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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and PASS-assisted evaluation of coumarin–benzimidazole derivatives as potential anti-inflammatory and anthelmintic agents

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Abstract Two series of novel derivatives have been designed by coupling medicinally important coumarin and benzimidazole nuclei through different linkers. These compounds have been predicted to be potent anti-inflammatory and anthelmintic by in silico studies using PASS (prediction of activity spectra for substances) software. The compounds are synthesized and evaluated for the predicted activities as well as for their in vitro antioxidant potential. Compounds of first series (**4a–4f**) are found good to moderate anti-inflammatory agents. Among these, compounds **4b** and **4f** exhibited maximum anti-inflammatory activity (45% inhibition), which is equivalent to the activity of indomethacin (48% inhibition) after 3 h (peak inflammatory response time). Compounds of second series (**5a–5f**) exhibit anthelmintic activity. Amongst these, compound **5f** has mortality activity marginally higher than albendazole (10–11 s). Compound **5e** is found to be the most potent antioxidant with remarkable EC_{50} value (0.08 µM/mL), which is though a little less than that of ascorbic acid (0.03 µM/mL). In addition, a comparative analysis of calculated Lipinski's parameters reveals that all test compounds have the propensity to be orally bioavailable. Based on these findings, compounds **4b**, **4f**, **5e**, and **5f** are identified as new leads to develop potent anti-inflammatory, anthelmintic, and antioxidant compounds.

Keywords Coumarin · Benzimidazole · Anti-inflammatory · Anthelmintic · Antioxidant

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Abbreviations

PASS	Prediction of activity spectrum of substances
OPD	o-Phenylenediamine
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DCU	Dicyclohexylurea
NCEs	Novel chemical entities
SCMC	Sodium carboxy methyl cellulose
DPPH	2,2-Diphenyl-1-picrylhydrazyl
BHT	Butylhydroxytoluene

Introduction

Benzimidazole nucleus is an integral structural component of many drugs belonging to different therapeutic categories such as anthelmintics (albendazole, mebendazole, and thiabendazole), proton pump inhibitors (omeprazole, lansoprazole, and pantoprazole), antihistaminic (astemizole), antiviral (enviradine), and antihypertensive (candesarten and telmisartan). An amino group at 2-position of benzimidazole generates a rigid form of guanidine, which is an indispensable pharmacophore for diverse therapeutic activities (Bansal and Silakari 2012). In addition, an aromatic moiety such as naphthyl, heteroaryl, *p*-nitrophenyl, or pyridyl at the same position is known to produce potent anthelmintics (Tsukamoto et al. 1980). Coumarin is another important heterocyclic system, which has been exploited to develop numerous therapeutic agents such as warfarin, phenprocoumon, and acenocoumarol as anticoagulant; armillarisin A as antibiotic; hymecromone as choleretic and

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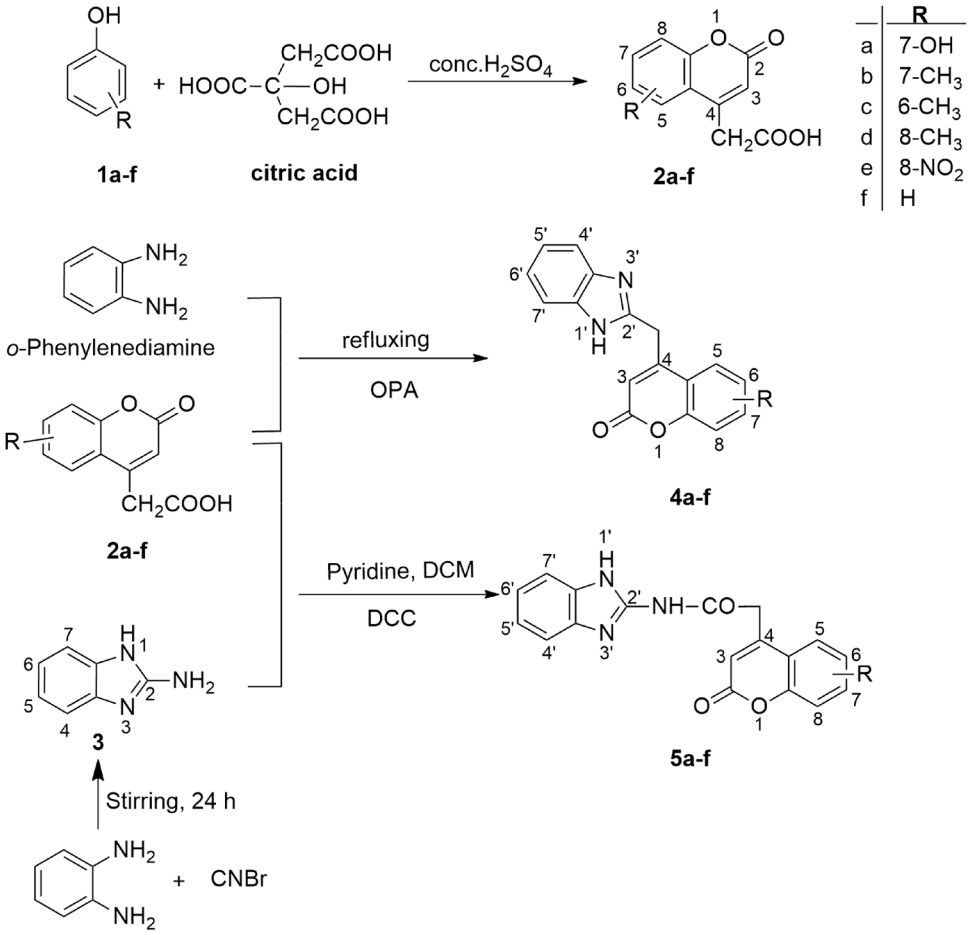
Scheme 1 Synthesis of test compounds of series **4** and **5**



antispasmodic; and cromakalim as antihypertensive (Wu et al. 2009). It is also explored for the development of anticancer (Riveiro et al. 2008), antimicrobial (Gormley et al. 1996; Manvar et al. 2011), anti-Alzheimer disease (Anand et al. 2012), antiviral (Curini et al. 2003), anti-hyperlipidemic (Madhavan et al. 2003), and antioxidant and anti-inflammatory (Bansal et al. 2013) agents. Hybridization of coumarin nucleus with other varied heterocyclics is being exploited to develop novel hybrid molecules that possess multiple pharmacological activities (Sandhu et al. 2014). Coumarin–benzimidazole hybrids are reported as potential antiviral and antitumour agents (Paul et al. 2013; Tsay et al. 2013). With an aim to further explore the potential of coumarin–benzimidazole hybrids as privileged medicinal scaffold, we have coupled variedly substituted coumarin nucleus with benzimidazole through a metabolizable or non-metabolizable linker to obtain two series of test compounds. The pharmacological activities spectrum of the test compounds was predicted by in silico method using prediction of activity spectrum of substances (PASS) software, and the compounds were evaluated for most likely pharmacological activities.

Results and discussion

Chemistry

The test compounds were synthesized by a four step synthetic scheme (Scheme 1). In first step, a substituted phenol (**1a–1f**) was condensed with citric acid to produce the corresponding coumarin-4-acetic acid derivative (**2a–2f**) as an intermediate. The other intermediate, 2-aminobenzimidazole (**3**), was synthesized by reactions between *o*-phenylendiamine and cyanogen bromide (CNBr). For synthesis of first series of test compounds (**4a–4f**), each of the intermediates **2a–2f** was refluxed with *o*-phenylendiamine for 3–6 h in the presence of orthophosphoric acid (OPA) as catalyst. For the second series of test compounds, **5a–5f**, each of the intermediates **2a–2f** was coupled with **3** under anhydrous conditions using dicyclohexylcarbodiimide (DCC) as coupling agent. All intermediates and test compounds in the scheme were purified by recrystallization from aqueous ethanol, and their structures were ascertained by infrared (IR), ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and high resolution mass spectral (HRMS) analyzes. Disappearance of stretching

Table 1 PASS predicted activity of test compounds

Compounds	Probability of a compound being active (P_a) or inactive (P_i)									
	Anthelmintic		Anti-inflammtory		Antineoplastic		Anti-ischemic		Kidney function stimulant	
	P_a	P_i	P_a	P_i	P_a	P_i	P_a	P_i	P_a	P_i
4a	0.547	0.013	0.688	0.051	0.712	0.020	0.624	0.043	0.732	0.016
4b	0.475	0.027	0.677	0.057	0.702	0.022	0.605	0.049	0.707	0.025
4c	0.440	0.039	0.695	0.048	0.721	0.019	0.601	0.050	0.728	0.017
4d	0.482	0.025	0.694	0.048	0.681	0.027	0.605	0.049	0.726	0.018
4e	–	–	0.621	0.091	0.660	0.032	–	–	0.637	0.070
4f	–	–	0.720	0.036	0.734	0.017	0.634	0.040	0.721	0.019
5a	0.797	0.003	–	–	0.707	0.021	–	–	0.686	0.036
5b	0.752	0.003	–	–	0.697	0.023	–	–	0.657	0.054
5c	0.757	0.003	–	–	0.715	0.020	–	–	0.681	0.039
5d	0.725	0.004	–	–	0.677	0.028	–	–	0.679	0.040
5e	0.654	0.005	–	–	0.657	0.033	–	–	0.633	0.073
5f	0.747	0.004	–	–	0.727	0.018	–	–	0.674	0.043

bands due to –COOH in IR spectra of compounds **4a–4f**, which were otherwise noted in IR spectra of coumarin carboxylic acids (**2a–2f**) ascertained that the latter is condensed with *o*-phenylenediamine to form benzimidazole–coumarin hybrids (**4**). Formation of compounds of series **5** was ascertained by the presence of a band in the range of 1630–1680 cm^{-1} corresponding to –CONH– linkage. In ^{1}H-NMR spectra, the –NH– protons were detected at δ values ranging from 4.5 to 5.0, which were confirmed by deuterium exchange experiments. Carbon atoms of benzene ring of coumarin moiety were detected as downfield or upfield signals depending upon the type of substituent present in the moiety. In HRMS analyzes, mass of parent ion [MH^+] of each compound was found to correspond to its theoretical mass.

In-silico studies

PASS predicted activity spectrum of test compounds

PASS predicted spectrum of activity of the test compounds (Table 1) showed that compounds **4a–4f** possess good probability of exhibiting anti-inflammatory activity (P_a values 0.621–0.720) whereas compounds **5a–5f** have propensity to act as good anthelmintics (P_a values 0.654–0.797). In addition, compounds of series **4** were also predicted to exhibit anti-ischemic activity (0.601–0.634) whereas compounds of series **5** were predicted to be anticancer (0.657–0.734) and kidney function stimulant (0.633–0.732).

Molecular properties calculations

Magnitude of all calculated Lipinski's parameters were within the acceptable range (Table 2), and hence as per Lipinski's rule of five, the compounds were suggested to be orally bioavailable. Total polar surface area (TPSA) is another parameter for optimizing ability of a drug to permeate cells. Molecules with polar surface area not greater than 140 Å sq can easily permeate cells, and all test compounds have TPSA less than 140. Therefore the compounds are predicted to have good cell membrane permeability.

Biological assay

Anti-inflammatory activity

Based on PASS prediction data, compounds **4a–4f** were evaluated for anti-inflammatory activity. All compounds exhibited moderate to good activity (35–45% inhibition of rat paw edema) compared to indomethacin (47% inhibition) (Table 3). The peak anti-inflammatory effect was observed after 3 h of carageenan injection. Compounds **4b** and **4f** were maximally active (45% inhibition), and equipotent to indomethacin (48% inhibition). Comparative evaluation of activity data of all six compounds in the series suggested that the activity is independent of the electronic effects of the substituents present on coumarin nucleus.

Table 2 Calculated Lipinski's parameters and TPSA of test compounds

Compounds	Log P[a]	TPSA[b]	MW[c]	nON[d]	nOHNH[e]	n viol.[f]
4a	3.00	79.12	292.30	5	2	0
4b	3.92	58.89	290.32	4	1	0
4c	3.92	58.89	290.32	4	1	0
4d	3.90	58.89	290.32	4	1	0
4e	3.41	104.71	321.29	7	1	0
4f	3.50	58.89	276.30	4	1	0
5a	2.14	108.22	321.29	7	3	0
5b	3.07	87.99	319.32	6	2	0
5c	3.07	87.99	319.32	6	2	0
5d	3.07	87.99	319.32	6	2	0
18	2.64	133.81	364.31	9	2	0
5f	2.65	87.99	305.29	6	2	0

[a] Calculated lipophilicity

[b] Total polar surface area

[c] Molecular weight

[d] Number of hydrogen bond acceptor

[e] Number of hydrogen bond donors

[f] Number of violations from Lipinski's rule of five

Anthelmintic activity

Earthworms are used for evaluation of anthelmintic activity as they resemble, both anatomically and physiologically, with the intestinal roundworm parasites in human beings. All compounds of series **5** showed paralytic activity and mortality against the helminths in a dose dependent manner (Figs. 1 and 2, respectively). Compound **5f** was found to be the most potent paralytic agent (time for paralysis 14 min) from the series, and equipotent to albendazole (time for paralysis 15 min) at a concentration of 0.1%. Also, its mortality activity was marginally greater than the activity of albendazole at all concentrations. The activity was in consonant with their PASS predicted scores that support the applicability of PASS in reliable prediction of activity spectrum of organic molecules.

Antioxidant activity

In vitro antioxidant activity of all compounds was evaluated as radical scavenging potential using DPPH method. A compound possessing antioxidant potential react with DPPH radical and decreases the color intensity due to the radical. The color intensity is measured spectrophotometrically and related to the radical scavenging activity. Kinetics of the reaction between test compound and DPPH revealed that absorbance plateau was attained at 30 min. Hence, 30 min was taken as optimum incubation

Table 3 Anti-inflammatory activity of the test compounds

Compounds	Paw volume (ml)[a]; % inhibition in paw volume[b]						
	0 (h)	0.5 (h)	1.0 (h)	2.0 (h)	3.0 (h)	4.0 (h)	6.0 (h)
4a	$0.09 \pm 0.008^{c,d}$; 25	0.16 ± 0.009^{c}; 27	$0.20 \pm 0.009^{c,d}$; 29	$0.22 \pm 0.012^{c,d}$; 31	$0.25 \pm 0.007^{c,d}$; 40	$0.22 \pm 0.010^{c,d}$; 35	$0.18 \pm 0.006^{c,d}$; 28
4b	0.11 ± 0.006; 8	0.16 ± 0.012^{c}; 27	0.18 ± 0.009^{c}; 36	$0.22 \pm 0.005^{c,d}$; 31	0.23 ± 0.005^{c}; 45	0.21 ± 0.008^{c}; 38	$0.17 \pm 0.008^{c,d}$; 32
4c	0.10 ± 0.005; 17	$0.14 \pm 0.008^{c,d}$;36	$0.21 \pm 0.007^{c,d}$; 25	$0.26 \pm 0.009^{c,d}$; 19	$0.25 \pm 0.007^{c,d}$; 41	$0.18 \pm 0.008^{c,d}$; 47	0.16 ± 0.009^{c}; 36
4d	0.11 ± 0.007; 8	$0.15 \pm 0.007^{c,d}$;31	0.19 ± 0.009^{c}; 32	0.20 ± 0.010^{c}; 38	$0.26 \pm 0.007^{c,d}$; 38	$0.26 \pm 0.004^{c,d}$; 23	0.22 ± 0.012; 12
4e	$0.09 \pm 0.007^{c,d}$; 25	0.16 ± 0.008^{c}; 27	$0.20 \pm 0.009^{c,d}$; 29	$0.23 \pm 0.007^{c,d}$; 28	$0.27 \pm 0.008^{c,d}$; 36	$0.23 \pm 0.008^{c,d}$; 32	$0.22 \pm 0.012^{c,d}$; 12
4f	$0.10 \pm 0.009^{c,d}$; 17	$0.15 \pm 0.108^{c,d}$;31	0.18 ± 0.007^{c}; 36	$0.22 \pm 0.011^{c,d}$; 31	0.23 ± 0.008^{c}; 45	$0.23 \pm 0.009^{c,d}$;32	$0.22 \pm 0.012^{c,d}$; 12
Control	0.12 ± 0.008	0.22 ± 0.008	0.28 ± 0.009	0.32 ± 0.010	0.42 ± 0.017	0.34 ± 0.012	0.25 ± 0.008
Standard	0.12 ± 0.005^{c}	0.17 ± 0.005^{c}; 22	0.18 ± 0.005^{c}; 36	0.19 ± 0.005^{c}; 41	0.22 ± 0.005^{c}; 48	0.20 ± 0.005^{c}; 41	0.16 ± 0.007^{c}; 36

Data is statistically analyzed using one way ANNOVA followed by multiple comparison (tukey) test

[a] Values are expressed as mean ± SD ($n = 6$)

[b] With respect to control

[c] Values are significantly different from control at $p < 0.05$

[d] Values are significantly different from indomethacin at $p < 0.05$

time for evaluating antioxidant activity of the compounds. All test compounds decreased the color intensity of DPPH solution, which suggested that the compounds possess radical scavenging ability. Compounds **5a–5f** were found better radical scavenger than compounds **4a–4f** (Table 4). Compound **5e** was more potent (EC_{50} 0.08 µM/mL) than BHT (EC_{50} 23.4 µM/mL), and less potent than ascorbic acid (EC_{50} 0.03 µM/mL). The results revealed that an electron withdrawing group increases the antioxidant potential, probably be increasing electrophilicity of the amidic nitrogen atom.

Conclusions

Two series of coumarin–benzimidazole hybrids (**4** and **5**) were designed and screened for pharmacological activities using PASS software, and structure activity relation (SAR) is proposed as shown in Fig. 3. The compounds were synthesized through a four-step reaction scheme. Based on the in silico studies, compounds of series **4** were evaluated for anti-inflammatory activity whereas those of series **5** were evaluated for anthelmintic activity. All compounds were also evaluated for in vitro antioxidant activity. Compounds **4b** and **4f** emerged as the most potent anti-inflammatory compounds, and equipotent to indomethacin. Compound **5f** was found to exhibit paralytic activity equivalent to albendazole and mortality marginally greater than albendazole. All compounds also exhibited moderate to excellent free radical scavenging activity. These findings support the hypothesis that coupling of two different pharmacophores can produce a single hybrid molecule that exhibits dual/multiple pharmacological activities. The most active compounds from these series (**4b**, **4f**, **5e**, and **5f**) can be taken as lead for design and development of novel, potent and safe anti-inflammatory and anthelmintic medicinal agents.

Materials and methods

The reactions were monitored by thin layer chromatography using silica-gel precoated aluminum plates (Merck, Germany) visualized in ultraviolet (UV) chamber at short and long wavelengths. Melting points were recorded with open capillary method, and were uncorrected. ^{1}H-NMR were

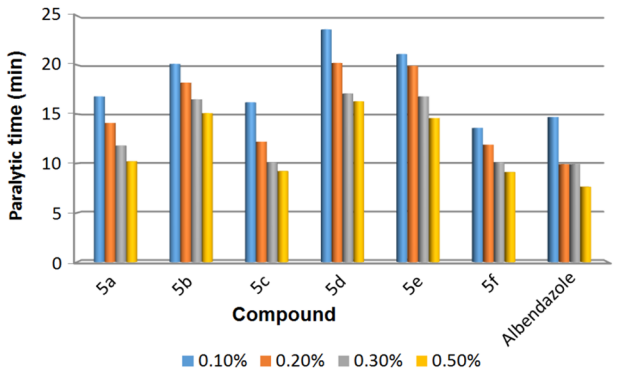


Fig. 1 Anthelmintic activity of compounds **5a–5f** as time of paralysis. Values are expressed as mean ± SD ($n = 6$)

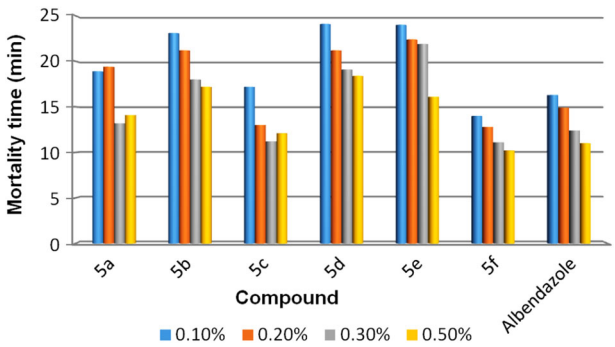


Fig. 2 Anthelmintic activity of compounds **5a–5f** as time of death. Values are expressed as mean ± SD ($n = 6$)

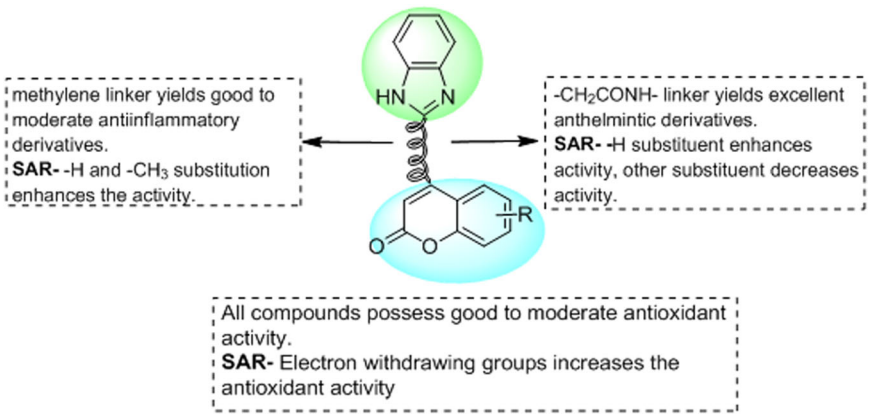


Fig. 3 Proposed SAR of the coumarin–benzimidazole coupled derivatives for varied activities

Table 4 Antioxidant activity of the test compounds

Compounds	EC_{50} (μM/mL)
4a	1.75
4b	4.42
4c	3.98
4d	4.13
4e	0.17
4f	2.24
5a	1.02
5b	3.01
5c	2.80
5d	2.42
5e	0.08
5f	1.80
BHT	23.4
Ascorbic acid	0.03

recorded in dimethyl sulfoxide (DMSO)-d_6 on Bruker Avance II, 400 MHz NMR spectrophotometer. For ^{13}C-NMR spectra, the instrument was operated at 100 MHz. Chemical shifts were reported as δ using tetramethylsilane as internal standard with multiplicities mentioned (br, broad; s, singlet; d, doublet; m, multiplet; dd, double doublet), and number of protons. Coupling constants (J) were expressed in Hz. Infrared spectra were recorded using KBr disks on a Perkin Elmer Fx IR Spectrophotometer. High resolution mass spectral analysis was performed on microTOF-Q11 mass spectrometer (Bruker Daltonics GmbH, Germany).

Albino rats (150–200 g) of either sex were used for evaluation of anti-inflammatory activity. The animals were housed in cages at room temperature 27 ± 3 °C with a 12 h light/dark cycle, and were allowed food and water ad libitum. These were randomly allocated into control, standard, and test groups. The study was approved by institutional animal ethical committee under CPCSEA guidelines. Red earthworms *Pheretima posthuma* were procured from a vermipost, located in village Jagatpur (Chandigarh, India), and were got authenticated from the Department of Zoology, Punjabi University, Patiala.

Chemistry

*Synthesis of coumarin-4-acetic acid analogs (**2a–2f**)*

Coumarin-4-acetic acid is a known compound (Manwar et al. 2008). The reaction conditions reported for this compound were modified and optimized (method A) to synthesis the analogs **2a–2f**. These analogs were also synthesized by microwave assisted method (method B).

Method A A mixture of citric acid (0.02 mol) and sulfuric acid (0.03 mol) was stirred at room temperature for 30 min. The mixture was kept in boiling water bath to remove carbon monoxide (CAUTION: Fume Cupboard). As soon as the evolution of CO gas was slackened, the flask was removed from the bath, kept aside for 15 min or till the reaction mixture was free from CO bubbles. The contents were cooled to 10 °C, and (un)substituted phenol (0.02 mol) cooled to 10 °C was added drop wise. The reaction mixture was stirred at room temperature for 48 h, and poured over crushed ice. The precipitates were filtered and dissolved in saturated sodium bicarbonate solution. The solution was acidified to afford the intermediates **2a–2f**.

Method B A mixture of citric acid (0.02 mol), sulphuric acid (0.03 mol) and (un)substituted phenol (0.02 mol) was heated in a microwave oven at 10% power for 4 min. The reaction mixture was poured over crushed ice and processed similarly as in method A to obtain the desired product.

7-Hydroxycoumarin-4-acetic acid (**2a**) Yield 67% (method A), 78% (method B); m.p. 170 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3210 (Ar C–H), 2924 (Al C–H), 1710 (C=O), 1701 (C=O lactone), 1458 (C=C), 1130 (C–O), 1290 (C–OH); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 11.08 (1H, s, COOH), 7.41–6.98 (2H, m, H−5, and H-6), 6.69–6.23 (2H, m H-8, and H-3), 4.93 (1H, dd, OH), 3.02–2.89 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 171.5 (C=O), 160.2 (C-2), 112.5 (C-3), 152.4 (C-4), 150.5 (C-8a), 135.2 (C-6), 124.8 (C-5), 122.9 (C-7), 120.1 (C-4a), 104.1 (C-8), 37.0 (–CH_2). HRMS (+ESI) $[M + H]^+$: 221.0445 (theoretical), 221.0464 (found).

7-Methylcoumarin-4-acetic acid (**2b**) Yield 72% (method A), 88% (method B); m.p. 190 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3210 (Ar C–H), 2916 (Al C–H), 1710 (C=O), 1693 (C=O lactone), 1530 (C=C), 1120 (C–O), 1280 (C–OH); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.42–7.18 (3H, m, H-5, H-6, and H-8), 6.59–6.51 (1H, m, H-3), 2.98 (2H, s, CH_2), 2.44–2.41 (3H, m, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 171.5 (C=O), 160.8 (C-2), 154.6 (8a), 143.1 (C-7), 125.7 (C-6), 125.6 (C-5), 112.5 (C-3), 118 (C-4), 117.4 (C-8), 115 (C-4), 37.0 (–CH_2), 21.3 (CH_3). HRMS (+ESI) $[M + H]^+$: 219.0652 (theoretical), 219.0681 (found).

6-Methylcoumarin-4-acetic acid (**2c**) Yield 64% (method A), 74% (method B); m.p. 192 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3172 (Ar C–H), 2932 (Al C–H), 1705 (C=O), 1688 (C=O lactone), 1527 (C=C), 1155 (C–O), 1230 (C–OH); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 11.25 (1H, s, COOH), 7.46–7.22 (2H, m, H-5, and H-7), 6.92–6.21 (2H, m, H-8, and H-3), 3.02–2.88 (2H, m, CH_2), 2.39–2.34 (3H, m, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 171.2 (C=O),

160.8 (C-2), 155.4 (C-4), 150.5 (C-8a), 127.8 (C-7), 127.6 (C-5), 125.1 (C-6), 120.9 (C-4a), 116.9 (C-8), 112.5 (C-3), 37.0 (–CH_2), 21.7 (CH_3). HRMS (+ESI) $[M + H]^+$: 219.0652 (theoretical), 219.0676 (found).

8-Methylcoumarin-4-acetic acid (**2d**) Yield 52% (method A), 64% (method B); m.p. 182 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3261 (Ar C–H), 2944 (Al C–H), 1712 (C=O), 1700 (C=O lactone), 1554 (C=C), 1182 (C–O), 1224 (C–OH); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 11.23 (1H, m, COOH), 7.53–7.42 (2H, m, H−5, and H-7), 7.26–7.09 (2H, m, H-6, and H-3), 2.91–2.87 (2H, s, CH_2), 2.42–2.31 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 171.5 (C=O), 160.8 (C-2), 155.0 (C-4), 150.4 (C-8a), 132.8 (C-7), 126.3 (C-8), 125.6 (C-5), 125.3 (C-6), 120.9 (C-4a), 112.5 (C-3), 37.0 (–CH_2), 15.7 (CH_3). HRMS (+ESI) $[M + H]^+$: 219.0652 (theoretical), 219.0679 (found).

8-Nitrocoumarin-4-acetic acid (**2e**) Yield 72% (method A), 81% (method B); m.p. 212 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3215 (Ar C–H), 2921 (Al C–H), 1709 (C=O), 1689 (C=O lactone), 1516 (C=C), 1146 (C–O), 1229 (C–OH), 1509 (N=O); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 10.86 (1H, s, COOH), 7.92–8.40 (2H, m, H-5, and H-7), 6.35–7.52 (2H, m, H-6, and H-3), 2.90–2.92 (2H, dd, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 160.8 (C-2), 112.5 (C-3), 155.0 (C-4), 121.9 (C-4a), 134.7 (C-5), 126.3 (C-6), 126.5 (C-7), 142.6 (C-8), 145.9 (C-8a), 37.0 (–CH_2), 171.5 (C=O); HRMS (+ESI) $[M + H]^+$: 250.0346 (theoretical), 250.0371 (found).

Coumarin-4-acetic acid (**2f**) Yield 63% (method A), 68% (method B); m.p. 182 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3155 (Ar C–H), 2924 (Al C–H), 1710 (C=O), 1688 (C=O lactone), 1499 (C=C), 1187 (C–O), 1250 (C–OH); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 11.60 (1H, m, COOH), 7.65–7.49 (2H, m, H-5, and H-7), 7.32–6.39 (3H, m, H-3, H-6, and H-8), 3.10 (2H, s, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 170.9 (C=O), 161.0 (C-2), 155.4 (C-4), 150.0 (C-8a), 137.7 (C-6), 128.5 (C-7), 128.2 (C-5), 128.1 (C-4a), 118.1 (C-8), 111.8 (C-3), 37.0 (–CH_2); HRMS (+ESI) $[M + H]^+$: 205.0496 (theoretical), 205.0513 (found).

Synthesis of 2-aminobenzimidazole ***3***

The intermediate **3** was synthesized by using the method as reported by Leonard et al (1947). Briefly, solutions of cyanogen bromide (0.03 mol) and *o*-phenylenediamine (0.02 mol), prepared separately in 25 mL of 50% aqueous methanol, were mixed in a 250 mL conical flask and stirred at room temperature for 24 h. Thereafter, methanol was recovered under vacuum on water bath. The remaining solution was cooled to room temperature and made alkaline with aqueous ammonia. Compound **3** was separated as precipitates, which were recrystallized as beige colored crystals from ethanol-water mixture. Yield: 88%; m.p. 234 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3434 (N–H), 3116 (Ar C–H), 1636 (C=N), 1483 (N–H), 1451 (C=C), 1239 (C–N), 846 (N–H), 719 (C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.22 (2H, dd, H-7, and H-4), 6.9 (2H, dd, H-6, and H-5), 5.4 (1H, br, NH), 4.8 (2H, br, NH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164.3 (C-2), 143.6 (3a′), 115.5 (C-4), 124.8 (C-5), 123.6 (C-6), 110.6 (C-7), 148.4 (C-7a′); HRMS (+ESI) $[M + H]^+$: 134.0713 (theoretical), 134.0728 (found).

Synthesis of test compounds ***4a–4f***

A synthetic method reported by Li et al (2006) was taken as lead to synthesize these compounds. In general, each of the intermediates **2a–2f** (0.002 mol) was refluxed with *o*-phenylenediamine (0.002 mol) at 200–250 °C for 3–6 h in the presence of OPA (5 mL). After completion of reaction, the mixture was poured in to 100–150 mL of cold water, and the compound was separated by addition of ammonia solution, which was recrystallized from hot aqueous ethanol mixture.

4-[(Benzimidazol-2-yl)methyl]-7-hydroxy coumarin (**4a**) Yield 59%; m.p. 233 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3192 (Ar C–H), 2911 (Al C–H), 1705 (C=O), 1608 (C=N), 1455 (Ar C=C), 1390 (C–OH), 1138 (C–O), 1270 (C–N), 744 (C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.73–7.74 (2H, m, H-4′, and H-7′), 7.42–7.36 (2H, m, H-5, and H-6), 7.06–7.04 (2H, M, H-5′, and H-6′), 7.03–6.92 (2H, m, H-2, and H-8), 4.68–4.81 (2H, m, OH, and H-1′), (2H, s, CH_2), 3.28–3.21; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 159.9 (C-2), 159.1 (C-7), 155.6 (C-4), 154.2 (C-8a), 151.9 (C-2′), 151.7 (C-7a′), 141.5 (C-3a′), 125.8 (C-5), 124.8 (C-5′), 123.6 (C-6′), 119.1 (C-4′), 116.1 (C-4a), 115.3 (C-6), 113.4 (C-3), 110.9 (C-7′), 104.0 (C-8), 40.4 (CH_2); HRMS (+ESI) $[M + H]^+$: 293.0921 (theoretical), 293.0946 (found).

4-[(Benzimidazol-2-yl) methyl]-7-methyl coumarin (**4b**) Yield 64%; m.p. 229 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3079 (Ar C–H), 2937 (Al C–H), 1726 (C=O), 1603 (Ar C=N), 1477 (Ar C=C), 1475 (C–O), 1272 (C–N), 1567 (N–H), 736 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.14–8.19 (m, 2H, H-4′, and H-7′), 7.81–7.84 (m, 2H, H-5, and H-7), 7.51–7.62 (m, 2H, H-5′, and H-6′), 7.21–6.79 (m, 2H, H-3, and H-8), 4.62 (s, 1H, NH), 3.34 (m, 2H, CH_2), 2.26–2.29 (m, 3H, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 161.3 (C-2), 111.4 (C-3), 155.6 (C-4), 119.9 (C-4a), 125.8 (C-5), 125.3 (C-6), 141.7 (C-7), 117.0 (C-8), 152.9 (C-8a), 21.0 (CH_3), 39.4 (CH_2), 152.4 (C-2′), 141.9 (C-3a′) 119.1 (C-4′), 124.6 (C-5′), 123.6 (C-6′), 111.0 (C-7′), 150.0 (7a′); HRMS

(+ESI) $[M + H]^+$: 291.1128 (theoretical), 291.1139 (found).

4-[(Benzimidazol-2-yl) methyl]-6-methyl coumarin (**4c**) Yield 69%; m.p. 231 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3079 (Ar C–H), 2937 (Al C–H), 1726 (C=O), 1603 (Ar C=N), 1477 (Ar C=C), 1475 (C–O), 1272 (C–N), 1567 (N–H), 736 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.19–8.14 (2H, m, H-4′, and H-7′), 7.84–7.81 (2H, m, H-5, and H-7), 7.62–7.51 (2H, m, H-5′, and H-6′), 7.21–6.79 (2H, m, H-3, and H-8), 4.62 (1H, s, NH), 3.34 (2H, m, CH_2), 2.29–2.26 (3H, m, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 160.8 (C-2), 155.0 (C-4), 152.4 (C-2′), 150.5 (C-8a), 150.1 (C-7a′), 140.9 (C-3a′), 135.3 (C-6), 131.7 (C-7), 126.8 (C-5), 124.2 (C-5′), 123.6 (C-6′), 121.4 (C-4a), 119.0 (C-4′), 116.0 (C-8), 112.5 (C-3), 110.3 (C-7′), 21.3 (CH_3); HRMS (+ESI) $[M + H]^+$: 291.1128 (theoretical), 291.1147 (found).

4-[(Benzimidazol-2-yl) methyl]-8-methyl coumarin (**4d**) Yield 47%; m.p. 236 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3054 (Ar C–H), 2918 (Al C–H), 1720 (C=O), 1621 (Ar C=N), 1452 (Ar C=C), 1488 (C–O), 1274 (C–N), 1537 (N–H), 743 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.19–8.14 (2H, m, H-4′, and H-7′), 7.84–7.81 (2H, m, H-5, and H-7), 7.62–7.51 (2H, m, H-5′, and H-7′), 7.21–6.79 (2H, m, H-3, and H-6), 4.71–4.62 (H, s, NH), 3.42–3.34 (2H, s, CH_2), 2.29–2.26 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 160.8 (C-2), 155.0 (C-4), 150.4 (C-8a), 139.9 (C-7a′), 141.5 (C-2′), 138.9 (C-3a′), 132.8 (C-7), 126.3 (C-8), 125.6 (C-5), 125.3 (C-6), 123.0 (C-5′), 123.0 (C-6′), 120.9 (C-4a), 115.2 (C-4′), 115.2 (C-7′), 112.5 (C-3), 40.0 (CH_2), 15.7 (CH_3); HRMS (+ESI) $[M + H]^+$: 291.1128 (theoretical), 291.1142 (found).

4-[(Benzimidazol-2-yl) methyl]-8-nitro coumarin (**4e**) Yield 76%; m.p. 239 °C; IR (FT-IR) ν_{max} (cm^{-1}): 2997 (Ar C–H), 2885 (Al C–H), 1761 (C=O), 1640 (Ar C=N), 1508 (C–O), 1509 (C–N=O), 1450 (Ar C=C), 1296 (C–N), 812 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.44–8.03 (2H, m, H-4′, and H-7′); 7.98–7.75 (2H, m, H-5, and H-7), 7.56–7.01 (2H, m, H-5′, and H-6′), 7.02–6.78 (2H, m, H-3, and H-6), 3.42 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 160.8 (C-2), 155.0 (C-4), 145.9 (C-8a), 142.6 (C-8), 141.5 (C-2′), 138.9 (C-7a′), 138.9 (C-3a′), 134.7 (C-5), 126.5 (C-7), 126.3 (C-6), 123.0 (C-5′), 123.0 (C-6′), 121.9 (C-4a), 115.2 (C-4′), 115.2 (C-7′), 112.5 (C-3), 40.0 (CH_2); HRMS (+ESI) $[M + H]^+$: 322.0822 (theoretical), 322.0849 (found).

4-[(Benzimidazol-2-yl) methyl] coumarin (**4f**) Yield 77%; m.p. 219 °C; IR (FT-IR) ν_{max} (cm^{-1}): 2991 (Ar C–H), 2845 (Al C–H), 1766 (C=O), 1642 (Ar C=N), 1458 (Ar C=C), 1510 (C–O),1503 (N–H), 1278 (C–N), 810 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.79–7.76 (2H, m, H-4′, and H-7′), 7.64–7.43 (2H, m, H-5, and H-7), 7.27–7.26 (2H, m, H-5′, and H-6′), 7.24–7.10 (2H, m, H-6, and H-8), 5.10 (1H, s, NH), 3.16 (2H, s, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 160.8 (C-2), 155.4 (C-4), 153.2 (C-8a), 152.1 (C-2′), 151.2 (C-7a′), 140.9 (C-3a′), 128.1 (C-5), 128.1 (C-7), 126.4 (C-5′), 126.3 (C-6′), 125.3 (C-6), 121.1 (C-4a), 116.1 (C-4′), 115.0 (C-8), 112.1 (C-3), 109.5 (C-7′), 39.8 (CH_2); HRMS (+ESI) $[M + H]^+$: 277.0972 (theoretical), 277.0995 (found).

*Synthesis of test compounds **5a–5f***

The reaction conditions reported by Montalbetti et al. (2005) to synthesize similar kind of compounds were modified and optimized to synthesize each of these test compounds using DCC as a coupling agent. In general, a suspension of each of the intermediate **2a–2f** (0.01 mol) and DCC (0.01 mol) in 100 mL of dried dichloromethane (DCM) was vigorously stirred under nitrogen for 30 min. A solution of **3** (0.01 mol) in dried DCM (30 mL) and freshly distilled pyridine (50 mL) along with 4-dimethylaminopyridine (DMAP) (4×10^{-4} mol) was added to the stirred reaction mixture. The contents were maintained at 0 °C for 15 min, than stirred at 0 °C for 2 h followed by stirring at room temperature for 12 h. Dicyclohexylurea (DCU) was filtered, and the filtrate was dried in vacuum to yield solid residue. The latter was mixed and stirred with dried ethyl acetate with heating on water bath. The ethyl acetate soluble portion was filtered, washed with distilled water, dried over magnesium sulfate, and evaporated under vacuum. The resulting crude solid was recrystallized from methanol to yield the test compound.

4-[(Benzimidazol-2-yl) amino carbonyl methyl] 7-hydroxy coumarin (**5a**) Yield 77%; m.p. 219 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3326 (N–H), 3036 (Ar C–H), 2929 (Al C–H), 1650 (C=O), 1462 (C–O), 1436 (Ar C=C), 1572 (Ar C–N=O), 1650 (CO–NH), 1219 (C–OH), 740 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.44–7.42 (2H, m, H-4′, and H-7′), 7.32–7.28 (2H, m, H-5, and H-6), 7.10–7.09 (2H, m, H-5′, and H-6′), 7.09–7.00 (2H, m, H-3, and H-8), 4.84 (2H, s, NH, and OH), 2.82–2.95 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164.0 (C=O), 160.8 (C-2), 158.1 (C-7), 155.0 (C-4), 154.7 (C-8a), 146.7 (C-2′), 136.6 (C-3a′), 126.2 (C-5), 123.0 (C-5′), 113.6 (C-4a), 112.6 (C-6), 112.5 (C-3), 115.2 (C-4′), 115.2 (C-6′), 102.0 (C-8), 43.1 (CH_2); HRMS (+ESI) $[M + H]^+$: 336.0979 (theoretical), 336.0994 (found).

4-[(Benzimidazol-2-yl) amino carbonyl methyl]-7-methyl coumarin (**5b**) Yield 55%; m.p. 287 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3327 (N–H), 3056 (Ar C–H), 2853 (Al C–H), 1726 (C=O), 1625 (CO–NH), 1462 (C–O), 1448 (Ar C=C), 787 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.94–7.89 (2H, m, H-7′, and H-4′), 7.79–7.76 (2H, m, H-6′, and H-5′), 7.09–7.00 (2H, m, H-3, and H-8), 7.07–7.05 (2H, m, H-5, and H-6), 4.69–4.57 (1H, m, NH), 2.89 (2H, m, CH_2), 2.24 (3H, m, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 165.4 (C=O), 160.6 (C-2), 154.9 (C-4), 153.8 (C-8a), 152.0 (C-2′), 149.0 (C-3a′), 143.8 (C-7a′), 143.8 (C-7), 112.7 (C-3), 118.1 (C-4a), 125.3 (C-6), 125.1 (C-5), 124.4 (C-6′), 122.4 (C-5′), 117.2 (C-8), 115.6 (C-7′), 110.5 (C-4′), 42.9 (CH_2), 21.3 (CH_3); HRMS (+ESI) $[M + H]^+$: 334.1186 (theoretical), 334.1203 (found).

4-[(Benzimidazol-2-yl) amino carbonyl methyl]-6-methyl coumarin (**5c**) Yield 64%; m.p. 290 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3327 (N–H), 3056 (Ar C–H), 2853 (Al C–H), 1726 (C=O), 1462 (C–O), 1448 (Ar C=C), 1672 (CO–NH), 742 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.84 (1H, s, NH), 7.56–7.49 (2H, m, H-7′, and H-4′), 7.47–7.32 (2H, m, H-5′, and H-6′), 7.19–7.10 (2H, m, H-5, and H-7), 7.05–6.95 (2H, m, H-3, and H-8), 4.96 (1H, s, NH), 2.89 (2H, s, CH_2) 2.39–2.34 (3H, m, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 165.1 (C=O), 161.0 (C-2), 154.9 (C-4), 153.8 (C-8a), 152.1 (C-2′), 148.9 (C-3a′), 143.2 (C-7a′), 135.3 (C-6), 131.8 (C-7), 127.1 (C-5), 124.3 (C-6′), 123.4 (C-5′), 120.1 (C-4a), 116.0 (C-8), 115.5 (C-7′), 112.1 (C-3), 110.1 (C-4′), 42.8 (CH_2), 21.9 (CH_3); HRMS (+ESI) $[M + H]^+$: 334.1186 (theoretical), 334.1211 (found).

4-[(Benzimidazol-2-yl) amino carbonyl methyl]-6-methyl coumarin (**5d**) Yield 39%; m.p. 282 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3300–3500 (N–H), 3038 (Ar C–H), 2933 (Al C–H),1682 (C=O), 1655 (CO–NH), 1457 (Ar C=C), 843 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 7.84–7.70 (m, 2H, H-4′, and H-7′), 7.51–7.43 (m, 2H, H-5′, and H-6′), 7.41–7.23 (m, 2H, H-5, and H-7), 7.11–6.93 (m, 2H, H-3, and H-6), 4.73 (s, 1H, NH), 2.50 (s, 2H, CH_2), 2.36–2.20 (m, 3H, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164 (C=O), 161.0 (C-2), 155 (C-4), 146.7 (C-2′), 145.9 (C-8a), 142.6 (C-8), 136.6 (C-7a′), 136.6 (C-3a′), 134.7 (C-5), 126.5 (C-7), 126.3 (C-6), 123 (C-5′), 123 (C-6′), 121.9 (C-4a), 115.2 (C-4′), 115.2 (C-7′), 112.1 (C-3), 43.1 (CH_2); HRMS (+ESI) $[M + H]^+$: 334.1186 (theoretical), 334.1211 (found).

4-[(Benzimidazol-2-yl) amino carbonyl methyl]-8-nitro-coumarin (**5e**) Yield 47%; m.p. 285 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3300–3500 (N–H), 3060 (Ar C–H), 2922 (Al C–H), 1713 (C=O), 1654 (CO–NH), 1512 (N=O), 1490 (C–O), 1419 (Ar C=C), 743 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.20–7.85 (2H, m, H-5, and H-7), 7.65–7.40 (2H, m, H-4′, and H-7′), 7.34–7.12 (3H, m, H-6, H-6′ and H-5′), 6.99–6.89 (1H, m, H-3), 5.13 (1H, s, NH), 2.01 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164.0 (C=O), 160.8 (C-2), 155 (C-4), 146.7 (C-2′), 145.9 (C-8a), 142.6 (C-8), 136.6 (C-3a′), 136.6 (C-7a′), 134.7 (C-5), 126.5 (C-7), 126.3 (C-6), 123.4 (C-5′), 123.3 (C-6′), 121.9 (C-4a), 115.2 (C-4′), 115.2 (C-7′), 112.5 (C-3), 43.1 (CH_2); HRMS (+ESI) $[M + H]^+$: 365.0881 (theoretical), 365.0902 (found).

4-[(Benzimidazol-2-yl) amino carbonyl methyl] coumarin (**5f**) Yield 66%; m.p. 288 °C; IR (FT-IR) ν_{max} (cm^{-1}): 3300–3500 (N–H), 3038 (Ar C–H), 2933 (Al C–H),1682 (C=O), 1655 (CO–NH),1457 (Ar C=C), 843 (Ar C–H); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 8.12 (1H, s, NH), 7.88–7.78 (2H, m, H-4′, and H-7′), 7.55–7.45 (2H, m, H-5, and H-7), 7.30–7.28 (2H, m, H-6, and H-8), 7.18–7.14 (2H, m, H-5′, and H-6′), 2.95–2.81 (2H, s, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 164.0 (C=O), 160.8 (C-2), 155 (C-4), 153.5 (C-8a), 146.7 (C-2′), 136.6 (C-3a′), 136.6 (C-7a′), 128.6 (C-5), 128.3 (C-7), 125.4 (C-6), 123 (C-5′), 123 (C-6′), 121 (C-4a), 116.3 (C-8), 115.2 (C-4′), 115.2 (C-7′), 112.5 (C-3), 43.1 (CH_2); HRMS (+ESI) $[M + H]^+$: 320.1030 (theoretical), 320.1052 (found).

In-silico studies

PASS predicted activity spectrum of test compounds

The biological activity of each test compound was predicted by the computer software PASS, which predicts the activity spectrum of a compound as probable activity (P_a) and probable inactivity (P_i) with accuracy of prediction as high as 85%. The activities having P_a of 0.5–0.7 for a compound are considered probable activities of the compound (Poroikov et al. 2000).

Molecular property calculations

Molecular properties such as lipophilicity (Log P), total polar surface area (TPSA), molecular weight (MW), hHydrogen bond acceptors (nON), hydrogen bond donors (nOHNH), number of violations (nviol), and number of rotatable bonds (nrotb) were calculated using molinspiration calculations software. These properties helped in prediction of intestinal absorption, blood brain barrier permeability, and oral bioavailability according to Lipinski's rule of 5 (Lipinski et al. 1997).

Biological assays

Anti-inflammatory activity

Anti-inflammatory activity of test compounds of series **4** was evaluated by carrageenan-induced rat paw edema method as reported by Winter et al. (1963). Edema was induced by sub-plantar injection of 0.1 mL of 1% carrageenan solution. The test compounds were administered i.p. as suspension in 0.5% sodium carboxy methyl cellulose (SCMC) 30 min prior to injection of carrageenan, at a dose equimolar to the standard drug. Indomethacin at a dose of 20 mg/kg was used as standard. SCMC (0.5%) was used as control. Volume of the paw was measured at 0, 0.5, 1, 2, 3, 4, and 6 h intervals (Fereidoni et al. 2000). Anti-inflammatory activity was calculated as percent inhibition of carrageenan induced paw edema using the following formula (Chu and Kovacs 1977).

Percent inhibition = 100−[(edema volume in treated animal/edema volume in control animal) × 100].

Anthelmintic activity

It was evaluated for each test compound of series **5** using the method as described by Ajaiyeoba et al. (2001). The worms were divided into different groups with six earthworms in each group, and washed with normal saline. The target compounds were evaluated at different concentrations (0.1, 0.2, 0.3, and 0.5% w/v) taking albendazole as standard drug. The helminthes were placed in a solution of each compound at each concentration in Petri plates. The time taken for worms to become motionless was noted as paralysis time, whereas the time taken for death of the worm was noted as lethal time. To ascertain death, each worm was frequently subjected to external stimuli that stimulate and induce movement in the worms, if alive. The mean paralysis time and mean lethal time were calculated for each compound at each concentration.

Antioxidant activity

It was evaluated as hydrogen donating or radical scavenging ability of the compound using DPPH method taking BHT and ascorbic acid as standard drugs (Locatelli et al. 2009). Briefly, a 700 µl solution of each test compound in methanol was mixed with the same volume of DPPH solution (100 µM in methanol). Mixture was shaken vigorously, allowed to stand in dark at room temperature for 30 min and absorbance was noted at 515 nm. For control reading, the test compound solution was replaced with methanol, and for standard reading, it was replaced with solutions of BHT or ascorbic acid. Antiradical activity was expressed as percent inhibition (I%) and calculated using the following equation: $[(Abs_{Control}-Abs_{Test})/Abs_{Control}] \times 100$. Different concentrations of each compound and standard were used in order to obtain calibration curves. Antioxidant activity of each compound and standard drug was evaluated in triplicate and EC_{50} values were reported as mean ± SD.

Compliance with ethical standards

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

References

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001) In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extract. Pharm Biol 39:217–220

Anand P, Singh B, Singh N (2012) A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. Bioorg Med Chem 20:1175–1180

Bansal Y, Sethi P, Bansal G (2013) Coumarin: a potential nucleus for anti-inflammatory molecules. Med Chem Res 22:3049–3060

Bansal Y, Silakari O (2012) The therapeutic journey of benzimidazoles: a review. Bioorg Med Chem 20:6208–6236

Chu D, Kovacs B (1977) Anti-inflammatory activity in oak gall extracts. Arch Int Pharmacodyn Ther 230:166–176

Curini M, Epifano F, Maltese F, Marcotullio MC, Gonzales SP, Rodriguez JC (2003) Synthesis of *Collinin*, an antiviral coumarin. Aust J Chem 56:59–60

Fereidoni M, Ahmadiani A, Semnanian S, Javan M (2000) An accurate and simple method for measurement of paw edema. J Pharmacol Toxicol Methods 43:11–14

Gormley NA, Orphanides G, Meyer A, Cullis PM, Maxwell A (1996) The interaction of coumarin antibiotics with fragments of the DNA Gyrase B protein. Biochemistry 35:5083–5092

Leonard NL, Daviy CY, Karl BM (1947) Sulfonate salts of substituted benzimidazoles. J Med Chem 69:2459–2461

Li YF, Wang GF, He PL, Huang WG, Zhu FH, Gao HY, Tang W, Luo Y, Feng CL, Shi LP (2006) Synthesis and anti-hepatitis B virus activity of novel benzimidazole derivatives. J Med Chem 49:4790–4794

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 23:3–25

Locatelli M, Gindro R, Travaglia F, Coïsson JD, Rinaldi M, Arlorio M (2009) Study of the DPPH-scavenging activity: development of a free software for the correct interpretation of data. Food Chem 114:889–897

Madhavan GR, Balraju V, Mallesham B, Chakrabarti R, Lohray VB (2003) Novel coumarin derivatives of heterocyclic compounds as lipid-lowering agents. Bioorg Med Chem Lett 13:2547–2551

Manvar A, Bavishi A, Radadiya A, Patel J, Vora V, Dodia N, Rawal K, Shah A (2011) Diversity oriented design of various hydrazides and their in-vitro evaluation against *Mycobacterium tuberculosis* $H37_{RV}$ strains. Bioorg Med Chem Lett 21:4728–4731

Manwar A, Malde A, Verma J, Virsodia V, Mishra A, Upadhyay K, Acharya H, Coutinho E, Shah A (2008) Synthesis, anti-tubercular activity and 3D-QSAR study of coumarin-4-acetic acid benzylidene hydrazides. Eur J Med Chem 43:2395–2403

Montalbetti Christian AGN, Falque V (2005) Amide bond formation and peptide coupling. Tetrahedron 61:10827–10852

Paul K, Bindal S, Luxami V (2013) Synthesis of new conjugated coumarin-benzimidazole hybrids and their anticancer activity. Bioorg Med Chem Lett 23:3667–3672

Poroikov VV, Filimonov DA, Borodina VY, Lagunin AA, Kos A (2000) Robustness of biological activity spectra predicting by computer program PASS for non-congeneric sets of chemical compounds. J Chem Inf Comput Sci 40:1349–1355

Riveiro ME, Moglioni A, Vazquez R, Gomez N, Facorro G, Piehl L, de Celis ER, Shayo C, Davio C (2008) Structural insights into hydroxycoumarin-induced apoptosis in U-937 cells. Bioorg Med Chem 16:2665–2675

Sandhu S, Bansal Y, Silakari O, Bansal G (2014) Coumarin hybrids as novel therapeutic agents. Bioorg Med Chem 22:3806–3814

Tsay S, Hwu JR, Singh R, Huang W, Chang YH, Hsu M, Shieh F, Lin C, Hwang KC, Horng J, Clercq ED, Vliegen I, Neyts J (2013) Coumarins hinged directly on benzimidazoles and their ribofuranosides to inhibit hepatitis C virus. Eur J Med Chem 63:290–298

Tsukamoto G, Yoshino K, Kohono T, Ohtaka H, Kagaya H, Ito K (1980) Synthesis and anti-inflammatory activity of some 2-(substituted-pyridinyl) benzimidazole derivatives. J Med Chem 23:734–738

Winter CE, Risley EA, Nuss GW (1963) Anti-inflammatory and antipyretic activities of indomethacin, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid. J Pharmacol 3:369–376

Wu L, Wang X, Xu W, Farzaneh F, Xu R (2009) The structure and pharmacological functions of coumarin and their derivatives. Curr Med Chem 16:4236–4260