

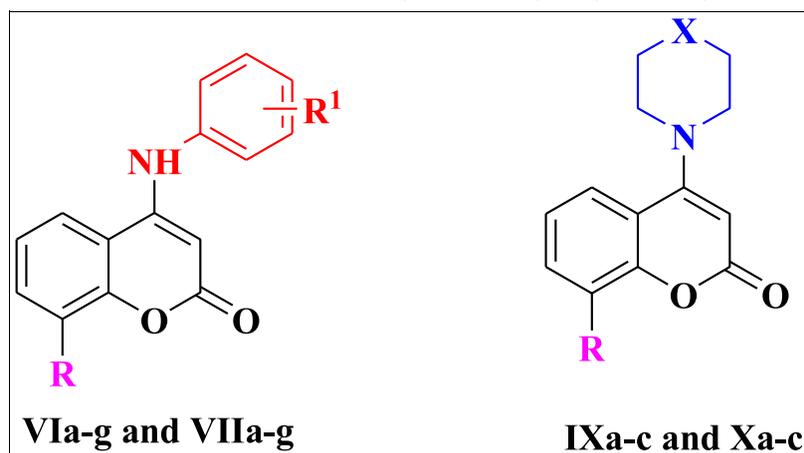
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Two series of coumarins possessing the aniline- and heterocyclic ring at 4th position have been synthesized and evaluated for their *in vitro* cytotoxic activity against MCF-7 cancer cell line in MTT assay. Structure activity relationship (SAR) studies reveal that the electron donor group at position-8 of coumarin played an important role in cytotoxic activity. Compound **VIIId** showed the potent cytotoxic activity followed by compound **Xa** with IC₅₀ = 6.25 and 6.50 μM, respectively. A docking study has also been carried out for the most potent compound to get an insight into molecular interactions with p50 subunit of NF-κB protein.

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INTRODUCTION

Cancer is a renegade system of growth that originates within living biosystem having one hallmark characteristic of uncontrolled growth that invades to other tissues [1]. It is one of the most vital health problems of the current era and is a leading cause of death [2]. Although, many efforts have been made to discover new agents endowed with cytotoxic actions, still these agents are suffering from serious limitations such as lack of selectivity and life-threatening side effects [3]. Hence, there is an urgent need for discovery of potent, safe, and selective cytotoxic agents. Natural products and their derivatives have historically served as invaluable source of drugs and also exist as mainstay for cancer chemotherapy. Naturally occurring compounds have an additional ability to interact with more than one target as exemplified by paclitaxel **1**, vincristine **2**, vinorelbine **3**, teniposide **4**, and various water-soluble analogs of camptothecin (e.g., topotecan **5**) [4] (Fig. 1).

Coumarin is a naturally occurring potent constituent having widespread distribution in nature and accompanied with low toxicity profile [5,6]. Because of their beneficial effects on

human health, coumarins are used for diverse range of biological activities such as anti-cancer [7,8], anti-HIV [9,10], anticoagulant [11], antimicrobial [12,13], antioxidant [14], dyslipidemic [15], and anti-inflammatory [16]. Significant anti-cancer effect has been shown by coumarin and its metabolite, 7-hydroxycoumarin **6** [17,18], along with scopoletin (6-methoxy-7-hydroxycoumarin **7**) and esculetin (6,7-dihydroxycoumarin **8**), which also exhibited potent anti-proliferative effects in leukemic cells by inducing apoptosis [19,20]. Lee *et al.* [21–23] have reported a coumarin-containing compound, neo-tanshinlactone **9**, which exhibited more potent and selective inhibition than tamoxifen, on ER⁺ human breast cancer cell lines (Fig. 2).

4-Substituted coumarins are well known for interesting anticancer activity especially 4-hydroxy-substituted coumarin [24]. Some of the 4-substituted coumarins known for anti-cancer activity (Fig. 1) include di-coumarinpolysulfides (compound **10–12**) that accumulate in the G2/M phase of the cell cycle and induce apoptosis in HCT116 colon cancer cells [25]. Various coumarins substituted with imidazole **13** and benzothiazole ring **14**, **15** have been reported as potent anticancer agents [26,27] (Fig. 3).

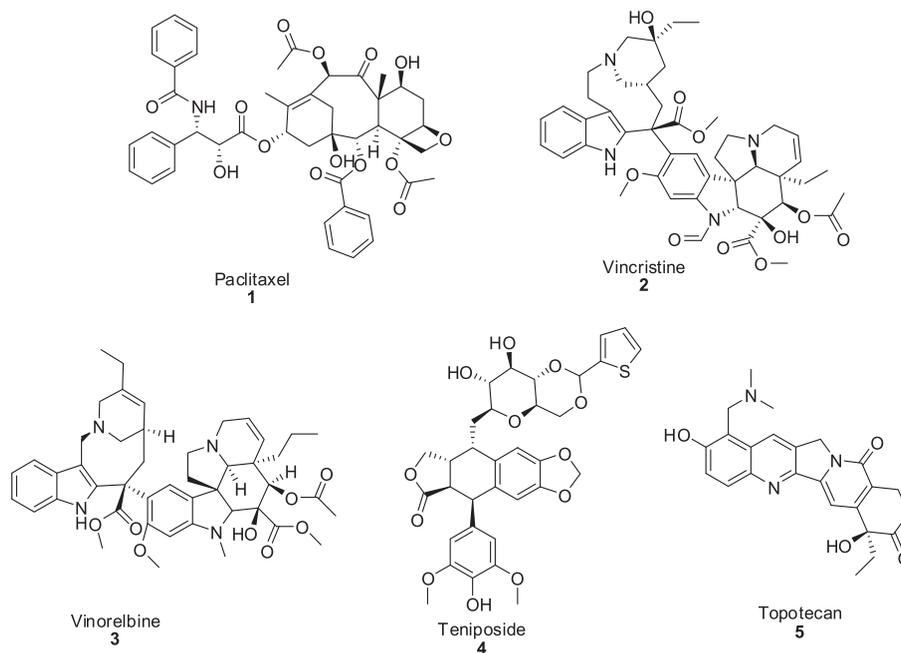


Figure 1. Naturally occurring cytotoxic compounds.

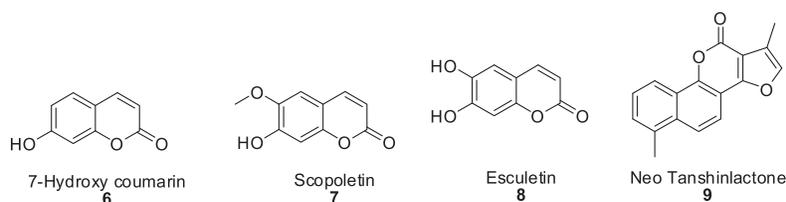


Figure 2. Coumarins as anticancer agents.

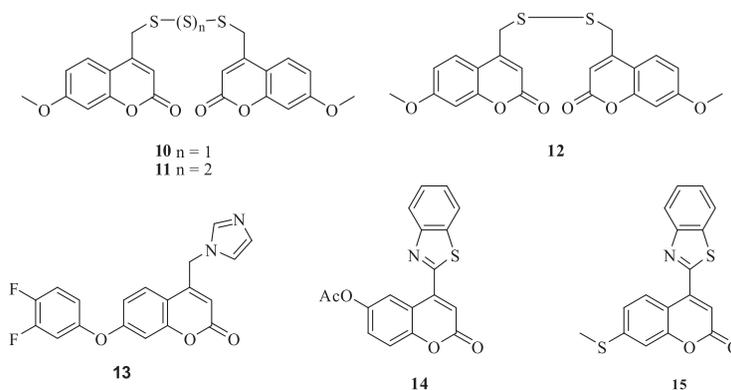


Figure 3. 4-Substituted coumarins as anticancer agents.

As a part of development in the field of coumarins as potent anti-cancer agents, we have decided to further explore coumarin as a core scaffold by synthesized two series of 4-substituted coumarins by replacing the 4-hydroxy group with various substituted amines as in Series-I and heterocyclic amines as in Series-II (Fig. 4). The synthesized compounds were further evaluated for cytotoxicity against breast cancer cell line.

RESULTS AND DISCUSSION

Chemistry. Synthesis of the target compounds was accomplished through slight modification of previously reported methods (Scheme 1) [28–30]. For this, treating of (un)substituted phenol with malonic acid in the presence of $POCl_3$ and anhydrous $ZnCl_2$ at $70^\circ C$ for 8–10 h yielded 4-hydroxy coumarin (**III**) [28]. Subsequent

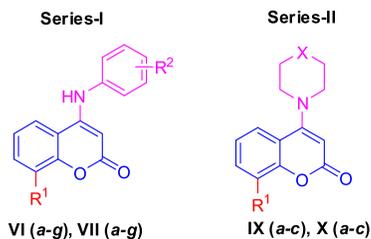


Figure 4. Proposed coumarin derivatives. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chlorination at 4-position with POCl_3 under refluxing conditions afforded the 4-chloro product **IV** [29]. The targeted compounds **VI(a-g)** and **VII(a-g)** were assessed by refluxing 4-chloro derivative **IV** with substituted anilines **V** in the presence of organic base such as triethylamine in ethanol [30]. Similarly, tertiary amines **IX(a-c)** and **X(a-c)** were obtained by reaction of 4-chloro derivative **IV** with secondary amines **VIII** in the presence of triethylamine. All the synthesized compounds were characterized by IR and ^1H NMR. The reaction time, melting point, and percentage yield of various synthesized compounds are mentioned in Table 1.

In vitro cytotoxic activity. All the synthesized compounds have been evaluated for cytotoxic activity against MCF-7 cancer cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [31]. Growth of the breast cancer cells was quantitated by the ability of living cells to reduce water soluble MTT to purple formazan in the presence of mitochondrial dehydrogenase. The amount of formazan product formed is directly proportional to the

number of living cells. Biological evaluation was carried out against MCF-7 breast cancer cell lines by using docetaxel as a positive control. The IC_{50} values were illustrated in Table 1.

Among both the series, compounds (**VIIa-g** and **Xa-c**) comprising methyl group at the 8th position of coumarin were found to be more potent than unsubstituted coumarins (**VIa-g** and **IXa-c**). Compound **VIIId** ($\text{R}=\text{CH}_3$, $\text{R}^1=4\text{-OC}_2\text{H}_5$) was found to be the most potent compound followed by **Xa** ($\text{R}=\text{CH}_3$, $\text{X}=\text{NH}$) with an IC_{50} value of 6.25 and 6.5 μM , respectively.

From C-8-substituted coumarins, except compound **VIIId** (4-ethoxy), compounds comprising methoxy group at the 3rd position of phenyl ring **VIIg** ($\text{IC}_{50}=8.5\ \mu\text{M}$) also possess excellent cytotoxic activity. Whereas, 4-Cl derivative **VIIb** ($\text{IC}_{50}=11.5\ \mu\text{M}$), unsubstituted **VIIa**, and 4-methoxy-substituted phenyl ring as in compound **VIIf** ($\text{IC}_{50}=12.5\ \mu\text{M}$) possess moderate cytotoxic activity. However, few compounds from this series such as compound **VIIc** (4-F) and **VIIe** (4- CH_3) were found to be relatively less cytotoxic with $\text{IC}_{50}=20.5$ and 21.0 μM , respectively, as compared to the standard drug, Docetaxel ($\text{IC}_{50}=42.5\ \mu\text{M}$). All the heterocyclic amine-containing candidates with C-8 methyl-substituted coumarins **Xa-c** possess significant cytotoxic activity (**Xa**, $\text{IC}_{50}=6.5\ \mu\text{M}$ > **Xb**, $\text{IC}_{50}=9.5\ \mu\text{M}$ > **Xc**, $\text{IC}_{50}=11.0\ \mu\text{M}$).

From these results, it seems that the electron-donating substitution at the 8th position of the coumarin plays an important role in the cytotoxic activity as all the active compounds have methyl group at the 8th position of the coumarin skeleton. Although, substitutions of heterocyclic

Scheme 1. Synthesis of 4-substituted coumarin derivatives VI, VII, IX, and X.

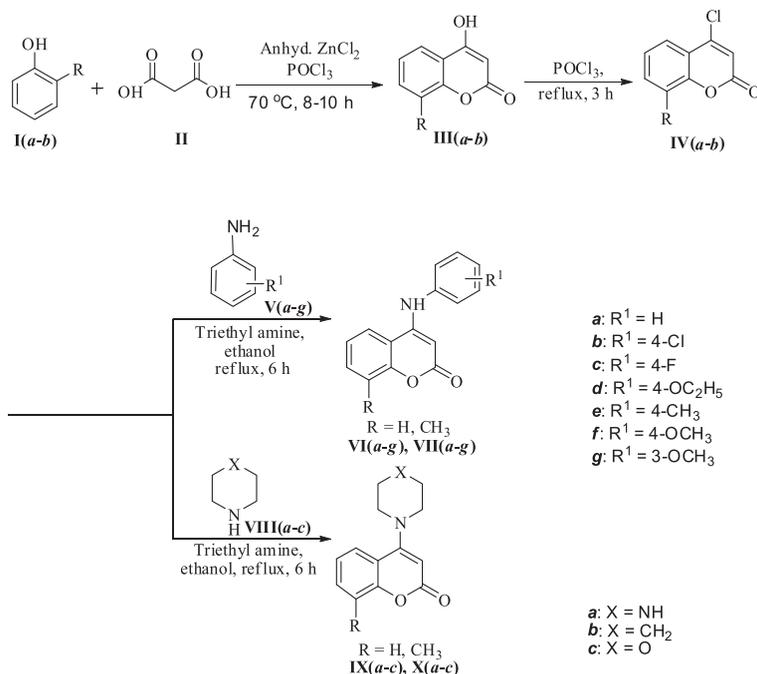
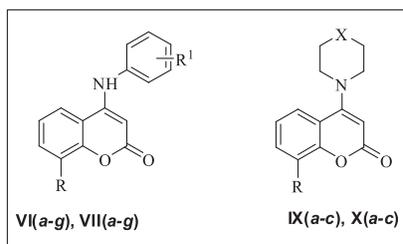


Table 1

Physico-chemical characterization and IC₅₀ value of 4-substituted coumarins (**VIa-g**, **VIIa-g**, **IXa-c**, and **Xa-c**) against MCF-7 cancer cell line.

S. no.	Code	R	R ¹ or X	% yield	Reaction time (h)	M.P. (°C)	IC ₅₀ MCF-7 (μM)
1	VIa	H	H	52	6	273–276	17.5
2	VIb	H	4-Cl	58	7.5	261–265	14.0
3	VIc	H	4-F	45	7	272–275	9.5
4	VI d	H	4-OC ₂ H ₅	71	6	218–220	Inactive
5	VIe	H	4-CH ₃	65	6.5	288–292	11.5
6	VI f	H	4-OCH ₃	69	7	239–243	8.5
7	VI g	H	3-OCH ₃	47	6.5	221–225	Inactive
8	VIIa	CH ₃	H	69	6	262–268	12.5
9	VIIb	CH ₃	4-Cl	63	8	245–250	11.5
10	VIIc	CH ₃	4-F	59	6.5	247–253	20.5
11	VII d	CH ₃	4-OC ₂ H ₅	81	6	233–235	6.25
12	VII e	CH ₃	4-CH ₃	58	6.5	284–286	21.0
13	VII f	CH ₃	4-OCH ₃	67	7.5	268–272	12.5
14	VII g	CH ₃	3-OCH ₃	52	6	213–218	8.5
15	IXa	H	NH	75	7	142–144	21.0
16	IXb	H	CH ₂	58	6	153–156	37.5
17	IXc	H	O	62	7.5	131–135	Inactive
18	Xa	CH ₃	NH	70	6.5	108–112	6.5
19	Xb	CH ₃	CH ₂	49	6	126–130	9.5
20	Xc	CH ₃	O	47	7	92–94	11.0
21	Docetaxel	–	–	–	–	–	42.5

ring system at the 4th position enhance the cytotoxic activity as in compounds **Xa**, **Xb**, and **Xc**. By comparing compounds, **VIIb** (IC₅₀ = 11.5 μM) and **VIIc** (IC₅₀ = 20.5 μM), it seems that substitution of more electronegative group at para position of aniline part leads to a decrease in potency. Moreover, compounds **VII d** and **VII f** have different substitutions at para position of substituted anilines such as ethoxy and methoxy, respectively, and **VII g** comprising of methoxy group at meta position of substituted anilines showed higher activity, probably because of the presence of two electron-donating groups.

From unsubstituted coumarins, compound **VI f** was found to be most potent in the series with IC₅₀ = 8.5 μM may be because of the presence of electron-donating methoxy group at the para position of the substituted aniline part. Compounds **VIa**, **VIb**, and **VIc** have shown moderate activity with IC₅₀ = 17.5, 14.0, and 9.5 μM, respectively; this explains that potency increases as electronegativity increases at the para position of substituted anilines. Compounds **VI d** and **VI g** having ethoxy group at para position and ethoxy group at meta position of

substituted anilines, respectively, were found to be inactive and in Series-II, compounds **IXa** and **IXb** exhibited moderate cytotoxic activity with IC₅₀ = 21.0, 37.5 μM respectively; this indicates that the methyl group at the 8th position of coumarin is necessary for the potent cytotoxicity of these compounds. However, compound **IXc** having piperidine ring at the 4th position of coumarin was found to be inactive.

DOCKING STUDIES

To rationalize the activity profile of 4-substituted coumarins, docking of most potent compound was performed. A flexible docking study of **VII d** was carried out at the binding site of p50 subunit of NF-κB protein (PDB ID: 1NFK) using GOLD software [32]. The highest scoring conformation was selected to study interaction.

The compound **VII d** was docked at the binding site of p50 subunit of NF-κB protein, and the best conformation was selected on the basis of score and visual inspection. Figure 5 shows the binding conformation of the PB1 at

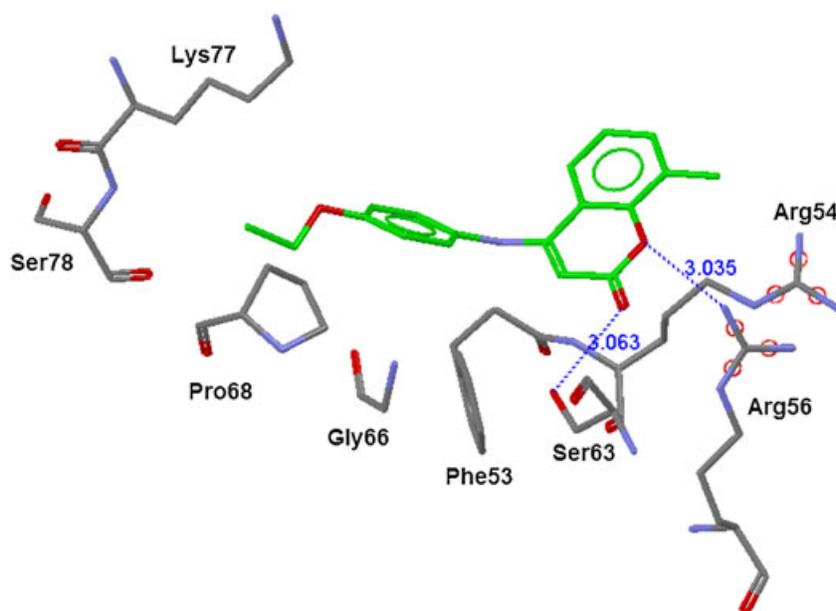


Figure 5. Binding conformation of **PB1** at binding site 1NFK. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the binding site of p50 subunit of NF- κ B protein. The **VIIId** occupies the cavity of p50 subunit of NF- κ B protein. The hydrogen bonding with binding residues helps in the stabilization of **VIIId** in the cavity. Two important hydrogen bonding were observed between the carbonyl and ring oxygen of coumarin with oxygen and nitrogen of Ser63 and Arg56, respectively. Further the **VIIId** was found to be involved in van der Waal interaction with residues at receptor site.

CONCLUSION

Two series of 4-substituted coumarins were prepared and screened for cytotoxic activity. In both series, compounds comprising electron-donating group (methyl) at the 8th position of coumarin **VIIa–g** and **Xa–c** showed excellent activity than unsubstituted compounds **VIa–g** and **IXa–c**. Although, substitution of anilino group at the position-4 possesses comparatively less potency than that of the heterocyclic ring-substituted compounds. The benchmark-active compounds are **VIIId**, followed by **Xa** with IC₅₀ values of 6.25 and 6.5 μ M, respectively, which opens the new avenue for the novel 4-substituted coumarin derivatives as promising anti-cancer lead candidates. The docking study revealed the crucial interactions which make **VIIId** a potential inhibitor and rationalize the cytotoxic activity of 4-substituted coumarins. Further, mechanistic study is underway and will be communicated in the near future.

EXPERIMENTAL

General. Melting points (mp) were uncorrected and were recorded in open capillaries on the Veego VMP-PM

melting point apparatus. Proton (¹H-NMR) and Carbon-13 nuclear magnetic resonance (¹³C-NMR) spectra were recorded in CDCl₃ or DMSO-*d*₆ as solvent on Bruker Avance II 400 NMR spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (TMS) for all recorded NMR spectra. IR spectra were recorded on Shimadzu 8400 S FT-IR spectrophotometer with KBr pellets. Mass spectra were recorded on a Q-ToF micro Mass Spectrometer. The progress of the reaction was monitored on precoated silica-gel plates using hexane:ethyl acetate as solvent and visualizing with ultraviolet light. Starting materials and reagents used in reactions were obtained commercially from Acros, Sigma Aldrich, SD fine chemicals and were used without purification, unless otherwise indicated.

General procedure for the synthesis of compound III [28]. (Un)substituted phenol (0.1 mole) and malonic acid (0.1 mole) were added to a mixture of phosphorous oxychloride (40 g) and freshly fused zinc chloride (40 g). The reaction mixture was heated on water bath at 60–70°C for 8–10 h until it become viscous and turned dark black and then poured into ice water mixture. The solid residue was filtered out and treated with solution of saturated sodium carbonate. The filtrate was slowly acidified with dil. HCl. Upon neutralization, the obtained precipitates were filtered, washed with water, dried, and recrystallized from methanol.

4-Hydroxy-2H-chromen-2-one (IIIa). Yield 48%, m.p. 208–212°C [Lit. 210–215°C]. ¹H NMR (300 MHz, DMSO-*d*₆) δ : 6.67 (s, 1H, coumarin-*H*), 7.42–7.56 (m, 3H, Ar*H*), 7.69 (d, *J* = 8.0 Hz, 1H, Ar*H*), 15.93 (s, 1H, OH).

4-Hydroxy-8-methyl-2H-chromen-2-one (IIIb). Yield 51%, m.p. 215–220°C. ^1H NMR (300 MHz, DMSO- d_6) δ : 2.34 (s, 3H, CH_3), 6.65 (s, 1H, coumarin- H), 7.36 (m, 2H, Ar H), 7.58 (d, $J=7.8$ Hz, 1H, Ar H), 15.93 (s, 1H, OH).

General procedure for the synthesis of compound IV [29]. (Un)substituted 4-hydroxy-2H-chromen-2-one **III** (0.185 mol) and 70-mL POCl_3 were refluxed for 3 h, cooled, and slowly poured into crushed ice (700 g) with vigorous stirring. The solid was collected by filtration and washed successively with ice water. Finally, azeotropic distillation with n -hexane, hot filtration of the by-product, followed by evaporation of solvent and final recrystallization with methanol give the desired product.

4-Chloro-2H-chromen-2-one (IVa). Yield 60%, m.p. 87–89°C [Lit.]. ^1H NMR (300 MHz, DMSO- d_6) δ : 6.80 (s, 1H, coumarin- H), 7.58–7.44 (m, 3H, Ar H), 7.69 (d, $J=8.0$ Hz, 1H, Ar H).

4-Chloro-8-methyl-2H-chromen-2-one (IVb). Yield 58%, m.p. 96–99°C. ^1H NMR (300 MHz, DMSO- d_6) δ : 2.34 (s, 3H, CH_3), 6.82 (s, 1H, coumarin- H), 7.34–7.28 (m, 2H, Ar H), 7.60 (d, $J=7.8$ Hz, 1H, Ar H).

SYNTHESIS OF 4-SUBSTITUTED COUMARINS

Preparation of 4-substituted coumarins: Series-I (VIa–g and VIIa–g) [30]. (Un)substituted 4-chloro-2H-chromen-2-one **IV** (0.05 mol) and (un)substituted aniline (0.05 mol) were dissolved in ethanol (30 mL), and catalytic amount of triethyl amine was added. The resulting solution was refluxed for 1 h and left at room temperature for 4–5 h. The separated precipitate were filtered off and recrystallized from ethanol. Using this procedure, the following compounds were synthesized.

4-(Phenylamino)-2H-chromen-2-one (VIa). Yield: 52%, m.p. 273–276°C. IR ν_{max} (cm^{-1}): 3299 (NH), 3027 (C—H), 1662 (C=O), 1609 (C=C). ^1H NMR (300 MHz, DMSO- d_6) δ : 5.29 (s, 1H, coumarin- H), 7.29–7.38 (m, 5H, Ar H), 7.47 (d, 2H, $J=6.9$ Hz, Ar H), 7.65 (t, 1H, $J=7.5$ Hz, Ar H), 8.24 (d, 1H, $J=7.5$ Hz, Ar H), 9.31 (s, 1H, NH). ^{13}C NMR (75 MHz, CDCl_3) δ : 161.58, 150.12, 148.26, 139.50, 134.08, 129.11, 129.01, 125.68, 124.12, 123.68, 123.01, 120.11, 119.45, 108.81, 84.21. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{11}\text{NO}_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.12; H, 4.73; N, 6.63.

4-(4-Chlorophenylamino)-2H-chromen-2-one (VIb). Yield: 58%, m.p. 261–265°C. IR ν_{max} (cm^{-1}): 3279 (NH), 1663 (C=O), 1609 (C=C), 747 (C—Cl). ^1H NMR (300 MHz, DMSO- d_6) δ : 5.29 (s, 1H, coumarin- H), 6.84 (d, 2H, $J=7.8$ Hz, Ar H), 7.31 (d, 2H, $J=7.8$ Hz, Ar H), 7.52–7.54 (m, 2H, Ar H), 7.76 (t, 1H, $J=7.8$ Hz, Ar H), 8.24 (d, 1H, $J=7.5$ Hz, Ar H), 9.31 (s, 1H, NH). ^{13}C NMR (75 MHz, CDCl_3) δ : 161.58, 155.24, 150.12, 140.23, 132.12, 130.69, 130.01, 129.56, 125.68, 124.79, 122.98, 122.01, 112.47, 110.22, 84.11. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{10}\text{ClNO}_2$: C, 66.31; H, 3.71; N, 5.16. Found: C, 67.75; H, 4.02; N, 5.01. MS: $m/z=271$ [M^+].

4-(4-Fluorophenylamino)-2H-chromen-2-one (VIc). Yield: 45%, m.p. 272–275°C. IR ν_{max} (cm^{-1}): 3285 (NH), 1666 (C=O), 1601 (C=C), 1218 (C—F). ^1H NMR (300 MHz, DMSO- d_6) δ : 5.31 (s, 1H, coumarin- H), 6.78 (d, 2H, $J=8.0$ Hz, Ar H), 7.19 (d, 2H, $J=8.0$ Hz, Ar H), 7.38–7.40 (m, 2H, Ar H), 7.70 (t, 1H, $J=8.0$ Hz, Ar H), 8.26 (d, 1H, $J=8.0$ Hz, Ar H), 9.34 (s, 1H, NH). *Anal.* Calcd. for $\text{C}_{15}\text{H}_{10}\text{FNO}_2$: C, 70.58; H, 3.95; N, 5.49. Found: C, 71.25; H, 3.56; N, 5.58.

4-(4-Ethoxyphenylamino)-2H-chromen-2-one (VI d). Yield: 71%, m.p. 218–220°C. IR ν_{max} (cm^{-1}): 3298 (NH), 2983 (C—H), 1663 (C=O), 1622 (C=C), 1246 (C—O). ^1H NMR (300 MHz, DMSO- d_6) δ : 2.01 (t, 3H, $J=7.3$ Hz, CH_3), 4.26–4.28 (m, 2H, CH_2), 5.8 (s, 1H, coumarin- H), 6.28 (d, 2H, $J=7.3$ Hz, Ar H), 6.87 (d, 2H, $J=7.3$ Hz, Ar H), 7.38–7.42 (m, 2H, Ar H), 7.70 (t, 1H, $J=7.3$ Hz, Ar H), 8.14 (d, 1H, $J=7.3$ Hz, Ar H), 9.19 (s, 1H, NH). *Anal.* Calcd. for $\text{C}_{17}\text{H}_{15}\text{NO}_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.43; H, 5.01; N, 4.67.

4-(*p*-Tolylamino)-2H-chromen-2-one (VIe). Yield: 65%, m.p. 288–292°C. IR ν_{max} (cm^{-1}): 3287 (NH), 1655 (C=O), 1617 (C=C). ^1H NMR (300 MHz, DMSO- d_6) δ : 2.38 (s, 3H, CH_3), 5.93 (s, 1H, coumarin- H), 7.16–7.26 (m, 4H, Ar H), 7.34–7.43 (m, 2H, Ar H), 7.60 (d, 1H, $J=8.1$ Hz, Ar H), 7.65 (d, 1H, $J=8.1$ Hz, Ar H). *Anal.* Calcd. for $\text{C}_{16}\text{H}_{13}\text{NO}_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.34; H, 5.66; N, 5.11.

4-(4-Methoxyphenylamino)-2H-chromen-2-one (VI f). Yield: 69%, m.p. 239–243°C. IR ν_{max} (cm^{-1}): 3292 (NH), 3071 (C—H), 1661 (C=O), 1622 (C=C), 1203 (C—O). ^1H NMR (300 MHz, DMSO- d_6) δ : 4.02 (s, 3H, OCH_3), 6.0 (s, 1H, coumarin- H), 6.24 (d, 2H, $J=7.5$ Hz, Ar H), 6.64 (d, 2H, $J=7.5$ Hz, Ar H), 7.42–7.58 (m, 3H, Ar H), 7.79 (d, 1H, $J=7.5$ Hz, Ar H), 8.02 (s, 1H, NH). *Anal.* Calcd. for $\text{C}_{16}\text{H}_{13}\text{NO}_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.02; H, 5.08; N, 5.00.

4-(3-Methoxyphenylamino)-2H-chromen-2-one (VI g). Yield: 47%, m.p. 221–225°C. IR ν_{max} (cm^{-1}): 3292 (NH), 2977 (C—H), 1655 (C=O), 1602 (C=C), 1134 (C—O). ^1H NMR (300 MHz, DMSO- d_6) δ : 4.02 (s, 3H, OCH_3), 5.64 (s, 1H, coumarin- H), 6.15–6.29 (m, 3H, Ar H), 7.21–7.28 (m, 3H, Ar H), 7.59 (t, 1H, $J=8.0$ Hz, Ar H), 7.91 (d, 1H, $J=8.0$ Hz, Ar H), 8.4 (s, 1H, NH). *Anal.* Calcd. for $\text{C}_{16}\text{H}_{13}\text{NO}_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.12; H, 4.26; N, 5.00.

4-(Phenylamino)-8-methyl-2H-chromen-2-one (VIIa). Yield: 69%, m.p. 262–268°C. IR ν_{max} (cm^{-1}): 3307 (NH), 2975 (C—H), 1659 (C=O), 1609 (C=C). ^1H NMR (300 MHz, DMSO- d_6) δ : 1.19 (s, 3H, CH_3), 5.34 (s, 1H, coumarin- H), 6.27 (d, 2H, $J=7.5$ Hz, Ar H), 6.67–7.21 (m, 5H, Ar H), 7.84 (d, 1H, $J=7.5$ Hz, Ar H), 9.31 (s, 1H, NH). ^{13}C NMR (75 MHz, CDCl_3) δ : 158.85, 149.12, 148.26, 139.50, 134.08, 131.23, 128.11, 126.22, 124.13, 122.52, 122.01, 120.81, 120.08, 108.81, 84.21, 16.01. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{13}\text{NO}_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.98; H, 5.26; N, 5.63.

4-(4-Chlorophenylamino)-8-methyl-2H-chromen-2-one (VIIf). Yield: 63%, m.p. 245–250°C. IR ν_{\max} (cm⁻¹): 3291 (NH), 1659 (C=O), 1608 (C=C), 726 (C—Cl). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.19 (s, 3H, CH₃), 5.45 (s, 1H, coumarin-*H*), 6.55 (d, 2H, *J*=7.5 Hz, Ar*H*), 7.19–7.21 (m, 3H, Ar*H*), 7.23 (t, 1H, *J*=7.5 Hz, Ar*H*), 8.20 (d, 1H, *J*=7.5 Hz, Ar*H*), 9.25 (s, 1H, NH). ¹³C NMR (75 MHz, CDCl₃): δ 159.56, 152.11, 150.42, 133.31, 128.39, 127.63, 127.01, 126.78, 125.23, 124.79, 122.01, 121.76, 117.34, 112.21, 84.11, 16.01. *Anal.* Calcd. for C₁₆H₁₂ClNO₂: C, 67.26; H, 4.23; N, 4.90. Found: C, 67.45; H, 4.13; N, 4.95.

4-(4-Fluorophenylamino)-8-methyl-2H-chromen-2-one (VIIfc). Yield: 59%, m.p. 247–253°C. IR ν_{\max} (cm⁻¹): 3295 (NH), 2976 (C—H), 1611 (C=O), 1600 (C=C), 1233 (C—F). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.20 (s, 3H, CH₃), 5.43 (s, 1H, coumarin-*H*), 6.78 (d, 2H, *J*=7.8 Hz, Ar*H*), 7.17 (d, 2H, *J*=7.8 Hz, Ar*H*), 7.23–7.25 (m, 2H, Ar*H*), 8.21 (d, 1H, *J*=7.8 Hz, Ar*H*), 9.25 (s, 1H, NH). *Anal.* Calcd. for C₁₆H₁₂FNO₂: C, 71.37; H, 4.49; N, 5.20. Found: C, 70.28; H, 5.20; N, 5.15.

4-(4-Ethoxyphenylamino)-8-methyl-2H-chromen-2-one (VIIfd). Yield: 81%, m.p. 233–235°C. IR ν_{\max} (cm⁻¹): 3306 (NH), 2974 (C—H), 1660 (C=O), 1610 (C=C), 1246 (C—O). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.47 (s, 3H, CH₃), 4.06 (m, 2H, CH₂), 4.77 (s, 3H, ArCH₃), 5.52 (s, 1H, coumarin-*H*), 6.92–6.95 (m, 2H, Ar*H*), 7.17–7.23 (m, 3H, Ar*H*), 7.41–7.48 (m, 2H, Ar*H*). *Anal.* Calcd. for C₁₈H₁₇NO₃: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.53; H, 4.73; N, 4.10.

4-(*p*-Tolylamino)-8-methyl-2H-chromen-2-one (VIIfe). Yield: 58%, m.p. 284–286°C. IR ν_{\max} (cm⁻¹): 3300 (NH), 2973 (C—H), 1658 (C=O), 1608 (C=C). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.05 (s, 3H, CH₃), 2.30 (s, 3H, ArCH₃), 5.61 (s, 1H, coumarin-*H*), 6.26 (d, 2H, *J*=8.0 Hz, Ar*H*), 6.82 (d, 2H, *J*=8.0 Hz, Ar*H*), 7.24–7.25 (m, 1H, Ar*H*), 7.58 (d, 1H, *J*=8.0 Hz, Ar*H*), 7.65 (d, 1H, *J*=8.0 Hz, Ar*H*). *Anal.* Calcd. for C₁₇H₁₅NO₂: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.44; H, 6.01; N, 5.15.

4-(4-Methoxyphenylamino)-8-methyl-2H-chromen-2-one (VIIfg). Yield: 67%, m.p. 268–272°C. IR ν_{\max} (cm⁻¹): 3301 (NH), 2952 (C—H), 1663 (C=O), 1591 (C=C), 1247 (C—O). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.12 (s, 3H, CH₃), 3.78 (s, 3H, OCH₃), 5.24 (s, 1H, coumarin-*H*), 6.00 (s, 1H, Ar*H*), 6.24 (d, 2H, *J*=7.5 Hz, Ar*H*), 6.64 (d, 2H, *J*=7.5 Hz, Ar*H*), 7.23–7.26 (m, 2H, Ar*H*), 8.00 (s, 1H, NH). *Anal.* Calcd. for C₁₇H₁₅NO₃: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.35; H, 5.12; N, 4.58.

4-(3-Methoxyphenylamino)-8-methyl-2H-chromen-2-one (VIIfh). Yield: 52%, m.p. 213–218°C. IR ν_{\max} (cm⁻¹): 3301 (NH), 2993 (C—H), 1662 (C=O), 1601 (C=C), 1264 (C—O). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.12 (s, 3H, CH₃), 4.10 (s, 3H, OCH₃), 5.24 (s, 1H, coumarin-*H*), 5.98–6.15 (m, 3H, Ar*H*), 6.75 (t, 1H, *J*=8.0 Hz, Ar*H*), 7.23–7.25 (m, 2H, Ar*H*), 7.67 (d, 1H, *J*=8.0 Hz, Ar*H*),

8.52 (s, 1H, NH). *Anal.* Calcd. for C₁₇H₁₅NO₃: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.22; H, 5.89; N, 4.22.

Preparation of 4-Substituted coumarins: Series-II (IXa-c and Xa-c). The 4-chloro-2H-chromen-2-one IVa/4-chloro-8-methyl-2H-chromen-2-one IVb (0.05 mol) and secondary amines (0.05 mol) were dissolved in ethanol (30 mL) and added catalytic amount of triethyl amine. The resulting solution was refluxed for 1 h and left at room temperature for 4–5 h. The separated precipitate was filtered off and recrystallized from ethanol.

4-(Piperazin-1-yl)-2H-chromen-2-one (IXa). Yield: 58%, m.p. 153–156°C. IR ν_{\max} (cm⁻¹): 3397 (NH), 2852 (C—H), 1712 (C=O), 1603 (C=C), 1321 (C—N). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.51 (t, 4H, *J*=4.1 Hz, piperazin-CH₂), 2.78 (t, 4H, *J*=4.1 Hz, piperazin-CH₂), 5.23 (s, 1H, Ar*H*), 7.79 (d, 1H, *J*=8.0 Hz, Ar*H*), 7.45–7.54 (m, 3H, Ar*H*). *Anal.* Calcd. for C₁₃H₁₄N₂O₂: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.05; H, 6.18; N, 12.76.

4-(Piperidin-1-yl)-2H-chromen-2-one (IXb). Yield: 62%, m.p. 131–135°C. IR ν_{\max} (cm⁻¹): 3063 (NH), 2940 (C—H), 1708 (C=O), 1604 (C=C), 1320 (C—N). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.58 (t, 4H, *J*=4.1 Hz, piperidin-CH₂), 1.62 (t, 2H, *J*=4.1 Hz, piperidin-CH₂), 3.21 (t, 4H, *J*=4.1 Hz, piperidin-CH₂), 5.25 (s, 1H, coumarin-*H*), 7.47–7.56 (m, 3H, Ar*H*), 7.79 (d, 1H, *J*=8.0 Hz, Ar*H*). *Anal.* Calcd. for C₁₄H₁₅NO₂: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.85; H, 6.24; N, 6.86.

4-Morpholino-2H-chromen-2-one (IXa). Yield: 75%, m.p. 142–144°C. IR ν_{\max} (cm⁻¹): 3032 (NH), 2862 (C—H), 1707 (C=O), 1600 (C=C), 1332 (C—N), 1137 (C—O). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 3.24 (t, 4H, *J*=3.9 Hz, morpholino-CH₂), 3.93 (t, 4H, *J*=3.9 Hz, morpholino-CH₂), 5.76 (s, 1H, coumarin-*H*), 7.24–7.29 (m, 1H, Ar*H*), 7.36 (d, 1H, *J*=8.4 Hz, Ar*H*), 7.50–7.55 (m, 1H, Ar*H*), 7.62 (d, 1H, *J*=7.8 Hz, Ar*H*). ¹³C NMR (75 MHz, CDCl₃): δ 158.20, 152.87, 150.58, 126.73, 123.24, 122.28, 115.07, 113.33, 74.98, 67.46, 67.46, 48.82, 48.82. *Anal.* Calcd. for C₁₃H₁₃NO₃: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.29; H, 5.20; N, 6.13.

4-(Piperazin-1-yl)-8-methyl-2H-chromen-2-one (Xa). Yield: 49%, m.p. 126–130°C. IR ν_{\max} (cm⁻¹): 3301 (NH), 2959 (C—H), 1692 (C=O), 1600 (C=C), 1322 (C—N). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 2.08 (s, 3H, CH₃), 2.65 (t, 4H, *J*=3.8 Hz, piperazin-CH₂), 3.08 (t, 4H, *J*=3.8 Hz, piperazin-CH₂), 5.50 (s, 1H, coumarin-*H*), 7.20 (t, 1H, *J*=7.5 Hz, Ar*H*), 7.28 (d, 1H, *J*=7.8 Hz, Ar*H*), 7.75 (d, 1H, *J*=7.8 Hz, Ar*H*). *Anal.* Calcd. for C₁₄H₁₆N₂O₂: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.12; H, 6.82; N, 11.11.

4-(Piperidin-1-yl)-8-methyl-2H-chromen-2-one (Xb). Yield: 47%, m.p. 92–94°C. IR ν_{\max} (cm⁻¹): 3299 (NH), 2937 (C—H), 1655 (C=O), 1603 (C=C), 1319 (C—N). ¹H NMR (300 MHz, DMSO-*d*₆) δ : 1.60–1.48 (m, 6H, piperidin-CH₂), 2.20 (s, 3H, CH₃), 2.88 (t, 4H, *J*=4.0 Hz, piperidin-CH₂), 5.45 (s, 1H, coumarin-*H*), 7.13 (t, 1H,

$J=7.8$ Hz, ArH), 7.33 (d, 1H, $J=7.8$ Hz, ArH), 7.45 (d, 1H, $J=7.8$ Hz, ArH). Anal. Calcd. for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76. Found: C, 74.85; H, 7.82; N, 5.21.

4-Morpholino-8-methyl-2H-chromen-2-one (Xc). Yield: 70%, m.p. 108–112°C. IR ν_{\max} (cm^{-1}): 3368 (NH), 2918 (C—H), 1710 (C=O), 1601 (C=C), 1318 (C—N), 1131 (C—O). 1H NMR (300 MHz, DMSO- d_6) δ : 2.46 (s, 3H, CH_3), 3.23 (t, 4H, $J=4.5$ Hz, morpholine- CH_2), 3.93 (t, 4H, $J=4.8$ Hz, morpholine- CH_2), 5.74 (s, 1H, coumarin-H), 7.14 (t, 1H, $J=7.5$ Hz, ArH), 7.35 (d, 1H, $J=7.2$ Hz, ArH), 7.44 (d, 1H, $J=7.8$ Hz, ArH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.92, 151.85, 149.16, 130.28, 122.10, 121.01, 114.42, 110.79, 75.26, 68.00, 68.00, 47.56, 47.56, 15.26. Anal. Calcd. for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.43; H, 6.18; N, 6.93. MS: $m/z=243$ [M^+]

In vitro cytotoxicity assay [31]. Cytotoxic effects were examined in MCF-7 breast cancer cell lines. The MCF-7 was at a concentration of 3×80 cells/mL, plated onto 96 well flat bottom culture plates for 24 h, in which cell number was kept at 4000–5000 using Neubauer cell counting chamber. After 24 h of incubation, cells were treated with various concentrations of test drugs. Then cultures were incubated for 72 h at 37°C in a humidified incubator. After 72 h of incubation (37°C, 5% CO_2 in humidified atmosphere), 5 μ L of MTT (5 mg/mL in DMSO) was added to each well, and the plate was incubated for a further 4 h at 37°C in dark. The resulting formazan was dissolved in 50- μ L dimethyl sulphoxide, and absorbance of the solution was read at 595 nm using an ELISA plate reader (Biorad, Model 680, Japan). All determinations were carried out in triplicate. Concentrations of test drugs showing 50% reduction in cell viability (i.e., IC_{50} values) were determined by a nonlinear regression analysis.

DOCKING

The coordinates of p50 subunit of NF- κ B protein were obtained from protein data bank (PDB code 1NFK) [33]. The structure of **PB1** was drawn in MOE v2010.10 and subjected to energy minimization using the MMFF94x forcefield [34]. The compound was docked in p50 subunit of NF- κ B protein using GOLD 5.0.1 software [32]. GOLD performs genetic algorithm-based ligand docking to optimize the conformation of ligand at the receptor binding site. To calculate the scoring function ChemPLP scoring function was used. The compound was docked ten times, and each pose was ranked according to its ChemPLP score. The conformation with highest score was selected for discussion.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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