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Synthesis of novel β-carboline based chalcones with high cytotoxic activity against breast cancer cells

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ABSTRACT

A series of novel β-carboline based chalcones was synthesized and evaluated for their cytotoxic activity against a panel of human cancer cell lines. Among them we found that two of the compounds **7c** and **7d**, showed marked anti-proliferative activity in a panel of solid tumor cell lines with highest effect in breast cancer. The compounds **7c** and **7d** showed an IC_{50} of 2.25 and 3.29 μM, respectively against human breast cancer MCF-7 cell line. Further, the compound **7c** markedly induced DNA fragmentation and apoptosis in breast cancer cells.

Cancer has become a major cause of death in the world and posed a great challenge to the fields of medicine and immunology. Approximately seven million people die from cancer every year due to which this disease has become responsible for 12.5% of death worldwide.[1] Chemotherapy has been widely employed for various cancer treatments. Most of the presently available chemotherapeutic agents work through the induction of apoptosis.[2] There are mainly two pathways by which cells undergo apoptosis; one is extrinsic and another one is intrinsic. Cancer cells are well known to aberrantly regulate either one or both of these pathways according to their need for survival and become resistant against most of the common chemotherapeutic drugs.[3] Therefore, development of new anticancer drugs and more effective treatment strategies for cancer are of utmost importance.

Natural anticancer pharmacophores play a major role in sensitizing the tumor cells toward apoptosis.[4] Among the natural products isolated from plants, chalcones, belonging to flavonoid family, represent one of the most interesting classes of biologically active compounds.[5–7] Anticancer properties of naturally occurring and synthetic chalcones continue to attract considerable interest.[8–10] Chalcones isolated from various plants species like *Angelica*, *Glycyrrhiza*, and *Scutellaria*, have been used in traditional medicine to treat various diseases including cancer.[11–14] Due to their high therapeutic index, ease of preparation, the potential for oral administration and safety, chalcones have emerged as potential anticancer agents.[15–17] Similarly, β-carboline alkaloids form a large group of natural and synthetic indole alkaloids. Their presence in medicinal plants such as *Peganum harmala* and *Eurycoma longifolia*, have drawn attention due to their antitumor activities.[18,19] Harmine, a β-carboline alkaloid isolated from the extracts of *Peganum harmala*, is highly cytotoxic and significantly inhibit tumor cell growth with apoptotic effects.[20] According to other reports, hyrtiocarboline, isolated from a Papua New Guinea marine sponge, *Hyrtios reticulates*, have also been identified as an antitumor agent.[21] Moreover, our group has also synthesized some β-carboline[22] derivatives that have shown good anticancer activity.[23]

Instigated with all the above facts and in continuation with our efforts towards natural product inspired synthesis of novel compounds of medicinal value,[24–26] we designed β-carboline based chalcones where one phenyl ring (ring B) was replaced with the β-carboline pharmacophore and discovered a new class of anticancer agents in this endeavor (Fig. 1), capable of targeting breast cancer cells.

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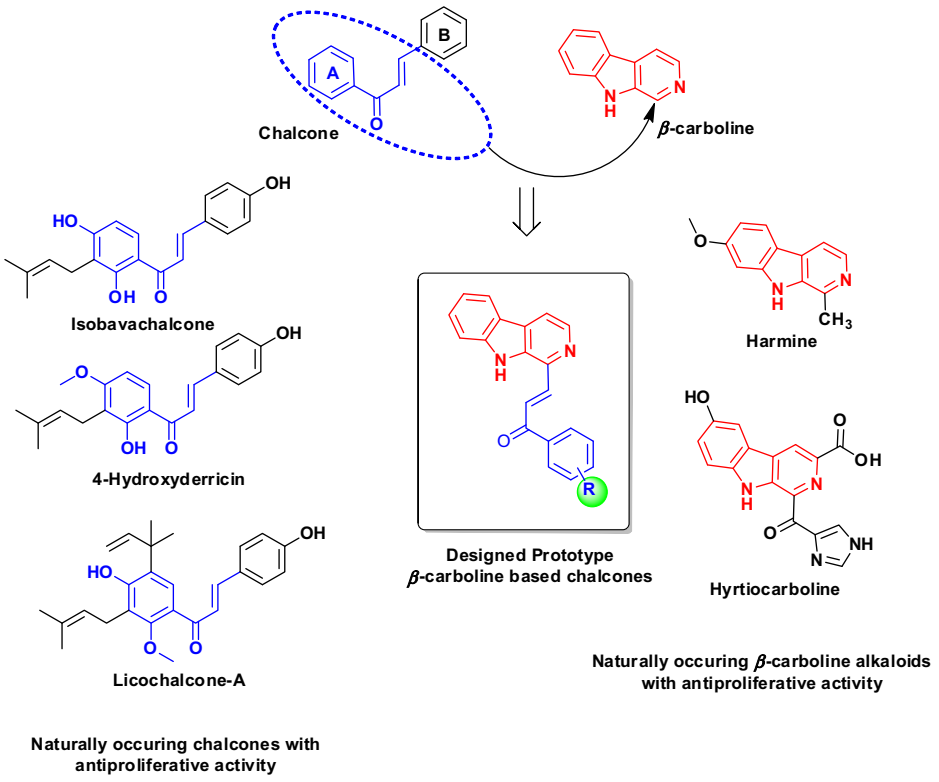
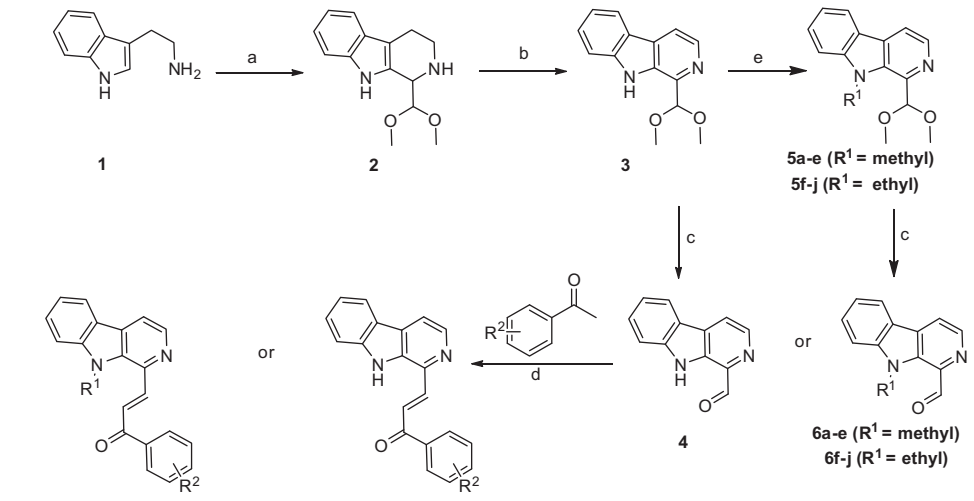


Figure 1. Designing of β-carboline based chalcones.



8a: R^1 = methyl, R^2 = H
8b: R^1 = methyl, R^2 = 4'-methoxy
8c: R^1 = methyl, R^2 = 3',4'-dimethoxy
8d: R^1 = methyl, R^2 = 3',4',5-trimethoxy
8e: R^1 = methyl, R^2 = 4'-methyl
8f: R^1 = ethyl, R^2 = H
8g: R^1 = ethyl, R^2 = 4'-methoxy
8h: R^1 = ethyl, R^2 = 3',4'-dimethoxy
8i: R^1 = ethyl, R^2 = 3',4',5'-trimethoxy
8j: R^1 = ethyl, R^2 = 4'-methyl

7a: R^2 = H
7b: R^2 = 4'-methoxy
7c: R^2 = 3',4'-dimethoxy
7d: R^2 = 3',4',5'-trimethoxy
7e: R^2 = 4'-methyl
7f: R^2 = 4'-bromo
7f: R^2 = 4'-chloro
7f: R^2 = 4'-fluoro

Scheme 1. Reagents and conditions: (a) $OHCCH(OMe)_2$, 5% TFA, CH_2Cl_2, rt, 17 h; (b) $KMnO_4$, THF, rt, 17 h; (c) $AcOH/H_2O$ (2:3 ratio), 120 °C, 1 h; (d) NaOH (10% in H_2O): MeOH, rt, 4 h; (e) RI, NaH, DMF, rt, 4 h.

Table 1
Percentage inhibition of compounds in various cancerous cell lines

Compd no.	MiaPaCa-2 (Pancreas)	PLC/PRF/5 (Liver)	SKOV-3 (Ovarian)	DU-145 (Prostate)	A-172 (Brain)	A549 (Lung)	MCF7 (Breast)	DLD-1 (Colon)
7a	21.74 ± 2.71	76.65 ± 5.11	0.91 ± 2.01	20.12 ± 3.22	78.44 ± 10.50	11.74 ± 6.22	32.87 ± 5.34	70.08 ± 5.55
7b	33.68 ± 2.40	70.78 ± 4.54	33.85 ± 2.71	61.86 ± 2.11	77.18 ± 6.20	29.2 ± 0.80	37.17 ± 3.11	74.19 ± 8.11
7c	87.53 ± 7.14	78.29 ± 5.44	41.57 ± 4.45	71.91 ± 11.05	74.32 ± 4.32	84.67 ± 2.44	94.55 ± 5.14	88.10 ± 9.70
7d	84.75 ± 9.11	73.93 ± 7.32	36.65 ± 6.50	76.09 ± 9.01	64.96 ± 4.24	73.44 ± 6.24	86.79 ± 10.14	80.58 ± 4.54
7e	2.10 ± 5.01	70.54 ± 5.14	5.10 ± 3.11	11.37 ± 4.09	60.47 ± 8.22	9.91 ± 5.21	20.92 ± 4.24	42.42 ± 2.44
7f	−4.72 ± 3.00	26.45 ± 3.55	−2.43 ± 3.14	−0.24 ± 3.22	5.41 ± 2.10	9.19 ± 3.32	7.15 ± 2.85	14.88 ± 2.22
7g	−13.08 ± 3.37	80.54 ± 5.44	0.20 ± 5.04	7.63 ± 2.04	75.78 ± 7.03	4.68 ± 2.34	15 ± 3.12	41.16 ± 5.02
7h	−4.04 ± 3.01	49.85 ± 1.55	8.15 ± 2.11	−5.2 ± 3.22	16.25 ± 2.87	6.73 ± 4.33	9.92 ± 1.97	25.64 ± 4.12
8a	25.49 ± 1.29	64.9 ± 4.12	28.63 ± 5.52	53.25 ± 4.31	75.46 ± 4.24	19.29 ± 3.11	27.24 ± 2.26	67.46 ± 6.62
8b	−0.03 ± 4.12	16.93 ± 1.34	37.43 ± 4.12	9.1 ± 3.25	0.18 ± 4.14	−3.88 ± 5.09	8.18 ± 3.24	28.25 ± 5.44
8c	7.71 ± 5.34	42.44 ± 6.54	68.67 ± 10.35	34.13 ± 4.45	15.54 ± 3.34	19.6 ± 6.68	12.94 ± 3.57	45.29 ± 4.45
8d	19.04 ± 8.93	40.09 ± 6.50	37.35 ± 9.21	59.49 ± 8.53	38.75 ± 3.59	33.84 ± 7.44	28.77 ± 5.53	49.45 ± .7.64
8e	13.72 ± 5.35	32.21 ± 4.42	46.16 ± 11.34	45.69 ± 5.65	19.19 ± 5.35	13.21 ± 7.46	20.79 ± 4.34	35.79 ± 5.67
8f	25.01 ± 5.59	75.13 ± 5.24	33.54 ± 11.24	54.23 ± 7.56	80.55 ± 8.58	12.54 ± 3.45	39.28 ± 7.59	77.57 ± 8.34
8g	9.18 ± 4.53	61.61 ± 4.30	43.74 ± 5.40	52.52 ± 8.93	39.24 ± 9.58	21.25 ± 7.49	27.24 ± 5.66	55.5 ± 8.24
8h	15.35 ± 8.36	56.2 ± 9.58	57.58 ± 4.59	56.83 ± 4.38	70.09 ± 2.35	35.63 ± 3.59	29.56 ± 2.59	66.04 ± 6.35
8i	24.73 ± 9.48	70.66 ± 4.91	68.54 ± 5.49	60.15 ± 7.39	81.12 ± 6.23	44.4 ± 2.21	32.7 ± 3.69	73.38 ± 5.24
8j	11.93 ± 3.29	34.09 ± 7.29	47.70 ± 9.69	35.03 ± 7.47	26.57 ± 6.35	16.43 ± 4.39	30.01 ± 6.36	44.14 ± 6.49
Doxorubicin	85.13 ± 4.54	90.66 ± 5.64	69.20 ± 5.45	73.92 ± 7.12	67.63 ± 8.58	69.05 ± 9.57	56.93 ± 6.48	73.22 ± 7.74

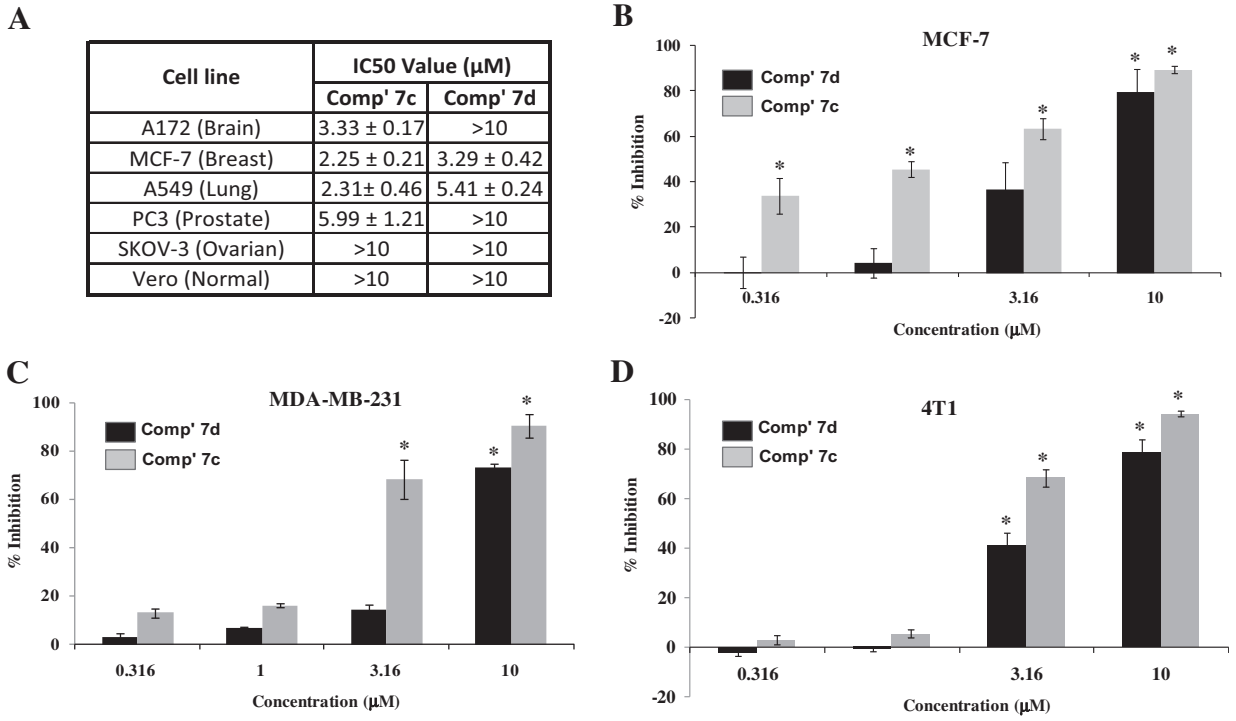


Figure 2. Selected β-carboline based chalcones (compounds **7c** and **7d**) inhibit cellular proliferation of different human carcinoma cells with varying potential and were found to be highly effective on breast cancer cells. Different human cancer cells (A172, MCF7, A549, PC3, SKOV-3 and normal Vero cells) were treated with varying concentrations of compounds for 48 h and the half maximal proliferation inhibitory concentration (IC_{50}) values were determined (Panel A) by SRB assay. Dose response histograms were shown for human breast cancer MCF-7 and MDA-MB-231 (Panel B and Panel C, respectively) and mouse breast cancer 4T1 (Panel D) cell lines. Results are representative of three independent experiments. *Columns*, average of triplicate readings of samples; *error bars*, ±SD $^{*}p$ <0.01, compared with vehicle treated cells.

In the present investigation, designed chalcones were prepared by the classical method that has been outlined in Scheme 1. The synthesis of chalcone involved Claisen–Schmidt condensation of substituted 1-phenylethanone and β-carboline-1-carbaldehyde or *N*-alkylated β-carboline-1-carbaldehyde in 10% aqueous solution of NaOH and methanol. Our synthetic strategy started with the Pictet–Spengler reaction of tryptamine **1** with dimethoxyglyoxal, to afford the tetrahydro-β-carboline derivative **2**, which was oxidized with $KMnO_4$ at room temperature overnight, resulting in acetal **3**. The acetal **3** was deprotected in aqueous acetic acid to give the corresponding aldehyde **4**. In order to obtain acetal **5a**–**j** the intermediate **3** was alkylated with methyl and ethyl iodide in DMF, after deprotonation with sodium hydride, to give *N*-alkylated indole. Acetals **5a**–**j** was further deprotected with the same methodology as followed for acetal **3** to give corresponding aldehydes **6a**–**j**.[27] The aldehydes **4** and **6a**–**j** were coupled with substituted 1-phenylethanone to provide desired products **7a**–**h** and **8a**–**h**, respectively. The products were characterized by analysis and comparison of

their spectral and physical data including ^{1}H NMR and HRMS given in the 'Supporting information' provided along with this manuscript.

To study the in vitro anticancer activity of β-carboline based chalcones, we screened a series of 18 compounds in eight different cancer cell lines representing eight cancer types. Representative cell lines from different cancer types are DLD-1 (Colorectal adenocarcinoma), MCF-7 (Breast adenocarcinoma), PLC/PRF/5 (Liver hepatoma), A549 (Lung carcinoma), SKOV-3 (Ovarian adenocarcinoma), MiaPaca-2 (Pancreatic adenocarcinoma), DU145 (Prostate carcinoma), A-172 (Brain glioblastoma). Detailed results of these investigations in terms of percentage inhibitions of cell growth at 10 μM of each compound are given in Table 1. As part of present investigation to determine the important features of β-carboline based chalcone influencing their anticancer activity, we disclose here additional structural requirements for the anti-proliferative activity of this newly designed chalcones. The substituents to be introduced on the chalcone scaffold were chosen among those frequently found in naturally occurring compounds, such as methoxy. Moreover, with the goal to explore the influence of electron density on the activity of enone, we introduced substituent with varied electron density on the phenyl ring of the chalcone counterpart. For that purpose, methyl, besides methoxy group was used as electron donating groups and halogens were used as electron withdrawing groups. We observed that compounds substituted with electron donating group at the phenyl ring of chalcone (**7b**, **7c**, **7d**, **7e**) had positive impact towards the inhibitory activity of the compounds in comparison to the unsubstituted compounds (**7a**) and the compounds with electron withdrawing groups (**7f**, **7g**, **7h**). Further, for the structural optimization of the compounds alkylation of the indole NH was done with methyl (**8a**–**e**) and ethyl groups (**8f**–**j**) with the goal to determine whether the modification at the indole NH can improve the efficacy of the compounds. Unfortunately, a significant lowering was observed in the activity of the compounds in this case. Although the ethyl substituted compounds were found to be more active than methyl substituted compounds but overall these compounds were not as good as the parent compounds. These results indicate the importance of free indole NH group in β-carboline moiety for its anticancer activity in the compounds and can be correlated with the occurrence of natural β-carboline alkaloid like harmine and hyrtiocarboline, with antitumor activities, having free indole NH.[20,21] The primary screening of

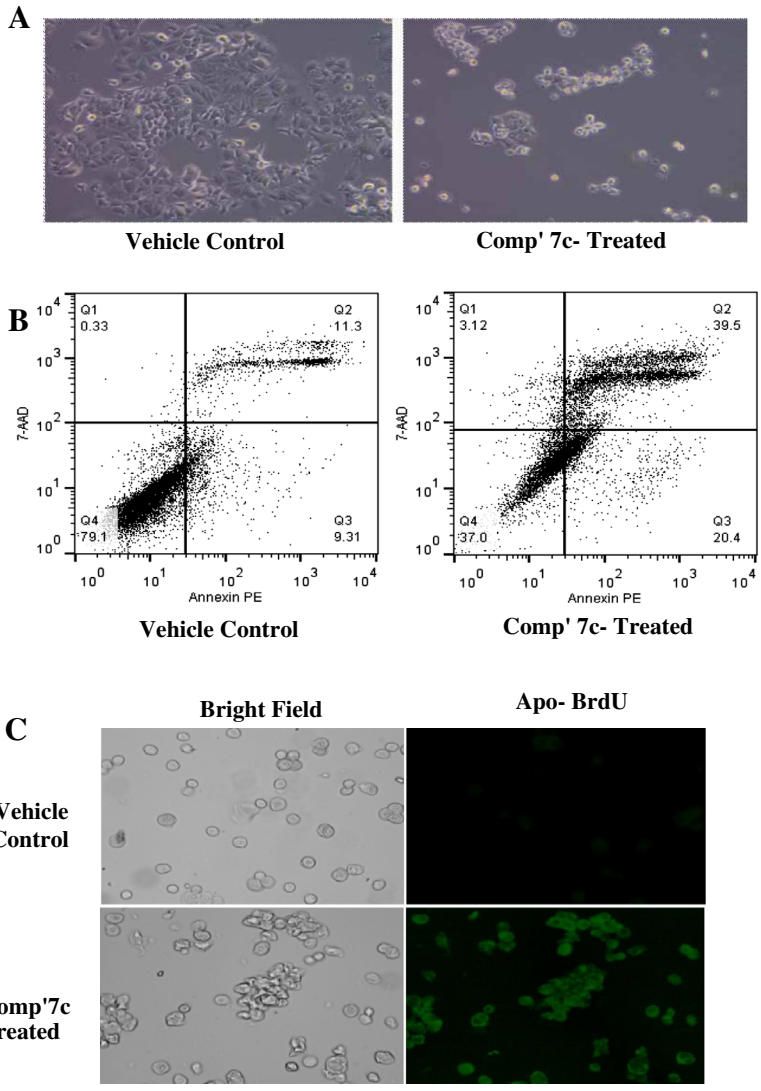


Figure 3. Effect of β-carboline based chalcone (compound **7c**) on breast cancer cell apoptosis. (A) Photo micrographs of confluency of MCF7 cells in vehicle treated and 24 h treatment of compound **7c**. (B) Flow cytometric analysis of MCF7 cells undergoing apoptosis after the treatment with compound **7c** at the indicated concentration for 24 h. The cells were harvested and labeled with Annexin-V-PE and 7-AAD and analyzed by Flow cytometry. (C) Apoptosis indicator DNA fragmentation was determined by Apo-BrdU TUNNEL assay. Bright Field (left panels) and fluorescent photo (right panels) micrographs of Alexa Flour 488-labeled anti-BrdU antibody for vehicle and compound **7c** treated MCF7 cells.

diversely substituted chalcones concluded that the OMe substituents are quite promising in providing the most active tumor cell growth inhibitors and relevant structure–activity relationship. Moreover, the activity of chalcones was found to be dependent on the presence, the number, and the positions of the methoxy group. The compounds with 3′,4′-dimethoxy (**7c**) and 3′,4′,5′-trimethoxy (**7d**) groups were found to be the most active among all the compounds screened against various cancer cell lines.

Therefore, based on the above observations compounds **7c** and **7d** were selected for further analysis and their respective IC_{50} (inhibitory concentration 50) values were calculated in six different cell lines by taking 4 different concentrations of 10, 3.16. 1 and 0.316 μM (half log scale). IC_{50} values of compounds **7c** and **7d** in different cancer cell lines are shown in Figure 2; Panel A. Consistent with our previous finding, here we observe that compounds **7c** and **7d** have shown IC_{50} values less than 5 μM in multiple cancer cell lines. However, based on the minimal IC_{50} values, we decided to perform rest of the experiments with compounds **7c** and **7d** on breast cancer cell lines. In this endeavor, dose response curves were prepared for compounds **7c** and **7d** at three different breast cancer cell lines MCF-7, MDA-MB 231 and 4T1 (Fig. 2; Panels B–D). Dose response curve clearly indicated that both compounds **7c** and **7d** were potentially active against all three breast cancer cell lines MCF-7, MDA-MB 231 and 4T1 in a dose dependent manner. However, at a same dose of compound **7c** and **7d**, we found that compound **7c** was more effective in killing three different types of breast cancer cells.

To determine the gross morphology change after the exposure of compound **7c** for 24 h, we examined treated and control cells under microscope and observed that there was change in structured cell morphology as well as marked reduction of cell numbers in treated groups compared to control (Fig. 3A). Reduction of cell numbers and gross phenotype alterations might be an indication of cellular apoptosis. To further validate that whether compound **7c** induces apoptosis in MCF-7 cells, we performed 7-AAD/Annexin-V staining and cells were analyzed by flow cytometry. Annexin-V binds to phosphatidylserine (PS) which is normally present in inner membrane and translocate to outer membrane as an early event in apoptotic cells whereas 7-AAD is a DNA labeling dye which does not enters the healthy cells as they are impermeable to it but enters the cells and label their DNA once their permeability changes in advance stages of apoptosis.

As shown in the Figure 3B, FACS-acquired dot plots of control and treated groups, percentage of Annexin-V^{low}/7-AAD^{low} (non apoptotic healthy cells), Annexin-V^{high}/7-AAD^{low} (early apoptotic cells), and Annexin-V^{high}/7-AAD^{high} (late apoptotic cells) cells were determined and found that after the treatment of compound **7c**, Annexin V^{high}/7-AAD^{low} population was increased. This indicates that treatment of compound **7c** enabled induction of apoptosis in MCF-7 cells.

To further confirm the apoptotic process in compound **7c** exposed cells, we assessed DNA fragmentation which is an essential step in the cells undergoing apoptosis. We performed APO-BrdU TUNNEL assay to detect DNA fragmentation as an indication of apoptosis in MCF-7 cells after **7c** treatment. This assay act on the basis that DNA breaks generated during apoptosis will expose a larger fraction of 3′-hydroxyl ends which can serve as starting point of terminal deoxynucleotidyl transferase (TdT) to label these breaks with deoxythymidine analog 5-bromo-2′-deoxyuridine 5′-triphosphate (BrdUTP) which is finally detected using anti-BrdU antibody using immouno-fluorescence techniques. Results of this experiment as shown in the Figure 3C clearly indicate that the number of MCF-7 cells showing enhanced green fluorescence in response to compound **7c** treatment compared to vehicle (DMSO) treated cells, which further proves its capability to induce apoptosis in breast cancer cells.

In conclusion, the present investigation led to the identification of newly designed β-carboline based chalcones with potential cytotoxic activities and brings into account the new structural elements that will aid in the development of more active chalcones. The compound **7c** demonstrated strong inhibition of the cellular proliferation of several cancer cell lines and found to be the highly effective in inducing apoptosis in breast cancer cells. Further, the easy and economical preparation opens a new avenue for the development of this new class of compounds as novel anticancer agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.109.

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