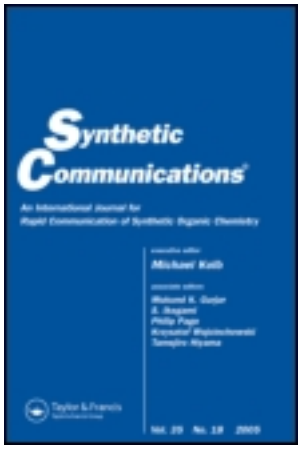

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LACCASE FROM *BASIDIOMYCETOUS* FUNGUS–CATALYZED SYNTHESIS OF SUBSTITUTED BENZOPYRANOCOUMARINS VIA DOMINO REACTION

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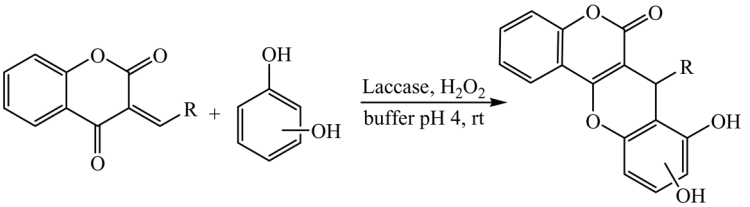
GRAPHICAL ABSTRACT



Abstract *The present investigation provides a simple and convenient route to organic synthesis of substituted benzopyranocoumarin, which is important because it is a probable HIV protease inhibitor. The reaction of α,β-unsaturated derivatives of coumarins with catechol or 1,4-hydroquinones was catalyzed using laccase in an aqueous medium. Quinones, generated in situ by the oxidation of the corresponding catechol or 1,4-hydroquinones, underwent a domino reaction with chalcones to produce benzopyranocoumarins.*

Keywords Aqueous medium; benzopyranocoumarin; domino reaction; laccase; reusability

INTRODUCTION

The rapid spread of acquired immunodeficiency syndrome (AIDS) has stimulated discovery of therapeutic agents to arrest the replication of the causative virus, human immunodeficiency virus (HIV). One promising possibility to interrupt the viral life cycle is the use of virus-encoded protease inhibitors, which are indispensable for viral maturation. The substituted coumarins and analogous compounds have been identified as active nonpeptide HIV protease inhibitors.[1,2] Benzopyranes

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themselves have also been ascribed biologically important skeletons owing to their intriguing molecular structure[3] and remarkable pharmacological efficiency. Their fusion with coumarin results in a superior biological moiety, benzopyranocoumarin.

Extensive research efforts have been dedicated to evaluating the new environmentally friendly redox reactions, an important pre-existing field in industrial chemistry. In this context, enzyme-catalyzed oxidation reactions are of considerable current interest.[4–6] Laccase, which belongs to the blue copper oxidase group of enzymes (benzenediol: oxygen oxidoreductase; EC 1.10.3.2), catalyzes the oxidation of a broad range of substrates and frequently exhibits high selectivity in aqua-mediated conditions, which provides a unique solution for a variety of green redox reactions.[7,8] This blue enzyme for green chemistry finds industrial applications in various biotechnological processes, including bleaching, detoxification, addition to washing powders, removal of phenolic browning products from food products, as well as in treatments of environment pollutants.[9,10] The major advantage attributed to the use of laccase catalyst is its redox activity, making it ecofriendly and eliminating water as the sole by-product.[11]

The effective use of biocatalysts is hampered by some peculiar properties of enzyme proteins, such as nonreusability, high sensitivity to several denaturating agents, and toxicological effects.[12] Many of these undesirable constraints have been removed by the use of an aqueous medium. The unique properties of an aqueous medium, such as high dielectric constant and cohensive energy density, showed an extraordinary effect on reaction rate. Moreover, its cost-effectiveness, high abundance, non-flammability, and nontoxic nature puts it in the domain of ecofriendly solvents.[13]

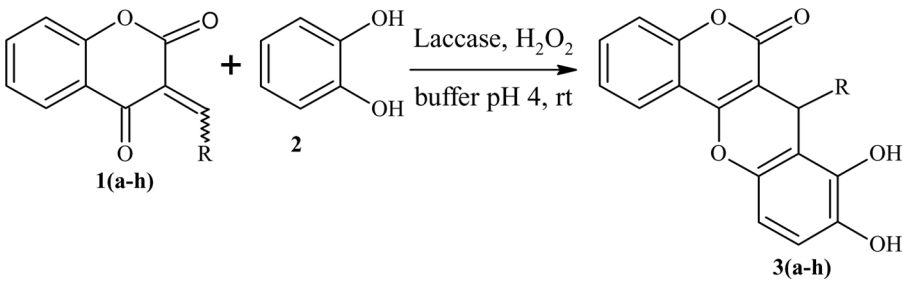
In continuation of our effort to explore chemoenzymatic reactions, it was thought worthwhile to employ water as a solvent in a biocatalytic reaction.[14] Herein, we report an efficient and ecofriendly laccase-catalyzed synthesis of benzopyranocoumarin. The reactions require stoichiometric oxidant in an aqueous medium.

RESULTS AND DISCUSSION

There has been a great deal of interest in the synthesis of benzopyranocoumarins owing to their biological importance and greater therapeutic potentiality as probable HIV protease inhibitors with great therapeutic index.[15] To get more potent inhibitors, we have successfully attempted a novel and green synthesis of benzopyranocoumarin though fusion of quinones with chalcones (Schemes 1 and 2).

Laccase is a multicopper oxidase that reduces oxygen (source of O_2 being a disproponation redox reaction of H_2O_2 in acidic medium, as handling of H_2O_2 is much easier than handling the oxygen gas) to water and simultaneously performs one-electron oxidation of many aromatic substrates such as phenol and aromatic amines.[16] Catechol/hydroquinones underwent 1,4-addition reaction with α,β-unsaturated derivatives of coumarins, leading to nonisolable **6** followed by its oxidation to give **7**, which further underwent an intramolecular 1,4-addition reaction to afford benzopyranocoumarins **3a–h**. In other words, the entire domino process consists of one oxidation and two 1,4-additions[17] (Fig. 1).

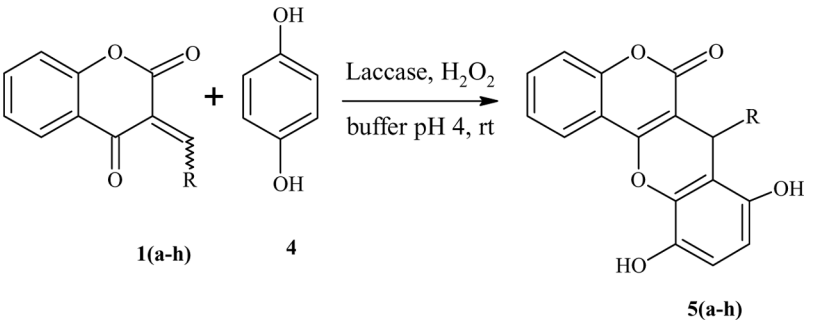
Our initial studies were focused on optimization of reaction conditions for the synthesis of benzopyranocoumarins. The enzymatic synthesis of **3a** and **5a** from **1a**



Scheme 1. Synthesis of benzopyranocoumarina derivatives **3a–h**.

were chosen as a model reaction, and the reaction conditions were optimized for enzymatic synthesis of **3a** and **5a** from **1a**. An experiment was carried out using 1 mmol of **1a** and 1.5 mmol of **3a/5a** in 15 mL of aqueous medium without the addition of laccase or H_2O_2. There was no product formation. The same was also observed when 0.5 mL of 30% of H_2O_2 and heat-killed laccase were also added. In another experiment, when 400 U laccase were added without H_2O_2, then only traces of product formation were observed on thin-layer chromatography (TLC). The yield of **3a/5a** was significantly improved with the addition of 0.5 mL of 30% H_2O_2 along with 400 U of laccase. An excess of chalcone was required for the reaction; quinones may undergo competing decomposition, dimerization, or polymerization because of intrinsic instability. Quinones and α,β-unsaturated derivatives of coumarins were used in a 1:1.5 ratio to tackle the problem.

The enzymes have been immobilized using several methodologies for their biocatalytic applicability, affecting their activity, effectiveness of utilization, deactivation, regeneration kinetics, and cost,[18] without considering the toxicity of immobilization reagents and waste disposal problem. In this study, the laccase-catalyzed synthesis was carried out in an aqueous medium for three consecutive runs and was monitored by TLC. Table 1 depicts the yield of products benzopyranocoumarin **3a** and **5a** catalyzed by laccase in three consecutive runs. The yield decreased slightly after each run.[19] For the model reaction of synthesis of **3a** and **5a** from **1a**, the catalyst could be recovered almost quantitatively and reused several times. In view of the



Scheme 2. R = phenyl, 4-methoxyphenyl, benzo[1,3]dioxol-5-yl, 2-chloroquinolin-3-yl, 2-thienyl, 3-nitrophenyl, 4-chlorophenyl, 2*H*-indol-3-yl.

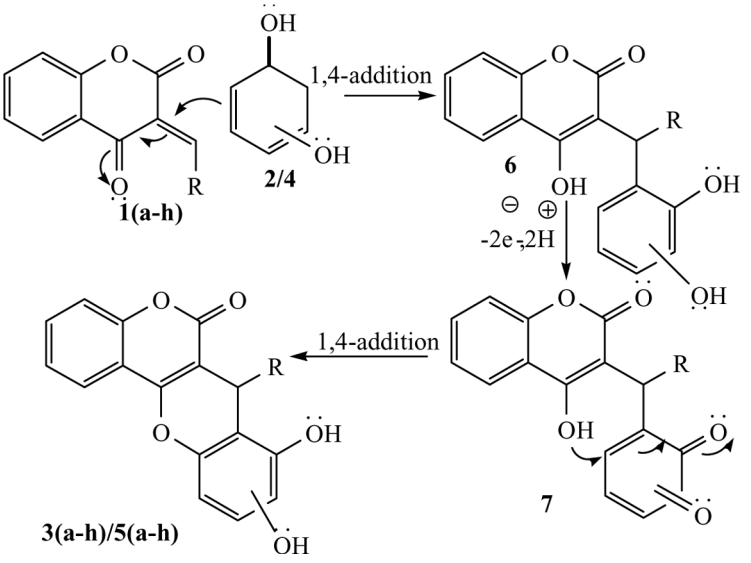


Figure 1. Proposed mechanism pathway.

environmentally friendly methodologies, recovery and reuse of the catalyst is highly preferable (Table 1).

To optimize the reaction condition, different solvents were tried. It was observed that the reaction in an aqueous buffer proceeded successfully using buffer tablets of NaH_2PO_4, Na_2HPO_4, and potassium phthalate with pH 4, the optimum pH for best laccase activity. While no reaction was observed in phosphate/citrate buffer medium with pH 5.5, poor yields were obtained in the case of acetic buffer. The lower percentage yields in other solvent systems were due to a decrease in laccase activity in nonintermiscible organic and aqueous phases. Moreover, the domino reaction was shown to exhibit better reactivity and selectivity in an aqueous medium than in organic solvent (Table 2).

Futher, the reaction temperature was shown to affect on the productivity. The reaction was studied at different temperatures varying from 5 °C to 60 °C. The best

Table 1. Benzopyranocoumarin derivatives **3a** and **5a** obtained in recycled laccase using the optimized conditions[a]

Runs	**3a** yield (%)[b]	**5a** yield (%)[b]
I (fresh)	68	59
II	62	54
III	57	51

[a]Reaction conditions: 1 mmol **2** or **4** and 1.5 mmol of **1a** were taken in 15 mL of buffer solution, and THF was added to dissolve the reactants; 0.5 mL of 30% H_2O_2 was added to the resultant solution and stirred well for 4 h.

[b]Yield refers to the isolated and unoptimized yield.

Table 2. Solvent optimization for the synthesis of benzopyranocoumarina[a]

Entry	Solvent	**3a** yield (%)[b]	**5a** yield (%)[b]
1	1 M acetete buffer pH 4	24	17
2	Water	—	—
3	THF	56	52
4	1 M phosphate/citric buffer pH 5.5	—	—
5	1 M buffer tablet of pH 4 + THF	68	59

[a]Reaction conditions: 1 mmol **2** or **4** and 1.5 mmol of **1a** were taken in 15 mL of solvents; 400 U of laccase and 0.5 mL of 30% H_2O_2 were added to the resultant solution and stirred well for 4 h.

[b]Yield refers to the isolated and unoptimized yield.

results were obtained at room temperature (25 °C). This result was attributed to the increase in rate of decomposition and polymerization of the in situ–generated quinones when higher temperature was employed, while at lower temperature the insolubility of α,β-unsaturated derivatives of coumarins was observed and finally retarded the reaction. To combat the insolubility and unstability, the reactions were carried out at ambient temperature (Fig. 2).

The structures of the synthesized benzopyranocoumarina derivatives **3a** were confirmed on the basis of their spectral data. The infrared (IR) spectrum of products **3a** showed two bands, one at 1650–1700 cm^{-1} for C=O stretching and one at 3350–3300 cm^{-1} for O-H stretching. The significant ^1H NMR and mass spectrum further satisfied the proposed product of benzopyranocoumarins. However, IR, mass, and especially ^1H NMR spectra did not confirm the structure of the final product of **3a** because there is not much difference in proton of H-7 in I structure and H-12a in structure II. Although the reaction pathway followed in the scheme resulted in type I product, there are also a chance of getting II (Fig. 2), thus ^{13}C NMR was found to be informative. ^{13}C NMR confirms the sole product (I) obtained in our protocol. This can be explained simply. Had C-12a bonded with oxygen on one side, this would have moved its signal upfield by 70–80 ppm, while the signal of C-6 carbon atom is downfield because of a double bond (i.e., 145–155 ppm) (structure II). Our spectra do not consist of such types of signals. Although the spectra show the signal of C-12a at 166.44 and C-6 signals at 36.15 ppm, that entirely diminishes the possibility of structure II. The purity of compounds was determined by CHN data.

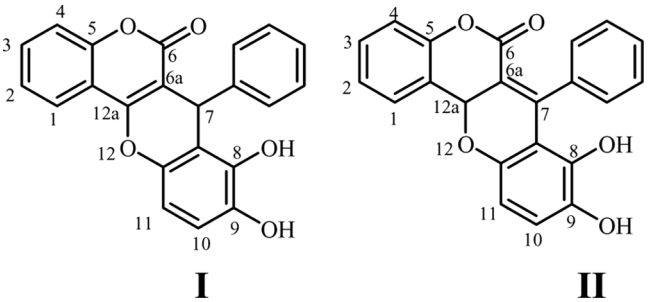


Figure 2. Probable structure of benzopyranocoumarina derivatives **3a**.

Table 3. Reaction time and yield for the laccase-catalyzed synthesis of benzopyranocoumarin derivatives **3a–h** and **5a–h**[a]

			3a–h		5a–h	
S. no.	Entry	R	Yield (%)[b]	Time (h)	Yield (%)[b]	Time (h)
1	a	Phenyl	68	4	45	4.5
2	b	4-Methoxyphenyl	63	4.5	26	6.5
3	c	Benzo[1,3]dioxol-5-yl	65	5.5	57	5
4	d	Thiophen-2-yl	55	3.5	24	4.5
5	e	3-Nitophenyl	25	7.5	48	8.5
6	f	4-Chlorophenyl	43	6	9.5	6
7	g	2*H*-Indol-3-yl	52	4	65	5
8	h	2-Chloroquinolin-3-yl	64	4.5	58	4

[a]Reaction conditions: 1 mmol **2** or **4** and 1.5 mmol of **1a** were taken in 15 mL of solvents; 400 U of laccase and 0.5 mL of 30% H_2O_2 were added to the resultant solution and stirred well for 4 h.
[b]Yield refers to the isolated and unoptimized yield.

After optimizing the reaction conditions and confirming product, the reaction was accomplished with diverse α,β-unsaturated derivatives of coumarins. The choices of aldehyde taken was based on biological activity. For example, in chlorobenzaldehyde and 2-chloroquinolin-3-yl aldehyde, the chloro group enhances the biological activity, whereas hetero aromatic aldehydes such as thiophenecarboxaldehde, benzo[1,3]dioxol-5-aldehyde, and 2*H*-indol-3-carboxaldehyde are employed for superior activity. Nitro- and methoxy groups also possess activity, so these mentioned aldehydes were taken to carry out this synthesis.[20–22] The reactivity of the α,β-unsaturated derivatives of coumarins with the in situ–generated quinone is shown in Table 3.

CONCLUSION

The laccase–aqueous system was explored for the domino reaction. This synthesis is tunable for catalyst and solvent to integrate the reaction and facilitate the reduction of waste, with use of more benign solvents. Hence, in addition to the simple reaction conditions, easy workup procedure and recyclability of catalyst make our methodology a valid contribution to the existing processes in the field of benzopyranocoumarin synthesis. The other benfit of this protocol is the use of environmentally benign biocatalyst and reaction medium.

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were recorded on a Burker Top Spin 300-MHz and 75.6-MHz spectrometer with chemical shift values (δ) in parts per million (ppm) downfield from tetramethylsilane (TMS) using dimethylsulfoxide (DMSO) as solvent. IR spectra of each sample were recorded on a model Perkin-Elmer Fourier transform FT–IR 1710 spectrometer using KBr. Elemental analyses were performed using a Heraeus CHN-Rapid Analyzer. Electrospray ionization (EI) mass spectra were recorded on a turnover frequency (TOF) MS mass

spectrometer. The purity of compounds was checked on silica-gel-coated aluminum plates (Merck TLC: mass particle size 10–12 μm; particle distribution 5–20 μm; layer thickness 250 μm; plate height 30 μm). The chalcones were prepared according to the literature procedure.[23] Guaiacol was purchased from Hi Media Laboratories Pvt. Ltd. (Mumbai, India). All media components and chemicals used were of analytical grade.

Microorganism

The *Basidiomycetous* fungus, RCK-1, isolated from deteriorated sugarcane bagasse collected from Sugar Mill Industry, Sonipat, Haryana, India, was grown and maintained on malt extract agar (MEA) containing (gL^{-1}) malt extract 20.0, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $Ca(NO_3)_2 \cdot 4H_2O$ 0.5, and agar 20.0 (pH 7.0) at 30 °C.[24,25] Pure cultures were stored at 4 °C and subcultured every fortnight.

Laccase Production

Static cultivation was carried out at 32 °C in 250-ml Erlenmeyer flasks with 50 ml malt extract broth (MEB) containing (gL^{-1}) malt extract 20.0, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, and $Ca(NO_3)_2 \cdot 4H_2O$ 0.5 (pH 7.0). The flasks were inoculated with two fungal discs (8 mm in diameter each) from the periphery of 4-day-old fungal culture and incubated at 30 °C. After 96 h of growth, 0.5 mL copper sulfate (1 mM) was added to MEB. The cultures were harvested at regular intervals by filtering through Whatman No. 1 filter paper after 264 h of growth. The culture filtrate was centrifuged at 12,000 g for 20 min at 4 °C, and the supernatant obtained was assayed for laccase activity.

Purification of Laccase

The culture filtrate (900 mL) containing laccase enzyme was precipitated by addition of ammonium sulfate (80% cutoff) and centrifuged at 10,000 g for 20 min at 4 °C. The precipitate was resuspended in 50 mM citrate phosphate buffer (pH 5.5) and dialyzed at 4 °C against the same buffer. This partially purified enzyme (78 mL) was concentrated to 20 ml using a 10-kDa filter membrane (Vivaspin, Vivascience, Sartorius Group, Stone house, UK) at 4 °C applied to an anion-exchange DEAE-sepharose (Amersham Pharmacia Biotech, Freiburg, Germany) column ($25 \times 2\,cm^2$), equilibrated with 10 mM citrate phosphate buffer (pH 7.0). The proteins were eluted by NaCl gradient (0–1 M in equilibrating buffer) at a flow rate of 0.5 mL min^{-1} with 1 mL fraction size. Fractions with laccase activity were pooled, desalted, and assayed for laccase activity.

Enzyme Assay

Guaiacol was used as a substrate for assaying laccase activity.[17] One unit (U) of laccase was defined as the change in absorbance of 0.01 ml^{-1} min^{-1} at 470 nm.

General Procedure for Synthesis of 9,10-Dihydroxy Benzopyranocoumarin and 8,11-Dihydroxy Benzopyranocoumarin

Catechol or hydroquinone (1 mmol, 100 mg) and 1.5 mmol of chalcone were taken in 15 mL of buffer solution, and THF was added to dissolve the reactants. Then purified laccase (400 U) was added to the resultant solution and stirred well, 0.5 mL of 30% H_2O_2 was added to oxygenate the reaction mixture. The progress of the reaction was checked by TLC examination every 30 min. Upon completion of the reaction, the reaction mixture was extracted with ethyl acetate (3×15). The organic layer was dried over Na_2SO_4. The product was purified by using column chromatography with a mobile phase of 80:20 hexane/ethylacetate and recrystallized with ethanol. The aqueous phase was reused without any treatment.

Selected Data

8,9-Dihydroxy-7-phenyl-7*H*-benzopyranocoumarin (3a). HRMS: M^+ 358.2322. Anal. calculated for $C_{22}H_{14}O_5$:C, 73.74, and H, 3.94%. Found:C, 73.85, and H, 4.02%. IR (υ, cm^{-1}, KBr pellet) 3322.44 (OH), 1683.12 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.51 (s, 1H, H-7), 6.69 (dd, 11 *J*, 4H, Ar), 7.25 (dt, 8 *J*, 4H, Ar), 7.61 (d, 9 *J*, 2H, Ar), 7.89 (d, 5 *J*, 1H, Ar), 8.90 (s, 2H, OH). δ_C (DMSO-d_6, 75 MHz) 36.38, 103.97, 115.79, 115.87, 118.97, 119.41, 123.55, 124.11, 125.41, 126.17, 126.78, 128.04, 130.84, 131.64, 132.93, 140.84, 145.36, 152.43, 165.02, 166.44, 168.83.

8,9-Dihydroxy-7-(4-methoxy-enyl)-7*H*-benzopyranocoumarin (3b). HRMS: M^+ 388.0881. Anal. calculated for $C_{23}H_{16}O_6$:C, 71.13, and H, 4.15%. Found: C, 71.56, and H, 4.28%. IR (υ, cm^{-1}, KBr pellet) 3326.24 (OH), 1679.44 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.51 (s, 1H, H-7), 3.71 (s, 3H, CH_3), 6.48 (dd, 6 *J*, 4H, Ar), 7.43 (dd, 6 *J*, 4H, Ar), 8.20 (dd, 2H, Ar), 8.60 (s, 2H, OH). δ_C (DMSO-d_6, 75 MHz) 35.32, 56.63, 102.12, 114.67, 115.76, 119.17, 120.19, 123.55, 124.11, 125.41, 126.17, 126.78, 128.04, 130.84, 131.64, 132.93, 141.08, 145.83, 151.24, 165.46, 167.79, 170.68.

8,9-Dihydroxy-7-(benzo[1,3]dioxol-phenyl)-7*H*-benzopyranocoumarin (3c). HRMS: M^+ 402.5658. Anal. calculated for $C_{23}H_{14}O_7$:C, 68.66, and H, 3.51%. Found:C, 68.35, and H, 3.62%. IR (υ, cm^{-1}, KBr pellet) 3312.83 (OH), 1675.84 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.51 (s, 1H, H-7), 5.96 (s, 2H, CH_2) 6.76 (m, 4H, Ar), 7.66 (dd, 6 *J*, 4H, Ar), 8.25 (d, 8 *J*, 1H, Ar), 9.02 (s, 2H, OH). δ_C (DMSO-d_6, 75 MHz) 34.63, 91.32, 104.35.

8,9-Dihydroxy-7-thiophen-7*H*-benzopyranocoumarin (3d). HRMS: M^+ 364.5324. Anal. calculated for $C_{20}H_{12}O_5S$:C, 65.93; H, 3.32; S, 8.80%. Found:C, 65.54; H, 3.62; S, 8.59%. IR (υ, cm^{-1}, KBr pellet) 3345.18 (OH), 1675.53 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.35 (s, 1H, H-7), 6.37–6.85 (m, 3H, Ar), 7.23–7.56 (dd, 8 *J*, 4H, Ar), 8.02 (d, 11 *J*, 2H, Ar), 9.02 (s, 2H, OH).

8,9-Dihydroxy-7-(4-nitro-phenyl)-7*H*-benzopyranocoumarin (3e). HRMS: M^+ 403.1564 Anal. calculated for $C_{22}H_{13}NO_7$:C, 65.51; H, 3.25; N, 3.47%. Found:C, 65.82; H, 3.38; N, 3.61%. IR (υ, cm^{-1}, KBr pellet) 3286.23 (OH), 1694.82 (C=O). δ_H

(DMSO-d_6, 300 MHz) 2.32 (s, 1H, H-7), 6. 61 (dd, 2H, Ar), 7.12 (m, 4H, Ar), 8.09 (t, 7 *J*, 2H, Ar), 8.45 (dd, 11 *J*, 2H, Ar), 8.90 (s, 2H, OH).

8,9-Dihydroxy-7-(4-chloro-phenyl)-7*H*-benzopyranocoumarin (3f). HRMS: M^+ 392.2354. Anal. calculated for $C_{22}H_{13}ClO_5$:C, 67.72; H, 3.34; Cl, 9.03%. Found:C, 67.68; H, 3.56; Cl, 9.21%. IR (υ, cm^{-1}, KBr pellet) 3269.89 (OH), 1698.84 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.63 (s, 1H, H-7), 6.58 (dd, 9 *J*, 3H, Ar), 7.35–7.45 (m, 4H, Ar), 8.20 (d, 12 *J*, 3H, Ar), 9.17 (s, 2H, OH).

8,9-Dihydroxy-7-(2*H*-indol-3-yl)-7*H*-benzopyranocoumarin (3g). HRMS: M^+ 427.1358. Anal. calculated for $C_{26}H_{21}NO_5$:C, 73.06; H, 4.95; N, 3.28%. Found:C, 73.18; H, 4.82, N, 3.48%. IR (υ, cm^{-1}, KBr pellet) 3298.36 (OH), 1668.38 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.70 (s, 1H, H-7), 6.38–6.85 (dd, 4H, Ar), 7.01–7.17 (m, 4H, Ar), 7.35–7.45 (dd, 5H, Ar), 8.37 (d, 2H, Ar), 9.02 (d, 2H, Ar), 930 (s, 2H, OH), 10.02 (s, 1H, NH).

8,9-Dihydroxy-7-(2-chloroquinolin-3-yl)-7*H*-benzopyranocoumarin (3h). HRMS: M^+ 473.1558. Anal. calculated for $C_{27}H_{20}ClNO_5$:C, 68.43; H, 4.25; Cl, 7.48; N, 2.96%. Found:C, 68.55; H, 4.36; Cl, 7.61; N, 2.66%. IR (υ, cm^{-1}, KBr pellet) 3310.94 (OH), 1682.66 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.62 (s, 1H, H-7), 6.76 (dd, 10 *J*, 4H, Ar), 7.05 (d, 2H, Ar), 7.23–7.45 (m,5H, Ar), 7.85–8.31 (dd, 4 h, Ar), 9.20 (d, 2H, Ar), 9.97 (s, 2H, OH).

8,11-Dihydroxy-7-phenyl-7*H*-benzopyranocoumarin (5a). HRMS: M^+ 358.0846. Anal. calculated for $C_{22}H_{14}O_5$:C, 73.74, and H, 3.94%. Found:C, 73.68, and H, 4.62%. IR (υ, cm^{-1}, KBr pellet) 3326.93 (OH), 1679.84 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.56 (s, 1H, H-7), 6.38 (d, 7 *J*, 3H, Ar), 7.14 (dd, 6 *J*, 4H, Ar), 7.56 (m, 4H, Ar), 8.20, 8.95 (s, 2H, OH). δ_C (DMSO-d_6, 75 MHz) 37.23, 105.74, 114.97, 115.78, 119.87, 120.21, 123.22, 123.67, 125.24, 126.45, 127.81, 128.38, 130.34, 131.22, 133.89, 141.24, 145.45, 153.56, 163.50, 166.56, 170.42.

8,11-Dihydroxy-7-(4-methoxy-phenyl)-7*H*-benzopyranocoumarin (5b). HRMS: M^+ 388.1532. Anal. calculated for $C_{23}H_{16}O_6$:C, 71.13, and H, 4.15%. Found:C, 71.56, and H, 4.28%. IR (υ, cm^{-1}, KBr pellet) 3326.83 (OH), 1675.62 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.51 (s, 1H, H-7), 3.71 (s, 3H, CH_3), 6.18 (d, 8 *J*, 3 H, Ar), 6.89 (t, 4 *J*, 2H, Ar), 7.34 (m, 3H, Ar), 8.20 (dd, 6 *J*, 3H, Ar), 8.63 (s, 2H, OH). δ_C (DMSO-d_6, 75 MHz) 35.89, 56.63, 105.79.

8,11-Dihydroxy-7-(benzo[1,3]dioxol-phenyl)-7*H*-benzopyranocoumarin (5c). HRMS: M^+ 402.2341. Anal. calculated for $C_{23}H_{14}O_7$:C, 68.66, and H, 3.51%. Found:C, 68.35, and H, 3.62%. IR (υ, cm^{-1}, KBr pellet) 3301.98 (OH), 1676.32 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.51 (s, 1H, H-7), 5.96 (s, 2H, CH_2), 6.27 (dd, 7 *J*, 3H, Ar), 6.98 (t, 4 *J*, 3H, Ar), 8.25 (m, 3H, Ar), 8.93 (s, 2H, OH). δ_C (DMSO-d_6, 75 MHz) 34.35, 90.65, 102.95, 114.97, 115.66, 119.34, 120.32, 123.43, 124.02, 125.14, 126.45, 126.87, 128.12, 130.45, 131.87, 133.78, 141.45, 146.89, 153.23, 166.89, 170.08, 171.56.

8,11-Dihydroxy-7-thiophen-7*H*-benzopyranocoumarin (5d). HRMS: M^+ 364.0405. Anal. calculated for $C_{20}H_{12}O_5S$:C, 65.93; H, 3.32; S, 8.80%. Found:C, 65.34; H, 3.62; S, 8.96%. IR (υ, cm^{-1}, KBr pellet) 3326.93 (OH), 1673.94 (C=O).

δ_H (DMSO-d_6, 300 MHz) 2.63 (s, 1H, H-7), 6.12 (dd, 3 *J*, 3H, Ar), 6.98 (t, 3 *J*, 2H, Ar), 8.20 (m, 3H, Ar), 9.05 (s, 2H, OH).

8,11-Dihydroxy-7-(4-nitro-phenyl)-7*H*-benzopyranocoumarin (5e). HRMS: M^+ 403.2624. Anal. calculated for $C_{22}H_{13}NO_7$:C, 65.51; H, 3.25; N, 3.47%. Found:C, 65.34; H, 3.60; N, 3.18%. IR (υ, cm^{-1}, KBr pellet) 3326.93 (OH), 1679.84 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.51 (s, 1H, H-7), 6.38 (t, 3 *J*, 2H, Ar), 7.04 (d, 7 *J*, 3H, Ar), 7.67 (m, 4H, Ar), 8.24 (d, 10 *J*, 1H, Ar), 8.90 (s, 2H. OH).

8,11-Dihydroxy-7-(4-chloro-phenyl)-7*H*-benzopyranocoumarin (5f). HRMS: M^+ 392.0265. Anal. calculated for $C_{22}H_{13}ClO_5$:C, 67.72; H, 3.34; Cl, 9.03%. Found:C, 67.89; H, 3.18; Cl, 8.85%. IR (υ, cm^{-1}, KBr pellet) 3307.63 (OH), 1679.84 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.51 (s, 1H, H-7), 6.25 (dd, 3 *J*, 3H, Ar), 7.05 (t, 5 *J*, 2H, Ar), 7.57 (m, 4H, Ar), 8.58 (t, 6 *J*, 1H, Ar), 8.78 (s, 2H, OH).

8,11-Dihydroxy-7-(2*H*-indol-3-yl)-7*H*-benzopyranocoumarin (5g). HRMS: M^+ 427.2131. Anal. calculated for $C_{26}H_{21}NO_5$:C, 73.06; H, 4.95; N, 3.28%. Found:C, 72.89; H, 4.86; N, 3.11%. IR (υ, cm^{-1}, KBr pellet) 3315.37 (OH), 1670.55 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.75 (s, 1H, H-7), 6.46 (d, 9 *J*, 2H, Ar), 7.34 (t, 4 *J*, 3H, Ar), 7.38 (dd, 3 *J*, 8H, Ar), 8.94 (m, 5H, Ar), 9.20 (s, 2H, OH), 10.13 (s, 1H, NH).

8,11-Dihydroxy-7-(2-chloroquinolin-3-yl)-7*H*-benzopyranocoumarin (5h). HRMS: M^+ 473.1558. Anal. calculated for $C_{27}H_{20}ClNO_5$:C, 68.43; H, 4.25; Cl, 7.48; N, 2.96%. Found:C, 68.33; H, 4.12; Cl, 7.33; N, 2.87%. IR (υ, cm^{-1}, KBr pellet) 3313.0112 (OH), 1683.546 (C=O). δ_H (DMSO-d_6, 300 MHz) 2.5 (s, 1H, H-7), 6.65 (t, 3 *J*, 4H, Ar), 7.15 (dd, 2 *J*, 5H, Ar), 7.85 (m, 4H, Ar), 8.68 (m, 4H, Ar), 9.85 (s, 2H, OH).

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