H, H_{coum} + H_{arom}), 7.07 (s, 1H, H''_{olfe}), 3.77–3.75 (t, 2H, H-13), 3.69–3.66 (t, 2H,H-15), 2.13 (s, 3H, Me), 2.09 (s, 3H, SMe), 1.89–1.83 (m, 2H, H-13). ^{13}C NMR (DMSO-d_6): δ 173.9 (CO-17), 166.5 (C_{arom}-4'), 161.3 (CO-11), 160.3 (CO_{coum}-2), 154.8 (C_{coum}-10a), 145.9 (C_{arom}-4''), 140.1 (C_{coum}-4), 136.5–116.7 (C_{coum} + C_{arom}), 44.1 (C-SMe) , 43.1 (C-13), 41.2 (C-15), 27.9 (C-14), 16.9 (CH_3). Anal. calculated for $C_{37}H_{33}FN_2O_5S$: C,69.79; H, 5.22; N, 4,40; Found: C, 68.85; H, 4.47; N, 4.26.

***N*-(3-(2-((2,3-dimethylphenyl)amino)benzamido)-propyl)-3-oxo-3*H*-benzo[*f*]chromene-3-carboxamide (VIII).** White crystals; yield: (0.46 g) 66%; mp: 215–218°C; TLC system: hexane : ethyl acetate (8 : 2); R_f : 0.63. FT-IR (KBr, cm^{-1}): ν 3348, 3317, 3209 (NH), 3047, 2931 (CH), 1723, 1697 (C=O), 1604, 1565 (C=C), 1513 (NH bending), 1172 (C–O). ^{1}H NMR (DMSO-d_6, δ, ppm): δ 10.45 (br s, 1H , $NH_{mefemanic}$), 8.95 (br s, 1H, NH-16), 8.90 (s, 1H, H_{coum}-4), 8.61 (br s, 1H, NH-12), 8.19 (d, 1H, 1H, $3'_{arom}$A), 8.02 (d, 1H, H-5), 7.98 (d, 1H, H-8), 7.92–7.09 (10H, H_{coum} + H_{arom}), 3.77–3.75 (t, 2H, H-13), 3.69–3.66 (t, 2H, H-15), 2.48 (s, 3H, Me), 2.42 (s, 3H, Me), 2.08 (m, 2H, H-13). ^{13}C NMR (DMSO-d_6): δ 170.8 (CO-17), 161.3 (CO-11), 160.3 (CO_{coum}-2), 153.9 (C_{coum}-10a), 147.9 (C-2A), 141.5 (C_{coum}-4), 141.2 (C-1B), 134.2–116.1 (C_{coum} + C_{arom}), 43.2 (C-13), 41.1 (C-15), 27.5 (C-14), 20.7 (Me-2B), 16.8 (Me-3B). Anal. calculated for $C_{32}H_{29}N_3O_4$: C, 73.97; H, 5.63; N, 8,09; Found: C, 72.88; H, 5.41; N, 7.81.

***N*-(3-(2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanamido)propyl)-3-oxo-3*H*-benzo[*f*]chromene-3-carboxamide (IX).** White crystals; yield: (0.35 g) 48%; mp: 223–226°C; TLC system: hexane : ethyl acetate (8 : 2); R_f : 0.51. FT-IR (KBr, cm^{-1}): ν 3332, 3277 (NH), 3047, 2981 (CH), 1724, 1662 (C=O), 1612, 1566 (C=C), 1527 (NH bending), 1241 (C-O), 911 (C-F). ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.97 (br s, 1H, NH-16), 8.90 (s, 1H, H_{coum}-4), 8.74 (br s, 1H, NH-12), 7.99 (d, 1H, H-5), 7.76 (d, 1H, H-8), 7.76–7.21 (12H, H_{coum} + H_{arom}), 4.30 (q, 1H, H-18), 3.77–3.75 (t, 2H, H-13), 3.70–3.67 (t, 2H, H-15), 2.33–2.29 (m, 2H, H-13), 1.40 (d, 3H,Me). ^{13}C NMR (DMSO-d_6): δ 174.9 (CO-17), 161.4 (CO-11), 160.3 (CO_{coum}-2), 159.8 (C_{arom}-3'), 153.9 (C_{coum}-10a), 148.0 (C_{coum}-4), 134.9–115.0 (C_{coum} + C_{arom}), 44.1 (C-18), 43.3 (C-13), 41.1 (C-15), 27.7 (C-14), 18.2 (CH_3). Anal. calculated for $C_{31}H_{27}FN_2O_4$: C,72.93; H, 5.33; N, 5,49; Found: C, 71.89; H, 5.08; N, 5.27.

***N*-(3-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetamido)propyl)-3-oxo-3*H*-benzo[*f*]-chromene-3-carboxamide (X)**. White powder; yield: (0.48 g) 62%; mp: 192–194°C; TLC system: hexane : ethyl acetate (7 : 3); R_f : 0.55; FT-IR (KBr, cm^{-1}): ν 3325, 3278 (NH), 3024, 2954 (CH), 1732, 1689, 1655 (C=O), 1604, 1566 (C=C), 1535 (NH bending), 1231 (C–O), 680 (C–Cl); ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.87 (s, 1H, H_{coum}-4), 8.83 (br s, 1H, NH-16), 8.72 (br s, 1H, NH-12), 7.97 (d, 1H, H-5), 7.90 (d, 1H, H-8), 7.75 (t, 1H, H_{coum}-6), 7.73 (d, 2H, H_{arom}-2 + H_{arom}-6), 7.72 (d, 2H, H_{arom}-3 + H_{arom}-5), 7.70 (t, 1H, H_{coum}-7), 7.49 (d, 1H, H_{coum}-9), 7.44 (d, 1H, H_{indom}-7), 7.42 (s, 1H, H_{indom}-4), 7.40 (d, 1H, H_{coum}-10), 7.37 (d, 1H, H_{indom}-6), 4.57 (s, 3H, OMe), 4.42 (s, 2H, H-18), 3.55–3.53 (t, 2H, H-13), 3.42–3.40 (t, 2H, H-15), 3.38 (s, 3H, Me), 1.57–1.53 (m, 2H, H-14). ^{13}C NMR (DMSO-d_6): δ 174.0 (CO-17) ,162.5 (CON_{indom}), 161.0 (CO-11), 160.4 (CO_{coum}-2), 154.5 (C_{arom}-5'), 153.8 (C_{coum}-10a), 148.6 (C_{coum}-4), 147.5 (C_{arom}-4''), 134.4–116.1 (C_{coum} + C_{arom}), 105.1 (C_{indom}-3'), 59.4 (OMe), 42.5 (C-13), 41.8 (C-15), 34.8 (C-18), 24.8 (C-14), 14.0 (CH_3). Anal. calculated for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9,45; Found: C, 68.02; H, 5.19; N, 9.08.

***N*-(3-(2-(3-benzoylphenyl)propanamido)propyl)-3-oxo-3*H*-benzo[*f*]chromene-3-carboxamide (XI)**. Brown powder; yield: (0.39 g) 52%; mp: 182–186°C; TLC system: hexane : ethyl acetate (7 : 3); R_f : 0.63. FT-IR (KBr, cm^{-1}): ν 3324, 3310 (NH), 3055, 2931 (CH), 1734, 1713, 1673 (C=O), 1612, 1566 (C=C), 1542 (NH bending), 1226 (C–O). ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.87 (br s, 1H, NH-16), 8.84 (s, 1H, H_{coum}-4), 8.51 (br s, 1H, NH-12), 7.97 (d, 1H, H-5), 7.75 (d, 1H, H-8), 7.73 (s, 1H, H_{arom}-2A), 7.72–7.29 (12H, H_{coum} + H_{arom}), 4.23 (q, 1H, CH), 3.77–3.72 (t, 2H, H-13), 3.69–3.60 (t, 2H, Hz, H-15), 2.75–2.59 (m, 2H, H-13), 1.31 (d, 3H, Me). ^{13}C NMR (DMSO-d_6): δ 193.6 (COPh), 176.8 (CO-17), 161.3 (CO-11), 160.3 (CO_{coum}-2), 153.9 (C_{coum}-10a), 147.9 (C_{coum}-4), 135.3–116.1 (C_{coum} + C_{arom}), 59.2 (C-18), 43.2 (C-13), 41.1 (C-15), 27.9 (C-14), 16.8 (CH_3). Anal. calculated for $C_{33}H_{28}N_2O_5$: C, 74.42; H, 5.30; N, 5,26; Found: C, 73.67; H, 5.06; N, 5.01.

***N*-(3-(3-(4,5-diphenyloxazol-2-yl)propanamido)-propyl)-3-oxo-3*H*-benzo[*f*]chromene-3-carboxamide (XII)**. White powder; yield: (0.36 g) 42%; mp: 226–229°C; TLC system: hexane : ethyl acetate (7 : 3); R_f : 0.45. FT-IR (KBr, cm^{-1}): ν 3327, 3304 (NH), 3047, 2984 (CH), 1721, 1696 (C=O), 1636 (C=N), 1612, 1566 (C=C), 1527 (NH bending), 1211 (C–O). ^{1}H NMR (DMSO-d_6, δ, ppm): δ 8.96 (br s, 1H, NH-16), 8.89 (s., 1H, H_{coum}-4), 8.32 (br s, 1H, NH-12), 7.99 (d, 1H, H-5), 7.88 (d, 1H, H-8), 7.76–7.22 (14H, H_{coum} + H_{arom}), 3.77–3.75 (t, 2H, H-13), 3.69–3.65 (t, 2H, H-15), 2.45–2.42 (t, 2H, 19), 2.29–2.22 (t, 2H, 18), 1.45–1.24 (m, 2H, H-13). ^{13}C NMR (DMSO-d_6): δ 172.0 (CO-17), 161.5 (CO-11), 160.4 (CO_{coum}-2), 155.5(C_{oxaz}-2' + C10a), 148.4 (C_{oxaz}-5'), 146.8 (C_{coum}-4), 137.6 (C-4'), 135.1–111.2 (C_{coum} + C_{arom}), 43.2 (C-13), 41.5 (C-15), 37.1 (C-18), 29.5 (C-19), 26.0 (C-14). Anal. calculated for $C_{35}H_{29}N_3O_5$: C, 73.54; H, 5.11; N, 7,35; Found: C, 72.12; H, 4.83; N, 7.09.

Acetylcholinesterase inhibition assay [14]: A stock concentration solution (0.01 M) concentration of each sample has been prepared and then different concentrations (10^{-2}, 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9} and 10^{-11} M) of each sample were prepared by dilution with dimethyl sulfoxide (DMSO) as solvent. Anti-AChE activity was measured in human serum as follows: (50 μL) of DTNB solution (0.001 M) was added to (2.25 mL) of

Na_3PO_4 buffer solution (pH 7.3, 0.2 M), 0.25 mL of inhibitor was mixed with 2 ml of the same buffer. Next step, (10 μL) of serum was added, mixed well and (2 mL) of the mixture was transferred to a measuring cell (1 cm), then (34 μL) of (AChI 0.06 M) was added. After adding the substrate, the changes in absorbency was measured at 430 nm. The inhibition percentage was calculated by comparing the activity presence and absence of the inhibitor under the same conditions according to the equation (1), The IC_{50} values were determined graphically from inhibition curves using excel program.

$$\% \text{ Inhibition} = \left(100 - \frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}}\right) \times 100. \quad (1)$$

CONCLUSIONS

A 5,6-benzomcomarin derivative was successfully synthesized and then was conjugated with anti-inflammatory drugs via alkylamide spacers, obtained yields were good using DCC as catalyst and MeCN as solvent. Fluorescence properties of synthesized compounds were expressed as quantum yield (Φ_F). Interestingly, ethyl 5,6-benzocoumarin-3-carboxylate compound gave a higher quantum yield ($\Phi_F = 0.96$), meanwhile, Sulindac, Mefenamic acid, Fluribiprofen and Ketoprofen analogues gave reasonable quantum yields (Φ_F) 0.60, 0.65, 0.78 and 0.84, respectively. Inhibitory activity of the Sulindac, Fluribiprofen, Indomethacin and Ketoprofen analogues against AChE was significantly higher by compare to other compounds. Antimicrobial activity of synthesized 5,6-benzomcomarin derivatives showed good results in compared for standard, this activity may be attributed to functional groups within structure of heterocyclic compounds.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals performed by any of the authors.

SUPPLEMENTARY MATERIALS

Supplementary materials are available for this article at https://doi.org/10.1134/S1068162020040032 and are accessible for authorized users.

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