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ORIGINAL ARTICLE

N-Benzylation of 6-aminoflavone by reductive amination and efficient access to some novel anticancer agents via topoisomerase II inhibition

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Abstract

Series of novel *N*-benzyl derivatives of 6-aminoflavone (**9a**–**n**) were synthesized and evaluated for anticancer and topoisomerase II enzyme inhibition activity. All the synthesized compounds were screened for in vitro anticancer activity against human breast cancer cell line (MCF-7) and human lung cancer cell line (A-549). Among the synthesized compounds, **9f** and **9g** were found to be the most potent anticancer agents against human breast cancer cell line (MCF-7) with IC_{50} values of 9.35 µM and 9.58 µM, respectively. Compounds **9b**, **9c** and **9n** exhibited promising anticancer activity against human lung cancer cell line (A-549) with 43.71%, 46.48% and 44.26% inhibition at the highest concentration of 10 µM, respectively. Compounds **9c**, **9f** and **9g** have ability to inhibit the topoisomerase II enzyme. Compound **9f** showed most potent topoisomerase II enzyme inhibition activity with IC_{50} value of 12.11 µM. Further, these compounds have a high potential to be developed as a promising topoisomerase II inhibitors.

Keywords Aminoflavones · Buchwald coupling · Reductive amination · Anticancer agent · Topoisomerase II enzyme inhibitor · Malic acid

Introduction

Processes of cell growth viz. proliferation, migration, and angiogenesis are regulated by the various growth factors and mitogen-stimulated signaling networks. Any dysregulation of these factors can lead to cancer. Flavones are known to have anticancer activity through modulation of these factors [1]. Scientific findings have shown that the flavones inhibit mitosis [2, 3], angiogenesis [4] and enzymes such as tyrosine kinase [5], topoisomerase [6–8], aromatase [9], epoxide hydrase [10], and tubulin polymerization [11–14]. Most of these compounds are either natural products or derivatives (Fig. 1). Aminoflavones are a class of compounds having nitrogen directly attached to aromatic sp^2 carbon, exhibits diverse biological activity. Substituted aminoflavones (Fig. 1) show anti-tubercular, cytotoxic and kinase inhibitory activity with anti-proliferative effects [15, 16]. Compound

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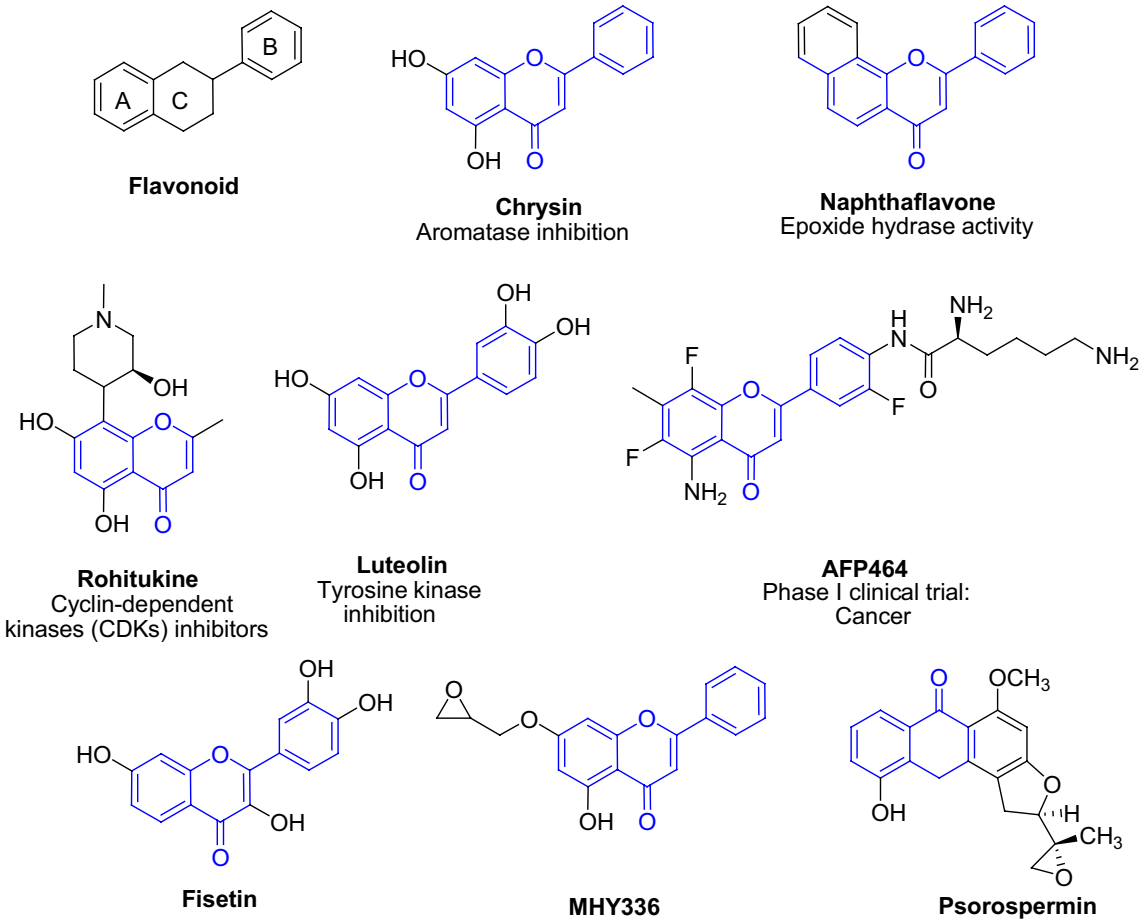

Fig. 1 Biologically active flavonoid structures

AFP646 is in phase-I clinical trial for cancer treatment [17]. A synthetic flavonoid MHY336 has ability to arrest the cell cycle in G2/M or S phase via Topo-II-dependent mechanism [18]. Dietary flavonoid fisetin acts as a dual inhibitor of Topo-I and Topo-II in cells [19]. Psorospermin, a natural antitumor antibiotic [20], intercalates DNA, and its alkylating potential is significantly increased in the presence of Topo-II [21, 22]. Unfortunate negligence of nature to aminoflavonoids as natural products might be attributed to intricacy in the synthesis of aminoflavones [15, 16], particularly those in which amino group is attached to **A** ring. This indeed limited structure activity relationship (SAR) studies of aminoflavones and related derivatives. Simon and co-workers have synthesized differently substituted 6-aminoflavones and shown that structural modifications particularly in B-ring would not have much impact on anticancer activity [23]. To improve inhibitory potency of 6-aminoflavone and to build SAR, a series of *N*-benzyl derivatives of 6-aminoflavones was designed as a novel anticancer agents. Herein, the present work reports the synthesis and anticancer activity of these novel flavones.

Results and discussion

Chemistry

Multistep synthetic protocol with synthesis of 1,3-diketone **5** from 4-bromophenol was initiated (**1**). 5-Bromo-2-hydroxyacetophenone (**3**) from **1** by methylation followed by Friedel–Craft acylation and in situ demethylation was prepared. To execute the Bekar–Venkataraman rearrangement, compound **3** was benzoylated by using benzoyl chloride and pyridine to get 5-bromo-2-benzoyloxy-acetophenone (**4**) which on further base catalyzed Bekar–Venkataraman rearrangement resulting in 1,3-diketone (**5**). Diketone **5** is then cyclized to 6-bromoflavone (**6**) employing malic acid under solvent free reaction condition [24]. The Buchwald coupling reaction of 6-bromoflavone (**6**) with benzophenone imine, Pd (II) catalyst and xantphos as ligand, Cs_2CO_3 as a base in dioxane at 90 °C delivered imine **7** in 72% yield. Hydrolysis of imine **7** with TFA in THF at room temperature gave 6-aminoflavone **8** in excellent yield. Kónya et al.

have reported direct amination of 6-bromoflavone; however, emphasized steric hindrance in the reacting amine is a limiting factor in this reaction [15]. α-Branching in reacting amine, hampers yield of the coupling reaction. However, to overcome this limitation, reductive amination protocol is used to synthesize novel 6-(1-arylmethanamino)-2-phenyl-*4H*-chromen-4-ones (**9a**–**n**). It improved the yields and efficacy of parallel synthetic protocol to intended target molecules. Various aldehydes were employed for synthesizing imines which were then reduced to corresponding arylmethanamino derivatives (**9a**–**n**) using $NaBH(OAc)_3$ in one-pot reaction protocol to get excellent yield (Table 1).

Biology

Initially in vitro anti-proliferative efficacy of the synthesized N-benzyl derivatives of 6-aminoflavone (**9a**–**n**) was evaluated against human breast cancer cell line (MCF-7) and human lung cancer cell line (A-549) using MTT assay. Doxorubicin (standard antitumor drug) was used as a positive control. Cancer cells were treated with synthesized derivatives at various concentrations such as 0.1 uM, 1 uM and 10 uM to determine the % cancer cell inhibition and % cancer cell viability so as to predict the in vitro anticancer activity.

Table 2 shows that most of the synthesized compounds have shown good anticancer activity against human breast (MCF-7) and lung (A-549) cancer cell lines. **9f** was found to be the most potent anticancer compound among the synthesized derivatives against human breast cancer cell line (MCF-7) with 52.90% cancer cell line inhibition. **9g** was found to be the second most potent anticancer compound among the synthesized derivatives against human breast cancer cell line (MCF-7) with 52.17% cancer cell line inhibition. Other compounds such as **9a**, **9k** and **9n** showed good anticancer activity against human breast cancer cell line (MCF-7) with 42.03%, 30.67% and 44.76% cancer cell line inhibition, respectively.

New derivatives **9(a–n)** also displayed good anti-proliferative activity against human lung cancer cell line (A-549). Thus, compound **9c** exhibited 46.48% inhibition against human lung cancer cell line (A-549), while compounds **9n, 9b** and **9j** showed 44.26%, 43.71% and 41.96% of human lung cancer cell line inhibition, respectively, at 10 uM concentration. Compounds **9f, 9g** and **9n** were found to be more potent anticancer agent than that of standard drug Doxorubicin against MCF-7 cells. Compound **9a** was found to be equipotent to that of Doxorubicin against MCF-7 cells. Compounds **9b, 9c,** and **9n** were found to be more potent anticancer agents than that of Doxorubicin against A-549 cells. Compound **9j** with *p*-methoxy group was found to be equipotent to that of Doxorubicin against A-549 cells.

From SAR (Table 2), it is confirmed that 2-phenyl-4*H*-chromen-4-one is essential for the anticancer activity. Various 6-(substituted benzyl amino)-2-phenyl-4*H*-chromen-4-one derivatives were synthesized to study the effect of substituent on benzyl amino ring. Compound **9f** i.e., 6-(4-Chlorobenzylamino)-2-phenyl-4H-chromen-4-one with 4-chloro substitution on the benzylamino group was found to be potent anticancer agent among all synthesized derivatives against human breast cancer cell line (MCF-7). Compound **9g** with 4-bromo substitution on the benzylamino group showed good anticancer activity against MCF-7 cell line. Substituents like *m*-NO_2 (**9a**), *p*-ethoxy (**9k**), 3, 4-dimethoxy (**9m**) and 3,4-dichloro (**9c**) on benzylamino group resulted in decrease in the activity.

Compound **9c** with 2,3-dichloro substituent on benzylamino group showed good (46.48%) inhibition against the human lung cancer cell line (A-549), while replacing 2,3-dichloro substituent either by nitrile (**9b,** 43.71%) or *p*-methoxy (**9j**, 41.96%) resulted in decrease in activity. Other substituents like 4-bromo (**9g**, 36.51), 5-Bromo-2-hydroxy (**9d**, 35.69%) and 3-NO_2 (**9a,** 21.08) exhibited lesser inhibition against human lung cancer cell line (A-549). Interestingly, when benzyl group was replaced by quinolin-2-ylmethyl group, compound **9n** showed good in vitro anticancer activity against both the selected cell lines. This means that the replacement of benzyl group by some other heterocyclic ring may result in good anticancer activity. In general, electron withdrawing groups such as chloro- and bromo substituents on benzyl group exhibit good anticancer activity against both cell lines. The benzyl substitution with dichloro group (**9c**) was found to be more potent than that of with p-chloro group (**9b**) against A-549 cell lines. The para-fluro (**9e**) substituted derivatives were not found to be as good anticancer agents as that of para-chloro (**9b**) and para-bromo (**9g**) substituted derivatives.

Measurement of topoisomerase II activity

In order to shed light on core antitumor mechanism, the most active compounds (**9b, 9c, 9f, 9g and 9n**) were further evaluated to predict their ability to inhibit topoisomerase II enzyme. The catalytic activity of Topo II was evaluated according to the procedure reported by Patra et al. [18]. Doxorubicin was used as a standard drug in this assay. Doxorubicin inhibits the progression of topoisomerase II, enzyme which relaxes supercoils in DNA for transcription. Results of measurement of topoisomerase II activity are given in Table 3.

The synthesized compounds **9b**, **9c, 9f, 9g** and **9n** have an ability to inhibit topoisomerase II enzyme. Compounds **9f** and **9g** were found to be potent topoisomerase II enzyme inhibitor among tested synthesized compounds. Compounds **9f** and **9g** have shown IC_{50} value of 12.11 μM and 12.79 μM,

Table 1 Synthesis of novel *N*-benzyl derivatives of 6-aminoflavones

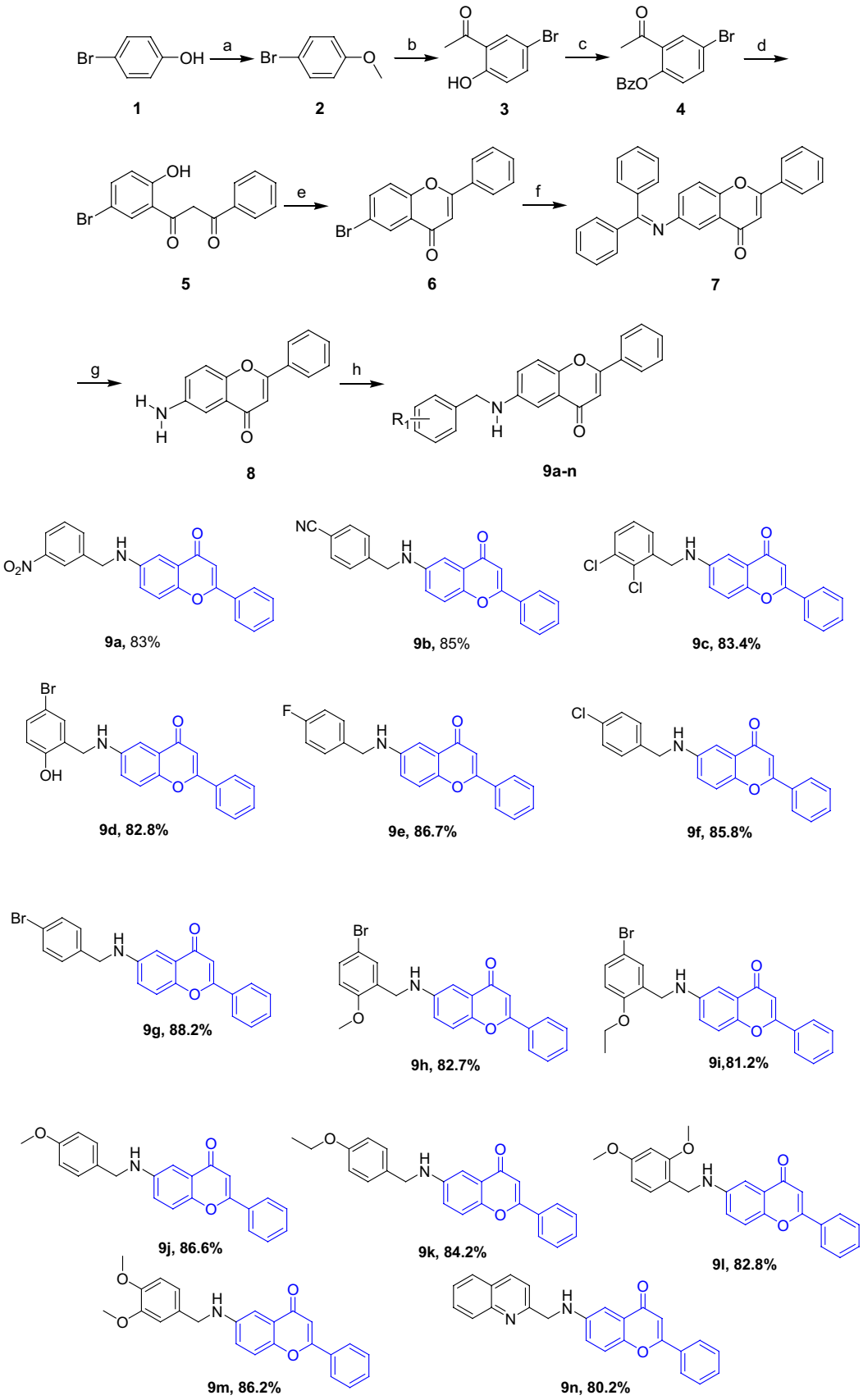

Table 1 (continued)

Reagents and Reaction Conditions: (a) K_2CO_3, Methyl iodide, Acetonitrile, 50 °C, 6 h; (b) acetyl chloride, anhy. $AlCl_3$, 6 h, r.t; (c) benzoyl chloride, C_5H_5N, 20 min, 3% HCl; (d) KOH, C_5H_5N, 15 min, 10% AcOH; (e) malic acid, oil bath preheated at 140 °C; (f) $Pd_2(dba)_3$, CS_2CO_3, benzophenone imine, and xantphos, dioxane, 16 h, 90 °C; (g) TFA, THF, r.t. 2 h; (h) aldehyde, sodium triacetoxy borohydride,TFA, DCM, 0 °C to r.t. 45 min

Table 2 Anticancer activity of novel *N*-benzyl derivatives of 6-aminoflavones (at 10uM)

	MCF-7			A-549		
	% Viability	% Inhibition	SD	% Viability	% Inhibition	SD
Control	100	0	1.08	100	0	2.00
9a	57.96	**42.03**	4.27	78.91	21.08	3.26
9b	92.77	7.22	1.66	56.28	**43.71**	0.67
9c	78.93	21.06	1.13	53.51	**46.48**	0.03
9d	92.47	7.52	1.09	64.30	**35.69**	2.72
9e	91.72	8.27	0.21	83.83	16.16	0.70
9f	47.09	**52.90**	1.09	90.25	9.749	0.49
9g	47.82	**52.17**	1.02	63.48	**36.51**	0.45
9h	102.95	0	3.88	90.88	9.11	4.65
9i	102.754	0	0.88	94.37	5.62	1.23
9j	103.91	0	3.57	58.03	**41.96**	1.02
9k	69.32	**30.67**	0.60	83.38	16.61	3.14
9l	99.49	0.50	0.91	92.96	7.03	1.55
9m	75.87	24.12	2.15	85.33	14.66	4.45
9n	55.23	**44.76**	0.03	55.73	**44.26**	3.32
Doxorubicin	57.78	42.22	0.98	56.33	43.67	1.34

SD standard deviation; human breast cancer cell line: MCF-7 and human lung cancer cell line: A-549

Table 3 Topoisomerase II enzyme inhibition, docking score and MM GBSA binding free energy of selected compounds

Compound	IC_{50} μM[a]	Docking score	MM GBSA Binding free energy
9b	35.46 ± 0.05	− 5.69	− 23.25
9c	28.18 ± 0.21	− 6.69	− 33.11
9f	12.11 ± 0.15	− 7.14	− 28.25
9g	12.79 ± 0.28	− 6.63	− 30.66
9n	30.92 ± 0.16	− 8.90	− 56.61
Doxorubicin	0.94 ± 0.05	− 11.18	− 42.33

[a] Three independent experiments were performed for each concentration

respectively, as compared to the Doxorubicin (0.94 μM). The synthesized compounds are not as strong topoisomerase II enzyme inhibitor as that of standard drug Doxorubicin.

Docking study

The activity of *N*-benzyl derivatives of 6-aminoflavone was justified by its predicted binding mode at the DNA cleavage site of topoisomerase II. Analysis of human Topo II co-crystal structure complexed with DNA and etoposide (PDB code 3QX3) showed that etoposide is incorporated between base pairs of DNA and forms H bonds with amino acid residue Asp479 and DNA guanine fragment (DG13). Certainly, the molecular docking study showed that all docked compounds had been placed in between DNA base pair and had good contact with Topo II residues and DNA fragments. Analysis of interaction of docked compounds with Topo II-DNA complex revealed existence of H-bonding interaction with amino acid residue Gln778, and pi–pi stacking interactions with DG13, DC8, and DG12 DNA fragments. It was also revealed that in most of the compounds, carbonyl group of flavone moiety stabilities ligand–protein complex by forming H-bond with amino acid residue Gln778. Similarly N-benzyl group support the complex by establishing pi–pi stacking interaction with various fragments of DNA. These results indicate that most of the compounds showed similar binding pattern with binding site amino acid residues as that of Doxorubicin (Figs. 2, 3).

Materials and methods

Chemistry

All reactions were performed in oven-dried glassware. All reagents and solvents were obtained from commercial

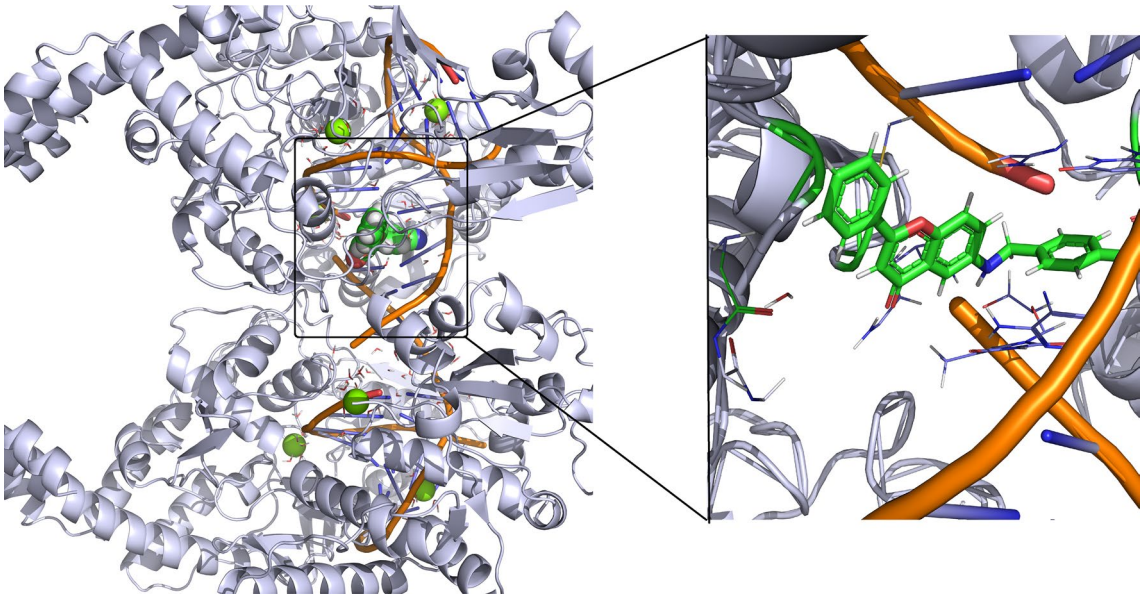

Fig. 2 Binding pose of *N*-benzyl derivative of 6-aminoflavone at DNA cleavage site of topoisomerase II

suppliers and used as received. Analytical thin-layer chromatography (TLC) was performed on precoated Merck silica gel plates (60F-254), visualized with a UV254 lamp and stained with $KMnO_4$. Melting points are uncorrected and were determined in open capillary tubes using paraffin oil bath. ^1H and ^{13}C NMR spectra were obtained as solutions in deuterated solvents. Standard ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Varian Mercury spectrometer in DMSO-d_6 and $CDCl_3$ solution and with tetramethylsilane as an internal standard. IR spectra were recorded on Perkin Elmer Model 1600 series FTIR instrument. Mass spectra were recorded on Agilent 1200SL-6100 LC/MS (ES-API) instrument. High-resolution mass spectra (HRMS) were performed with a QTOF Micromass Mass Spectrometer in electro spray ionization mode.

1-Bromo-4-methoxybenzene (2) Methyl iodide (1.31 g, 9.24 mmol) was added at RT to a stirred solution of 4-bromophenol (**1**, 1.0 g, 5.78 mmol) and K_2CO_3 (1.59 g, 11.56 mmol) in 10 mL acetonitrile. Reaction mixture was refluxed for 6 h at 50 °C. After completion of the reaction (TLC check), the mixture was cooled to room temperature, filtered through Celite bed and washed with ethyl acetate (3 × 20 mL). Organic layer was washed with water (3 × 40 mL), dried over anhydrous Na_2SO_4 concentrated on vaccuo to get colorless oil (0.91 g, 84%); b.p. 223 °C; IR (KBr): 1657, 1339, 840, 670 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40 (d, $J = 8$ Hz, 2H), 6.81 (d, $J = 8$ Hz, 2H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.1, 132.2, 115.7, 112.8, 55.4; LCMS (ES-API) m/z: 186.96 $(M+H)^+$.

5-Bromo-2-hydroxyacetophenone (3) To a stirred solution of 4-bromo anisole (**2**, 1.0 g, 5.37 mmol) in 10 mL dichloromethane, acetyl chloride (0.42 g, 5.37 mmol) was added at 0 °C. After 5 min of stirring anhydrous $AlCl_3$ (0.78 g, 5.91 mmol) was added portion wise, then reaction mixture was stirred for 6 h. After completion of the reaction (TLC check), the mixture was poured on crushed ice, product precipitates out. The precipitate thus obtained was filtered off, washed with diethyl ether and dried to get white solid. (0.9 g, 79%); m.p. 58–60 °C; IR (KBr): 3363, 1663, 845, 671 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 12.70 (s, 1H), 7.84 (s, 1H), 7.55 (t, $J = 8$ & 4 Hz, 1H), 6.90 (d, $J = 8$ Hz, 1H), 2.64 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.5, 161.3, 139.1, 132.9, 120.9, 120.5, 110.4, 26.7; LCMS (ES-API) m/z: 214.87 $(M+H)^+$.

5-Bromo-2-benzoyloxy-acetophenone (4) To a stirred solution of 5-bromo-2-hydroxyacetophenone (**3**, 1.0 g, 4.67 mmol) in 10 mL pyridine benzoyl chloride (0.78 g, 5.60 mmol) was added at 0 °C. After 20 min of stirring, reaction mixture was poured into ice cooled solution of 3% hydrochloric acid, product precipitates out. The crude product was filtered off washed with water and recrystallized from methanol to give yellow solid; m.p. 82–85 °C; IR (KBr): 3363, 1755, 1663, 845, 671 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.20–8.18 (m, 2H), 7.96 (d, $J = 2.4$ Hz, 1H), 7.69–7.64 (m, 2H), 7.55–7.51 (m, 2H), 7.13 (d, $J = 8.8$ Hz, 1H), 2.53 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 196.0, 164.8, 148.3, 136.1, 134.1, 132.9, 130.5, 130.3, 128.9, 125.8, 114.3, 29.7; LCMS (ES-API) m/z: 318.0 $(M+H)^+$.

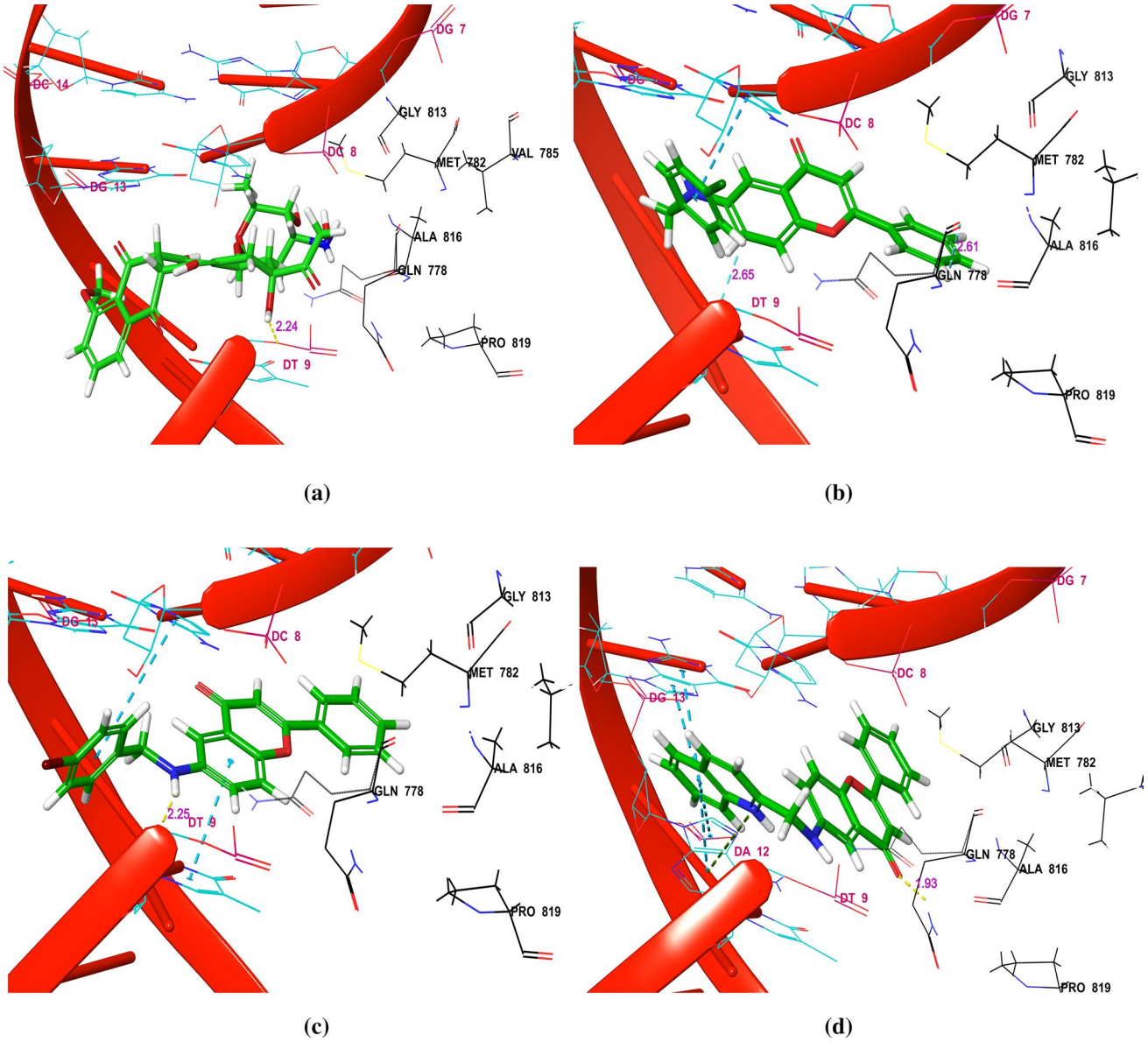

Fig. 3 Binding pose of compounds at DNA cleavage site of topoisomerase II. **a** Doxorubicin, **b** compound **9f**, **c** compound **9g** and **d** compound **9n**. Black colored wire representation of atoms indicates amino acid residues, cyan colored wire representation of atoms are fragments of DNA, yellow colored dotted line indicates H-bonding and cyan colored dotted line indicates pi–pi stacking

1-(5-Bromo-2-hydroxyphenyl)-3-phenylpropane-1,3-dione (5) To a solution of 5-bromo-2-benzoyloxy-acetophenone (**4**, 1.0 g., 3.14 mmol) in 7.5 mL of pyridine, potassium hydroxide (0.26 g., 4.71 mmol) was added and reaction mixture was stirred at 50 °C for 15 min. After completion of the reaction (TLC check), the mixture was poured in ice cooled solution of 10% aq. acetic acid (50 mL) product precipitates out. The solid thus obtained was filtered off, washed with 10% aq. acetic acid and dried to get yellow solid (0.89 g, 80%), m.p. 108–109 °C; IR (KBr): 3363, 1663, 845, 671 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 15.4 (bs, 1H), 12.05 (s, 1H), 7.97–7.95 (m, 2H), 7.86 (d, $J = 2$ Hz, 1H), 7.60–7.49 (m, 4H), 6.91 (d, $J = 8.8$ Hz, 1H), 6.76 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 186.5, 183.4, 172.7, 159.1, 138.4, 137.1, 135.1, 133.1, 131.7, 129.2, 128.9, 128.2, 127.5, 122.5, 121.0, 120.3, 110.8, 96.0; LCMS (ES-API) m/z: 318.0 $(M+H)^+$.

6-Bromo-2-phenyl-4*H*-chromen-4-one (6) The mixture of 1-(2-hydroxyphenyl)-3-aryl-1,3-propanedione (**5**, 1.0 g., 3.14 mmol) and malic acid (0.42 g., 3.14 mmol) was heated in a preheated oil bath at 140 °C for 10 min. After completion of the reaction (TLC check), the mixture was allowed to cool at room temperature, water (10 mL) and ethyl acetate

(20 mL) were added. Organic layer was washed with aq. $NaHCO_3$ (3 × 40 mL) and dried over anhydrous Na_2SO_4 then concentrated in vaccuo to get crude product. The crude product was purified by column chromatography on silica gel using hexane-ethylacetate (85:15) solvent system to give white solid (0.82 g, 87%); m.p. 194–196 °C; IR (KBr): 1645, 1604, 1568, 1130, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.20–8.10 (m, 3H), 8.01 (dd, $J = 10.8$ & 2.4 Hz, 1H), 7.83 (d, $J = 8.8$ Hz, 1H), 7.65–7.55 (m, 3H), 7.12 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.3, 163.4, 155.1, 137.3, 132.5, 131.3, 129.6, 127.3, 126.9, 125.3, 121.7, 118.3, 107.4; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{15}H_{10}BrO_2$: 300.9864, found: 300.9867.

6-(Benzhydrylidene-amino)-2-phenyl-chromen-4-one (7) The 6-bromo flavone (**6**, 1.0 g., 3.33 mol), CS_2CO_3 (1.62 g., 5.65 mmol), benzophenone imine (0.6 g., 3.33 mmol) was dissolved in dioxane (10 mL). Then it was degassed by argon gas for 20 min, xantphos (50 mg., 0.10 mmol) and $Pd_2(dba)_3$ (9 mg., 0.01 mmol) was added. The reaction mixture was stirred for 16 h at 90 °C. After completion of the reaction (TLC check), the mixture was cooled to room temperature, diluted with ethyl acetate and filtered through Celite bed. The filtrate was concentrated in vacuo. To this residue MeOH was added, the solid obtain was filtered and washed with diethyl ether. The crude product was purified by column chromatography on silica gel using hexane-ethylacetate (80:20) solvent system to give yellow solid (0.96 g, 72%); m.p. 248–249 °C; IR (KBr): 3442, 3063, 1637, 1616, 1455, 1361, 1290, 1025, 908, 775, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.89 (d, $J = 8$ Hz 2H), 7.80–7.77 (m, 2H), 7.60–7.54 (m. 1H), 7.50–7.52 (m, 4H), 7.45 (t, $J = 8$ & 5 Hz, 2H), 7.40–7.38 (m, 1H), 7.28–7.29 (m, 1H), 7.17 (t, $J = 8$ & 4 Hz, 3H), 7.11 (t, $J = 8$ & 4 Hz, 2H), 6.76 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.3, 169.9, 163.0, 152.6, 148.8, 139.7, 135.7, 131.9, 131.4, 129.4, 129.0, 128.3, 126.2, 124.1, 118.3, 116.0, 107.1; LCMS (ES-API) m/z: 402.14 $(M+H)^+$: HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{28}H_{20}NO_2$: 402.1489, found: 402.1492.

6-Amino-2-phenyl-4*H*-chromen-4-one (8) To a solution of 6-(benzhydrylidene-amino)-2-phenyl-chromen-4-one (**7**, 1.0 g., 2.49 mmol) in 40 mL of THF, trifluoroacetic acid (0.28 g., 2.49 mmol) was added at 0 °C. The reaction mixture was stirred for 2 h at room temperature. After completion of the reaction (TLC check), the mixture was concentrated in vacuo. The residue was neutralized by addition of aq. $NaHCO_3$ solution and then extracted in ethyl acetate (2 × 10 mL). Combined organic extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using hexane-ethylacetate (95:5) solvent system to give yellow solid (1.37 g, 81%); m.p. 200–202 °C; IR (KBr): 3416, 3342, 1733, 1609, 1565, 768, 677 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.10–8.01 (m, 2H), 7.55–7.58 (m, 3H), 7.54 (d, $J = 8.8$ Hz, 1H), 7.06–7.11 (m, 2H), 6.87 (s, 1H), 3.30 (s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.4, 163.0, 150.0, 144.1, 132.1, 131.3, 128.9, 126.2, 124.7, 122.2, 119.0, 107.9, 106.7; LCMS (ES-API) m/z: 238.9 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{15}H_{12}NO_2$: 238.0868, found: 238.0874.

General procedure for the synthesis of 6-amino-2-phenyl-4*H*-chromen-4-ones (9a–9n) To a solution of 6-amino-2-phenyl-4H-chromen-4-one (**8**, 1 g., 4.21 mmol) and substituted aromatic aldehydes (4.21 mmol) in 1,2-dichloroethane (10 mL), sodium triacetoxy borohydride (1.34 g., 6.34 mmol) was added at 0 °C. Trifluoroacetic acid (0.96 g., 8.43 mmol) was added to the above solution and reaction mixture was stirred at 0 °C to RT for 45 min. After completion of the reaction (TLC check), the mixture was diluted by adding DCM, organic layer was first washed with aq. NH_4Cl and then with saturated solution of brine. Organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified through column chromatography on silica gel using hexane-ethylacetate (85:15) solvent system to give the corresponding flavones derivatives in high yields.

6-(3-Nitrobenzylamino)-2-phenyl-4*H*-chromen-4-one (9a) Yellow solid, yield 82.6%, m.p. 157–160 °C; IR (KBr): 3280, 3062, 1762, 1222, 1188, 830, 504 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.25 (s, 1H), 8.12–8.10 (m, 1H), 8.05–8.03 (m, 2H), 7.87–7.85 (m, 1H), 7.67–7.63 (m, 1H), 7.59–7.57 (m, 4H), 7.23–7.20 (m, 1H), 6.98–6.96 (m, 1H), 6.91–6.90 (m, 1H), 6.88 (s, 1H), 4.54–4.52 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 177.3, 172.7, 159.2, 144.3, 134.0, 132.0, 130.0, 129.0, 128.4, 127.6, 126.2, 124.9, 121.2, 119.9, 118.0, 105.4, 103.1, 46.4; LCMS (ES-API) m/z: 372.0 $(M+H)^+$ HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{22}H_{17}N_2O_4$: 373.1188, found: 373.1195.

6-(4-Cyanobenzylamino)-2-phenyl-4*H*-chromen-4-one (9b) Yellow solid, yield 85.2%, m.p. 167–168 °C; IR (KBr): 3287, 3043, 2222, 1613, 1497, 671, 543 cm^{-1}; ^{1}HNMR (DMSO-d_6, 400 MHz) δ 8.05–8.03 (m, 2H), 7.81 (d, $J = 8.4$ Hz, 2H), 7.62–7.56 (m, 6H), 7.18 (dd, $J = 9.6$ & 3.2 Hz, 1H), 6.92 (d, $J = 2.8$ Hz, 1H), 6.88–6.86 (m, *2* H), 4.48–4.47 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.5, 163.1, 149.9, 144.9, 144.4, 132.7, 132.1, 131.5, 129.1, 127.9, 126.3, 124.9, 121.2, 119.4, 118.9, 111.3, 106.8, 104.8, 48.0; LCMS (ES-API) m/z: 353.0 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{23}H_{17}N_2O_2$: 353.1290, found: 353.1286.

6-(2,3-Dichlorobenzylamino)-2-phenyl-4*H*-chromen-4-one (9c) Yellow solid, yield 83.4%, m.p. 168–171 °C; IR (KBr): 3299, 3068, 1685, 1288, 1096, 811, 770 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.06–8.04 (m, 2H), 7.60–7.55 (m, 5H), 7.36–7.33 (m, 2H), 7.21–7.18 (m, 1H), 6.86–6.90 (m, 3H), 4.47–4.47 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.3, 162.6, 149.3, 145.5, 138.6, 134.3, 132.9, 131.9, 131.3, 129.0, 128.9, 127.3, 126.7, 126.0, 124.5, 121.1, 119.0, 106.3, 103.5, 46.1; LCMS (ES-API) m/z: 396,397.9, 400 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{22}H_{16}Cl_2NO_2$: 396.0558, found: 396.0552.

6-(5-Bromo-2-hydroxybenzylamino)-2-phenyl-4*H*-chromen-4-one (9d) Yellow solid, yield 82.8%, m.p. 188–191 °C; IR (KBr): 3287, 3105, 1719, 1643, 1413, 808, 548 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.99 (s, 1H), 8.10–7.90 (m, 2H), 7.57–7.54 (m, 4H), 7.31–7.10 (m, 3H), 6.99–6.93 (m, 2H), 6.92 s, 1H), 6.61 (d, $J=6.4$ Hz, 1H), 4.27–4.27 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.4, 162.6, 154.6, 149.4, 146.0, 131.9, 131.2, 131.1, 130.7, 128.9, 127.3, 126.0, 124.5, 121.7, 118.9, 117.2, 111.0, 106.3, 104.0, 43.51; LCMS (ES-API) m/z: 423.7 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{22}H_{17}BrNO_3$: 422.0392, found: 422.0398.

6-(4-Fluorobenzylamino)-2-phenyl-4*H*-chromen-4-one (9e) Yellow solid, yield 86.7%, m.p. 132–135 °C; IR (KBr): 3288, 3066, 1613, 1537, 1431, 842 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.91 (s, 2H), 7.63–7.26 (m, 8H), 7.08–7.02 (m, 3H), 6.78 (s, 1H), 4.38 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.6, 162.9, 149.8, 145.4, 137.7, 132.2, 131.9, 131.4, 129.3, 129.1, 126.3, 124.9, 121.4, 121.2, 119.2, 106.8, 104.6, 47.9; LCMS (ES-API) m/z: 346.2 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{22}H_{17}FNO_2$: 345.1165, found: 345.1172.

6-(4-Chlorobenzylamino)-2-phenyl-4*H*-chromen-4-one (9f) Yellow solid, yield 85.8%, m.p. 164–166 °C; IR (KBr): 3285, 3061, 1791, 1611, 1047, 999, 578 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.94–7.92 (m, 2H), 7.55–7.54 (m, 4H), 7.53–7.43 (m, 1H), 7.34–7.29 (m, 5H), 7.04 (t, $J=8$ Hz, 1H), 6.81 (s, 1H), 4.41 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.5, 162.9, 149.7, 145.4, 137.1, 132.1, 131.3, 129.0, 128.9, 128.8, 126.2, 124.8, 121.1, 119.1, 106.9, 104.4, 100.0, 47.7; LCMS (ES-API) m/z: 362.8$(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{22}H_{17}ClNO_2$: 362.0869, found: 362.0873.

6-(4-Bromobenzylamino)-2-phenyl-4*H*-chromen-4-one (9g) White solid, yield 88.2%, m.p. 174–176 °C; IR (KBr): 3287, 3037, 1745, 1611, 1135, 793 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.05–8.03 (m, 2H), 7.61–7.51 (m, 6H), 7.38 (s, 1H), 7.34 (s, 1H), 7.18 (dd, $J=8.8$ & 2.8 Hz, 1H), 6.94 (d, $J=2.8$ Hz, 1H), 6.89 (s, 1H), 6.80–6.77 (m, 1 H), 4.35–4.33 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.6, 162.9, 149.8, 145.4, 137.7, 132.2, 131.9, 131.4, 129.3, 129.1, 126.3, 124.9, 121.4, 121.2, 119.2, 106.8, 104.6, 47.9; LCMS (ES-API) m/z: 408.0 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{22}H_{17}BrNO_2$: 406.0442, found: 406.0446.

6-(5-Bromo-2-methoxybenzylamino)-2-phenyl-4*H*-chromen-4-one (9h) Yellow solid, yield 82.7%, m.p. 173–176 °C; IR (KBr): 3366, 3067, 1684, 1450, 1029, 708, 549 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.05–8.03 (m, 2H), 7.58–7.56 (m, 4H), 7.42- 7.32 (m, 2H), 7.18 (dd, $J=5.6$ & 3.2 Hz, 1H), 7.01 (d, $J=8$ Hz, 1H), 6.92–6.89 (m, 2H), 6.67–6.65 (m, 1H), 4.30–4.29 (m, 2H), 3.89 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.6, 162.8, 156.5, 149.6, 145.6, 132.1, 131.2, 131.2, 131.0, 129.0, 128.9, 128.8, 126.2, 124.8, 121.2, 119.0, 110.9, 106.6, 104.6, 55.6, 43.2; LCMS (ES-API) m/z: 437.9 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{23}H_{19}BrNO_3$: 436.0558, found: 436.0548.

6-(5-Bromo-2-ethoxybenzylamino)-2-phenyl-4*H*-chromen-4-one (9i) Yellow solid, yield 81.2%, m.p. 180–184 °C; IR (KBr): 3374, 3038, 1618, 1506, 910, 753 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.90 (s, 2H), 7.51–7.27 (m, 8H), 7.07–7.03 (m, 1H), 6.77 (s, 2H), 4.39 (s, 2H), 4.07 (d, $J=5.4$ Hz, 2H), 1.45 (m, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.6, 162.8, 155.9, 149.7, 145.7, 132.2, 131.3, 131.0, 129.2, 128.4, 128.9, 126.2, 124.9, 122.2, 121.2, 119.0, 112.9, 106.7, 104.7, 63.9, 43.3, 14.8; LCMS (ES-API) m/z: 450.1 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{24}H_{21}BrNO_3$: 450.0705, found: 450.0707.

6-(4-Methoxybenzylamino)-2-phenyl-4*H*-chromen-4-one (9j) Yellow solid, yield 86.6%, m.p. 134–136 °C; IR (KBr): 3296, 3107, 1613, 1484, 1177, 769 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.06–8.03 (m, 2H), 7.60–7.52 (m, 4H), 7.30 (d, $J=8$ Hz, 2H), 7.18 (dd, $J=8.8$ & 2.8 Hz, 1H), 6.96 (d, $J=2.8$ Hz, 1H), 6.92–6.89 (m, 3H), 6.66–6.64 (m, 1 H), 4.28–4.26 (m, 2H), 3.66 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.1, 162.2, 150.1, 146.1, 132.1, 131.8, 131.1, 130.7, 128.8, 128.6, 125.9, 124.4, 121.4, 118.7, 113.7, 106.1, 102.8, 55.1, 47.2; LCMS (ES-API) m/z: 358.0 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{23}H_{20}NO_3$: 358.1443, found: 358.1441.

6-(4-Ethoxybenzylamino)-2-phenyl-4*H*-chromen-4-one (9k) Yellow solid, yield 84.2%, m.p. 159–162 °C; IR (KBr): 3306, 3107, 1683, 1452, 1292, 767, 686 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.08–8.01 (m, 2H), 7.60–7.51 (m, 4H), 7.28 (d, $J=8.4$ Hz, 2H), 7.17 (dd, $J=6.4$ & 2.4 Hz, 1H), 6.96 (d, $J=6.8$ Hz, 1H), 6.91–6.88 (m, 3H), 6.64 (d, $J=3.6$ Hz, 1H), 4.27–4.25 (d, $J=8$ Hz, 2H), 3.98 (q, $J=14$ & 6.8 Hz, 2H), 1.30 (t, $J=6.8$ Hz, 3H); ^{13}C NMR

(DMSO-d_6, 100 MHz) δ 178.6, 162.7, 158.4, 149.5, 145.7, 132.2, 131.2, 130.4, 128.9, 128.1, 126.2, 124.8, 121.2, 118.9, 114.7, 106.6, 104.1, 63.4, 48.0, 14.8; LCMS (ES-API) m/z: 372.9 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{24}H_{21}NO_3$: 372.1599, found: 372.1599.

6-(2, 4-Dimethoxybenzylamino)-2-phenyl-4*H*-chromen-4-one (9l) Yellow solid, yield 82.8%, M.p. 126–129 °C; IR (KBr): 3359, 3038, 1613, 1523, 1274, 1120, 719 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.09–8.01 (m, 2H), 7.57–7.53 (m, 4H), 7.18–7.13 (m, 2H), 6.93 (d, $J=2.8$ Hz, 1H), 6.88 (s, 1H), 6.59 (d, $J=2.4$ Hz, 1H), 6.47–6.45 (m, 2H), 4.21–4.20 (m, 2H), 3.84 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.3, 162.4, 160.1, 158.3, 149.4, 146.2, 131.9, 131.1, 129.5, 126.9, 126.0, 124.5, 121.5, 118.9, 118.7, 106.2, 103.9, 103.5, 98.3, 55.3, 55.2, 42.8; LCMS (ES-API) m/z: 388.2 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{24}H_{22}NO_4$: 388.1549, found: 388.1544.

6-(3,4-dimethoxybenzylamino)-2-phenyl-4*H*-chromen-4-one (9m) Yellow solid, yield 86.2%, m.p. 138–141 °C; IR (KBr): 3288, 3106, 1613, 1484, 1152, 936, 541 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.08–8.01 (m, 2H), 7.57–7.53 (m, 4H), 7.18 (dd, $J=8.8$ & 2.8 Hz, 1H), 7.01–6.99 (d, $J=2.8$ Hz, 2H), 6.92–6.89 (m, 3H), 6.68–6.61 (m, 1 H), 4.27–4.25 (m, 2H), 3.74 (s, 3H), 3.71 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.6, 162.6, 160.4, 158.5, 149.4, 146.0, 132.2, 131.2, 129.9, 128.9, 126.1, 124.7, 121.4, 119.0, 118.8, 106.6, 104.5, 103.9, 98.7, 65.8, 55.4, 43.5; LCMS (ES-API) m/z: 388.2 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{24}H_{22}NO_4$: 388.1548, found: 388.1551.

6-((Quinolin-2-yl)methylamino)-2-phenyl-4*H*-chromen-4-one (9n) Brown solid, yield 80.3%, m.p. 146–149 °C; IR (KBr): 3347, 3037, 1696, 1198, 773 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.42 (d, $J=8.6$ Hz, 1H), 8.10–7.89 (m, 4H), 7.82–7.69 (m, 1H), 7.70–7.50 (m, 7H), 7.26–7.21 (m, 1H), 7.03–7.01 (m, 1H), 6.84 (s, 1H), 4.68–4.70 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 178.4, 162.0, 159.6, 153.3, 149.8 145.3, 138.3, 132.2, 132.08, 130.6, 129.1, 126.3, 126.2, 124.9, 122.2, 119.3, 124.9, 122.2,119.3, 118.7, 106.7, 103.7, 48.8; LCMS (ES-API) m/z: 379.0 $(M+H)^+$; HRMS (ESI) *m/z* $[M+H]^+$: calcd for $C_{25}H_{19}N_2O_2$: 379.1450, found: 379.1446.

Docking study

Molecular docking study was performed in Maestro 9.1 using Glide v. 6.8 (Schrodinger LLC). All compounds were built using Maestro build panel and optimized to lower energy conformers using Lig prep v3.5 (Schrodinger, Inc., New York, NY, USA). The X-ray crystallographic structure of the human topoisomerase II beta (PDB ID: 3QX3), in which drugs simultaneously interact with both DNA and enzymes [25] was taken from RCSB Protein Data Bank. The protein was prepared by using protein preparation wizard for docking using 'protein preparation wizard' in Maestro v10.3. It contains two chains, a chain along with DNA and Mg^{2+} ion. The ligand within active site and all water molecules were removed, while magnesium ion was allowed to remain with the charge of + 2. The active site was defined to include all atoms within 6.5A° radius of native ligand. The bond orders and formal charges were added to hetero-groups and hydrogen's were added to all atoms in the structure. Side chains that are not close to the binding cavity and do not participate in salt bridges were neutralized and termini were capped by adding ACE and NMA residue. After preparation, structure was refined to optimize hydrogen bond network using OPLS_2005 force field. The minimization was terminated when the energy converged or the RMSD reached a maximum cut off of 0.30 Å. The extra precision (XP) docking mode for all compounds was performed on generated grid of protein structure [26]. Final evaluation of ligand–protein binding was done with Glide score [27–29].

Docked pose of ligands was further rescored using Prime MM-GBSA approach which is used to estimate free binding energy of enzyme–ligands complex. Energies of ligand–receptor complexes were calculated using Prime MM-GBSA with flexibility set for residue surrounding 5 Å region of ligand binding site. Binding energy of receptor and ligand is as calculated by Prime Energy, a Molecular Mechanics + Implicit Solvent Energy Function (kcals/mol).

The binding free energy ΔG_{bind} is estimated as

$$\Delta G_{bind} = G(C) - G(P) - G(L)$$

where ΔG_{Bind} is total binding free energy, $G(C)$ is binding energy of protein ligand complex, $G(L)$ is binding energy of ligand and $G(P)$ is binding energy of protein.

Biological assay

In vitro anticancer screening

All newly synthesized compounds (**9a**–**n**) were evaluated for their in vitro anticancer activity against human breast cancer cell line (MCF-7) and human lung cancer cell line (A-549) by MTT assay. Two cell lines MCF-7 and A-549 (NCCS, Pune, India) were grown in DMEM medium containing 10% fetal bovine serum [Life technologies (Gibco)] and 0.7% antibiotics. Cells were seeded into 96 well microtiter plates in 100 µL of media at plating density of 5000 cells/well. Seeded cells were incubated at 37 °C, 5% CO_2, 95% air and 100% humidity for 24 h. At 24 h, old media was changed with fresh media followed by treatment with each compound

at 10 µM, 1 µM, and 0.1 µM. After 24 h treatment, cell viability was assessed by 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT), cells were incubated with 20 µL of MTT (5 mg/mL) in PBS for 4 h at 37 °C. The medium was removed and formazan crystals dissolved in DMSO. MTT reduction was quantified by measurement of absorbance at 570 nm using a multimode reader, Synergy Mx of BioTek [30]. Statistical employed methods were Sigma Plot 10.0 and Microsoft Excel 2010.

Measurement of topoisomerase II inhibitory activity

The most active anticancer agents from the synthesized series were further evaluated for prediction of their mode of action using Topo II drug screening kit (TopoGEN, Inc., Columbus) according to procedure reported by Patra et al. [18]. Doxorubicin was used as standard in this evaluation.

The reaction was started with incubation of a mixture consisted of human Topo II (2 µl), substrate super coiled pHot1 DNA (0.25 µg), 50 µg/mL test compound (2 µl), and assay buffer (4 µl) in 37 °C for 30 min. In order to terminate reaction, 10% sodium dodecylsulphate (2 µl) and proteinase K (50 µg/mL) were added at 37 °C for 15 min. followed by incubation for 15 min at 37 °C. Then, DNA was run on 1% agarose gel in Bio Rad gel electrophoresis system for 1–2 h followed by staining with GelRedTM stain for 2 h and destained for 15 min with TAE buffer. The gel was imaged via Bio Rad's Gel Doc TMEZ system. Both super coiled and linear strands DNA were incorporated in the gel as markers for DNA-Topo II intercalators. The results were reported as IC_{50} (50% inhibition concentration) values [31].

Conclusions

In conclusion, we have efficiently developed reductive amination protocol as an alternative route for the synthesis of novel *N*-benzyl derivatives of 6-aminoflavone which leads to increase in yield of the desired substituted aminoflavones. In vitro anti-proliferative activity against two human cancer cell lines (MCF-7 and A-549) and topoisomerase II inhibitory activity was performed. Among the synthesized derivatives, aminoflavones **9f** and **9g** were found to be most active anti-proliferative compounds against human breast cancer cell line (MCF-7) and aminoflavones **9c**, **9n**, **9b**, and **9j** demonstrated good anti-proliferative activity against human lung cancer cell line (A-549). However, compound **9n** has shown promising anti-proliferative activity against both cell lines. We found that compounds **9f** and **9g** exhibited robust inhibition of enzyme topoisomerase II with IC_{50} values 12.11 and 12.79 µM, respectively. The in silico docking studies of synthesized compounds showed that all compounds have good binding affinity toward topoisomerase II enzyme and have placed in between DNA base pair at active site of enzyme. Consequently, these *N*-benzyl derivatives of 6-aminoflavone may serve as lead scaffold for development of novel anti-proliferative agents. Our efforts in this regard are underway.

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Compliance with ethical standards

Conflict of interest There are no conflicts to declare.

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