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SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW CHALCONES AS POTENTIAL CYTOTOXIC AGENTS

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ABSTRACT

Chalcones, a group of compounds with two aromatic rings connected by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids exhibiting a wide spectrum of biological activities. The presence of a reactive α, β-unsaturated keto functional group is partly responsible for their activity. The main objective present study is to synthesize and characterize some new chalcones of 4-bromoacetophenone by reaction with various aromatic and heteroaromatic aldehydes. The structure of the synthesized compounds were confirmed by means of IR, ^{1}HNMR ^{13}C NMR, mass spectra and by elemental analysis. To screen the synthesized chalcones for their cytotoxic activities. Total compounds (B_2 to B_{25}) were synthesized. All these compounds are new and the characteristic physical and spectral data was presented separately in detail. All the chalcones exhibited characteristic absorption bands in the IR spectra (cm^{-1}), ^{1}H NMR and ^{13}C NMR spectra shows standard characteristic absorption bands. The chalcones (B_1-B_{25}) have been evaluated for their cytotoxicity against HT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines. Methotrexate was used as the reference standard. The results clearly revealed that most of the compounds possessed cytotoxic activity as evidenced by the IC_{50} values. Of all the compounds tested against HT-29 cell lines, the compound B_{22} having a thienyl moiety in its structure showed maximum activity with a IC_{50} value of 42 µg/mL. This is followed by compounds, B_{15} having a bromofuran moiety (IC_{50} 67 µg/mL), B_5 having a difluoro phenyl moiety (IC_{50} 68 µg/mL), B_{21} having a pyrrolyl moiety (IC_{50} 73 µg/mL), B_1 having a 4-methylphenyl moiety (IC_{50} 76 µg/mL) and B_6 having a dichlorophenyl moiety (IC_{50} 80 µg/mL). From this study, it can be concluded that the chalcones were found to have more cytotoxic activities.

Key words: Chalcones, cytotoxic activities, IR Spectra, NMR Spectra, Mass Spectra.

INTRODUCTION

Chalcones [1], a group of compounds with two aromatic rings connected by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids exhibiting a wide spectrum of biological activities. The presence of a reactive α, β-unsaturated keto functional group is partly responsible for their activity. Chalcones occur widely in nature particularly in colored flowers. All the Chalcones give pink coloration with concentrated H_2SO_4 (positive Wilson test) [2] and violet coloration with alcoholic ferric chloride solution when substituted with a phenolic hydroxyl. Chalcones on heating with traces of iodine in dimethyl sulphoxide (DMSO) for 2 hrs give the corresponding flavones. Chalcones were converted to the corresponding flavonols by their oxidation using hydrogen peroxide in methanolic sodium hydroxide solution and these flavonols showed characteristic greenish yellow fluorescence in ethanolic solution as well as with concentrated sulphuric acid [3]. A number of

Chalcones with novel substituents were isolated earlier in our laboratories from different Tephrosia species possessing significant antimicrobial and anticancer activities. This has prompted us to synthesize a number of other chalcones having aromatic/ heteroaromatic rings with number of substituent's and was also screened for these biological activities. The present work is a continuation of the earlier work with a view to prepare a large number of chalcones and to screen them for antimicrobial and cytotoxic activities. It is believed that such a focused approach would result in the identification of some promising leads at one or the other stage during the course of our systematic academic research work. Chalcones afford a facile route of access to many of the heterocyclic systems containing oxygen and nitrogen. An attempt is therefore made to synthesize chalcones from 4-bromoacetophenone by reaction with either aromatic or heteroaromatic aldehydes using Claisen-Schmidt condensation. The resulting chalcones were obtained after purification and characterization by physical and spectral methods. Since the chalcones were also reported to possess antimicrobial and cytotoxic activities, they were also screened for these activities.

The main objective present study is to synthesize and characterize some new chalcones of 4-bromoacetophenone by reaction with various aromatic and heteroaromatic aldehydes. Synthesized chalcones were characterizing the synthesized chalcones using IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses data. To screen the synthesized chalcones for their cytotoxic activities. To carry out the atom based 3D-QSAR studies in the case of cytotoxic activity on the synthesized chalcones in order to draw some useful correlations between the observed and predicted biological activities.

MATERIALS AND METHODS

The organic solvents such as methanol, acetone, chloroform and ethyl acetate were of spectral grade and used as such without further purification. Anhydrous methanol was obtained by fractional distillation and storing over type 4A molecular sieves. The acetone present in methanol was removed by using the following procedure. A mixture of 500 mL of methanol, 25 mL of furfural and 60 mL of 10% sodium hydroxide solution was refluxed for 12 h, then the mixture was distilled and the first few milliliters of the distillate was rejected as it contains trace amount of formaldehyde. Ethanol obtained by distillation of commercial ethyl alcohol was refluxed over ignited calcium oxide for 6 h and distilled at atmospheric pressure and then used. Some of the solvents were purchased from the local manufacturers and S.D Fine Chem. Ltd, Mumbai, India. All the chemicals used in the synthesis were obtained from standard commercial sources. 4-bromoacetophenone was purchased from Aldrich Chemical Co. (Melwaukee, Wisconsin, USA). Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography.

Synthesis of chalcones of 4-bromoacetophenone: A mixture of 4-bromoacetophenone (0.001mole) and the appropriate aryl aldehydes (0.001 mole) was stirred in ethanol (7.5 mL) and to it aqueous solution of KOH (50%, 7.5 mL) was added. The mixture was kept for 24 h and it was acidified with 1:1 mixture of hydrochloric acid and water, then it was filtered under vacuum and the product was washed with water **(Scheme 1).**

Synthesis of 1-(4'-bromophenyl)-3-(4"-methyl phenyl)-2-propen-1-one (B_1): A mixture of 4-bromoacetophenone (0.001 mole) and 4-methyl benzaldehyde (0.001 mole) was stirred in ethanol (7.5 mL) and to it aqueous solution of KOH (50%, 7.5 mL) was added. The mixture was kept for 24 hrs and it was acidified with 1:1 HCl and H_2O. Then it was filtered under vacuum and the product was washed with water. The obtained solid was purified by column chromatography and recrystallized from a mixture of ethyl acetate and hexane (1:1). By adopting the same procedure, compounds (B_2 to B_{25}) were also synthesized and characterized.

Spectral Properties of Chalcones: The oxygenated chalcones usually possess U.V absorption maxima in the range 340-390 nm and chalcones lacking B-ring oxygenation may have their absorption at considerably shorter wavelengths and a minor peak usually appears in the range 220-270 nm[4]. The infrared spectra of chalcones show usually a band near 1625-1650 cm^{-1}, characteristic of an α, β-unsaturated carbonyl group[5,6]. The α-H and β-H of chalcones resonate at δ 6.7 – 7.4 and δ 7.3 -7.7 as two doublets (J=17 Hz) respectively in the ^{1}H NMR spectra [7]. This large J value shows that the olefinic bond has trans geometry. In the ^{13}C NMR spectra of chalcones, the carbonyl carbon appears between δ 188.6 and 194.6[8]. The α and β carbon atoms give rise to signals in between δ 116.1 – 128.1 and δ 136.9 – 145.4 respectively and can be readily identified by their characteristic appearance as a six line multiple in the off resonance decoupled spectrum [9]. The

presence of the 2'-hydroxy group shifts the carbonyl carbon value to downfield by 3ppm relative to corresponding acetoxy and methoxy compounds, presumably owing to hydrogen bonding.

Cytotoxicity studies: The in-vitro cytotoxicity of the test compounds (**B_1 to B_{25}**) was evaluated by the MTT assay. This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with DMSO and the released, solubilized frozamine dye or formazan reagent is measured spectrophotometrically at 570 nm. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells [10-12].

Materials: HT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. DMEM (Dulbecco's Modified Eagels Medium), MEM (Minimum Essential Media Eagle), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, EDTA were purchased from Sigma chemicals (St.Louis,MO). Fetal bovine serum (FBS) was purchased from Arrow Labs, 96 well flat bottom tissue culture plates were purchased from Tarson.

Method:

a) **Maintenance of cell lines:** HT-29 and DU-145 cell lines were grown as adherent in DMEM media, whereas MCF-7 was grown in MEM media supplemented with 10% fetal bovine serum. The cultured was maintained in a humidified atmosphere with 5% CO_2.

b) **Preparation of samples for cytotoxicity:** Stock solutions of test compounds (B_1 to B_{25}**)** were prepared (10 mg/mL) in DMSO and from them various dilutions were made with sterile water to get the final drug concentrations of 10, 50, 100 and 200 mg/mL.

c) **Cytotoxicity evaluation:** The cells were seeded in 96 well plates at a density of $1x10^4$ (counted by Tryphan blue exclusion dye method) per well and were incubated for 24 hrs to recover. After incubation the medium was replaced with fresh media containing different dilutions of the test compounds. Then the plated were incubated for additional 48 hrs at 37^0C in DMEM/MEM with 10% FBS medium. Following incubation, the medium was removed and replaced with 90 µl of fresh DMEM without FBS. To the above wells, 10 µl of MTT reagent (5 mg/mL of stock solution in DMEM without FBS) was added and incubated at 37^0C for 3-4 hrs, there after the above media was replaced by adding 200 µl of DMSO to each well (to dissolve the blue formazan crystals) and incubated at 37^0C for 10 min. The absorbance at 570 nm was measured on a spectrophotometer.

Methotrexate was used as reference drug for comparison. Assay was performed in triplicate for three independent determinations. The cytotoxicity was expressed as IC_{50} (µg/mL) which is the concentration of the compound that inhibited proliferation rate of the tumor cells by 50% as compared to the control untreated cells. IC_{50} values were determined from the plot: % inhibition versus concentration.

$$\% \text{ inhibition at the given concentration} = 1 - \frac{\text{(Absorbance average)}}{\text{(Control absorbance average)}} \times 100$$

IC_{50} = Inv.log (50-c) / m; c and m derived from y = mx+c of plot of % inhibition Vs log C.

RESULTS AND DISCUSSION:

Characterization of Chalcones:

Compound B_1, analyzed for $C_{16}H_{13}BrO$, M.P. 166^o C, exhibited a $[M+H]^+$ at m/z 302 along with its isotope satellite signal at m/z 303 in its positive ion mode electron spray ionization mass spectrum (**Fig 1**). The I.R (cm^{-1}) spectrum, showed the characteristic absorption bands at 1659 (C=O), 1602 (C=C of Ar), 1504 (CH=CH) and 1115 (C-O-C).

The ^{1}H NMR spectrum (400 MHz, DMSO-d_6, showed the characteristic signals of CO-CH= and =CH-Ar at δ 7.26 and 7.35 as doublets (J=17 HZ) respectively confirming the trans geometry at the ethylenic double bond of the molecule. The spectrum also showed one aromatic methyl group integrating for three protons at δ 2.40. The peaks in between 7.18-7.79 integrated for ten protons, of which two were already accounted for the ethylenic protons and the other eight must be the aromatic protons.

The ^{13}C NMR(δ PPM) spectrum of compound B_1 exhibited the characteristic signals at 184.90 (C-1), 125.20 (C-2), 141.30 (C-3), 146.80 (C-2'), 136.50 (C-3'), 130.20 (C-4') and 134.80 (C-5'), 132.90 (C-1'), 126.80 (C-2'' and 6''), 128.70 (C-3'' and 5''), 134.90 (C-

4") and 24.20 (C of CH_3 at C-4") consistent with the proposed structure for the compound.

By adopting the above the synthetic procedure, compounds (B_2 to B_{25}) were also synthesized. All these compounds are new and the characteristic physical and spectral data was presented separately in detail. The physical characterization data of chalcones (B_1 - B_{25}) were given in **Table 1-2.** Elemental Analysis data of chalcones (B_1-B_{25}) results were given in **Table 3**.

All the (B_1 to B_{25}) chalcones exhibited characteristic absorption bands in the IR spectra (cm^{-1}) in between 1640-1660 (C=O), 1570-1605 (C=C quadrant of Ar), 1500-1550 (CH=CH), 1120-1240 (C-O-C) and at other regions of the spectrum depending upon the specific substituent present in each compound. The IR spectral data of chalcones (B_1-B_{12}) were given in **Table 4**.

The ^1H NMR spectra of the chalcones revealed the characteristic ethylenic protons of the chalcone system in between δ 6.85 and 8.10. The spectra also showed the peaks accounting for the aromatic protons and for the different substituent protons in between the corresponding regions of the spectrum. The ^{13}C NMR spectra of the chalcones exhibited the characteristic peaks of the carbonyl carbon in between δ 185-192, apart from the peaks corresponding to the other carbons. The IR spectral data of chalcones (B_1-B_{12}) were given in **Table 5.**
The mass spectra obtained by positive mode ionization method revealed the $[M+H]^+$ ions, whereas the spectra obtained by EI method revealed the molecular ion. In both the cases, the spectra also revealed the isotope satellite signals due to sulphur and halogens like chlorine and bromine, apart from the relevant fragment ions. The elemental analyses carried out for all the compounds supported the given molecular formulae.

The chalcones (B_1 - B_{25}) have been evaluated for their cytotoxicity against (Results were mentioned in **Table 6** HT-29 (colon cancer), MCF-7 (breast cancer) and DU-145 (prostate cancer) cell lines. Methotrexate was used as the reference standard.
The results clearly revealed that most of the compounds possessed cytotoxic activity as evidenced by the IC_{50} values. Of all the compounds tested against HT-29 cell lines, the compound B_{22} having a thienyl moiety in its structure showed maximum activity with a IC_{50} value of 42 µg/mL. This is followed by compounds, B_{15} having a bromofuran moiety (IC_{50} 67 µg/mL), B_5 having a difluorophenyl moiety (IC_{50} 68 µg/mL), B_{21} having a pyrrolyl moiety (IC_{50} 73 µg/mL), B_1 having a 4-methylphenyl moiety (IC_{50} 76 µg/mL) and B_6 having a dichlorophenyl moiety (IC_{50} 80 µg/mL). The other compounds also showed activity but at a higher IC_{50} values. The results indicated the importance of heteryl rings in enhancing the cytotoxic activity apart from the contribution of halogen substituent's on the aromatic ring.

Among the compounds tested for cytotoxicity on MCF-7 cell lines, again the compound B_{22} having the thienyl moiety showed maximum activity (IC_{50} 38 µg/mL). This is followed by compounds, B_{15} having a bromofuran moiety (IC_{50} 58 µg/mL) and B_1 having the methyl phenyl moiety (IC_{50} 83 µg/mL). All the other compounds showed cytotoxicity at higher values.

Among the compounds tested for cytotoxicity on DU-145 cell lines, it is interesting to note that the compound again with thieny unit (B_{22}) showed maximum activity (IC_{50} 18 µg/mL) and this is much more potent on these cell lines than the other two cell lines tested. This is followed by compounds, B_{15} (IC_{50} 23 µg/mL), B_5 (IC_{50} 43 µg/mL), B_6 (IC_{50} 61 µg/mL), B_{11} having nitro and methyl substituent's on the aromatic ring (IC_{50} 68 µg/mL), B_1 having a methyl phenyl moiety (IC_{50} 70 µg/mL) and B_2 having a fluorophenyl moiety (IC_{50} 72 µg/mL). It was also observed that among all the compounds tested on these three cell lines, most of the compounds showed maximum activity on prostate cancer cell lines (DU-145). This was consistent with our earlier observation on the cytotoxic activities of the chalcones synthesized in our laboratory, which demonstrated significant activity against prostate cancer cell lines (unpublished work).

CONCLUSION

From this study, it may conclude that, among the compounds tested for cytotoxicity on DU-145 cell lines, it is interesting to note that the compound again with thieny unit (B_{22}) showed maximum activity (IC_{50} 18 µg/mL). It was also observed that among all the compounds tested on these three cell lines, most of the compounds showed maximum activity on prostate cancer cell lines (DU-145).

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provides the facilities to carrying out this research work.

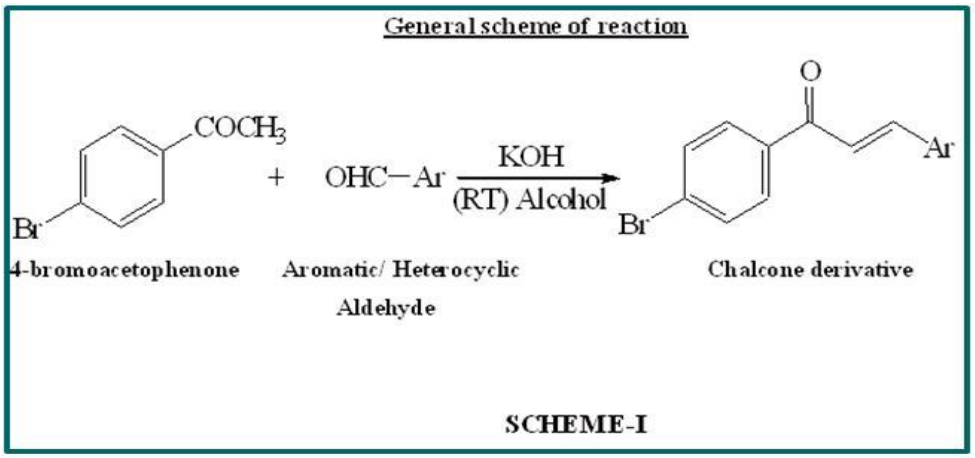
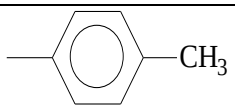
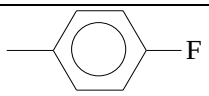
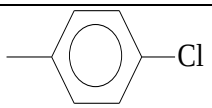
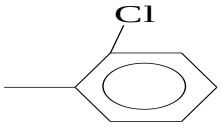
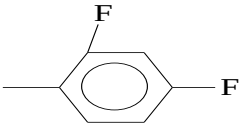
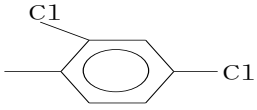
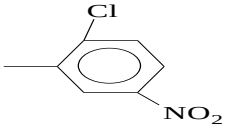
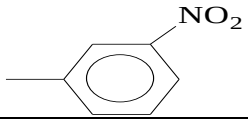


Table 1: Physical characterization data of chalcones (B_1-B_{12}).

Compound	Ar	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (°C)	Yield %
B_1		$C_{16}H_{13}BrO$	301	166	85
B_2		$C_{15}H_{10}BrFO$	305	132	89
B_3		$C_{15}H_{10}BrClO$	321	145	87
B_4		$C_{15}H_{10}BrClO$	321	149	73
B_5		$C_{15}H_9BrF_2O$	323	173	83
B_6		$C_{15}H_9BrCl_2O$	356	189	79
B_7		$C_{15}H_9BrClNO_3$	366	125	84
B_8		$C_{15}H_{10}BrNO_3$	332	175	78

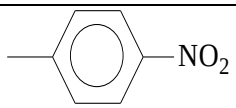
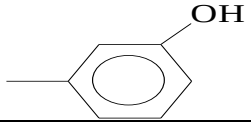
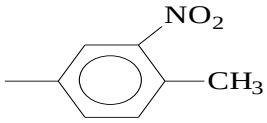
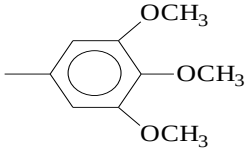
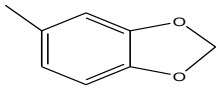
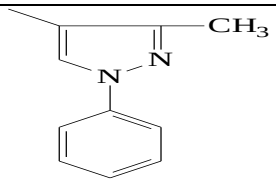
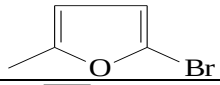
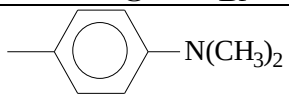
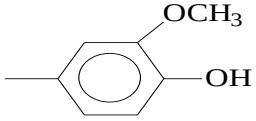
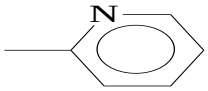
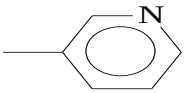
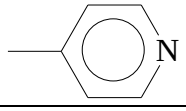
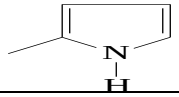
B_9		**$C_{15}H_{10}BrNO_3$**	**332**	**189**	**77**
B_{10}		**$C_{15}H_{11}BrO_2$**	**303**	**228**	**79**
B_{11}		**$C_{16}H_{12}BrNO_3$**	**346**	**174**	**83**
B_{12}		**C18H17BrO4**	**377**	**145**	**87**

Table 2: Physical characterization data of chalcones (B_{13}-B_{25})

B_{13}		**$C_{16}H_{11}BrO_3$**	**331**	**192**	**84**
B_{14}		**$C_{19}H_{15}BrN_2O$**	**367**	**145**	**79**
B_{15}		**$C_{13}H_8Br_2O_2$**	**356**	**163**	**83**
B_{16}		**$C_{17}H_{16}BrNO$**	**330**	**132**	**85**
B_{17}		**$C_{16}H_{13}BrO_3$**	**333**	**211**	**81**
B_{18}		**$C_{14}H_{10}BrNO$**	**288**	**153**	**88**
B_{19}		**$C_{14}H_{10}BrNO$**	**288**	**181**	**82**
B_{20}		**$C_{14}H_{10}BrNO$**	**288**	**182**	**86**
B_{21}		**$C_{13}H_{10}BrNO$**	**276**	**138**	**90**

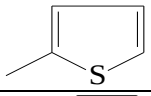
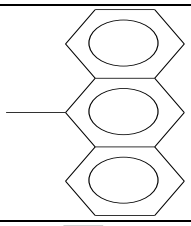
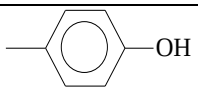
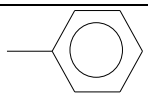
B_{22}		**$C_{13}H_9BrOS$**	**293**	**143**	**78**
B_{23}		**$C_{23}H_{15}BrO$**	**387**	**165**	**77**
B_{24}		**$C_{15}H_{11}BrO_2$**	**303**	**189**	**77**
B_{25}		**$C_{15}H_{11}BrO$**	**287**	**128**	**81**

Table 3: Elemental Analysis data of chalcones (B_1-B_{25})

Compound	(% Calc.)			C,H,N (% found)		
	C	H	N	C	H	N
B_1	63.81	4.35	-	63.85	4.31	-
B_2	59.04	3.30	-	59.13	3.26	-
B_3	56.02	3.13	-	56.12	3.18	-
B_4	56.02	3.13	-	56.04	3.13	-
B_5	55.75	2.81	-	55.71	2.84	-
B_6	50.60	2.55	-	50.62	2.51	-
B_7	49.14	2.47	3.82	49.11	2.42	3.87
B_8	54.24	3.03	4.22	54.23	3.13	4.21
B_9	54.24	3.03	4.22	54.21	3.10	4.21
B_{10}	59.43	3.66	-	59.41	3.61	-
B_{11}	55.51	3.49	4.05	55.53	3.43	4.01
B_{12}	57.31	4.54	-	57.32	4.52	-
B_{13}	58.03	3.35	-	58.05	3.31	-
B_{14}	62.14	4.12	7.63	62.12	4.11	7.61
B_{15}	43.86	2.26	-	43.82	2.22	-
B_{16}	61.83	4.88	4.24	61.81	4.82	4.22
B_{17}	57.68	3.93	-	57.65	3.95	-
B_{18}	58.36	3.50	4.86	58.33	3.51	4.82
B_{19}	58.36	3.50	4.86	58.32	3.52	4.82
B_{20}	58.36	3.50	4.86	58.35	3.54	4.81

B_{21}	56.55	3.65	5.07	56.52	3.63	5.03
B_{22}	53.26	3.09	-	53.21	3.07	-
B_{23}	71.33	3.90	-	71.31	3.94	-
B_{24}	59.43	3.66	-	59.41	3.62	-
B_{25}	62.74	3.86	-	62.73	3.82	-

Table 4: IR (KBr disc) spectral data of chalcones (B_1-B_{14})

Compound	Position of absorption band (cm^{-1})
B_1	1655 (C=O), 1602 (C=C of Ar), 1505(CH=CH), 1085 (C-O-C)825 (C-Br)
B_2	1664 (C=O), 1580 (C=C of Ar), 1524 (CH=CH), 1065 (C-O-C), 925 (C-F) 828 (C-Br)
B_3	1653 (C=O), 1585 (C=C of Ar), 1505 (CH=CH),1083 (C-O-C), 835 (C-Cl)823 (C-Br)
B_4	1652 (C=O), 1583 (C=C of Ar), 1502 (CH=CH), 1076 (C-O-C), 833 (C-Cl) 823 (C-Br)
B_5	1655 (C=O), 1581 (C=C of Ar), 1510 (CH=CH), 1073 (C-O-C), 925 (C-F)826 (C-Br)
B_6	1663 (C=O), 1578 (C=C of Ar), 1506 (CH=CH), 1082 (C-O-C), 833 (C-Cl)821 (C-Br)
B_7	1658 (C=O), 1603 (C=C of Ar), 1515 (CH=CH), 1048 (C-O-C), 824 (C-Cl), 1525 (N=O, asymmetric), 1348 (N=O, symmetric), 829 (C-Br)
B_8	1655 (C=O), 1605 (C=C of Ar), 1508 (CH=CH), 1045 (C-O-C), 1533 (N=O, asymmetric), 1345 (N=O, symmetric), 825 (C-Br)
B_9	1652 (C=O), 1610 (C=C of Ar), 1502 (CH=CH), 1063 (C-O-C), 1541 (N=O, asymmetric), 1346 (N=O, symmetric), 823 (C-Br)
B_{10}	3520 (O-H), 1648 (C=O), 1612 (C=C of Ar), 1505 (CH=CH), 1058 (C-O-C),
B_{11}	1655 (C=O), 1605 (C=C of Ar), 1500 (CH=CH), 1048 (C-O-C), 1545 (N=O, asymmetric), 1343 (N=O, symmetric), 822 (C-Br)
B_{12}	1652 (C=O), 1585 (C=C of Ar), 1462 (CH=CH), 1127 (-O-CH_3), 996 (C-O-C)827 (C-Br)
B_{13}	1643 (C=O), 1574 (C=C of Ar), 1500 (CH=CH), 1240 (O-CH_2-O),1036 (C-O-C) 829 (C-Br)
B_{14}	1663 (C=O), 1610 (C=N), 1588 (C=C of Ar), 1510 (CH=CH), 1391 (C-N), 1060 (C-O-C), 821 (C-Br)
B_{15}	1652 (C=O), 1585 (C=C of Ar), 1503 (CH=CH), 1082 (C-O-C), 829 (C-Br)
B_{16}	1650 (C=O), 1586 (C=C of Ar),1505 (CH=CH), 1178 (-N(CH_3)$_2$),1076 (C-O-C)821 (C-Br)
B_{17}	3450 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1510 (CH=CH), 1225 (-OCH_3), 1083 (C-O-C), 825 (C-Br)
B_{18}	1653 (C=O), 1605 (C=C of Ar), 1595 (C=N), 1508 (CH=CH), 1385 (C-N), 1079 (C-O-C), 822 (C-Br)
B_{19}	1645 (C=O), 1603 (C=C of Ar), 1590 (C=N), 1502 (CH=CH), 1370 (C-N), 1085 (C-O-C), 823 (C-Br)
B_{20}	1650 (C=O), 1605 (C=C of Ar), 1581 (C=N), 1505 (CH=CH), 1373 (C-N), 1083 (C-O-C), 829 (C-Br)
B_{21}	1652 (C=O), 1605 (C=C of Ar), 1588 (C=N), 1506 (CH=CH), 1375 (C-N), 1085 (C-O-C), 821 (C-Br)
B_{22}	1655 (C=O), 1610 (C=C of Ar), 1505 (CH=CH), 1078 (C-O-C), 624 (C-S)823 (C-Br)
B_{23}	1658 (C=O), 1605 (C=C of Ar), 1503 (CH=CH), 1085 (C-O-C), 823 (C-Br)
B_{24}	3460 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1505 (CH=CH), 1083 (C-O-C)824 (C-Br)
B_{25}	1650 (C=O), 1605 (C=C of Ar), 1502 (CH=CH), 1075 (C-O-C), 829 (C-Br)

Table 5: ^{1} H NMR spectral data of chalcones (B_1-B_{14})

Compounds	Chemical shift (δ) in ppm
B_1	2.40 (3H, s, Ar-CH_3), 7.23 (1H, d,J= 17 Hz, -CO-CH=), 7.73 (1H, d, J=17 Hz, =CH-Ar), 7.20-7.78 (8H, Ar-H)
B_2	7.15 (1H, d, J= 17 Hz, -CO-CH=), 7.62 (1H, d, J=17 Hz, =CH-Ar), 7.05-7.71 (8H, Ar-H)
B_3	7.45 (1H, d, J= 17 Hz, -CO-CH=), 7.82 (1H, d, J=17 Hz, =CH-Ar), 7.38-8.20 (8H, Ar-H)
B_4	7.43 (1H, d, J= 17 Hz, -CO-CH=), 7.80 (1H, d, J=17 Hz, =CH-Ar), 7.36-8.21 (8H, Ar-H)
B_5	7.40 (1H, d, J= 17 Hz, -CO-CH=), 7.73 (1H, d, J=17 Hz, =CH-Ar), 7.15-8.10 (7H, Ar-H)
B_6	7.68 (1H, d, J= 17 Hz, -CO-CH=), 7.85 (1H, d, J=17 Hz, =CH-Ar), 7.42-8.20 (7H, Ar-H)
B_7	7.49 (1H, d, J= 17 Hz, -CO-CH=), 7.65 (1H, d, J=17 Hz, =CH-Ar), 7.12-8.60 (7H, Ar-H)
B_8	7.40 (1H, d, J= 17 Hz, -CO-CH=), 7.62 (1H, d, J=17 Hz, =CH-Ar), 7.20-8.55 (8H, Ar-H)
B_9	7.43 (1H, d, J= 17 Hz, -CO-CH=), 7.68 (1H, d, J=17 Hz, =CH-Ar), 7.21-8.59 (8H, Ar-H)
B_{10}	7.38 (1H, d, J= 17 Hz, -CO-CH=), 7.52 (1H, d, J=17 Hz, =CH-Ar), 6.89 (1H, s, Ar-OH), 7.18-7.79 (8H, Ar-H)
B_{11}	2.50 (3H. s, Ar-CH_3), 7.40 (1H, d, J= 17 Hz, -CO-CH=), 7.65 (1H, d, J=17 Hz, =CH-Ar),7.15-8.53 (7H, Ar-H)
B_{12}	7.15 (1H, d, J= 17 Hz, -CO-CH=), 7.64 (1H, d, J=17 Hz, =CH-Ar), 7.12-7.58 (6H, Ar-H), 3.78 (3H,s,Ar-OCH_3), 3.88 (6H,s,2x Ar-OCH_3)
B_{13}	6.10 (2H,s,-O-CH_2O-), 6.88 (1H, d, J= 17 Hz, -CO-CH=), 7.69 (1H, d, J=17 Hz, =CH-Ar), 7.10-7.29 (7H, Ar-H)
B_{14}	2.45 (3H, s, Ar-CH_3), 6.85 (1H, d, J= 17 Hz, -CO-CH=), 7.65 (1H, d, J=17 Hz, =CH-Ar), 6.58-7.90 (9H, Ar-H)
B_{15}	7.23 (1H, d, J= 17 Hz, -CO-CH=), 7.71 (1H, d, J=17 Hz, =CH-Ar), 7.18-7.95 (6H, Ar-H)
B_{16}	3.10 (6H,s,-$N(CH_3)_2$, 6.88 (1H, d, J= 17 Hz, -CO-CH=), 7.75 (1H, d, J=17 Hz, =CH-Ar), 6.65-7.90 (8H, Ar-H)
B_{17}	7.21 (1H, d, J= 17 Hz, -CO-CH=), 7.68 (1H, d, J=17 Hz, =CH-Ar), 7.20-7.93 (7H, Ar-H), 6.75 (1H.s, Ar-OH), 3.82 (3H,s,Ar-OCH_3)
B_{18}	7.15 (1H, d, J= 17 Hz, -CO-CH=), 7.65 (1H, d, J=17 Hz, =CH-Ar), 6.30-8.15 (8H, Ar-H)
B_{19}	7.18 (1H, d, J= 17 Hz, -CO-CH=), 7.70 (1H, d, J=17 Hz, =CH-Ar), 7.12-8.20 (8H, Ar-H)
B_{20}	7.15 (1H, d, J= 17 Hz, -CO-CH=), 7.75 (1H, d, J=17 Hz, =CH-Ar), 7.20-8.15 (8H, Ar-H)
B_{21}	7.10 (1H, d, J= 17 Hz, -CO-CH=), 7.70 (1H, d, J=17 Hz, =CH-Ar), 6.35-7.90 (8H, Ar-H)
B_{22}	7.12 (1H, d, J= 17 Hz, -CO-CH=), 7.70 (1H, d, J=17 Hz, =CH-Ar), 6.62-8.10 (7H, Ar-H)
B_{23}	7.35 (1H, d, J= 17 Hz, -CO-CH=), 7.60 (1H, d, J=17 Hz, =CH-Ar), 7.20-8.90 (13H, Ar-H)
B_{24}	7.28 (1H, d, J= 17 Hz, -CO-CH=), 7.59 (1H, d, J=17 Hz, =CH-Ar), 6.85 (1H,s,Ar-OH),7.21-7.89 (8H, Ar-H)
B_{25}	7.21 (1H, d, J= 17 Hz, -CO-CH=), 7.62 (1H, d, J=17 Hz, =CH-Ar), 7.11-7.90 (9H, Ar-H)

Table 6: Cytotoxicity of the new chalcones (B_1 to B_{25}):

(IC_{50} values in µg/mL)

Compound	Ar	Cell line		
		HT-29	MCF-7	DU-145
B_1	4"-methyl phenyl	76 ± 2	83 ± 1	70 ± 2
B_2	4"-fluorophenyl	90 ± 2	NA	72 ± 1
B_3	4"-chlorophenyl	120 ± 1	172 ± 2	105 ± 2

B_4	2"-chlorophenyl	137 ± 2	183 ± 2	144 ± 1
B_5	2",4"-difluorophenyl	68 ± 2	89 ± 1	43 ± 2
B_6	2",4"-dichlorophenyl	80 ± 2	95 ± 2	61 ± 1
B_7	2"-chloro-5"-nitro phenyl	110 ± 2	NA	101 ± 2
B_8	3"-nitro phenyl	NA	NA	155 ± 2
B_9	4"-nitro phenyl	146 ± 2	167 ± 2	120 ± 1
B_{10}	3"-hydroxyphenyl	164 ± 2	182 ± 2	176 ± 2
B_{11}	3"-nitro-4"methylphenyl	85 ± 2	92 ± 2	68 ± 3
B_{12}	3",4",5"-trimethoxyphenyl	124 ± 2	133 ± 1	78 ± 2
B_{13}	3",4"-methelenedioxyphenyl	146 ± 2	153 ± 2	82 ± 2
B_{14}	1"-phenyl-3"-methylpyrazole-4"-yl	NA	NA	174 ± 2
B_{15}	5"-bromofuran-2"-yl	67 ± 2	58 ± 2	23 ± 1
B_{16}	4"-dimethylaminophenyl	132 ± 1	117 ± 2	105 ± 2
B_{17}	3"-methoxy-4"-hydroxyphenyl	93 ± 2	88 ± 1	74 ± 2
B_{18}	2"-pyridinyl	NA	148 ± 2	107 ± 2
B_{19}	3"-pyridinyl	NA	NA	123 ± 2
B_{20}	4"-pyridinyl	190 ± 2	NA	116 ± 1
B_{21}	2"-pyrrolyl	73 ± 2	104 ± 2	87 ± 1
B_{22}	2"-thienyl	42 ± 2	38 ± 1	18 ± 1
B_{23}	9"-anthracenyl	NA	NA	186 ± 2
B_{24}	4"-hydroxyphenyl	93 ± 2	109 ± 2	76 ± 2
B_{25}	Phenyl	NA	NA	185 ± 2

Data presented as mean ± SD (n=3). All the compounds and the standard dissolved in DMSO, diluted with culture medium containing 0.1% DMSO. The control cells were treated with culture medium containing 0.1% DMSO.
NA- No Activity (i.e. $IC_{50} > 200$ µg/mL).

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