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Short communication

Synthesis, pharmacological evaluation and docking studies of coumarin derivatives

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ABSTRACT

We synthesized coumarin derivatives using various aromatic and heterocyclic amines, and tested the target compound for its analgesic, anti-inflammatory, antimicrobial activities. Compounds **3l**, **3m** and **3n** showed significant anti-inflammatory, analgesic and antimicrobial activities. The synthesized compounds, then docked on COX-2 to predict the binding affinity and orientation at the active site of the receptor. It was found that the active compounds **3l**, **3m** and **3n** intact mainly with Arg 44 amino acid, which may be involved in COX-2 inhibition. The compounds which bind with Arg 44 have significant anti-inflammatory activity. This could be due to the formation of more effective hydrogen bond with the receptor. Comparing pharmacological activity and docking results, we conclude that heterocyclic derivatives linked with nitrogen at 7-position of coumarin seem to be potentially active drug.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are mainly used in the treatment of rheumatoid arthritis and various types of inflammatory conditions. Most of NSAIDs exhibit their anti-inflammatory effects by inhibiting cyclooxygenase (COX) which catalyzes the bioconversion of arachidonic acid to prostaglandins. However, inhibition of COXs may lead to undesirable side effects such as gastric ulceration, bleeding and renal function suppression. The two isozymes of COX involved in the prostaglandin biosynthesis are COX-1 and COX-2. COX-2 is mainly involved in inflammation condition [1–4]. Coumarin derivatives are anticoagulant [5], and have been shown to exert its anti-inflammatory effects through prostaglandin biosynthesis [6]. Coumarin, the potent basic pharmacodynamic nucleus has been reported to possess a wide variety of biological properties viz., anti-inflammatory [7–9], analgesic [10,11] anticancer [12,13], antimicrobial [14], antifungal [15], anthelmintic [16] and anticonvulsant activities [17,18].

Due to the importance of coumarin derivatives and amino-substituted derivatives several investigators have been investigating various compounds bearing single substituent or more complicated substituent in heterocyclic ring system mainly in 3-,4- and/or 7-position [6].

Encouraged by the above observations we synthesized newer 7-substituted coumarin derivatives in hope of obtaining better anti-inflammatory agents for a long period of time. We targeted to study the structure–activity relationship by altering different amines and substitutions at the 7-position of the coumarin. Herein we report the screening results of the anti-inflammatory, analgesic and antimicrobial activity of the synthesized compounds.

2. Results and discussion

The synthetic route of the compounds is outlined in Scheme 1. Bromination of 4-methyl-2-oxo-2*H*-chromene-7yl acetate (**1**) [19] with bromine in acetic acid yielded 4-methyl-2-oxo-2*H*-chromen-7ylbromoacetate (**2**). Further treatment of the compound **2** with different amines yielded 4-methyl-2-oxo-2*H*-chromen-7yl substituted acetates (**3a**–**n**). This reaction was carried out in presence of acetone as solvent. The progress of the reaction was being monitored by thin layer chromatography (TLC) on silica-G (Merck) coated glass plates, visualized by iodine vapor. IR, NMR and mass spectral data confirmed the formation of the final compound. These results will be reported in due course.

Anti-inflammatory and analgesic activity screening indicated that some of the tested compounds showed good activity for the

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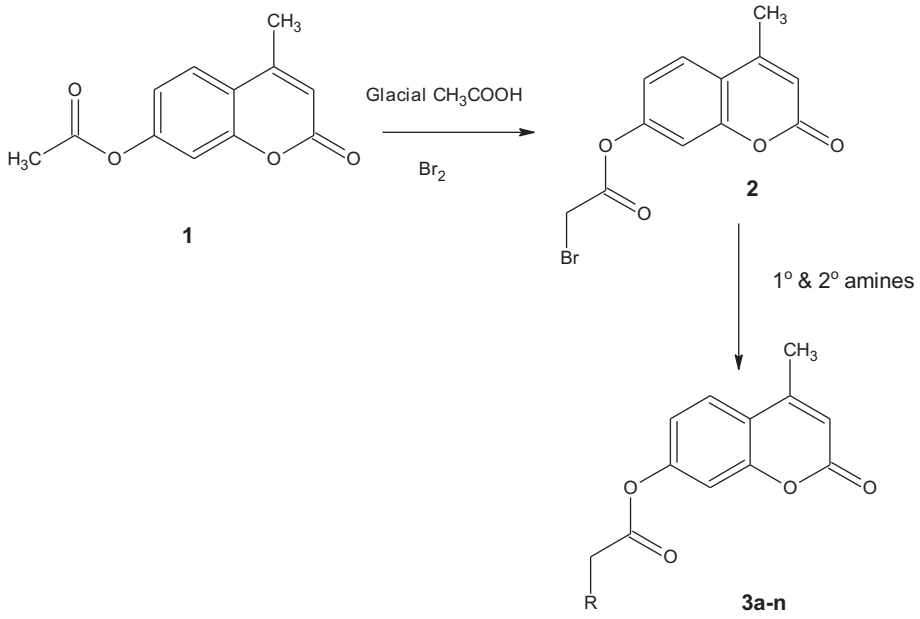

Compound	R
a	C_6H_5NH-
b	*o*-NO_2-C_6H_5NH-
c	*m*-NO_2-C_6H_5NH-
d	*p*-NO_2-C_6H_5NH-
e	*o*-Cl-C_6H_5NH-
f	*p*-Cl-C_6H_5NH-
h	*o*-OCH_3-C_6H_5NH-

Compound	R
h	*p*-OCH_3-C_6H_5NH-
i	*o*-CH_3-C_6H_5NH-
j	*m*-CH_3-C_6H_5NH-
k	*p*-CH_3-C_6H_5NH-
l	1,5-dihydro-4*H*-1,2,4-triazol-4-amino
m	1,3-benzothiazol-2-amino
n	1,3-thiazol-2-amino

Scheme 1. Synthesis of coumarin derivatives.

long period. The results are specified in Tables 1 and 2. Compounds **3l**, **3m** and **3n** showed moderate anti-inflammatory and analgesic activities. But compound **3l** is found to be active in 1 h of drug administration then it starts decreasing after 1 h. It could be due to metabolic instability of the compound. Derivatives **3c** and **3h** showed moderate activity and started decreasing after 3 h. Results revealed that, good anti-inflammatory and analgesic activity could be due to the presence of heterocyclic substituent in the coumarin derivative. Replacement of a heterocyclic ring with another substituted phenyl group decreases the activity. This clearly indicates that, analgesic and anti-inflammatory activity increases by modification of heterocyclic ring in 7-position of coumarin nucleus.

In vitro study of antibacterial effects against *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*) and antifungal activity against *Candida albicans* (*C. albicans*), *Aspergillus niger* (*A. niger*) indicated that not all the compounds exhibited good activity. Table 3 shows the inhibitory effects of the compounds against these organisms. It was evident from the screening results that the heterocyclic group substituted in 7-position of coumarin nucleus increase activity.

To compare the binding affinity of the newly synthesized compounds, we docked compounds in the empty binding site of COX-2 (PDB entry 4COX), with its bound inhibitor indomethacin which was shown in Fig. 1. Fig. 2 shows the compounds having more hydrogen bonding with the receptor.

Table 1
Anti-inflammatory activity of compounds (**3a**–**n**).

Compound	Change in paw volume (in ml) after drug administration (±SE)				Percentage inhibition of edema volume after			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Standard	0.26 ± 0.03	0.25 ± 0.02	0.23 ± 0.04	0.50 ± 0.02	53.5	67.9	68.8	57.6
3a	0.50 ± 0.02	0.55 ± 0.01	0.65 ± 0.03	0.90 ± 0.02	10.7	29.4	30.8	13.7
3b	0.44 ± 0.02	0.65 ± 0.04	0.79 ± 0.04	1.00 ± 0.03	21.4	16.6	15.9	15.2
3c	0.40 ± 0.01	0.54 ± 0.02	0.62 ± 0.03	0.80 ± 0.02	28.5	30.7	34.0	32.2
3d	0.49 ± 0.02	0.64 ± 0.04	0.75 ± 0.03	0.90 ± 0.03	12.5	17.9	20.2	23.7
3e	0.44 ± 0.03	0.69 ± 0.03	0.82 ± 0.03	0.90 ± 0.04	21.4	11.5	12.8	23.7
3f	0.50 ± 0.03	0.60 ± 0.03	0.79 ± 0.02	0.99 ± 0.05	10.7	23.0	15.9	16.1
3g	0.50 ± 0.02	0.66 ± 0.03	0.69 ± 0.02	0.95 ± 0.04	10.7	15.3	26.6	19.4
3h	0.39 ± 0.03	0.5 ± 0.04	0.59 ± 0.02	0.84 ± 0.05	30.3	33.3	37.2	28.8
3i	0.49 ± 0.02	0.7 ± 0.03	0.78 ± 0.02	0.95 ± 0.05	12.5	15.3	17.0	19.4
3j	0.49 ± 0.03	0.7 ± 0.02	0.79 ± 0.02	0.90 ± 0.04	12.5	15.3	15.9	23.7
3k	0.48 ± 0.02	0.6 ± 0.00	0.75 ± 0.03	1.10 ± 0.03	14.2	20.5	20.2	6.7
3l	0.32 ± 0.03	0.5 ± 0.03	0.68 ± 0.03	0.92 ± 0.03	42.8	42.3	27.6	22.2
3m	0.39 ± 0.04	0.50 ± 0.03	0.56 ± 0.03	0.70 ± 0.03	30.3	35.9	40.4	40.6
3n	0.34 ± 0.03	0.50 ± 0.02	0.49 ± 0.03	0.80 ± 0.03	39.2	37.1	47.8	32.3

Table 2
Analgesic activity of compounds (**3a**–**n**).

Compounds	Mean number of writhes in 30 min period after treatment ± SE	% Decrease relative to control
Control	46.2 ± 2.36	–
3a	35.3 ± 1.85	24
3b	33.4 ± 2.36	28
3c	40.1 ± 3.45	13
3d	33.3 ± 2.22	28
3e	36.4 ± 1.26	21
3f	40.9 ± 2.12	11
3g	33.4 ± 1.12	28
3h	38.4 ± 2.11	17
3i	35.4 ± 1.25	23
3j	40.2 ± 1.11	13
3k	33.2 ± 3.21	28
3l	29.3 ± 2.85	37
3m	30.2 ± 1.25	35
3n	25.3 ± 2.25	45
Std	20.3 ± 2.45	56

Indomethacin (standard drug) reveals MolDock score of −155.202 and form three hydrogen bonds between carboxylic moiety and Tyr 130 with a bond distance of 3.20 Å and another two bonds with oxygen of methoxy group and carbonyl oxygen with Asn 43 and Gln 42 with a bond distance of 3.57 and 3.29 Å. Compounds **3a**–**n** exhibited binding scores ranging from −135.58 to −159.65. Compounds **3l**, **3m**, and **3n** with MolDock score −135.58, −159.165 and −143.6 respectively, where they have significant biological activities. Compound **3m** is the most active one among the series. It forms eight hydrogen bonds, three hydrogen bonds with O-1 of coumarin moiety, two with Arg 44 of distance 3.10 and 3.03 Å and one hydrogen bond of distance 2.79 Å with Arg 469, O-2 of coumarin moiety bonded with Arg 469 with a distance of 3.16 Å, two hydrogen bonds between ester oxygen and Ser 421, and Tyr 122 with a distance of 2.58 Å and 3.23 Å respectively, one hydrogen bond between N of benzothiazole ring with Asn 43 with a distance of 3.38 Å, NH in aliphatic chain forms one hydrogen bond with a distance of 2.79 Å with Lys 468.

Docking studies on COX-2 reveals that the coumarin oxygen is mainly responsible for binding with Arg 44 amino acid. Attachment of nitrogen containing heterocyclic ring increases hydrogen bond formation. Comparison of the docking result with that of anti-inflammatory activity, heteroatom in the ring could be responsible for the good anti-inflammatory activity.

3. Experimental

3.1. Chemistry

The reagents used in the present work were of analytical grade and when necessary, were purified and dried by standard methods. All melting points were determined on open capillaries using Thermonik precision apparatus (Model-C-PMP-2, Mumbai, India) and were uncorrected. The IR spectrum was recorded on Tensor 27 spectrophotometer, Bruker Optic (Germany) using ATR method. All ^{1}H NMR spectra were recorded in Bruker spectrophotometer AMX-400 (400 MHz), Bruker Optic (Germany) in CD_3OD or DMSO. Chemical shifts were reported in parts per million (δ). All Mass spectra were recorded using Agilent 5973n GC/MS.

3.1.1. Synthesis of 4-methyl-2-oxo-2H-chromen-7yl bromo acetate (**2**)

4-Methyl-2-oxo-2*H*-chromen-7yl acetate (2.18 g, 0.01 M) was dissolved in 10 ml of glacial acetic acid; bromine (0.52 ml, 0.01 M)

Table 3
Antibacterial and antifungal activity (**3a**–**n**).

Compound	*E. coli* (MTCC[a] No.: 4315)			*B. subtilis* (MTCC[a] No.: 1789)			*C. albicans* (MTCC[a] No.: 183)			*A. niger* (MTCC[a] No.: 282)		
	Zone of inhibition in mm			Zone of inhibition in mm			Zone of inhibition in mm			Zone of inhibition in mm		
	10 μg/ml	25 μg/ml	50 μg/ml	10 μg/ml	25 μg/ml	50 μg/ml	10 μg/ml	25 μg/ml	50 μg/ml	10 μg/ml	25 μg/ml	50 μg/ml
3a	–	–	10.4 ± 0.88	10.4 ± 1.44	11.8 ± 0.99	12.6 ± 0.85	–	–	–	–	10.2 ± 1.44	–
3b	–	10.1 ± 1.21	–	11.2 ± 1.02	12.4 ± 0.85	13.7 ± 0.85	–	10.4 ± 1.24	–	10.1 ± 1.44	–	–
3c	10.3 ± 0.85	11.3 ± 0.22	11.1 ± 1.22	–	11.2 ± 1.58	13.4 ± 0.53	10.5 ± 1.45	–	–	–	10.5 ± 2.20	11.4 ± 1.25
3d	12.2 ± 0.88	14.2 ± 0.25	16.9 ± 0.24	10.2 ± 1.02	12.1 ± 1.22	13.4 ± 1.11	–	10.2 ± 1.42	11.4 ± 1.30	–	–	10.2 ± 1.20
3e	–	10.8 ± 1.22	12.6 ± 0.99	–	–	–	–	–	10.4 ± 1.42	–	10.2 ± 1.25	12.4 ± 1.41
3f	–	10.8 ± 0.44	12.6 ± 1.02	–	–	12.3 ± 0.62	10.6 ± 2.24	12.2 ± 2.19	13.4 ± 1.40	–	10.2 ± 1.22	12.1 ± 0.52
3g	–	–	10.2 ± 1.00	10.2 ± 0.99	11.4 ± 1.26	–	–	–	12.4 ± 0.95	10.4 ± 1.11	–	12.4 ± 2.1
3h	10.5 ± 0.92	11.2 ± 0.55	–	11.2 ± 1.42	13.3 ± 1.46	14.4 ± 1.2	10.2 ± 1.42	12.4 ± 1.62	13.2 ± 1.92	–	13.2 ± 1.42	14.1 ± 1.26
3i	–	10.5 ± 0.65	–	–	–	11.6 ± 1.25	–	–	12.2 ± 1.02	10.6 ± 1.11	12.4 ± 1.04	13.1 ± 1.02
3j	11.0 ± 1.55	14.0 ± 1.02	16.9 ± 1.99	10.5 ± 1.02	12.4 ± 1.00	13.6 ± 1.25	11.4 ± 0.95	13.4 ± 1.95	14.4 ± 2.02	–	12.2 ± 1.11	13.4 ± 1.89
3k	11.2 ± 2.01	13.0 ± 1.02	14.0 ± 1.06	–	–	12.3	–	–	12.4 ± 1.69	–	10.5 ± 1.25	11.4 ± 1.12
3l	–	14.1 ± 1.09	16.2 ± 0.95	12.5 ± 1.26	14.3 ± 1.95	18.1 ± 1.02	12.4 ± 2.3	13.4 ± 1.05	15.4 ± 1.42	11.2 ± 1.02	13.6 ± 0.99	–
3m	–	–	14.5 ± 1.22	10.4 ± 1.44	–	18.1 ± 1.42	13.4 ± 1.44	12.2 ± 1.82	–	–	–	12.4 ± 1.4
3n	–	10.2 ± 0.95	18.4 ± 0.95	10.4 ± 2.1	14.4 ± 1.8	16.9 ± 1.2	10.1 ± 0.95	–	12.4 ± 1.26	13.4 ± 1.42	15.4 ± 1.44	16.5 ± 2.45
Amikacin	14.0 ± 0.95	16.2 ± 1.20	20.0 ± 1.55	–	–	–	–	–	–	–	–	–
Vancomycin	–	–	–	14.2 ± 1.14	18.2 ± 1.8	20.1 ± 1.42	–	–	–	–	–	–
Griseofulvin	–	–	–	–	–	–	12.6 ± 1.42	14.2 ± 1.99	18.2 ± 1.89	–	–	–

[a] MTCC = Microbial Type Culture Collections and gene bank.

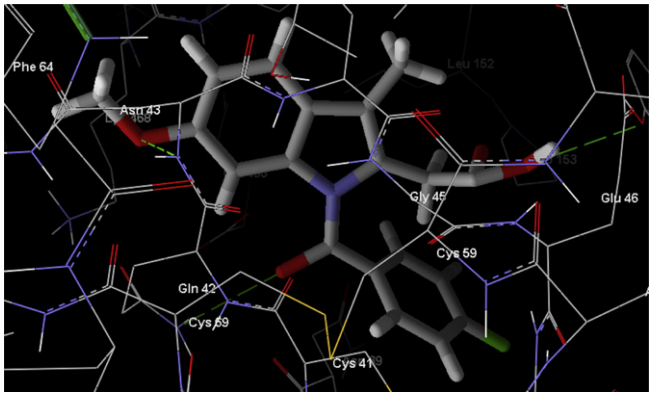

Fig. 1. Binding mode of original ligand indomethacin into its binding site of COX-2. It has MolDock score of −155.202 and forms three hydrogen bonds shown as green dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

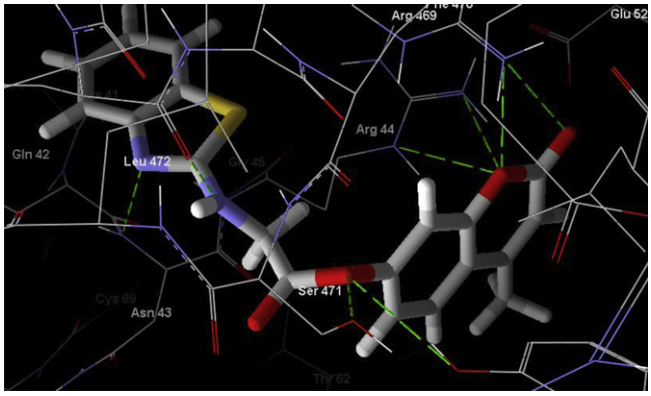

Fig. 2. Binding mode of compound **3m** into its binding site of COX-2. It has MolDock score of 159.165 and forms eight hydrogen bonds shown as green dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in glacial acetic acid mixture was added drop wise with constant stirring. The reaction mixture was stirred at 40 °C, for 4 h and cooled. After cooling the reaction mixture was poured into crushed ice. The precipitate was washed with water to remove excess of bromine.

Color: pale brown, recrystallization solvent: ethanol, yield: 84%, MP 118–120 °C; IR (cm^{-1}): 3022 (C–H aryl), 2939 (C–H alkyl), 1621 (C=O), 1261 (C–O); ^{1}H NMR (CD_3OD, δ ppm): 2.0 (s, 3H, CH_3); 4.0 (d, 2H, CH_2); 6.0 (s, 1H, CH); 6.8–7.4 (m, 3H, Ar–H). GC–MS (*m*/*z*) 298 [M + 1].

*3.1.2. General method for the synthesis of 4-methyl-2-oxo-2H-chromen-7yl substituted acetate (**3a**–**n**)*

4-Methyl-2-oxo-2*H*-chromen-7yl bromo acetate (2.97 g, 0.01 M) and different amines (0.01 M) were dissolved in solvent acetone. The reaction mixture was refluxed for 6 h. Finally the reaction mixture was cooled and the contents were poured into crushed ice. The solid separated out was filtered, washed with water and recrystallized from ethanol.

*3.1.2.1. 4-Methyl-2-oxo-2H-chromen-7-yl N-phenylglycinate (**3a**).* It is obtained by the reaction of bromo derivative **2** with aniline. Color: pale yellow powder, recrystallization solvent: ethanol, yield: 77%, MP 158–160 °C; IR (cm^{-1}): 3341 (NH), 3031 (C–H aryl), 2923 (C–H alkyl), 1629 (C=O), 1266 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.63 (s, 3H, CH_3); 4.22 (s, 1H, NH); 4.2 (d, 2H, CH_2); 6.3 (s, 1H, CH); 6.8–7.8 (m, 8H, Ar–H). GC–MS (*m*/*z*) 310 [M + 1].

*3.1.2.2. 4-Methyl-2-oxo-2H-chromen-7-yl N-(o-nitrophenyl)glycinate (**3b**).* It is obtained by the reaction of bromo derivative **2** with *o*-nitro aniline. Color: yellow powder, recrystallization solvent: ethanol, yield: 83%, MP 203–205 °C; IR (cm^{-1}): 3350 (NH), 3029 (C–H aryl), 2920 (C–H alkyl), 1774, 1729 (C=O), 1250 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.6 (s, 3H, CH_3); 4.1 (s, 1H, NH); 4.0 (d, 2H, CH_2); 6.2 (s, 1H, CH); 6.9–7.9 (m, 7H, Ar–H). GC–MS (*m*/*z*) 354 [M^+].

*3.1.2.3. 4-Methyl-2-oxo-2H-chromen-7-yl N-(m-nitrophenyl)glycinate (**3c**).* It is obtained by the reaction of bromo derivative **2** with *m*-nitro aniline. Color: yellow powder, recrystallization solvent: ethanol, yield: 80%, MP 210–213 °C; IR (cm^{-1}): 3363 (NH), 3044 (C–H aryl), 2935 (C–H alkyl), 1772, 1710 (C=O), 1252 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.8 (s, 3H, CH_3); 4.3 (s, 1H, NH); 4.2 (d, 2H, CH_2); 6.2 (s, 1H, CH); 7.2–8.2 (m, 7H, Ar–H). GC–MS (*m*/*z*) 354 [M^+].

*3.1.2.4. 4-Methyl-2-oxo-2H-chromen-7-yl N-(p-nitrophenyl)glycinate (**3d**).* It is obtained by the reaction of bromo derivative **2** with *p*-nitro aniline. Color: yellow powder, recrystallization solvent: ethanol, yield: 85%, MP 213–215 °C; IR (cm^{-1}): 3352 (NH), 3030 (C–H aryl), 2921 (C–H alkyl), 1761, 1729 (C=O), 1256 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.7 (s, 3H, CH_3); 4.2 (s, 1H, NH); 4.0 (d, 2H, CH_2); 6.2 (s, 1H, CH); 7.0–7.9 (m, 7H, Ar–H). GC–MS (*m*/*z*) 354 [M^+].

*3.1.2.5. 4-Methyl-2-oxo-2H-chromen-7-yl N-(o-chlorophenyl)glycinate (**3e**).* It is obtained by the reaction of bromo derivative **2** with *o*-chloro aniline. Color: brown crystal, recrystallization solvent: ethanol, yield: 66%, MP 185–187 °C; IR (cm^{-1}): 3342 (NH), 3034 (C–H aryl), 2920 (C–H alkyl), 1763, 1725 (C=O), 1252 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.7 (s, 3H, CH_3); 4.2 (s, 1H, NH); 4.0 (d, 2H, CH_2); 6.1 (s, 1H, CH); 6.9–7.9 (m, 7H, Ar–H). GC–MS (*m*/*z*) 344 [M^+].

*3.1.2.6. 4-Methyl-2-oxo-2H-chromen-7-yl N-(p-chlorophenyl)glycinate (**3f**).* It is obtained by the reaction of bromo derivative **2** with *p*-chloro aniline. Color: brown powder, recrystallization solvent: ethanol, yield: 69%, MP 198–199 °C; IR (cm^{-1}): 3344 (NH), 3033 (C–H aryl), 2922 (C–H alkyl), 1764, 1723 (C=O), 1252 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.4 (s, 3H, CH_3); 4.1 (s, 1H, NH); 4.0 (d, 2H, CH_2); 6.1 (s, 1H, CH); 7.0–7.9 (m, 7H, Ar–H). GC–MS (*m*/*z*) 344 [M^+].

*3.1.2.7. 4-methyl-2-oxo-2H-chromen-7-yl N-(o-methoxyphenyl)glycinate (**3g**).* It is obtained by the reaction of bromo derivative **2** with *o*-methoxy aniline. Color: brown crystal, recrystallization solvent: ethanol, yield: 65%, MP 186–189 °C; IR (cm^{-1}): 3346 (NH), 3034 (C–H aryl), 2924 (C–H alkyl), 1764, 1725 (C=O), 1254 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.4 (s, 3H, CH_3); 3.5 (s, 3H, OCH_3); 4.1 (s, 1H, NH); 4.0 (d, 2H, CH_2); 6.1 (s, 1H, CH); 7.1–7.9 (m, 7H, Ar–H). GC–MS (*m*/*z*) 339 [M^+].

*3.1.2.8. 4-Methyl-2-oxo-2H-chromen-7-yl N-(p-methoxyphenyl)glycinate (**3h**).* It is obtained by the reaction of bromo derivative **2** with *p*-methoxy aniline. Color: brown powder, recrystallization solvent: ethanol, yield: 72%, MP 198–199 °C; IR (cm^{-1}): 3344 (NH), 3033 (C–H aryl), 2922 (C–H alkyl), 1764, 1723 (C=O), 1252 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.4 (s, 3H, CH_3); 3.5 (s, 3H, OCH_3); 4.1 (s, 1H, NH); 4.0 (d, 2H, CH_2); 6.1 (s, 1H, CH); 7.0–8.0 (m, 7H, Ar–H). GC–MS (*m*/*z*) 339 [M^+].

*3.1.2.9. 4-Methyl-2-oxo-2H-chromen-7-yl N-(o-methylphenyl)glycinate (**3i**).* It is obtained by the reaction of bromo derivative **2** with *o*-methyl aniline. Color: yellow powder, recrystallization solvent: ethanol, yield: 62%, MP 155–159 °C; IR (cm^{-1}): 3341 (NH), 3032

(C–H aryl), 2929 (C–H alkyl), 1761, 1721 (C=O), 1251 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.4 (s, 3H, CH_3); 2.0 (s, 3H, CH_3); 4.0 (s, 1H, NH); 3.7 (d, 2H, CH_2); 6.1 (s, 1H, CH); 7.1–8.0 (m, 7H, Ar–H). GC–MS (*m*/*z*) 324 [M + 1].

*3.1.2.10. 4-Methyl-2-oxo-2H-chromen-7-yl N-(m-methylphenyl)glycinate (**3j**).* It is obtained by the reaction of bromo derivative **2** with *m*-methyl aniline. Color: yellow powder, recrystallization solvent: ethanol, yield: 64%, MP 168–169 °C; IR (cm^{-1}): 3343 (NH), 3032 (C–H aryl), 2920 (C–H alkyl), 1761, 1727 (C=O), 1251 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.4 (s, 3H, CH_3); 1.9 (s, 3H, CH_3); 4.2 (s, 1H, NH); 4.1 (d, 2H, CH_2); 6.0 (s, 1H, CH); 7.0–8.0 (m, 7H, Ar–H). GC–MS (*m*/*z*) 324 [M + 1].

*3.1.2.11. 4-Methyl-2-oxo-2H-chromen-7-yl N-(p-methylphenyl)glycinate (**3k**).* It is obtained by the reaction of bromo derivative **2** with *p*-methyl aniline. Color: brownish yellow powder, recrystallization solvent: ethanol, yield: 64%, MP 188–189 °C; IR (cm^{-1}): 3345 (NH), 3035 (C–H aryl), 2923 (C–H alkyl), 1764, 1723 (C=O), 1252 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.3 (s, 3H, CH_3); 2.0 (s, 3H, CH_3); 4.1 (s, 1H, NH); 4.0 (d, 2H, CH_2); 6.1 (s, 1H, CH); 7.2–8.1 (m, 7H, Ar–H). GC–MS (*m*/*z*) 324 [M + 1].

*3.1.2.12. 4-Methyl-2-oxo-2H-chromen-7-yl N-(1,5-dihydro-4H-1,2,4-triazol-4-yl)glycinate (**3l**).* It is obtained by the reaction of bromo derivative **2** with 1,5-dihydro-4*H*-1,2,4-triazol-4-amine. Color: reddish powder, recrystallization solvent: ethanol, yield: 62%, MP 210–212 °C; IR (cm^{-1}): 3348 (NH), 3358 (NH), 3034 (C–H aryl), 2923 (C–H alkyl), 1763, 1722 (C=O), 1250 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.4 (s, 3H, CH_3); 3.4 (s, 2H, CH_2); 4.0 (d, 2H, CH_2); 4.1 (s, 1H, NH); 4.4 (s, 1H, NH); 6.1 (s, 1H, CH); 7.2–8.1 (m, 4H, Ar–H). GC–MS (*m*/*z*) 302 [M$^+$].

*3.1.2.13. Synthesis of 4-methyl-2-oxo-2H-chromen-7-yl N-1,3-benzothiazole-2yl-glycinate (**3m**).* It is obtained by the reaction of bromo derivative **2** with 1,3-benzothiazole-2-amine. Color: yellow powder, recrystallization solvent: ethanol, yield: 56%, MP 198–200 °C; IR (cm^{-1}): 3333 (NH), 3031 (C–H aryl), 2921 (C–H alkyl), 1752, 1722 (C=O), 1251 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.1 (s, 3H, CH_3); 4.0 (s, 2H, CH_2); 4.3 (s, 1H, NH); 5.8 (s, 1H, CH); 6.9–8.1 (m, 7H, Ar–H). GC–MS (*m*/*z*) 366 [M$^+$].

*3.1.2.14. Synthesis of 4-methyl-2-oxo-2H-chromen-7-yl N-1,3-thiazol-2-ylglycinate (**3n**).* It is obtained by the reaction of bromo derivative **2** with 1,3-thiazole-2-amine. Color: pale yellow powder, recrystallization solvent: ethanol, yield: 67%, MP 198–200 °C; IR (cm^{-1}): 3306 (NH), 3041 (C–H aryl), 2902 (C–H alkyl), 1761, 1721 (C=O), 1570 (C=N) 1251 (C–O); ^{1}H NMR (DMSO-d_6, δ ppm): 2.4 (s, 3H, CH_3); 4.0 (d, 2H, CH_2); 4.1 (s, 1H, NH); 6.1 (s, 1H, CH); 7.2–8.1 (m, 5H, Ar–H). GC–MS (*m*/*z*) 316 [M$^+$].

3.2. Pharmacology

3.2.1. Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds (**3a**–**n**) was evaluated by carrageenan-induced acute paw edema method. Male Wistar albino rats weighing 150–200 g were divided into six animals in each group. One group was treated with 0.1 ml of 1% gum acacia suspension orally (control), second group was administered with a dose of 20 mg/kg of the suspension of Indomethacin (standard) and the other groups (test compounds) were treated with 20 mg/kg of the suspension of test compounds. After 30 min the animals were injected with 0.1 ml of 1% carrageenan in normal saline subcutaneously to the sub-plantar region of right hind paw. The paw volume was measured immediately ('0' h) and after 1 h, 2 h, 3 h and 4 h, respectively, by using plethysmograph. The amount of edema in the drug-treated groups was compared in relation to the control group with the corresponding time intervals. The results were expressed as percentage inhibition of edema over the untreated control group and represented in Table 1.

3.2.2. Analgesic activity

Acetic acid writhing test was performed on mice for calculating analgesic activity. Groups of six mice (body weight 20–30 g) of both sexes, pregnant female excluded, were given a dose of a test compound. Thirty minutes later, the animals were injected intraperitoneally with 0.25 ml/mouse of 0.5% acetic acid solution and writhes were counted during the following 30 min. The mean number of writhes for each experimental group and percent decrease compared with control group (mice not treated with test compounds) were calculated. The results were expressed in percentage decrease relative to control and tabulated in Table 2.

3.2.3. Antimicrobial activity

The newly synthesized compounds were tested for their antibacterial against *E. coli* (Gram negative), *B. subtilis* (Gram positive) and antifungal activity against *C. albicans*, *A. niger*. The results were compared to Amikacin, Vancomycin for antibacterial activity and Griseofulvin for antifungal activity as a reference drug. Experimental results were listed in Table 3.

3.3. Molecular modeling and docking studies

To assess the anti-inflammatory behavior of our coumarin derivatives on structural basis, automated docking studies were carried out using Molegro Virtual Docker program, the scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the COX-2 enzyme. The protein-ligand complex was constructed based on the X-ray structure (PDB entry 4COX) COX-2 with its bound inhibitor of Indomethacin. The scoring functions of the compounds were calculated with reference to indomethacin.

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