Synthesis and Biological Evaluation of Indolyl Bis-chalcones as Anti-Breast Cancer and Antioxidant Agents

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Abstract: A series of novel α-cyano substituted indolyl bis-chalcones (3a−l) has been synthesized and evaluated for their in vitro antitumor activity against the human breast cancer MCF7 (estrogen receptor-positive) and normal Vero cell lines using sulforhodamine B (SRB) assay method. Compounds 3a, 3c and 3d showed potent activity (GI50 = 11.7, 15.3 and 17.9 µM respectively) against the human breast cancer MCF7 cell line, which was almost as good as that of adriamycin (GI50 = < 0.1 µM) whereas, screening against the normal Vero Monkey cell line showed moderate selectivity. Furthermore, all the synthesized compounds screened for their antioxidant potential against DPPH, NO, SOR, and H2O2 radicals. Most of the bis-chalcones exhibited significant DPPH (51.09−12.72 %) and NO (64.11−34.43 %) radical scavenging activity and modest activity against SOR (88.08−43.14 %) and H2O2 (80.13−56.0 %) radicals compared to the reference standard ascorbic acid (40.78 %, 42.63 %, 87.05 %, and 79.42 % respectively). Current study provides impetus for the development of highly potent indolyl bis-chalcone derivatives as anticancer leads.

Keywords: indolyl bis-chalcone, breast cancer, anti-cancer activity, antioxidant activity.

INTRODUCTION

RECENTLY cancer is deemed to a principal worldwide health problem that leads to death.[1] Although considerable progress is made in controlling the progression of this devastating disease, till the date an entire cure for cancer remains a dream. Most of the cancer treatment is the use of surgery, radiation and chemotherapy.[2] Most of the marketed chemotherapeutic agents suffer from serious and sometimes intolerable toxic effects. So, the development of novel anticancer agents is a crucial need of time.[3,4] Chalcone is one of the important scaffold exhibiting diverse biological activities such as anti-inflammatory,[5] antimalarial,[6] antileishmanicidal, antiviral, antifungal, antibacterial and anticancer.[7,8] Different type of structural alterations was performed in the chalcones primary structure either by varying the aryl moieties or the enone linker. Another tactic which is not that typical in literature is to change the α-position of the α,β-unsaturated carbonyl system. This is a promising idea since it should have a direct and straightforward influence on the reactivity (Figure 1). Examples of the effect of α-alteration on biological activity are also present. First time Edwards et al. reported that α-substituted chalcones are more potent than their unsubstituted counter parts.[9] Lawrence et al. also improved cytotoxic effects of α-substituents such as phenyl, ester, cyano and fluoro groups on α,β-unsaturated carbonyl system.[10] Kumar et. al also reported α-cyano bis-indolyl chalcones as novel anticancer agents.[11] Recently, our research group reported α-cyano substituted bis-indolyl chalcone[12] and extended conjugated indolyl chalcones as potent anti-breast cancer agents.[13] In continuation of our constant efforts to discover a potent anticancer agents,[14−18] herein we have synthesized a series of novel α-cyano substituted indolyl bis-chalcone having phenyl ring as a spacer and in vitro evaluated for their anti-breast cancer and anti-oxidant activity (Scheme 1).
by dissolution of cyanoacetic acid (0.085 g, 1 mmol) in Ac₂O.

Indole

[@1]

NMR and 75 MHz for 13C NMR relative to TMS as an internal standard. The IR spectra were recorded on Shimadzu FT-IR instrument Bruker AV 400 MHz, for 1H NMR and 13C NMR spectra were recorded in DMSO- d6. Melting points were determined by open end capillary method and are uncorrected. All the 1H NMR spectra were obtained with UV light and or iodine vapors. All the solvents were dried using appropriate drying agents before use. The completion of reaction, the desired indolyl bis-chalcone (3a–I) was obtained as precipitate. The obtained precipitate was filtered, washed with water and oven dried. It was column purified by column chromatography using silica gel mesh size, 100–200 and elution with 10 % ethyl acetate in hexane.

(2E,2′E)-3,3′-(1,3-PHENYLENE)BIS(2-(1H-INDOLE-3-CARBONYL)ACRYLONITRILE) (3a)

Pale yellow solid; 88 %; 264–266 °C; IR (cm⁻¹): 3251 (NH), 2218 (CN), 1652 (C=O), 1593(C=C); 1H NMR (DMSO-d6, 400 MHz): δ = 11.69 (s, 2H, NH), 8.29–8.27 (m, 4H), 7.62 (d, J = 8.4 Hz, 2H), 7.48 (d, J = 8.4 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 7.20–7.16 (m, 5H), 6.64 (s, 1H); 13C NMR (DMSO-d6, 75 MHz): δ = 184.5, 152.1, 138.0, 137.1, 135.2, 127.8, 127.1, 122.4, 121.6, 121.0, 119.5, 119.1, 115.5, 111.0, 110.3, 108.2; HRMS: 467.4052 (M+H²⁺).

(2E,2′E)-3,3′-(1,3-PHENYLENE)BIS(2-(2-METHYL-1H-INDOLE-3-CARBONYL)ACRYLONITRILE) (3b)

Yellow solid; 91 %; 302–304 °C; IR (cm⁻¹): 3258 (NH), 2200 (CN), 1608 (C=O), 1545 (C=C); 1H NMR (DMSO-d6, 400 MHz): δ = 11.23 (s, 2H, NH), 8.18–8.15 (m, 2H), 7.65 (d, J = 7.6 Hz, 2H), 7.31–7.33 (m, 2H), 7.16–7.13 (m, 4H), 7.10–6.88 (m, 4H), 2.30 (s, 6H, -CH3); 13C NMR (DMSO-d6, 75 MHz): δ = HRMS: 495.1816 (M+H²⁺).

(2E,2′E)-3,3′-(1,3-PHENYLENE)BIS(2-(5-BROMO-1H-INDOLE-3-CARBONYL)ACRYLONITRILE) (3c)

Yellow solid; 89 %; 288–290 °C; IR (cm⁻¹): 3290 (NH), 3251 (NH), 2212 (CN), 2163 (CN), 1702 (C=O), 1690 (C=C); 1H NMR (DMSO-d6, 400 MHz): δ = 11.52 (s, 2H, NH), 8.48 (s, 2H), 8.34 (s, 2H), 7.69–7.63 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.09–6.99 (m, 3H), 6.94 (s, 1H), 6.79 (d, J = 8.8 Hz, 2H); 13C NMR (DMSO-d6, 75 MHz): δ = 185.3, 153.6, 138.2, 136.8, 135.0, 128.0, 128.2, 127.7, 124.5, 122.6, 121.0, 115.4, 113.2, 113.0, 110.6, 108.2; HRMS: 622.8913 (M+H²⁺).

General Procedure for the Preparation of Indolyl Bis-Chalcone (3a–I)

To a mixture of 3-cyanoacetylindole (0.184 g, 1 mmol) in ethanol (15 mL) was added piperidine (0.3 mL) and stirred for 5 min. Then isophthalaldehyde (0.134 g, 1 mmol) was added and this mixture was heated to 80 °C for 1–3 h. After completion of reaction, the desired indolyl bis-chalcone (3a–I) was obtained as precipitate. The obtained precipitate was filtered, washed with water and oven dried. It was column purified by column chromatography using silica gel mesh size, 100–200 and elution with 10 % ethyl acetate in hexane.

(2E,2′E)-3,3′-(1,3-PHENYLENE)BIS(2-(5-METHOXY-1H-INDOLE-3-CARBONYL)ACRYLONITRILE) (3d)

Pale yellow solid; 92 %; 264–266 °C; IR (cm⁻¹): 3281 (NH), 3245 (NH), 2220 (CN), 1635 (C=O); 1H NMR (DMSO-d6, 400 MHz): δ = 11.63 (broad s, 2H, NH), 8.63 (s, 2H), 8.43 (s, 2H), 7.83–7.76 (m, 1H), 7.67 (d, J = 2.0 Hz, 2H), 7.48–7.46 (m, 2H), 7.33 (d, J = 8.8 Hz, 2H), 7.22–7.18 (m, 3H), 3.83 (s, 6H, OCH3); 13C NMR (DMSO-d6, 75 MHz): δ = HRMS: 527.1714 (M+H²⁺).

(2E,2′E)-3,3′-(1,3-PHENYLENE)BIS(2-(5-CYANO-1H-INDOLE-3-CARBONYL)ACRYLONITRILE) (3e)

Yellow solid; 87 %; 252–254 °C; IR (cm⁻¹): 3392(NH), 2221 (CN), 2185 (CN), 1592(C=C); 1H NMR (DMSO-d6, 400 MHz): δ = 11.81 (broad s, 2H, NH), 8.62 (s, 2H), 7.76–7.69 (m, 4H), 7.47 (s, 2H), 7.34 (d, J = 7.4 Hz, 2H), 7.26–7.13 (m, 3H), 6.52

Figure 1. Chalcone framework.
(s, 1H); 13C NMR (DMSO-d6, 75 MHz): δ = 186.2, 153.3, 141.7, 138.7, 135.0, 128.4, 127.4, 127.0, 125.3, 123.6, 122.9, 118.5, 115.5, 111.4, 110.7, 108.2, 101.6; HRMS: 517.1323 (M+H)

**(2E,2'E)-3,3'-(1,3-PHENYLENE)BIS[2-(5-NITRO-1H-INDOLE-3-CARBONYL)ACRYLONITRILE]** (3f)

Yellow solid; 89%; 270–272 °C; IR (cm⁻¹): 3165(NH), 2216 (CN), 1607 (C=O), 1515 (NO₂); 1H NMR (DMSO-d6, 400 MHz): δ = 11.61 (broad s, 2H, NH), 8.68 (s, 2H), 8.60 (s, 2H), 8.31–8.28 (m, 2H), 7.77–7.76 (m, 1H), 7.48–7.44 (m, 2H), 7.25–7.19 (m, 5H); 13C NMR (DMSO-d6, 75 MHz): δ = 184.7, 154.0, 143.2, 138.7, 135.2, 132.1, 128.5, 127.8, 127.0, 126.1, 122.8, 115.4, 114.2, 112.0, 110.3, 108.4; HRMS: 557.4122 (M+H)

**(2E,2'E)-3,3'-(1,3-PHENYLENE)BIS[2-(1-METHYL-1H-INDOLE-3-CARBONYL)ACRYLONITRILE]** (3g)

Pale yellow solid; 90%; 264–266 °C; IR (cm⁻¹): 2219 (CN), 1614 (C=O), 1593 (C=C); 1H NMR (DMSO-d6, 400 MHz): δ = 8.62 (d, J = 6.5 Hz, 2H), 8.36 (s, 2H), 7.45 (d, J = 7.6 Hz, 2H), 7.57–7.52 (m, 4H), 7.40 (d, J = 8.0 Hz, 4H), 7.21 (t, J = 7.6 Hz, 1H), 6.62 (s, 1H), 3.65 (s, 6H, NCH₃); 13C NMR (DMSO-d6, 75 MHz): δ = 185.8, 153.7, 144.5, 139.3, 135.2, 128.5, 127.7, 124.5, 123.0, 122.7, 121.7, 119.8, 115.6, 110.7, 109.6, 108.2, 32.5; HRMS: 495.1816 (M+H)

**(2E,2'E)-3,3'-(1,3-PHENYLENE)BIS[2-(1,2-DIMETHYL-1H-INDOLE-3-CARBONYL)ACRYLONITRILE]** (3h)

Yellow solid; 92%; 238–240 °C; IR (cm⁻¹): 2225 (CN), 1614 (C=O), 1576(C=C); 1H NMR (DMSO-d6, 400 MHz): δ = 8.14 (d, J = 6.4 Hz, 2H), 7.50 (s, 2H), 7.50–7.42 (m, 6H), 7.26–7.20 (m, 3H), 6.70 (s, 1H), 3.63 (s, 6H, NCH₃), 2.51 (s, 6H, CH₂N); 13C NMR (DMSO-d6, 75 MHz): δ = 185.3, 186.8, 154.0, 140.3, 135.8, 128.5, 127.5, 126.2, 122.8, 121.8, 121.0, 119.7, 119.1, 115.5, 108.7, 103.0, 29.4, 12.2; HRMS: 523.4807 (M+H)

**(2E,2'E)-3,3'-(1,3-PHENYLENE)BIS[2-(1-METHYL-5-NITRO-1H-INDOLE-3-CARBONYL)ACRYLONITRILE]** (3i)

Yellow solid; 88%; 246–248 °C; IR (cm⁻¹): 2224 (CN), 1588 (C=O), 1563(C=C), 1534 (NO₂); 1H NMR (DMSO-d₆, 400 MHz): δ = 9.00 (s, 2H), 8.32 (s, 2H), 8.10 (d, J = 8.0 Hz, 2H), 7.93 (d, J = 8.0 Hz, 2H), 7.46 (s, 2H), 7.48 (d, J = 7.6 Hz, 2H), 7.22 (t, J = 7.6 Hz, 1H), 6.39 (s, 3H), 3.56 (s, 6H, NCH₃); 13C NMR (DMSO-d₆, 75 MHz): δ = 186.1, 154.2, 144.2, 140.5, 135.0, 128.5, 127.4, 120.7, 125.2, 123.6, 122.8, 118.4, 115.8, 111.7, 110.4, 108.1, 101.4, 32.5; HRMS: 545.4930 (M+H)

**PROCEDURE OF THE SRB-ASSAY FOR ANTICANCER SCREENING**

Tumor cells (human breast cancer cell line MCF-7, Source: NCI, USA and NCCS, Pune) were grown in tissue culture flasks in growth medium (RPMI-1640 with 2 mM glutamine, pH 7.4, 10 % fetal calf serum, 100 μg mL⁻¹ streptomycin, and 100 units mL⁻¹ penicillin) at 37 °C under the atmosphere of 5 % CO₂ and 95 % relative humidity employing a CO₂ incubator. The cells at sub confluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02 % EDTA) and placed in flasks in growth medium. The cells with more than 97 % viability were allowed to grow for one day at 37 °C in a CO₂ incubator. Suitable blanks and positive controls were added to the wells and cells were further allowed to grow for another 48 h. Suitable blanks and positive controls were also included. Each test was performed in triplicate. The cell growth was stopped by gently layering of 50 μL of 50 % trichloroacetic acid. The plates were incubated at 4 °C for 24 h; 

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an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were gently pipette out and discarded. The plates were washed five times with doubly distilled water to remove TCA, growth medium, etc. and were air-dried. 100 μL of SRB solution (0.4 % in 1 % acetic acid) was added to each well and the plates were incubated at ambient temperature for half an hour. The unbound SRB was quickly removed by washing the wells five times with 1 % acetic acid. Plates were air-dried, tris-buffer (100 μL of 0.01 M, pH 10.4) was added to all the wells and plates were gently stirred for 5 min on a mechanical stirrer. The optical density was measured on ELISA reader at 540 nm. The cell growth at absence of any test material was considered 100 % and in turn growth inhibition was calculated. GI50 values were determined by regression analysis.

2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) RADICAL SCAVENGING ACTIVITY

In this method, 0.1 mM DPPH solution was prepared in methanol by adding 39.4 mg of DPPH in 1000 mL of methanol, and to 0.5 mL of this solution, 1.5 mL of test compounds of the dissolved in DMSO were added at various concentrations of all (1, 10, 100, 500 and 1000 μg mL−1). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV–VIS spectrophotometer. Synthetic compound (1 mM) in DMSO dimer was added to H2O2 and absorbance was measured at 230 nm using a spectrophotometer. Synthetic compound (1 mM) in DMSO was added to H2O2 and absorbance was measured at 230 nm after 10 min against a blank solution containing phosphate buffer without H2O2. The percentage inhibition of H2O2 was calculated by formula,

\[
\% \text{ inhibition} (\text{H}_{2}\text{O}_2) = \frac{A_0 - A_1}{A_0} \cdot 100 \tag{1}
\]

where \(A_0\) is the absorbance of the control reaction, and \(A_1\) is the absorbance test compound and vitamin C.

NITRIC OXIDE (NO) RADICAL SCAVENGING ACTIVITY

The various concentrations of test compounds (as 1, 10, 100, 500, and 1000 μg mL−1) were prepared in ethanol. To 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline, to this, 1 mL of various concentrations of test compounds were mixed, and to this equal volume of freshly prepared Griess reagent was added, solution was then incubated at 25 °C for 3 h. Form this, 100 μL of the reaction mixture was transferred to a 96-well plate, and the absorbance was read at 546 nm using a microplate reader (Biotek, Italy). Ascorbic acid was used as standard control.

The percentage of nitrite radical scavenging activity of test compounds was calculated by

\[
\text{NO} \text{scavenging activity} = \frac{A_0 - A_1}{A_0} \cdot 100 \tag{2}
\]

where \(A_C\) is the absorbance of control, and \(A_I\) is absorbance of test compounds.

SUPEROXIDE RADICAL (SOR) SCAVENGING ASSAY

The reaction mixture consisting of 1 mL of nitro blue tetrazolium (NBT) solution (156 mM NBT in phosphate buffer, pH 7.4), 1 mL NADH solution (468 mM NADH in phosphate buffer, pH 7.4), and 1 mL of synthetic compound (1 mM) solution was mixed. The reaction was started by adding 1 mL of phenazine methosulfate (PMS) solution (60 mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance was measured at 560 nm against blank sample and compared with standards and percentage of inhibition was calculated using the same formula as above. Decreased absorbance of reaction mixture indicated increased SOR scavenging activity.

HYDROGEN PEROXIDE SCAVENGING (H2O2) ASSAY

A solution of H2O2 (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4). The concentration of H2O2 was determined by measuring absorption at 230 nm using a spectrophotometer. Synthetic compound (1 mM) in DMSO was added to H2O2 and absorbance was measured at 230 nm after 10 min against a blank solution containing phosphate buffer without H2O2. The percentage inhibition of H2O2 was calculated by formula,

\[
\% \text{ inhibition} (\text{H}_2\text{O}_2) = \frac{A_0 - A_1}{A_0} \cdot 100 \tag{3}
\]

where \(A_0\) is the absorbance of control and \(A_1\) is the absorbance of test sample.

RESULTS AND DISCUSSION

Chemistry

In the current study, syntheses of novel α-cyano substituted bis-chalcones (3a−l) were accomplished by the Knoevenagel condensation of 3-cyanoacetyl indoles 2 with substituted 3-isophthalaldehyde in the presence of piperidine in ethanol (Scheme 1). The starting compound, namely 3-cyanoacetyl indoles 2 were synthesized in good yield from the reaction of substituted indoles 1 with cyanoacetic acid in presence of acetic anhydride using the method described in the literature with minor modifications. The obtained crude products were purified by column chromatography using silica gel mesh size, 100–200 and elution with 10 % ethyl acetate in hexane. The structures of target molecules were analyzed by IR, 1H NMR and MS spectroscopic techniques.
A comparison of the TGI and LC50 concentrations of the drug adriamycin (GI50 = < 0.1 µM). On the other hand, cytotoxic against MCF-7 compared to the standard drug and LC50 drug development. Therefore, we have ensured the selectivity which is a major disadvantage in the progress of anticancer compounds. The cytotoxicity study showed that the GI50 against the normal Vero Monkey cell line. This cellular level screening results help to reveal the safety profile of active compounds with Adriamycin were also done. All the α-cyano substituted bis-chalcones were inactive (TGI and LC50 > 100 µM) like adriamycin against the MCF-7 cell line, however, decrease in activity was observed with cyano substitution. Comparing of GI50 values of 3a-d (GI50 = 11.7, 15.3 and 17.9 µM, respectively) and 3g-j (GI50 = 79.1 – >100 µM), we may presume that free NH of indole is essential for activity.

To confirm the effect α-cyano substituted chalcone and α-cyano substituted bis-chalcone on cytotoxic potential, we have prepared three simple α-cyano substituted chalcone analogues of compounds 3a, 3c and 3d by reacting suitable substituted 3-cyanoacetyl indole 2 with 3-(trifluoromethyl)benzaldehyde by refluxing in ethanol with the presence of piperidine. Comparison of the GI50 values against MCF-7 cancer cell line of α-cyano substituted chalcone and α-cyano substituted bis-chalcone were done. Bis-chalcone 3a, 3c and 3d havingphenyl ring as a spacer have increased the cytotoxic potential over their α-cyano substituted mono-indolyl chalcone analogues (Figure 2).

**IN VITRO ANTIOXIDANT ACTIVITY**

The series of bis-chalcone (3a–l) were evaluated for their direct scavenging activity against a variety of reactive oxygen and nitrogen species such as 2,2-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide (SOR), hydrogen peroxide (H2O2). Free radical scavenging substitution, compounds 3c and 3d containing bromo and methoxy group at 5-position of indole ring exhibited significant activity (GI50 = 15.3 and 17.9 µM) against MCF-7 cell line, however, decrease in activity was observed with cyano substitution. Comparing of GI50 values of 3a-d (GI50 = 11.7, 47.2, 15.3 and 17.9 µM, respectively) and 3g-j (GI50 = 79.1 – >100 µM), we may presume that free NH of indole is essential for activity.

**Biological Evaluation**

**IN VITRO ANTICANCER ACTIVITY**

All the synthesized novel α-cyano substituted bis-chalcones (3a–l) were evaluated for their in vitro anticancer activity against human breast cancer cell line MCF-7 by employing the sulforhodamine B (SRB) assay method. It is worth mentioning that most of the compounds were significantly cytotoxic against MCF-7 compared to the standard drug adriamycin (GI50 = < 0.1 µM). On the other hand, all other α-cyano substituted bis-chalcones showed weak cytotoxicity (GI50 = 79.1 – >100 µM) against MCF-7 cell line. A comparison of the TGI and LC50 Concentrations of the compounds with Adriamycin were also done. All the α-cyano substituted bis-chalcones 3a–l were inactive (TGI and LC50 > 100 µM) like adriamycin against the MCF-7 cell line.

Many reported drugs impact the normal cell growth, which is a major disadvantage in the progress of anticancer drug development. Therefore, we have ensured the selectivity of some active compounds by in vitro screening against the normal Vero Monkey cell line. This cellular level screening results help to reveal the safety profile of active compounds. The cytotoxicity study showed that the GI50 values for 3a, 3c and 3d are 65.1, 70.6 and 55.3 µM, respectively (Table 1). This novel α-cyano substituted bis-chalcones showed moderate selectivity against cancer lines over normal cell line.

Structure activity relationship (SAR) study reveals that the presence of electron donating groups at 5-position of indole holds better anticancer potential over electron withdrawing groups. Compound 3a with no substitution at 5-position of indole ring exhibited potent activity (GI50 = 11.7 µM) against MCF-7 cell line. Considering the type of

**Scheme 1.** Synthesis of novel α-cyano substituted bis-chalcones. Reagents and conditions: i) CNCH2COOH, (CH3CO)2O, reflux; ii) piperidine, ethanol, 80 °C, 1–3 h.

**Figure 2.** Comparison of anticancer activity of bis-indolyl chalcones over mono-indolyl chalcones.

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activity was measured in terms of percent inhibition by using reported procedure in literature and results are presented in Table 2. All the synthesized α-cyano substituted bis-chalcone have shown good to excellent scavenging activity against DPPH and NO radicals (Figure 3). The compounds 3d, 3a, 3c and 3b showed excellent DPPH free radical scavenging activity (51.09, 50.04, 46.90 and 41.10 %, respectively) as compared to standard ascorbic acid (AA) (40.78 %). Remaining compounds 3e-l showed moderate to weak DPPH free radical scavenging activity (12.72−37.90 %). Compounds 3a–g showed excellent NO free radical scavenging activity (42.67−63.11 %) as compared to standard ascorbic acid (42.63 %). All other compounds 3h–l showed moderate NO free radical scavenging activity (34.43−41.30). Compound 3b exhibited excellent activity (88.08 %) against SOR radical as compared to standard ascorbic acid (87.05 %). All other compounds were moderate SOR scavengers (43.14−85.92 %). Compound 3f have shown excellent H2O2 radical scavenging activity (80.13 %), whereas all other compounds showed moderate activity (56.00−73.23 %).

Table 1. In vitro anticancer screening of α-cyano substituted bis-chalcones (3a−l) against human breast cancer cell line MCF-7a and monkey normal kidney cell line Vero.

| Compound | R1 | R2 | R3 | MCF-7 | Vero (normal) |
|-----------|----|----|----|-------|---------------|
|           | LC50 | TGI | GI50 | LC50 | TGI | GI50 |
| 3a        | H   | H  | H  | >100 | >100 | 11.7 |
| 3b        | H   | CH3| H  | >100 | >100 | 15.3 |
| 3c        | H   | H  | Br | >100 | >100 | >100 |
| 3d        | H   | H  | OCH3 | >100 | >100 | >100 |
| 3e        | H   | H  | CN | >100 | >100 | >100 |
| 3f        | H   | H  | NO2 | >100 | >100 | >100 |
| 3g        | CH3| H  | H  | >100 | >100 | >100 |
| 3h        | CH3| CH3| H  | >100 | >100 | 79.1 |
| 3i        | CH3| H  | Br | >100 | >100 | >100 |
| 3j        | CH3| H  | OCH3| >100 | >100 | >100 |
| 3k        | CH3| H  | CN | >100 | >100 | >100 |
| 3l        | CH3| H  | NO2 | >100 | >100 | >100 |
| Adriamycin|     |     |    | 100  | 11.0 | <0.1 |
|           |     |     |    | >100 | >100 | 10.0 |

Concentrations in µM.

Concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) calculated from \(\frac{(T_i - T_z)}{T_z}\) × 100 = −50.

Drug concentration resulting in total growth inhibition (TGI) will calculated from \(T_i = T_z\).

Growth inhibition of 50% (GI50) calculated from \(\frac{(T_i - T_z)}{(C - T_z)}\) × 100 = 50.

Table 2. In vitro antioxidant activity of curcumin analogues (3a–l).

| Entry | % inhibition at 1 mM |
|-------|----------------------|
|       | DPPH | NO  | SOR | H2O2 |
| 3a    | 50.04 | 62.64 | 80.16 | 66.11 |
| 3b    | 41.10 | 59.97 | 88.08 | 71.00 |
| 3c    | 46.90 | 44.18 | 60.55 | 69.05 |
| 3d    | 51.09 | 63.11 | 79.12 | 56.00 |
| 3e    | 37.90 | 59.25 | 54.99 | 59.12 |
| 3f    | 31.32 | 51.11 | 54.00 | 80.13 |
| 3g    | 30.11 | 42.67 | 81.40 | 71.14 |
| 3h    | 27.18 | 36.01 | 85.92 | 73.23 |
| 3i    | 29.13 | 37.17 | 43.14 | 64.57 |
| 3j    | 30.09 | 34.43 | 54.21 | 60.16 |
| 3k    | 12.72 | 40.00 | 65.12 | 56.98 |
| 3l    | 17.43 | 41.30 | 43.43 | 63.19 |
| AA    | 40.78 | 42.63 | 87.05 | 79.42 |

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CONCLUSION

We designed and synthesized a series of novel α-cyano substituted bis-indolyl chalcone derivatives and in vitro evaluated them for their cytotoxic potential against breast cancer (MCF-7) and normal Vero Monkey cell line. Compound 3a, 3c and 3d showed strong activity against breast cancer as good as adriamycin. In general, the presence of electron donating groups at 5-position of indole ring over electron donating groups and free NH of indole are essential for activity. Antioxidant potential of synthesized compounds was also evaluated and most of the compounds exhibited significant DPPH and NO radical scavenging activity. The present investigation has thus provided impetus for the design and development of potent bis-indolyl chalcone derivatives as anticancer leads.

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