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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 (GI_{50} = 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 (GI_{50} = < 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 H_2O_2 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 H_2O_2 (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

PRESENTLY 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).

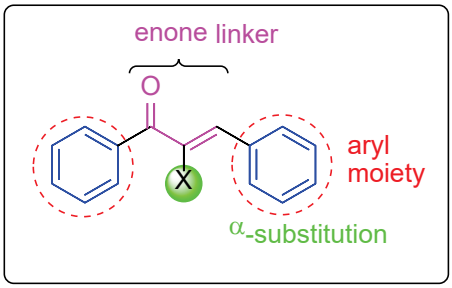


Figure 1. Chalcone framework.

EXPERIMENTAL

Materials and Methods

All the chemicals used for the synthesis were of synthetic grade and obtained from commercial sources. Development of the reactions was supervised by thin layer chromatography (TLC) using TLC plates (silica gel 60 F254, aluminum back, Merck). Visualization of TLC plate was achieved with UV light and or iodine vapors. All the solvents were dried using appropriate drying agents before use. Melting points were determined by open end capillary method and are uncorrected. All the ^{1}H NMR spectra were recorded in DMSO-*d*6 / $CDCl_3$ and chemical shifts in ppm were reported on instrument Bruker AV-400 MHz, for ^{1}H NMR and 75 MHz for ^{13}C NMR relative to TMS as an internal standard. The IR spectra were recorded on Shimadzu FT-IR spectrophotometer by using 1 % potassium bromide discs. Mass spectra were obtained with a Shimadzu LCMS-2010EV (Shimadzu, Japan). Anticancer activities were performed under the supervision of Dr.JyotiKode, Scientific Officer, Tata Memorial Centre, Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai-410210.

Synthesis

GENERAL PROCEDURE FOR THE PREPARATION OF 3-CYANOACETYLINDOLE (2)

Indole **1** (0.117g, 1 mmol) was added to a solution prepared by dissolution of cyanoacetic acid (0.085g, 1 mmol) in Ac_2O (10 ml) at 50 °C. The solution was heated at 85 °C for 5 min. During that period 3-cyanoacetylindole **2** started to crystallize. After 5 more min, the mixture was allowed to cool and solid was collected, washed with methanol, and dried.[12,13]

GENERAL PROCEDURE FOR THE PREPARATION OF INDOLYL BIS-CHALCONE (3a–l)

To a mixture of 3-cyanoacetylindole **2** (0.184g, 1 mmol) in ethanol (15 mL) was added piperidine (0.3 mL) and stirred for 5 min. Then isophthalaldehyde (0.134g, 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–l)** 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.

(2*E*,2'*E*)-3,3'-(1,3-PHENYLENE)BIS(2-(1*H*-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); ^{1}H NMR (DMSO-d_6, 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);^{13}C NMR (DMSO-d_6, 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+H)

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

Yellow solid; 91 %; 302–304 °C; IR (cm^{-1}): 3258 (NH), 2200 (CN), 1608 (C=O), 1545 (C=C); ^{1}H NMR (DMSO-d_6, 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, -CH_3);^{13}C NMR (DMSO-d_6, 75 MHz): δ = HRMS: 495.1816 (M+H)

(2*E*,2'*E*)-3,3'-(1,3-PHENYLENE)BIS(2-(5-BROMO-1*H*-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=O); ^{1}H NMR (DMSO-d_6, 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); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 185.3, 153.6, 138.2, 136.8, 135.0, 128.2, 128.0, 127.7, 124.5, 122.6, 121.0, 115.4, 113.2, 113.0, 110.6, 108.2; HRMS: 622.8913 (M+H)

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

Pale yellow solid; 92 %; 264–266 °C; IR (cm^{-1}): 3281 (NH), 3245 (NH), 2220 (CN), 1635 (C=O); ^{1}H NMR ($CDCl_3$, 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, OCH_3);^{13}C NMR ($CDCl_3$, 75 MHz): δ = HRMS: 527.1714 (M+H)

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

Yellow solid; 87 %; 252–254 °C; IR (cm^{-1}): 3392(NH),2221 (CN), 2185 (CN), 1592(C=O); ^{1}H NMR (DMSO-d_6, 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

(s, 1H); ^{13}C NMR (DMSO-d_6, 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)

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

Yellow solid; 89 %; 270–272 °C; IR (cm^{-1}): 3165(NH), 2216 (CN), 1607 (C=O), 1515 (NO_2);^{1}H NMR (DMSO-d_6, 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); ^{13}C NMR (DMSO-d_6, 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); ^{1}H NMR (DMSO-d_6, 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, NCH_3); ^{13}C NMR (DMSO-d_6, 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)

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

Yellow solid; 92 %; 238–240 °C; IR (cm^{-1}): 2215 (CN), 1594 (C=O), 1576(C=C); ^{1}H NMR (DMSO-d_6, 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, NCH_3), 2.51 (s, 6H, CCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 185.3, 168.6, 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)

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

Yellow solid; 87 %; 268–270 °C; IR (cm^{-1}): 2226 (CN), 1642 (C=O), 1526(C=C); ^{1}H NMR (DMSO-d_6, 400 MHz): δ= 8.40 (s, 2H), 7.59–7.50 (m, 4H), 7.46 (d, J = 7.6 Hz, 2H), 7.31–7.23 (m, 4H), 7.24 (t, J = 7.6 Hz, 1H), 6.60 (s, 1H), 3.61 (s, 6H, NCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 185.9, 154.4, 144.5, 135.3, 1351.1, 128.7, 128.2, 127.5, 124.3, 122.7, 121.0, 115.6, 113.2, 110.7, 110.0, 108.5, 32.6; HRMS: 653.0009 (M+1), 654.9952 (M+2), 655.2287 (M+3)

(2*E*,2'*E*)-3,3'-(1,3-PHENYLENE)BIS(2-(5-METHOXY-1-METHYL-1*H*-INDOLE-3-CARBONYL)ACRYLONITRILE) (3j)

Pale yellow solid; 90%; 270–272 °C; IR (cm^{-1}): 2216 (CN), 1621 (C=O), 1595(C=C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 8.40 (d, J = 4.0 Hz, 3H), 8.36 (s, 2H), 8.27 (dd, J = 8.0, 1.6 Hz, 2H), 8.00 (d, J = 2.8 Hz, 2H), 7.72 (t, J = 8.0, 7.6 Hz, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.04 (dd, J = 8.8, 2.4 Hz, 2H), 3.93 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 185.8, 154.3, 154.0, 144.2, 135.1, 128.7, 128.3, 127.6, 127.2, 122.7, 115.5, 112.0, 110.4, 109.3, 108.3, 104.2, 55.4, 32.1; HRMS: 555.2027 (M+H)

(2*E*,2'*E*)-3,3'-(1,3-PHENYLENE)BIS(2-(5-CYANO-1-METHYL-1*H*-INDOLE-3-CARBONYL)ACRYLONITRILE) (3k)

Yellow solid; 92 %; 260–262 °C; IR (cm^{-1}): 2226 (CN), 2217 (CN), 1661 (C=O), 1621(C=C); ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.32 (s, 2H), 7.80 (d, J = 6.8 Hz, 2H), 7.49 (s, 2H), 7.41–7.30 (m, 6H), 7.16 (t, J = 7.6 Hz, 1H), 6.67 (s, 1H), 3.68 (s, 6H, NCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 186.1, 154.2, 144.2, 140.5, 135.0, 128.5, 127.4, 127.0, 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)

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

Yellow solid; 88 %; 246–248 °C; IR (cm^{-1}): 2284 (CN), 1588 (C=O), 1563(C=C), 1534 (NO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ= 9.10 (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, 1H), 3.56 (s, 6H, NCH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 185.3, 154.1, 144.0, 142.5, 135.6, 132.2, 128.5, 127.2, 127.0, 126.2,122.4, 115.2, 114.0, 112.0, 110.3, 108.3, 32.6; HRMS: 585.5001 (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 % CO_2 and 95 % relative humidity employing a CO_2 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 growth medium. The cells with more than 97 % viability (trypan blue exclusion) were used for cytotoxicity studies. An aliquot of 100 µL (5×10^3 cells/well) of cells were transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for one day at 37 °C in a CO_2 incubator as mentioned above. The test materials at different concentrations (10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M) were then 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

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. GI_{50} 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 (Shimadzu, spectrophotometer). Vitamin-C was used as standard compound. Reduction in absorbance by test compounds and indicates radical scavenging activity. The scavenging activity by the DPPH radical was determined by

$$\mathrm{DPPH}_{\text{scavenging effect (\% inhibition)}} = \frac{A_0 - A_1}{A_0} \cdot 100 \quad (1)$$

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

$$\mathrm{NO}_{\text{scavenging activity}} = \frac{A_c - A_1}{A_C} \cdot 100 \quad (2)$$

where A_C is the absorbance of control, and A_1 is absorbance of test compounds.

SUPEROXIDE RADICAL (SOR) SCAVENGING ASSAY

The reaction mixture consisting of 1mL 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 1mL 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 (H_2O_2) ASSAY

A solution of H_2O_2 (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4). The concentration of H_2O_2 was determined by measuring absorption at 230 nm using a spectrophotometer. Synthetic compound (1 mM) in DMSO was added to H_2O_2 and absorbance was measured at 230 nm after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage inhibition of $H_2O_2$was calculated by formula,

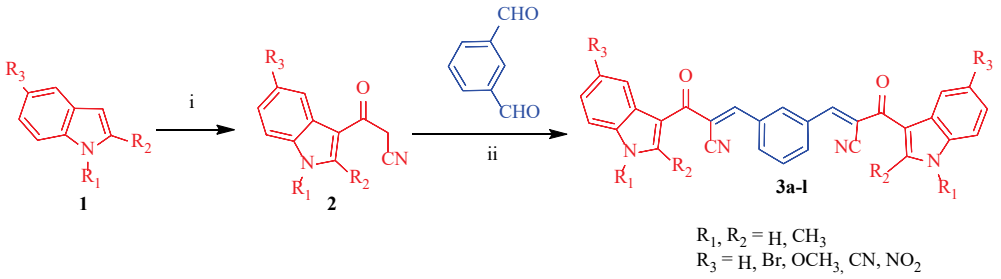
$$\%\ \text{inhibition}\ (H_2O) = \frac{A_0 - A_1}{A_0} \cdot 100 \quad (3)$$

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.[19] 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, ^{1}H NMR and MS spectroscopic techniques.



Scheme 1. Synthesis of novel α-cyano substituted bis-chalcones. Reagents and conditions: i) $CNCH_2COOH$, $(CH_3CO)_2O$, reflux; ii) piperidine, ethanol, 80 °C, 1–3 h.

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.[20] It is worth mentioning that most of the compounds were significantly cytotoxic against MCF-7 compared to the standard drug adriamycin, with the concentration of the drug that produced 50 % inhibition of cell growth (GI_{50}). Three parameters such as GI_{50}, TGI and LC_{50} were determined during the screening process and the results summarized in Table 1.

Compound **3a**, **3c** and **3d** exhibited potent activity (GI_{50} = 11.7, 15.3 and 17.9 μM, respectively) against the MCF-7 cell line which was almost as good as that of standard drug adriamycin (GI_{50} = < 0.1 μM). On the other hand, all other α-cyano substituted bis-chalcones showed weak cytotoxicity (GI_{50} = 79.1 – >100 μM**)** against MCF-7 cell line. A comparison of the TGI and LC_{50} concentrations of the compounds with Adriamycin were also done. All the α-cyano substituted b is-chalcones**3a–l** were inactive (TGI and LC_{50} > 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 GI_{50} 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 (GI_{50} = 11.7 μM) against MCF-7 cell line. Considering the type of substitution, compounds **3c** and **3d** containing bromo and methoxy group at 5-position of indole ring exhibited significant activity (GI_{50} = 15.3 and 17.9 μM) against MCF-7 cell line, however, decrease in activity was observed with cyano substitution. Comparing of GI_{50} values of **3a-d** (GI_{50} = 11.7, 47.2, 15. 3 and 17.9 μM, respectively) and **3g-j** (GI_{50} = 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 GI_{50} 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** having phenyl 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 (H_2O_2). Free radical scavenging

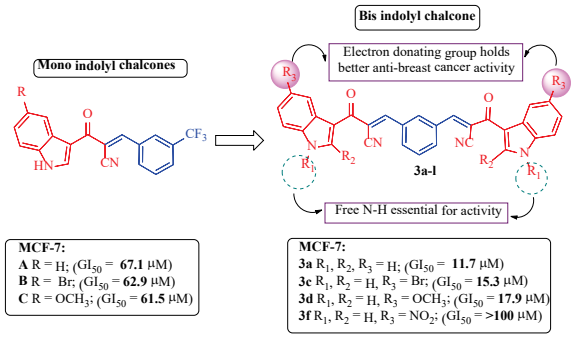
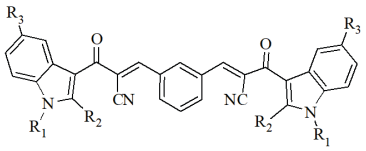


Figure 2. Comparison of anticancer activity of bis-indolyl chalcones over mono-indolyl chalcones.

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



Compound	R_1	R_2	R_3	MCF-7			Vero (normal)		
				LC_{50}[(b)]	TGI[(c)]	GI_{50}[(d)]	LC_{50}	TGI	GI_{50}
3a	H	H	H	> 100	> 100	11.7	> 100	> 100	65.1
3b	H	CH_3	H	> 100	> 100	47.2	> 100	> 100	> 100
3c	H	H	Br	> 100	> 100	15.3	> 100	> 100	70.6
3d	H	H	OCH_3	> 100	> 100	17.9	> 100	> 100	55.3
3e	H	H	CN	> 100	> 100	> 100	> 100	> 100	> 100
3f	H	H	NO_2	> 100	> 100	> 100	> 100	> 100	> 100
3g	CH_3	H	H	> 100	> 100	> 100	> 100	> 100	95.2
3h	CH_3	CH_3	H	> 100	> 100	79.1	> 100	> 100	> 100
3i	CH_3	H	Br	> 100	> 100	> 100	> 100	> 100	> 100
3j	CH_3	H	OCH_3	> 100	> 100	88.0	> 100	> 100	78.1
3k	CH_3	H	CN	> 100	> 100	> 100	> 100	> 100	> 100
3l	CH_3	H	NO_2	> 100	> 100	> 100	> 100	> 100	> 100
Adriamycin				100	11.0	< 0.1	>100	10.0	< 0.1

[(a)] Concentrations in μM.

[(b)] 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 $[(Ti - Tz) / Tz] \times 100 = -50$.

[(c)] Drug concentration resulting in total growth inhibition (TGI) will calculated from $Ti = Tz$.

[(d)] Growth inhibition of 50% (GI_{50}) calculated from $[(Ti - Tz) / (C - Tz)] \times 100 = 50$.

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 H_2O_2 radical scavenging activity (80.13 %), whereas all other compounds showed moderate activity (56.00–73.23 %).

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

Entry	% inhibition at 1 mM			
	DPPH	NO	SOR	H_2O_2
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

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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Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3758.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

REFERENCES

[1] M. H. El-Wakil, H.M. Ashour, M.N. Saudi, A. M. Hassan, I. M. Labouta, *Bioorg. Chem*. **2017**, *73*, 154–169. http://dx.doi.org/10.1016/j.bioorg.2017.06.009

[2] U. Bughani, S. Li, H.C. Joshi, *Recent Pat. Anti-Infect. Drug Discov.* **2009**, *4*, 164–182. https://doi.org/10.2174/157489109789318532

[3] E. Espinosa, P. Zamora, J. Feliu, M. G. Barón, *Cancer Treat. Rev.* **2003**, *29*, 515–523. https://doi.org/10.1016/S0305-7372(03)00116-6

[4] B. Mansoori, A. Mohammadi, S. Davudian, S. Shirjang, B. Baradaran, *Adv. Pharm. Bull*. **2017**, *7*, 339–348. http://dx.doi.org/10.15171/apb.2017.041

[5] C. Kontogiorgis, M. Mantzanidou, D. Hadjipavlou-Litina, *Mini. Rev. Med. Chem*. **2008**, *8*, 1224–1242. http://dx.doi.org/10.2174/138955708786141034

[6] A. Agarwal, K. Srivastava, S. Puri, P. M. S. Chauhan, *Bioorg. Med. Chem. Lett*. **2005**, *15,* 3133–3136. https://doi.org/10.1016/j.bmcl.2005.04.011

[7] X.-F. Liu, C.-J. Zheng, L.-P. Sun, X.-K. Liu, H.-R. Piao, *Eur. J. Med. Chem.* **2011**, *46*, 3469–3473. https://doi.org/10.1016/j.ejmech.2011.05.012

[8] M. Zoldakova, Z. Kornyei, A. Brown, B. Biersack, E. Madarász, R. Schobert, *BiochemPharmacol*. **2010**, *80*, 1487–1496. https://doi.org/10.1016/j.bcp.2010.07.046

[9] M. L. Edwards, D. M. Stemerick, P.S. Sunkara, *J. Med. Chem.* **1990**, *33*, 1948–1954. https://doi.org/10.1021/jm00169a021

[10] N. J. Lawrence, R. P. Patterson, L.-L. Ooi, D. Cook, S. Ducki, S., *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5844–5848. https://doi.org/10.1016/j.bmcl.2006.08.065

[11] D. Kumar, N. Maruthi Kumar, M. P. Tantak, M. Ogura, E. Kusaka, T. Ito, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5170–5174. https://doi.org/10.1016/j.bmcl.2014.09.085

[12] P. S. Bhale, H. V. Chavan, S. B. Dongare, S. N. Shringare, Y. B. Mule, P. B. Choudhari, B. P. Bandgar, *Current Bioactive Compounds* **2018**, *14*, 299–308. https://doi.org/10.2174/1573407213666170428112855

[13] P. S. Bhale, H. V. Chavan, S. B. Dongare, S. N. Shringare, Y. B. Mule, S. S. Nagane, B. P. Bandgar, *Bioorg. Med. Chem. Lett*. **2017**, *27*, 1502–1507. https://doi.org/10.1016/j.bmcl.2017.02.052

[14] S. N.Shringare, H.V. Chavan, P. S.Bhale, S.B.Dongare, Y. B. Mule, N. D.Kolekar, B. P.Bandgar, *Croat. Chem. Acta* **2018**, *91*, 357–366. https://doi.org/10.5562/cca3393

[15] S. B. Dongare, B. P. Bandgar, P. S. Bhale, S. N. Shringare, H. V. Chavan, *Croat. Chem. Acta* **2019**, *92*, 1–9. https://doi.org/10.5562/cca3418

[16] S. N. Shringare, H.V. Chavan, P. S. Bhale, S.B. Dongare, Y. B. Mule, S. B. Patil, B. P. Bandgar, *Med. Chem. Res*. **2018**, *27*, 1226–1237. https://doi.org/10.1007/s00044-018-2142-8

[17] P. S. Bhale, B. P. Bandgar, S. B. Dongare, S. N. Shringare, D. M. Sirsat, H. V. Chavan, *Phosphorus, Sulfur, and Silicon and the Related Elements* **2019**, *194*, 843–849. https://doi.org/10.1080/10426507.2019.1565760

[18] P. S. Bhale, H. V. Chavan, S. B. Dongare, S. T. Sankpal, B. P. Bandgar, *Anti-Cancer Agents in Medicinal Chemistry* **2018**, *18*, 757–764. https://doi.org/10.2174/1871520617666170912124258

[19] J. Slatt, I. Romero, J. Bergman, *Synthesis*, **2004**, *16*, 2760–2765. https://doi.org/10.1055/s-2004-831164

[20] P. Skehan, R. Strong, D. Scadiaro, A. Monks, J. Mc-Mahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyed, *J. Natl. Cancer Inst*. **1990**, *82*, 1107–1112. https://doi.org/10.1093/jnci/82.13.1107