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ORIGINAL PAPER

Synthesis and biological activities of novel ethers of quinolinone linked with coumarins

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Abstract A series of new ethers of quinolinone linked with different substituted coumarins and benzofurans were synthesized from 4-(bromomethyl)quinolinones. All newly synthesized compounds were screened for their in vitro antibacterial and antifungal activities. Most of the compounds with chloro substitution at the C-6 or C-7 position in quinolinone showed potent antibacterial and antifungal activities. In pharmacological evaluations, some of these chloroquinolinones also showed 70–77% inhibition of inflammation after 8 h, whereas the other compounds showed 51–55% inhibition. Most of the compounds showed potent analgesic activity compared to the standard and control. The structures of all newly synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and EI-MS.

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Introduction

Coumarins and their nitrogen analogues 1-azacoumarins (also known as quinolinones and carbostyrils) are heterocyclic units, lactones and lactams, respectively, which are indispensable to both chemists and biochemists. The natural occurrence, antimicrobial, anti-inflammatory, anticancer, and miscellaneous other properties of these two systems were recently reviewed and compared [1]. Many coumarin derivatives are known for their ability to scavenge free radicals, especially reactive oxygen species (ROS), and they have been used as inhibitors of cyclooxygenase and lipoxygenase in the arachidonic acid pathway of inflammation suppression [2]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have a broad spectrum of effects in acute pain management and target the cyclooxygenase enzyme. Several coumarin derivatives have significant anti-inflammatory activities and inhibit various enzymes involved in modulating inflammation [3–5]. The potential of coumarin derivatives as anti-inflammatory agents has been explored in our laboratory, by incorporating biocompatible pharmacophores like *p*-vanillyl, cyanoester, and paracetamol at position 4 of bromomethylcoumarin [6, 7]. We recently reported the synthesis and preliminary biological evaluation of an array of novel angularly fused polycyclic heterocycles with coumarin, benzofuran, and pyridine [8].

Quinolinone derivatives are metabolized to the corresponding 8-hydroxycoumarins in biological systems and were therefore found to be very good anti-inflammatory and analgesic agents [9]. The triheterocyclic

thiazoles synthesized from 4-(aminomethyl)quinolinone and 3-(bromoacetyl)coumarins [10] in our laboratory were found to exhibit promising anti-inflammatory and analgesic activities even after 24 h. We also reported that the introduction of halogens in general, and fluorine in particular, at position 4 in the aryloxy and arylamino moieties of coumarin and quinolinone [11–13] enhances their antimicrobial, analgesic, and anti-inflammatory activities. Interest in coumarins and quinolinones as antibiotics is due to the observation that they are potent inhibitors of bacterial DNA gyrase, which is involved in cell growth [14, 15].

We have documented the very good antibacterial activity associated with many coumarin and quinolinone derivatives bearing a variety of substituents at position 4 [16, 17]. In view of the biological importance of coumarin and quinolinone, we have designed new templates linking these two biolabile heterocycles together through an ether linkage in order to study the biological properties of these heterocycles as a single entity. This paper reports the synthesis and preliminary biological evaluation of ethers derived from quinolinone linked with different substituted coumarins and benzofurans.

Results and discussion

Syntheses

The basic synthetic route employed for the construction of various ethers is outlined in Scheme 1. The 4-(bromomethyl)quinolinones **1a–1d** required for the construction of the target molecules were synthesized by the bromination of acetoacetanilides and cyclizing the intermediate ω-bromoacetoacetanilides in sulfuric acid [11, 18]. The ethers **2a–2d**, **3a–3d**, **4a–4d**, and **5a–5d** were synthesized by the reaction of **1a–1d** and anhydrous K_2CO_3 with 6-hydroxy-4-methylcoumarin, 7-hydroxy-4-methylcoumarin, 7-hydroxy-4,5-dimethylcoumarin, and 2,4-dihydroxyacetophenone, respectively, in dry ethanol [19]. Further reaction of **5d** with various 4-(bromomethyl)coumarins and anhydrous K_2CO_3 in dry ethanol afforded the benzofuranyl ethers **6a–6e** via an intramolecular aldol condensation followed by dehydration. All the products gave satisfactory analytical and spectroscopic data, which are in full accordance with their assigned structures.

Antimicrobial activity

The in vitro antibacterial and antifungal activities of all newly synthesized compounds were assessed by the cup-plate method [20–22], and the results are shown in Table 1. Compounds **2b**, **2c**, **3b**, **3c**, **4b**, **4c**, **5d**, **5c**, and **6c** with chloro substitution at the C-6 or C-7 position in quinolinone showed potent antibacterial activity of 62–74% inhibition of *Escherichia coli* at 100 μg cm^{-3} and 10–27% inhibition at 25 μg cm^{-3}. Similar compounds showed 68–79% inhibition of *Bacillus cirrhosis* at 100 μg cm^{-3} and up to 20–35% inhibition at 25 μg cm^{-3}, and the remaining compounds showed moderate activity against both the bacteria.

Compounds **2b–2d**, **3b–3d**, **4b–4d**, **5b**, **5c**, and **6c** showed very good antifungal activity of 52% (**3d**) to 74% (**2b**) inhibition of *Aspergillus niger* at 100 μg cm^{-3} and 12% (**2d**) to 41% (**2b**) inhibition at 25 μg cm^{-3} (Table 2). Similar compounds showed 53% (**3d**) to 78% (**4b**) inhibition of *Rhizoctonia bataticola* at 100 μg cm^{-3} and 14% (**2d**) to 44% (**4b**) inhibition at 25 μg cm^{-3}.

Acute toxicity studies

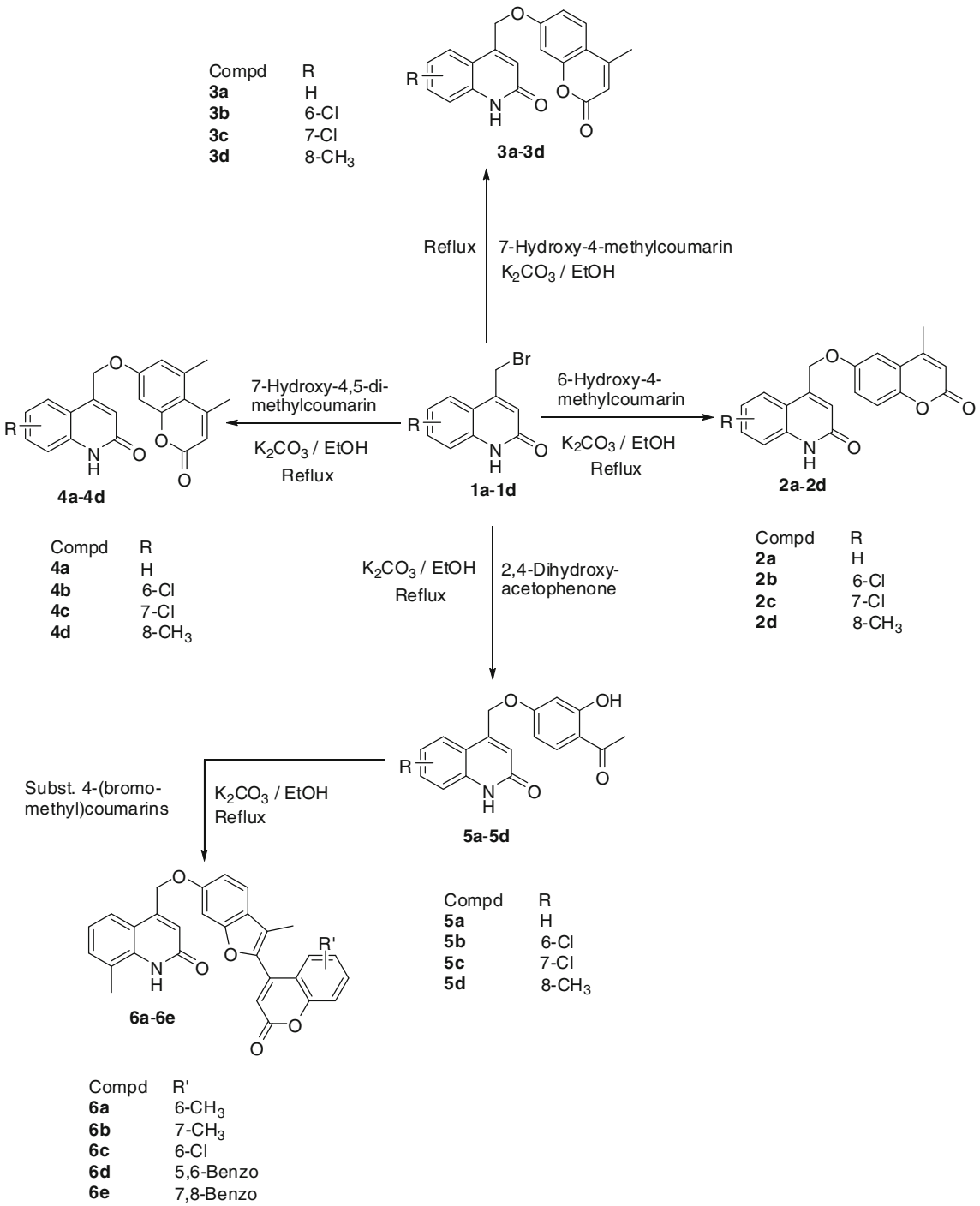
The acute toxicity studies of the test compounds were performed on albino mice fasted for 24 h. The test compounds were administered orally and intraperitoneally. The animals were watched for mortality and symptoms for 8 days [23, 24]. All the compounds possess a good safety profile and no mortality of animals was observed even after 24 h.

Analgesic activity

Abdominal constriction response induced by acetic acid is a sensitive procedure to establish efficacy of peripherally acting analgesics. Intraperitoneal (i.p.) administration of acetic acid causes an increase in the level of PGE2 and PGF 2α [25]. The results of the analgesic activity tests (Table 3) indicate that compounds **3b**, **2b**, **4b**, **5c**, and **6c** with chloro substitution in the quinolinone moiety showed the highest analgesic activity, even greater than that of the standard. Compounds **3c**, **2c**, **4c**, **5d**, and **6d** showed moderate analgesic activity. A graphical representation of the analgesic activity of selected compounds is given in Fig. 1. Compounds **2b**, **3b**, **4b**, **5c**, and **6c** with chloro substitution in the quinolinone moiety were found to be potent analgesic agents as compared to the standard.

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was assessed by the formalin-induced rat-paw edema method [26]. The results in Table 3 indicate that most of the compounds showed an extremely significant anti-inflammatory activity when compared to the control and the standard group and their onset of action was much quicker than the standard, because they showed significant anti-inflammatory activity at 2 h after administration. Compounds **5b**, **5c**, and **6b** showed a maximum of 77% inhibition of inflammation after 8 h. Compound **5c** was even more active than the other compounds after 12 and 24 h. The metabolites of compound **5c** might also show



Scheme 1

some anti-inflammatory activity. Compound **5c** may be more potent than the standard. Similarly compounds **6c** and **5d** showed a maximum of 74% inhibition of inflammation and compound **6d** showed a maximum of 70% inhibition of inflammation after 8 h, whereas the rest of the compounds showed moderate activity of 51–55% inhibition of inflammation after 8 h. A graphical representation of the anti-inflammatory activity of selected compounds is given in Figs. 2 and 3. Compounds **4c**, **5c**, and **6b** were active even after 24 h.

Table 1 Antibacterial activity of selected compounds

Compd.	Compd. conc. (μg cm^{-3})						Compd. conc. (μg cm^{-3})					
	100		50		25		100		50		25	
	E. coli (gram negative)						*B. cirrhosis* (gram positive)					
	Zone of inhibition						Zone of inhibition					
	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%
2b	184	74	98	43	41	27	210	79	128	54	71	35
2c	178	67	88	38	32	14	202	75	122	51	64	30
2d	102	37	51	18	–	–	138	49	72	26	–	–
3b	172	69	81	34	28	12	198	74	118	49	58	26
3c	177	62	78	27	25	10	188	70	108	44	52	23
3d	98	35	44	14	–	–	122	42	68	24	–	–
4b	170	68	81	34	32	14	190	70	110	45	58	26
4c	164	65	72	29	26	10	184	68	94	37	40	20
4d	114	42	58	21	–	–	136	48	72	26	–	–
5a	118	44	62	24	–	–	132	46	84	32	32	10
5b	162	64	84	36	32	14	186	69	98	39	48	20
5c	178	67	92	40	44	22	192	71	104	42	62	29
5d	113	42	43	13	–	–	128	45	72	26	–	–
6a	102	37	61	24	–	–	130	46	70	25	–	–
6b	114	42	51	18	–	–	138	49	74	27	–	–
6c	165	65	80	34	30	13	190	71	110	45	40	20
6d	100	36	40	12	–	–	140	47	68	24	–	–
Standard	240	100	200	100	160	100	260	100	220	100	170	100
Control	20	–	20	–	20	–	20	–	20	–	20	–

Standard, norfloxacin (100% inhibition at each concentration); control, dimethylformamide (DMF)

Conclusion

This study has shown that the presence of a chlorine substituent in the quinolinone or coumarin moiety enhances the antimicrobial, analgesic, and anti-inflammatory activities. Most of the compounds, viz. **2b**, **2c**, **3b**, **3c**, **4b**, **4c**, **5b**, **5c**, and **6c** with chloro substitution at the C-6 or C-7 position in quinolinone, showed potent antibacterial and antifungal activities. In the pharmacological evaluation, most of the compounds with chloro substitution showed potent analgesic activity as compared to the standard and control. Compounds **5b**, **5c**, and **5d** showed 74–77% inhibition of inflammation after 8 h. The introduction of a benzofuran moiety between the quinolinone and coumarin also enhanced the biological properties: compound **6c** was very active against both the bacteria and fungi. In pharmacological studies, compounds **6b**, **6c**, and **6d** showed 70–77% inhibition of inflammation after 8 h, and compounds **2b**, **2c**, **3b**, **3c**, **4b**, and **4c** showed 51–55% inhibition of inflammation after 8 h. In conclusion, nearly all of the compounds with chloro substitution in quinolinone or coumarin showed the highest activity after 8 h and some of them were active even at 12 and 24 h after administration, i.e., these compounds can act as slow-release anti-inflammatory agents. Our findings will have a big impact on chemists and biochemists conducting further investigations in this field in search of potent antimicrobial, analgesic, and anti-inflammatory agents.

Experimental

The melting points of the products were determined in open capillaries on a Buchi apparatus. The IR spectra were recorded on a Nicolet Impact-410 FT-IR spectrophotometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-300F spectrometer in $CDCl_3$, DMSO-d_6, or a mixture of $CDCl_3$ and CF_3COOH using TMS as an internal standard at 300 MHz (^{1}H) and 75 MHz (^{13}C) resonance frequency. D_2O exchange was applied to confirm the assignment of the signals of NH protons. The mass spectra were recorded on an Autospec ESI-MS. The elemental analysis was carried out using a Heraus CHN rapid analyzer. C, H, and N values of all compounds were found to be within ±0.4% of the theoretical values. The homogeneity of the compounds was assessed by TLC on

Table 2 Antifungal activity of some selected compounds

Compd.	Compd. conc. (μg cm^{-3})						Compd. conc. (μg cm^{-3})					
	100		50		25		100		50		25	
	A. niger						*R. bataticola*					
	Zone of inhibition						Zone of inhibition					
	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%
2b	192	74	126	55	82	41	180	72	120	55	74	38
2c	188	73	120	52	78	38	172	69	112	50	63	31
2d	148	55	78	30	38	12	142	55	70	27	40	14
3b	184	71	118	51	78	38	173	69	118	54	74	38
3c	187	72	110	47	69	32	168	67	110	50	65	32
3d	140	52	78	30	42	14	138	53	68	26	40	14
4b	187	72	118	51	75	36	192	78	128	60	82	44
4c	170	65	110	47	70	33	178	71	120	55	74	38
4d	150	56	78	30	40	13	148	58	74	30	48	20
5a	110	39	62	22	–	–	100	36	–	–	–	–
5b	170	65	104	44	64	29	162	64	110	50	68	34
5c	164	62	98	41	60	26	158	62	104	46	65	32
5d	128	46	70	26	–	–	118	44	60	18	–	–
6a	150	56	62	22	–	–	118	44	68	26	–	–
6b	165	62	80	32	38	12	102	37	–	–	–	–
6c	172	66	118	51	70	33	178	71	105	46	66	32
6d	155	57	74	28	–	–	110	39	–	–	–	–
Standard	250	100	210	100	170	100	240	100	200	100	160	100
Control	20	–	20	–	20	–	20	–	20	–	20	–

Standard, griseofulvin (100% inhibition at each concentration); control, DMF

aluminum silica gel 60 F_{254} (Merck) detected by UV light (254 nm) and iodine vapors. The reagents were all analytical reagent grade or chemically pure. All solvents were dried, deoxygenated, and redistilled before use by standard procedures [19].

General procedure for the synthesis of compounds ***2a–2e***

A mixture of substituted 4-(bromomethyl)quinolinone **1a–1d** (4.0 mmol), 6-hydroxy-4-methylcoumarin (4.0 mmol), and anhydrous potassium carbonate (4.0 mmol) in 20 cm^3 dry ethanol was refluxed on a water bath for 8 h. The separated solid was filtered, washed with 20% HCl and with an excess of cold water, dried, and crystallized from a suitable solvent.

4-[(4-Methyl-2-oxo-2H-chromen-6-yloxy)methyl]-quinolin-2(1H)-one (**2a**, $C_{20}H_{15}NO_4$)
Yield 74%; colorless crystals (acetic acid); m.p.: 308–310 °C; IR (KBr): $\bar{\nu} = 3{,}433$ (N–H stretching), 1,707 (C=O stretching, lactone), 1,657 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3 + CF_3COOH$): $\delta = 2.56$ (s, 3H, C4′-CH_3 of coumarin), 5.50 (s, 2H, C4-CH_2 of quinolinone), 6.26 (s, 1H, C3-H of coumarin), 6.56 (s, 1H, C3-H of quinolinone), 7.34–7.94 (m, 7H, Ar–H), 13.03 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3 + CF_3COOH$): $\delta = 163.50, 160.22, 157.28, 156.34, 154.28, 136.89, 133.48, 132.80, 127.08, 124.60, 119.40, 116.30, 116.21, 114.47, 114.30, 112.65, 110.98, 109.10, 65.98, 18.34$ ppm; EI-MS: $m/z = 345$ (M + H).

6-Chloro-4-[(4-methyl-2-oxo-2H-chromen-6-yloxy)-methyl]quinolin-2(1H)-one (**2b**, $C_{20}H_{14}ClNO_4$)
Yield 84%; colorless crystals (acetic acid); m.p.: 284–286 °C; IR (KBr): $\bar{\nu} = 3{,}428$ (N–H stretching), 1,720 (C=O stretching, lactone), 1,660 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3 + CF_3COOH$): $\delta = 2.50$ (s, 3H, C4′-CH_3 of coumarin), 5.56 (s, 2H, C4-CH_2 of quinolinone), 6.30 (s, 1H, C3-H of coumarin), 6.48 (s, 1H, C3-H of quinolinone), 7.30–7.90 (m, 6H, Ar–H), 13.08 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3 + CF_3COOH$): $\delta = 164.30, 161.20, 158.23, 156.74, 155.88,$

Table 3 Anti-inflammatory and analgesic activities of some selected compounds

Compd.	Anti-inflammatory activity[a]							Analgesic activity (number of writhings)
	Edema volume at different time intervals (% of inhibition)							
	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h	
Standard	0.66 (4)	0.81 (9)	0.88 (12)	0.68* (36)	0.63* (42)	0.81 (16)	0.98 (14)	22*
Control	0.71	0.91	1.01	1.06	1.07	0.89	1.01	35
2b	0.64 (5)	0.78 (8)	0.69* (32)	0.59* (45)	0.53* (51)	0.77 (12)	1.14 (0)	28*
2c	0.60 (5)	0.80 (9)	0.66* (31)	0.59* (45)	0.53* (51)	0.76 (12)	1.14 (0)	19*
3b	0.73 (0)	1.19 (0)	0.88 (12)	0.57* (44)	0.48* (55)	0.54** (39)	1.00 (0)	29*
3c	0.71 (0)	1.04 (0)	0.70 (30)	0.58* (46)	0.52* (51)	0.77 (11)	1.13 (0)	20*
4b	0.78 (0)	1.10 (0)	0.66* (31)	0.43* (60)	0.48* (55)	0.76 (12)	0.88 (7)	27*
4c	0.65 (5)	0.77 (10)	0.88 (12)	0.58* (46)	0.52* (51)	0.54** (39)	0.98 (14)	18*
5b	0.61 (7)	0.76 (10)	0.70 (30)	0.28* (72)	0.23* (77)	0.50* (39)	0.86 (9)	13*
5c	0.60 (7)	0.78 (8)	0.78 (18)	0.75*** (25)	0.23* (77)	0.45* (45)	0.98 (14)	27*
5d	0.58 (11)	0.75 (12)	0.75 (21)	0.58* (42)	0.26* (74)	0.56*** (32)	0.88 (7)	18*
6b	0.60 (8)	0.77 (10)	0.55* (42)	0.28* (72)	0.23* (77)	0.50* (39)	0.96 (12)	13*
6c	0.63 (5)	0.78 (8)	0.78 (18)	0.75*** (25)	0.26* (74)	0.56*** (32)	0.86 (9)	26*
6d	0.64 (4)	0.78 (8)	0.75 (21)	0.58* (42)	0.29* (70)	0.45* (45)	0.88 (7)	18*
F value	2.86	8.16	12.3	21.7	27.3	10.6	2.54	86.44

Standard error of the mean (SEM) 0.05–0.15. Standard, indomethacin at a dose of 10 mg kg^{-1} (i.p.). Test compounds at a dose of 100 mg kg^{-1} (i.p.). Data were analyzed by one-way ANOVA followed by Tukey comparison of all pairs

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared to control

[a] Assessed in a formalin-induced (3.5%) acute inflammation model in male Wistar rats ($n = 6$)

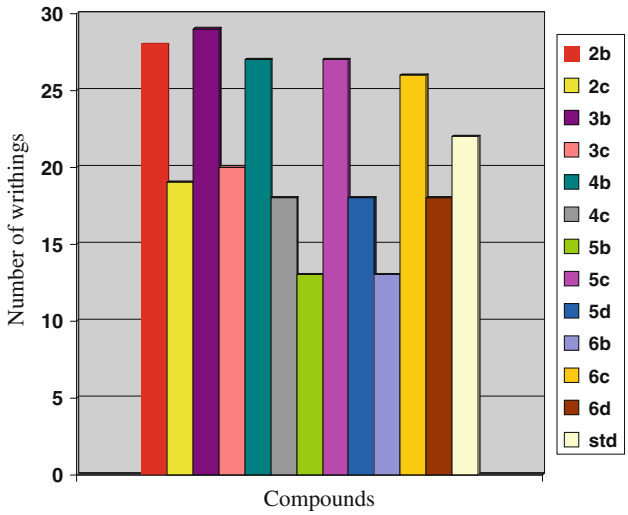


Fig. 1 Analgesic activity of selected compounds and standard

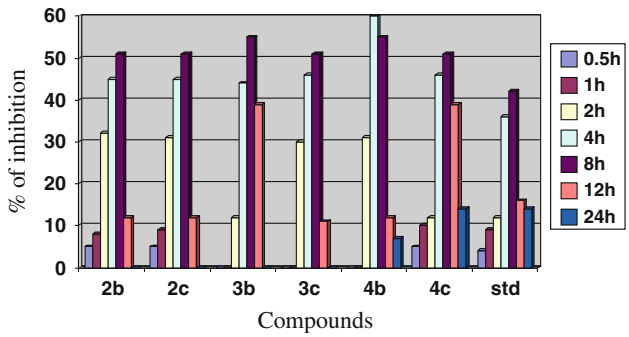


Fig. 2 Anti-inflammatory activity of selected compounds and standard

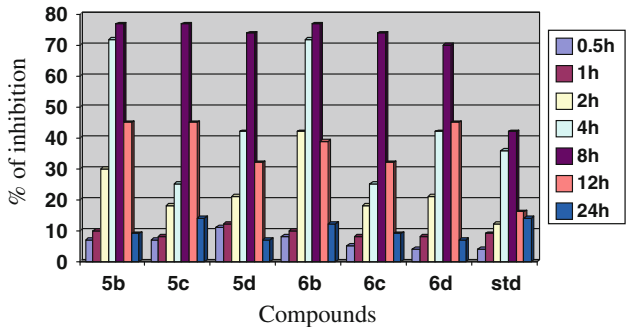


Fig. 3 Anti-inflammatory activity of selected compounds and standard

136.89, 134.48, 132.80, 127.08, 124.60, 118.40, 116.33, 116.21, 115.47, 114.30, 111.60, 110.98, 108.14, 64.18, 19.14 ppm; EI-MS: $m/z = 368$ (M + H).

7-Chloro-4-[(4-methyl-2-oxo-2H-chromen-6-yloxy)-methyl]quinolin-2(1H)-one (**2c**, $C_{20}H_{14}ClNO_4$)
Yield 80%; colorless crystals (acetic acid); m.p.: 320–322 °C; IR (KBr): $\bar{\nu} = 3{,}434$ (N–H stretching),

1,707 (C=O stretching, lactone), 1,669 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + CF_3COOH): $\delta = 2.58$ (s, 3H, C4′-CH_3 of coumarin), 5.60 (s, 2H, C4-CH_2 of quinolinone), 6.20 (s, 1H, C3-H of coumarin), 6.58 (s, 1H, C3-H of quinolinone), 7.37–7.95 (m, 6H, Ar–H), 13.02 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + CF_3COOH): $\delta = 164.10, 160.40, 158.23, 156.44, 155.58, 136.85, 134.45, 132.80, 127.08, 124.60, 118.40, 116.30, 116.21, 115.47, 114.10, 111.67, 110.98, 109.10, 65.10, 18.34$ ppm; EI-MS: $m/z = 368$ (M + H).

8-Methyl-4-[(4-methyl-2-oxo-2H-chromen-6-yloxy)-methyl]quinolin-2(1H)-one (**2d**, $C_{21}H_{17}NO_4$)
Yield 88%; colorless crystals (acetic acid); m.p.: 296–298 °C; IR (KBr): $\bar{\nu} = 3{,}440$ (N–H stretching), 1,708 (C=O stretching, lactone), 1,658 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + CF_3COOH): $\delta = 2.48$ (s, 3H, C8-CH_3 of quinolinone), 2.56 (s, 3H, C4′-CH_3 of coumarin), 5.55 (s, 2H, C4-CH_2 of quinolinone), 6.32 (s, 1H, C3-H of coumarin), 6.50 (s, 1H, C3-H of quinolinone), 7.35–7.88 (m, 6H, Ar–H), 13.08 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + CF_3COOH): $\delta = 166.00, 161.20, 158.23, 156.48, 154.28, 136.85, 134.45, 130.80, 128.08, 124.60, 118.40, 116.30, 116.21, 114.47, 114.10, 111.67, 110.98, 108.10, 65.10, 20.30, 18.10$ ppm; EI-MS: $m/z = 348$ (M + H).

General procedure for the synthesis of compounds ***3a–3d***

A mixture of substituted 4-(bromomethyl)quinolinone **1a–1d** (4.0 mmol), 7-hydroxy-4-methylcoumarin (4.0 mmol), and anhydrous potassium carbonate (4.0 mmol) in 20 cm^3 dry ethanol was refluxed on a water bath for 8 h. The separated solid was filtered, washed with 20% HCl and with an excess of cold water, dried, and crystallized from a suitable solvent.

4-[(4-Methyl-2-oxo-2H-chromen-7-yloxy)methyl]-quinolin-2(1H)-one (**3a**, $C_{20}H_{15}NO_4$)
Yield 78%; colorless crystals (acetic acid); m.p.: 310–312 °C; IR (KBr): $\bar{\nu} = 3{,}435$ (N–H stretching), 1,717 (C=O stretching, lactone), 1,657 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + CF_3COOH): $\delta = 2.57$ (s, 3H, C4′-CH_3 of coumarin), 5.55 (s, 2H, C4-CH_2 of quinolinone), 6.28 (s, 1H, C3-H of coumarin), 6.43 (s, 1H, C3-H of quinolinone), 7.08–7.96 (m, 7H, Ar–H), 13.07 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + CF_3COOH): $\delta = 164.54, 161.02, 158.18, 156.34, 154.28, 136.89, 133.18, 132.10, 127.08, 124.60, 119.40, 116.30, 116.21, 114.47, 114.30, 111.65, 110.18, 108.10, 64.38, 19.32$ ppm; EI-MS: $m/z = 334$ (M + H).

6-Chloro-4-[(4-methyl-2-oxo-2H-chromen-7-yloxy)-methyl]quinolin-2(1H)-one (**3b**, $C_{20}H_{14}ClNO_4$)
Yield 70%; colorless crystals (acetic acid); m.p.: 315–317 °C; IR (KBr): $\bar{\nu} = 3{,}440$ (N–H stretching), 1,720 (C=O stretching, lactone), 1,660 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + CF_3COOH): $\delta = 2.48$ (s, 3H, C4′-CH_3 of coumarin), 5.65 (s, 2H, C4-CH_2 of quinolinone), 6.22 (s, 1H, C3-H of coumarin), 6.40 (s, 1H, C3-H of quinolinone), 7.10–8.08 (m, 6H, Ar–H), 12.98 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + CF_3COOH): $\delta = 165.14, 160.11, 158.18, 155.34, 154.28, 137.89, 134.18, 132.10, 127.08, 125.60, 120.40, 116.30, 116.20, 114.47, 114.30, 110.65, 109.18, 107.13, 65.28, 18.18$ ppm; EI-MS: $m/z = 368$ (M + H).

7-Chloro-4-[(4-methyl-2-oxo-2H-chromen-7-yloxy)-methyl]quinolin-2(1H)-one (**3c**, $C_{20}H_{14}ClNO_4$)
Yield 72%; colorless crystals (acetic acid); m.p.: 292–294 °C; IR (KBr): $\bar{\nu} = 3{,}436$ (N–H stretching), 1,719 (C=O stretching, lactone), 1,667 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + CF_3COOH): $\delta = 2.50$ (s, 3H, C4′-CH_3 of coumarin), 5.45 (s, 2H, C4-CH_2 of quinolinone), 6.30 (s, 1H, C3-H of coumarin), 6.48 (s, 1H, C3-H of quinolinone), 7.05–7.96 (m, 6H, Ar–H), 12.88 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + CF_3COOH): $\delta = 164.34, 161.20, 159.18, 154.14, 153.18, 137.89, 134.18, 132.10, 128.08, 125.60, 119.40, 116.30, 115.20, 114.47, 114.20, 110.65, 109.18, 108.13, 64.22, 19.28$ ppm; EI-MS: $m/z = 368$ (M + H).

8-Methyl-4-[(4-methyl-2-oxo-2H-chromen-7-yloxy)-methyl]quinolin-2(1H)-one (**3d**, $C_{21}H_{17}NO_4$)
Yield 82%; colorless crystals (acetic acid); m.p.: 288–290 °C; IR (KBr): $\bar{\nu} = 3{,}442$ (N–H stretching), 1,710 (C=O stretching, lactone), 1,658 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + CF_3COOH): $\delta = 2.48$ (s, 3H, C8-CH_3 of quinolinone), 2.60 (s, 3H, C4′-CH_3 of coumarin), 5.65 (s, 2H, C4-CH_2 of quinolinone), 6.32 (s, 1H, C3-H of coumarin), 6.50 (s, 1H, C3-H of quinolinone), 7.10–7.98 (m, 6H, Ar–H), 12.98 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + CF_3COOH): $\delta = 165.24, 160.10, 159.28, 155.10, 152.18, 138.80, 134.18, 132.10, 128.08, 126.66, 119.40, 116.30, 115.20, 114.47, 114.10, 110.65, 109.18, 108.30, 64.22, 20.22, 19.28$ ppm; EI-MS: $m/z = 348$ (M + H).

General procedure for the synthesis of compounds ***4a–4d***

A mixture of substituted 4-(bromomethyl)quinolinone **1a–1d** (4.0 mmol), 7-hydroxy-4,5-dimethylcoumarin (4.0 mmol), and anhydrous potassium carbonate (4.0 mmol) in 20 cm^3

dry ethanol was refluxed on a water bath for 8 h. The separated solid was filtered, washed with 20% HCl and with an excess of cold water, dried, and crystallized from a suitable solvent.

4-[(4,5-Dimethyl-2-oxo-2H-chromen-7-yloxy)methyl]-quinolin-2(1H)-one (**4a**, $C_{21}H_{17}NO_4$)
Yield 80%; colorless crystals (acetic acid); m.p.: 285–287 °C; IR (KBr): $\bar{\nu} = 3{,}535$ (N–H stretching), 1,720 (C=O stretching, lactone), 1,666 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + CF_3COOH$): $\delta = 2.48$ (s, 3H, C4′-CH_3 of coumarin), 2.54 (s, 3H, C5′-CH_3 of coumarin), 5.56 (s, 2H, C4-CH_2 of quinolinone), 6.27 (s, 1H, C3-H of coumarin), 6.75 (s, 1H, C3′-H of quinolinone), 6.98 (s, 1H, C6′-H of coumarin), 7.16 (s, 1H, C8′-H of coumarin), 7.55–7.90 (m, 4H, Ar–H), 13.04 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3 + CF_3COOH$): $\delta = 163.44$, 160.22, 159.18, 156.34, 154.28, 136.89, 134.10, 132.10, 127.08, 124.60, 119.40, 116.30, 116.12, 114.47, 114.10, 111.65, 110.10, 108.10, 65.40, 20.12, 18.32 ppm; EI-MS: $m/z = 348$ (M + H).

6-Chloro-4-[(4,5-dimethyl-2-oxo-2H-chromen-7-yloxy)-methyl]quinolin-2(1H)-one (**4b**, $C_{21}H_{16}ClNO_4$)
Yield 78%; colorless crystals (acetic acid); m.p.: 282–284 °C; IR (KBr): $\bar{\nu} = 3{,}434$ (N–H stretching), 1,721 (C=O stretching, lactone), 1,668 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + CF_3COOH$): $\delta = 2.47$ (s, 3H, C4′-CH_3 of coumarin), 2.55 (s, 3H, C5′-CH_3 of coumarin), 5.54 (s, 2H, C4-CH_2 of quinolinone), 6.28 (s, 1H, C3-H of coumarin), 6.76 (s, 1H, C3′-H of quinolinone), 6.96 (s, 1H, C6′-H of coumarin), 7.15 (s, 1H, C8′-H of coumarin), 7.54–7.90 (m, 3H, Ar–H), 13.02 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3 + CF_3COOH$): $\delta = 165.14$, 160.20, 158.18, 157.34, 153.28, 135.89, 133.10, 131.10, 126.08, 120.60, 118.40, 116.30, 115.12, 114.47, 114.00, 111.65, 110.10, 109.10, 64.40, 20.82, 19.22 ppm; EI-MS: $m/z = 382$ (M + H).

7-Chloro-4-[(4,5-dimethyl-2-oxo-2H-chromen-7-yloxy)-methyl]quinolin-2(1H)-one (**4c**, $C_{21}H_{16}ClNO_4$)
Yield 75%; colorless crystals (acetic acid); m.p.: 281–282 °C; IR (KBr): $\bar{\nu} = 3{,}428$ (N–H stretching), 1,718 (C=O stretching, lactone), 1,660 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + CF_3COOH$): $\delta = 2.47$ (s, 3H, C4′-CH_3 of coumarin), 2.54 (s, 3H, C5′-CH_3 of coumarin), 5.57 (s, 2H, C4-CH_2 of quinolinone), 6.26 (s, 1H, C3-H of coumarin), 6.75 (s, 1H, C3′-H of quinolinone), 6.97 (s, 1H, C6′-H of coumarin), 7.16 (s, 1H, C8′-H of coumarin), 7.56–7.91 (m, 3H, Ar–H), 13.08 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3 + CF_3COOH$): $\delta = 164.14$, 161.20, 159.18, 158.34, 154.28, 136.89, 134.10, 130.10, 125.08, 120.60, 119.40, 117.30, 115.12, 114.40, 114.10, 111.65, 110.10, 108.10, 65.40, 20.12, 19.22 ppm; EI-MS: $m/z = 304$ (M^+).

4-[(4,5-Dimethyl-2-oxo-2H-chromen-7-yloxy)methyl]-8-methylquinolin-2(1H)-one (**4d**, $C_{22}H_{19}NO_4$)
Yield 88%; colorless crystals (acetic acid); m.p.: 244–246 °C; IR (KBr): $\bar{\nu} = 3{,}415$ (N–H stretching), 1,721 (C=O stretching, lactone), 1,659 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3 + CF_3COOH$): $\delta = 2.47$ (s, 3H, C4′-CH_3 of coumarin), 2.55 (s, 3H, C5′-CH_3 of coumarin), 2.62 (s, 3H, C8-CH_3 of quinolinone), 5.58 (s, 2H, C4-CH_2 of quinolinone), 6.25 (s, 1H, C3-H of coumarin), 6.68 (s, 1H, C3′-H of quinolinone), 6.96 (s, 1H, C6′-H of coumarin), 7.12 (s, 1H, C8′-H of coumarin), 7.34–7.90 (m, 3H, Ar–H), 13.02 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3 + CF_3COOH$): $\delta = 165.10$, 160.22, 159.10, 158.24, 154.48, 136.80, 134.15, 130.10, 125.08, 120.60, 119.40, 117.30, 115.19, 114.44, 114.10, 111.66, 110.10, 108.10, 66.40, 20.12, 19.22, 18.30 ppm; EI-MS: $m/z = 362$ (M + H).

General procedure for the synthesis of compounds ***5a–5d***

A mixture of substituted 4-(bromomethyl)quinolinone **1a–1d** (4.0 mmol), 2,4-dihydroxyacetophenone (4.0 mmol), and anhydrous potassium carbonate (4.0 mmol) in 20 cm^3 dry ethanol was refluxed on a water bath for 8 h. The separated solid was filtered, washed with 20% HCl and with an excess of cold water, dried, and crystallized from a suitable solvent.

4-[(4-Acetyl-3-hydroxyphenoxy)methyl]quinolin-2(1H)-one (**5a**, $C_{18}H_{15}NO_4$)
Yield 68%; colorless crystals (acetic acid); m.p.: 280–282 °C; IR (KBr): $\bar{\nu} = 3{,}430$ (N–H stretching), 1,718 (C=O stretching, lactone), 1,664 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ = 2.58 (s, 3H, CH_3 of acetyl), 5.49 (s, 2H, C4-CH_2 of quinolinone), 6.61–7.90 (m, 8H, Ar–H), 10.88 (s, 1H, NH), 12.62 (s, 1H, OH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 166.44$, 162.22, 159.18, 156.34, 154.28, 136.89, 134.10, 132.10, 127.08, 124.60, 119.40, 116.30, 116.12, 114.10, 111.65, 108.10, 66.40, 23.12 ppm; EI-MS: $m/z = 310$ (M + H).

4-[(4-Acetyl-3-hydroxyphenoxy)methyl]-6-chloroquinolin-2(1H)-one (**5b**, $C_{18}H_{14}ClNO_4$)
Yield 65%; colorless crystals (acetic acid); m.p.: 294–296 °C; IR (KBr): $\bar{\nu} = 3{,}434$ (N–H stretching), 1,710 (C=O stretching, lactone), 1,650 (C=O stretching, amide) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): $\delta = 2.68$ (s, 3H, CH_3 of acetyl), 5.58 (s, 2H, C4-CH_2 of quinolinone), 6.60–7.80 (m, 7H, Ar–H), 11.34 (s, 1H, NH), 12.43

(s, 1H, OH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.45, 161.32, 158.18, 156.34, 154.28, 136.89, 134.10, 132.10, 127.08, 124.60, 119.40, 116.30, 116.10, 114.10, 111.64, 108.10, 65.42, 25.32 ppm; EI-MS: m/z = 344 (M + H).

4-[(4-Acetyl-3-hydroxyphenoxy)methyl]-7-chloroquinolin-2(1H)-one (**5c**, $C_{18}H_{14}ClNO_4$)
Yield 60%; colorless crystals (acetic acid); m.p.: 290–292 °C; IR (KBr): $\bar{\nu}$ = 3,431 (N–H stretching), 1,711 (C=O stretching, lactone), 1,652 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + CF_3COOH): δ = 2.69 (s, 3H, CH_3 of acetyl), 5.56 (s, 2H, C4-CH_2 of quinolinone), 6.67–7.90 (m, 7H, Ar–H), 11.54 (s, 1H, NH), 13.14 (s, 1H, OH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$ + CF_3COOH): δ = 166.75, 160.22, 159.18, 156.34, 154.28, 136.89, 134.10, 132.10, 127.08, 124.60, 120.40, 116.30, 116.10, 114.10, 111.64, 109.10, 64.42, 24.32 ppm; EI-MS: m/z = 344 (M + H).

4-[(4-Acetyl-3-hydroxyphenoxy)methyl]-8-methylquinolin-2(1H)-one (**5d**, $C_{19}H_{17}NO_4$)
Yield 75%; colorless crystals (acetic acid); m.p.: 270–272 °C; IR (KBr): $\bar{\nu}$ = 3,445 (N–H stretching), 1,700 (C=O stretching, lactone), 1,660 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.49 (s, 3H, C8-CH_3 of quinolinone), 2.60 (s, 3H, CH_3 of acetyl), 5.35 (s, 2H, C4-CH_2 of quinolinone), 6.53–7.72 (m, 7H, Ar–H), 8.94 (s, 1H, NH), 12.75 (s, 1H, OH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.70, 162.12, 159.18, 156.34, 154.28, 136.89, 134.10, 132.10, 127.08, 124.60, 120.40, 116.30, 116.10, 113.10, 111.64, 108.18, 65.42, 25.32, 18.30 ppm; EI-MS: m/z = 324 (M + H).

General procedure for the synthesis of compounds ***6a–6e***

A mixture of 4-[(4-acetyl-3-hydroxyphenoxy)methyl]-8-methylquinolin-2(1*H*)-one **5d** (4.0 mmol), the appropriate substituted 4-(bromomethyl)coumarin (4.0 mmol), and anhydrous potassium carbonate (8.0 mmol) in 20 cm^3 dry ethanol was refluxed on a water bath for 14 h. The separated solid was filtered, washed with 20% HCl and with an excess of cold water, dried, and crystallized from a suitable solvent.

8-Methyl-4-[[3-methyl-2-(6-methyl-2-oxo-2H-chromen-4-yl)benzofuran-6-yloxy]methyl]quinolin-2(1H)-one
(**6a**, $C_{30}H_{23}NO_5$)
Yield 58%; colorless crystals (acetic acid); m.p.: 270–272 °C; IR (KBr): $\bar{\nu}$ = 3,430 (N–H stretching), 1,722 (C=O stretching, lactone), 1,665 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.42 (s, 3H, C3-CH_3 of benzofuran), 2.51 (s, 3H, C6-CH_3 of coumarin), 2.60 (s, 3H, C8-CH_3 of quinolinone), 5.35 (s, 2H, C4-CH_2 of quinolinone), 6.53–7.72 (m, 11H, Ar–H), 9.23 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.44, 160.22, 159.18, 158.90, 156.34, 154.28, 138.87, 136.89, 134.10, 132.10, 130.30, 128.83, 127.08, 125.87, 124.60, 123.45, 120.56, 119.40, 116.30, 116.12, 115.54, 114.10, 111.65, 110.90, 108.10, 63.40, 23.12, 21.12, 18.90 ppm; EI-MS: m/z = 478 (M + H).

8-Methyl-4-[[3-methyl-2-(7-methyl-2-oxo-2H-chromen-4-yl)-benzofuran-6-yloxy]methyl]quinolin-2(1H)-one
(**6b**, $C_{30}H_{23}NO_5$)
Yield 68%; colorless crystals (acetic acid); m.p.: 252–254 °C; IR (KBr): $\bar{\nu}$ = 3,433 (N–H stretching), 1,727 (C=O stretching, lactone), 1,675 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.47 (s, 3H, C3-CH_3 of benzofuran), 2.54 (s, 3H, C7-CH_3 of coumarin), 2.62 (s, 3H, C8-CH_3 of quinolinone), 5.42 (s, 2H, C4-CH_2 of quinolinone), 6.60–7.78 (m, 11H, Ar–H), 9.33 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.40, 162.12, 159.88, 158.90, 156.34, 154.28, 138.87, 136.80, 134.10, 132.10, 131.30, 128.83, 127.08, 125.87, 124.60, 123.45, 121.56, 119.40, 118.30, 116.12, 115.54, 114.10, 111.65, 110.90, 109.10, 64.40, 24.12, 21.10, 18.92 ppm; EI-MS: m/z = 478 (M + H).

8-Methyl-4-[[2-(6-chloro-2-oxo-2H-chromen-4-yl)-3-methylbenzofuran-6-yloxy]methyl]quinolin-2(1H)-one
(**6c**, $C_{29}H_{20}ClNO_5$)
Yield 63%; colorless crystals (acetic acid); m.p.: 240–242 °C; IR (KBr): $\bar{\nu}$ = 3,428 (N–H stretching), 1,730 (C=O stretching, lactone), 1,662 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.63 (s, 3H, C3-CH_3 of benzofuran), 2.64 (s, 3H, C8-CH_3 of quinolinone), 5.46 (s, 2H, C4-CH_2 of quinolinone), 7.28–7.75 (m, 11H, Ar–H), 11.49 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.40, 160.12, 159.00, 158.90, 156.34, 154.28, 138.87, 135.80, 134.10, 132.10, 131.30, 128.83, 127.08, 125.87, 124.60, 123.45, 121.56, 120.40, 118.30, 116.12, 115.54, 114.10, 111.65, 110.90, 108.10, 65.40, 24.12, 18.92 ppm; EI-MS: m/z = 498 (M + H).

8-Methyl-4-[[3-methyl-2-(3-oxo-3H-naphtho[2,1-b]pyran-1-yl)benzofuran-6-yloxy]methyl]quinolin-2(1H)-one
(**6d**, $C_{34}H_{23}NO_5$)
Yield 62%; colorless crystals (acetic acid); m.p.: 248–250 °C; IR (KBr): $\bar{\nu}$ = 3,425 (N–H stretching), 1,718 (C=O stretching, lactone), 1,659 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.54 (s, 3H, C3-CH_3 of benzofuran), 2.66 (s, 3H, C8-CH_3 of quinolinone), 5.48 (s, 2H, C4-CH_2 of quinolinone), 7.20–7.89 (m, 14H, Ar–H), 11.38 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 166.80, 161.10, 160.12,

158.98, 157.40, 156.30, 154.22, 144.09, 138.80, 135.18, 133.90, 132.10, 132.23, 128.86, 127.14, 125.80, 124.67, 123.55, 123.50, 122.60, 122.40, 120.41, 118.37, 117.45, 116.10, 115.50, 114.14, 111.75, 110.87, 109.20, 66.38, 24.10, 18.82 ppm; EI-MS: $m/z = 526$ (M+H).

8-Methyl-4-[[3-methyl-2-(2-oxo-2H-naphtho-[1,2-b]pyran-4-yl)benzofuran-6-yloxy]methyl]-quinolin-2(1H)-one (**6e**, $C_{34}H_{23}NO_5$)
Yield 60%; colorless crystals (acetic acid); m.p.: 260–262 °C; IR (KBr): $\bar{\nu} = 3{,}435$ (N–H stretching), 1,723 (C=O stretching, lactone), 1,675 (C=O stretching, amide) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.58$ (s, 3H, C3-CH_3 of benzofuran), 2.60 (s, 3H, C8-CH_3 of quinolinone), 5.55 (s, 2H, C4-CH_2 of quinolinone), 7.16–7.88 (m, 14H, Ar–H), 11.52 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta =$ 166.47, 161.00, 159.44, 158.76, 157.44, 156.37, 155.32, 144.10, 138.96, 135.78, 134.76, 130.70, 129.73, 128.66, 128.14, 125.71, 124.22, 124.10, 123.80, 123.60, 122.60, 120.11, 118.17, 117.67, 116.18, 115.54, 114.55, 111.95, 110.70, 109.25, 65.40, 24.50, 18.20 ppm; EI-MS: $m/z = 526$ (M + H).

Antimicrobial assay

The in vitro antimicrobial activity of the target compounds was tested against the two bacterial microorganisms *E. coli* (gram negative) and *B. cirrhosis* (gram positive) and the two fungal microorganisms *A. niger* and *R. bataticola*. DMF was used as a solvent control, and the reference drugs used were norfloxacin and griseofulvin. The tests were carried out by the cup-plate method [20–22], at concentrations of 100, 50, and 25 μg cm^{-3}. After 48 h of incubation at 37 °C the zone of inhibition was measured. The percentage inhibition of test compounds was calculated by relating the zone of inhibition of the test compound ($ZOI_{test\ compound}$) to those of the standard ($ZOI_{standard}$, taken as 100%) and control ($ZOI_{control}$) as follows:

$$\%\ \text{inhibition} = \frac{(ZOI_{\text{test compound}} - ZOI_{\text{control}})}{(ZOI_{\text{standard}} - ZOI_{\text{control}})} \times 100\%.$$

Acute toxicity

Groups of six albino mice weighing 20–25 g were fasted overnight and treated orally and intraperitoneally with the test compounds [23, 24]. The dose was varied from 1,000 to 100 mg kg^{-1} body weight. The animals were observed for 24 h for any signs of acute toxicity such as increased or decreased motor activity, tremors, convulsion, sedation, or lacrimation. No mortality of the animals was observed even after 24 h. Hence the LD_{50} cutoff value of the test compounds was fixed as 1,000 mg kg^{-1}. Thus, 100 mg kg^{-1}, i.e., 1/10 of the cutoff value, was taken as the screening dose for the evaluation of anti-inflammatory activity.

Analgesic activity

The analgesic activity of the test compounds was assessed in vivo using the abdominal constriction test induced by acetic acid 0.6% (0.1 cm^3/10 g) in mice [25]. Albino mice of both sexes (18–22 g) were used. Compounds were administered orally (10 mg kg^{-1}) as a suspension in 5% carbethoxymethyl cellulose (CMC, vehicle). Indomethacin (10 mg kg^{-1}) was used as the standard drug under the same conditions.

Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was assessed by the formalin-induced rat paw edema inhibition method according to Winter et al. [26], by employing 3.5% of formalin as the phlogistic agent. All test compounds were administered orally as suspensions in 2% CMC, 30 min before the injection of the phlogistic agent, at a dose of 100 mg kg^{-1} body weight. Indomethacin was used as a standard at a dose of 10 mg kg^{-1} body weight. Groups of six Sprague–Dawley rats of either sex were used in each experiment. Plain CMC (2%) served as a control. The paw edema volume was measured with the help of a plethysmograph by the mercury displacement method at 0 h (immediately after injection of formalin), 1, 2, 3, 4, and 5 h.

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