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IN SILICO DESIGN, SYNTHESIS AND *IN VITRO* ANTICANCER SCREENING OF NOVEL CHALCONE DERIVATIVES

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ABSTRACT

Chalcone are the central core for a large variety of biological compounds. Chalcones show antibacterial, antifungal, anticancer and anti-inflammatory properties. The present work involved the design and development of Chalcone derivatives as anticancer agents. The preliminary *in silico* screening was done using molecular docking. All the proposed derivatives were docked with various protein targets obtained from PDB, using ARGUS LAB software and satisfactory docking energy scores were obtained. From the analogues showing highest energy score, eleven compounds were synthesized by conventional method. Purity of the compounds thus synthesized was ascertained by consistency in melting point and R_f value and were characterized by IR and NMR spectral studies. The anticancer activities of the selected analogues were determined by MTT Assay method using Human Colorectal Adenocarcinoma (HCT116) and Human Breast cancer Cell lines (MDA-MB-468). The derivatives showed moderate activity on both cell lines. It has been noticed that derivatives like BCCL and BCF having electro negative atoms like Chlorine and Fluorine as their substituent, possess more activity than derivatives like BCMO and BCM which possess electro positive groups like Methoxy and Methyl groups.

KEYWORDS: Chalcone, MTT assay, MDA-MB-468, HCT116.

INTRODUCTION

Heterocyclic chemistry add-up-to almost half of all organic chemistry research worldwide. Heterocyclic structures form the basis of many agrochemical, veterinary and pharmaceutical products. Although a number of drugs are available in the market, thirst for discovering newer drug s with better pharmacokinetic profile, and lesser toxicity has become main objectives in the field of medicinal chemistry because of the fast development of microbial resistance towards the existing molecules. Studies on benzimidazole chalcone compounds have served as a feasible field of research in the perusal of biologically active compounds.

Chalcone is an aromatic ketone and an enone. They form the central core for a variety of important biological compounds, that are collectively known as chalcones or chalconoids. They are well known intermediates for synthesizing various heterocyclic compounds.[1] Chalcone derivatives, displayed a broad spectrum of pharmacological activities, among which antitumor,[2] antibacterial,[3,4] antifungal,[5] anti-inflammatory,[6] antiviral.[7] antioxidant, anticancer,[8] and antiamoebic[9,10,11] activities have been reported. They have also shown to inhibit the enzymes mainly mammalian alpha-amylase, cyclo-oxygenase (COX) and monoamine oxidase (MAO). Chalcones can be synthesized through cross aldol condensation between acetophenone and benzaldehyde in the presence of catalyst such as sodium hydroxide. Over the last few years, chalcones and derivatives received significant attention as potential anticancer agents and tubulin polymerization inhibitors.[12,13,14] since its antimitotic properties were discovered by Edwards et al in the 1990s.[15]

MATERIALS AND METHODS

The purity of the newly synthesized compounds were checked by Thin Layer Chromatography (TLC) using silica gel-G as stationary phase and the spot was visualized by Iodine vapour. The melting point of synthesized compounds was determined by open ended capillary tube method on a Thomas Hoover melting point apparatus and the values are found and uncorrected. The newly synthesized compounds were characterized by IR and ^{1}H NMR spectral analysis. IR spectra of the synthesized compounds were recorded using KBr pellets in the range of 4000-500cm^{-1} on Jasco FT/IR Model

4100. Proton NMR of the synthesised compounds was recorded in $CDCl_3$ on Bruker Ultra Shield DPX 400 in Indian institute of Science, Bangalore and in Banaras Hindu University. Chemical shifts were reported in δ (ppm) relative to Tetra Methyl Slilane (TMS) as internal standard.

***In silico* molecular modelling**

In silico molecular modification is the most important step in the rational drug designing of novel drugs, which act on specific therapeutic target.

Screening of proposed chalcone derivatives for different physicochemical properties using different software. Various physicochemical properties of the proposed molecules were calculated using ACD Labs Chemsketch 10.00 software.

Molinspiration software is used to calculate the "Lipinski Rule of Five" and drug likeness analysis. All of the*se in silico* properties will be closely evaluated and compared. Only the analogues with desired physicochemical properties, obeying Lipinski Rule of Five and those with not more than one violation will be selected for docking studies.

The selected Chalcone derivatives were then synthesised by conventional method using standard graded reagents and chemicals by Claisen-Schimidt condensation mechanism.

SCHEME

Scheme of Present Work

Step-1: ***Synthesis of 2-(α-hydroxy ethyl) benzimidazole: (compound I)***

A mixture of 27 g (0.25mol) of o-phenylene diamine, 25.5ml (30.6g, 0.34mol) of lactic acid was refluxed for 2½ hours. Reaction mixture was cooled and made alkaline by the gradual addition of 10% sodium hydroxide solution .The crude product was dissolved in 400 ml of boiling water. To this, add 2 g of activated charcoal and digested for 15 min. The digested solution was filtered rapidly at the pump through a pre heated Buchner funnel, the filtrate was cooled to about 10^0 C .The product obtained was filtered and washed with 25 ml of cold water and dried at 100^0C.

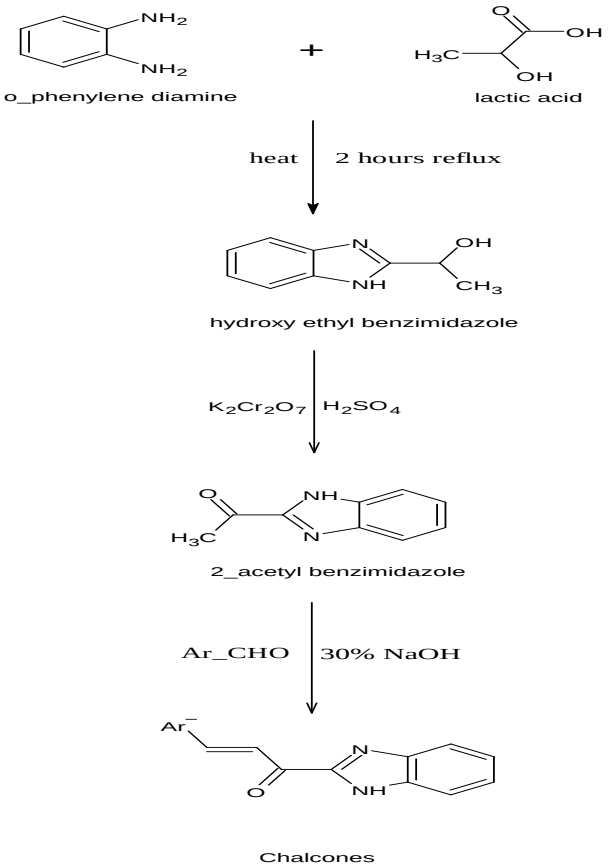


Figure 1: Scheme of work.

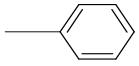
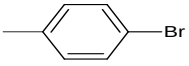
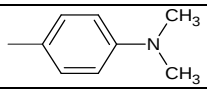
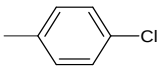
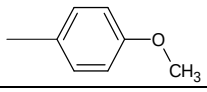
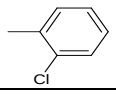
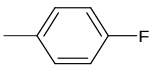
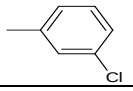
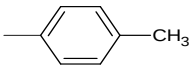
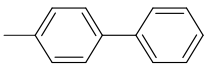
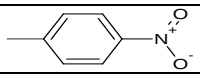
Step-2*: Synthesis of 2-acetyl benzimidazole: (compound II)*

At room temperature, a solution of $K_2Cr_2O_7$ (0.15 mol) and H_2SO_4 (25%, 80 ml) was drop wise added to a solution of 2-(α-hydroxy) ethyl benzimidazole 2a (0.01 mol) in dil. H_2SO_4 (5%, 40 ml) with constant stirring over a period of 20 min. After 20 minutes, the reaction mixture was further stirred at room temperature for 2 hours. After completion of the reaction (monitored by TLC), the reaction mixture was neutralized with aqueous NH_3 solution (1:1) and resultant orange solid was filtered, washed with water and dried.[16]

Step-3: ***Synthesis of Chalcone derivatives (Compounds)***

A solution of Compound - II (10mmol) in aq.NaOH (10 %, 30ml) was added to the respective aldehydes given below (10mmol) at room temperature. The reaction mixture was stirred for 30 min and the separated solid was filtered, washed with water and the crude product was re-crystallized from ethanol. (Scheme 1, Table 2) The physical constants of the synthesised compounds are presented in table 1.

Table 1: **Synthesized compounds with their compound code.**

Compound Code	—Ar	Compound Code	—Ar
BC		BCBR	
BCDM		BCCL	
BCMO		BCCL2	
BCF		BCCL3	
BCM		BCP	
BCN			

***In Vitro* Anticancer screening**

The derivatives BCF, BCCL, BCM, BCMO which showed good docking energy score were selected. The anticancer activity of these analogues were screened by MTT Assay method using Human Colorectal Adenocarcinoma (HCT116) and Human Breast cancer Cell lines (MDA-MB-468).

Cell treatment procedure

With trypsin-ethylene diamine tetraacetic acid (EDTA), the monolayer cells were detached to make single cell suspensions. Viable cells were counted using a hemocytometer. It is then diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred micro litres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 hours, the cells were treated with serial concentrations of the test samples which were initially dissolved in neat dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and stored frozen prior to use. Just before the drug addition, an aliquot of frozen concentrate was thawed and diluted with serum free medium to twice the desired final maximum test concentration. Additional four, 10 fold serial dilutions were made to provide a total of four drug concentrations. Aliquots of 100 µl of these different drug dilutions were added to the appropriate wells that already contains 100 µl of medium, and obtained the required final drug concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium without samples served as control. Triplicate was maintained for all concentrations.

MTT Assay

MTT is a water soluble tetrazolium salt which is yellow in colour. succinate-dehydrogenase, a mitochondrial enzyme in living cells, cleaves the tetrazolium ring and convert the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well after 48hours of incubation. It is then incubated at 37^0C for 4hours. The medium with MTT was then turned off. The formazan crystals formed were solubilized in 100µl of DMSO and then the absorbance was measured at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100 - Abs (sample) / Abs (control) x100.

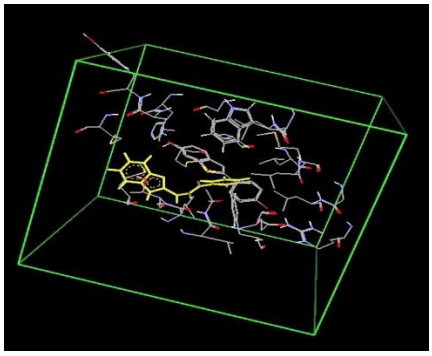
Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using GraphPad Prism software.

RESULT

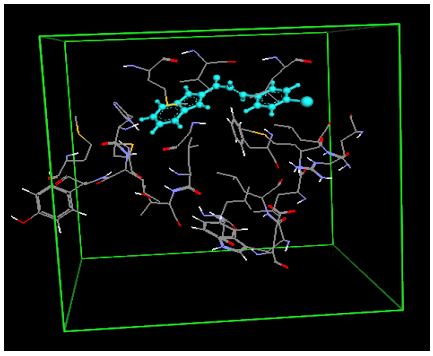
The **docking** studies were performed by using **Argus lab software**. The protein or enzyme target for anticancer studies were selected and the ligands which selected were docked with the selected targets and the docking scores were calculated. The standard inhibitor was also been docked to correlate the values of it with the docked ligands and the ligands which shows a higher docking score than the standard drug's docking energy score were identified and selected for wet lab synthesis.

Table 2: Docking Energy Scores of proposed derivatives against various Receptors.

Compound Code	PPAR-Gamma Energy Score	Estrogen Energy Score	Proteasome Energy Score	Tyrosine kinase Energy Score
BC	-8.52	-8.93	-8.34	-9.09
BCCL	**-11.48**	**-10.35**	**-11.55**	**-10.45**
BCDC	-9.78	-8.56	-9.51	-8.77
BCCB	-8.49	-8.55	-9.78	-8.63
BCCN	-9.38	-9.27	-7.81	-8.91
BCCL3	**-9.73**	**-8.85**	**-10.01**	**-9.23**
BCN	-8.85	-8.59	-8.12	-7.12
BCDN	-8.34	-9.28	-9.23	-8.23
BCNF	-8.51	-9.35	-9.35	-7.35
BCCL2	**-9.99**	**-9.90**	**-10.69**	**-9.99**
BCM	**-10.76**	**-10.73**	**-10.76**	**-11.76**
BCM2	-8.23	-9.73	-7.33	-8.86
BCMC	-8.48	-9.81	-8.65	-7.79
BCCF	-9.06	-8.95	-8.23	-8.88
BCFC	-8.01	-8.87	-8.14	-8.75
BCF	**-11.65**	**-11.82**	**-10.22**	**-10.65**
BCDF	-7.94	-8.85	-7.99	-7.96
BCFN	-8.05	-7.82	-8.34	-8.78
BCBR	-8.57	**-9.43**	-8.97	**-9.78**
BCDB	-8.73	-8.19	-8.32	-8.86
BCDM	-7.65	-8.82	-7.94	-8.86
BCMO	**-9.94**	**-9.74**	**-10.34**	**-10.26**
BCMO2	-7.43	-8.79	-9.12	-8.76
BCMOC	-8.43	-7.13	-9.23	-7.89
BCMON	-8.73	-8.35	-8.54	-8.90
BCP	-8.63	-7.46	-9.02	-8.55

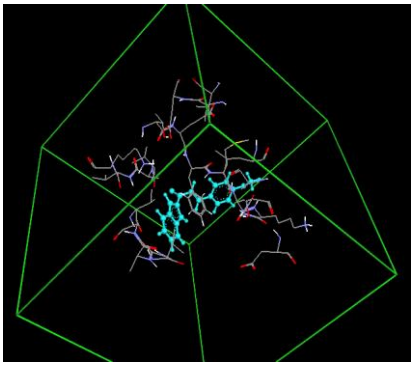


BCM

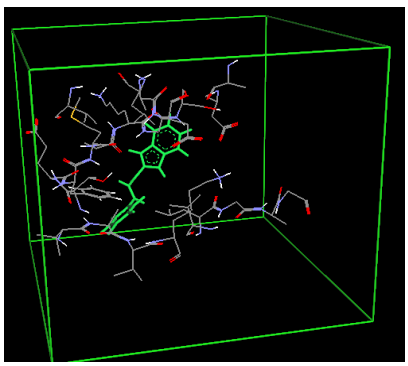


BCF

Figure 2: Docking images of different derivatives with estrogen receptor.

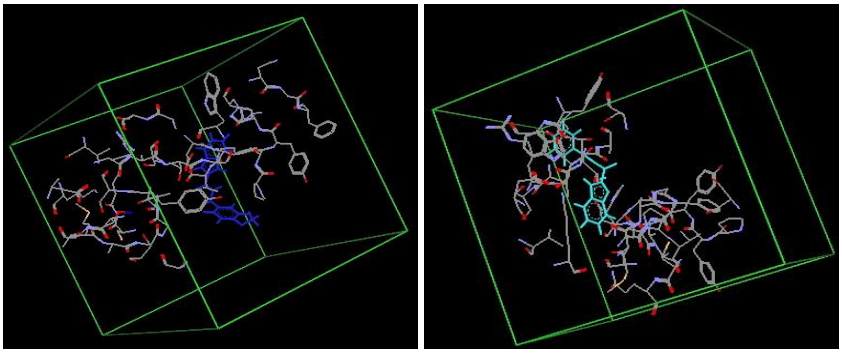


BCMO



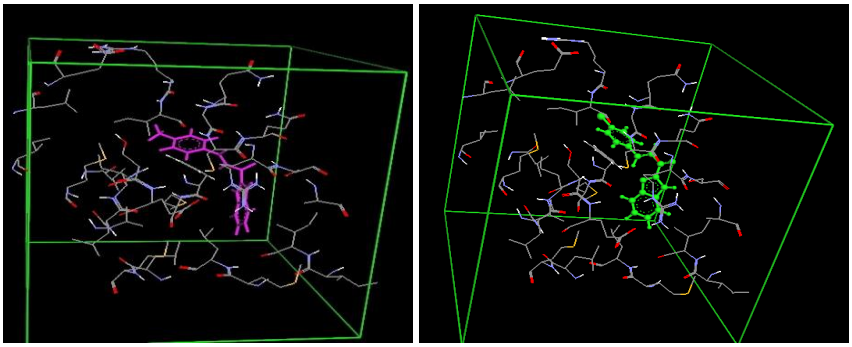
BCCL

Figure 3: Docking images of different derivatives with tyrosine.



BCCL **BCMO**

Figure 4: Docking images of different derivatives with proteasome receptor.



BCM **BCF**

Figure 5: Docking images of different derivatives with ppar gamma receptor.

Table 3: Physical Constant of Synthesized Compounds.

Compound Code	Molecular Formula	Molecular Weight	Percentage Yield (%w/w)	Melting Point ^{0}C	Rf Value
BC	$C_{16}H_{12}N_2O$	248	81.96	195	0.79
BCDM	$C_{18}H_{17}N_3O$	291	79.7	171	0.77
BCMO	$C_{16}H_{11}N_2O_2$	278	74.44	186	0.76
BCBR	$C_{16}H_{11}N_2OBr$	327	67.42	182	0.83
BCM	$C_{17}H_{14}N_2O$	262	75.38	140	0.74
BCN	$C_{16}H_{11}N_3O_3$	293	75.09	188	0.82
BCF	$C_{16}H_{11}N_2OF$	266	74.61	175	0.86
BCCL	$C_{16}H_{11}N_2OCl$	282	60.87	176	0.81
BCCL2	$C_{16}H_{11}N_2OCl$	282	69.73	165	0.78
BCCL3	$C_{16}H_{11}N_2OCl$	282	61.96	178	0.85
BCP	$C_{22}H_{16}N_2O$	324	52.24	122	0.71

Table 4: Spectral data of synthesized compounds.

COMPOUND CODE	FTIR Interpretation	^{1}HNMR($CDCl_3$) δppm
BC	1670 (C=0), 3250(NH), 1660(-CH=CH-), 1597(C=N), 749(Ar-CH)	5.07 (s, 1H, NH of benzimidazole) 7.73 (s, 1H, CH of ethylene (*Cis*)) 8.07 (s, 1H, CH of ethylene (*Trans*)) 7.28 – 7.49 (m, 4H, Ar*H* of benzimidazole) 7.81 – 7.83 (m, 5H, ArH)
BCDM	3292{-N-(CH_3)}, 1670(CH=CH), 1541 (C=N), 729 (Ar-CH)	3.83 (s, 3H, OCH_3) 4.93 (s, 1H, NH of imidazole) 7.37 (s, 1H, ethylene (*Cis*)) 8.03 (s, 1H, ethylene (*Trans*)) 6.81 – 7.36 (m, 4H, Ar*H* of benzimidazole) 7.64 – 7.97 (m, 3H, ArH)
BCMO	1680 (C=O), 1612(-CH=CH-), 1564 (C=N),	2.10 (s, 3H, CH_3) 4.97 (s, 1H, NH of imidazole)

	1217 (Ar-C-O-), 765 (Ar-H).	7.37 (s, 1H, ethylene (*Cis*)) 8.03 (s, 1H, ethylene (*Trans*)) 6.81 – 7.36 (m, 4H, Ar*H* of benzimidazole)7.64 – 8.03 (m, 4H, Ar*H* of toluene)
BCF	3292 (-NH), 1747 (C=0), 1666 (-CH=CH-), 1585 (C=N), 513(C-F), 746 (Ar-CH).	
BCM	3265 (N-H), 1735 (C=O), 1659 (-CH=CH-), 1514 (C=N), 736 (Ar-H).	
BCN	1666 (-CH=CH-), 1514 (C=N), 746 (Ar-CH)	
BCBR	3547 (N-H), 1689 (-C=O), 1689 (-CH=CH-), 1514 (C=N), 520 (C-Br).	
BCCL	3269 (N-H), 1660(-CH=CH-), 1514 (C=N), 736 (Ar-H), 470 (C-Cl).	
BCCL2	3269 (N-H), 1660(-CH=CH-), 1514 (C=N), 736 (Ar-H), 470 (C-Cl).	
BCCL3	3269 (N-H), 1660(-CH=CH-), 1514 (C=N), 736 (Ar-H), 470 (C-Cl).	
BCP	1683 (C=O), 1602(-CH=CH-), 1562 (C=N), 763 (Ar-H).	

Table 5: Anticancer Data for Compounds in MDA-MB-468 Cells.

Compound Code	% Growth Inhibition				IC_{50} Value (µM)
	0.1 (µM)	1.0 (µM)	10 (µM)	100 (µM)	
BCM	1.60	10.66	29.56	75.22	26.45
BCF	1.92	11.14	39.71	84.28	**15.26**
BCCL	1.06	9.13	37.75	82.60	**17.22**
BCMO	1.21	8.36	25.49	71.31	33.99

Table 6: Anticancer Data for Compounds in HCT-116 Cell Lines.

Compound Code	% growth inhibition				IC_{50} Value (µM)
	0.1 (µM)	1.0 (µM)	10(µM)	100 (µM)	
BCM	1.13	9.66	23.18	66.34	42.48
BCCL	1.72	12.14	25.71	73.77	**30.52**
BCF	1.06	14.75	29.75	78.53	**23.16**
BCMO	1.19	8.93	20.49	62.42	52.68

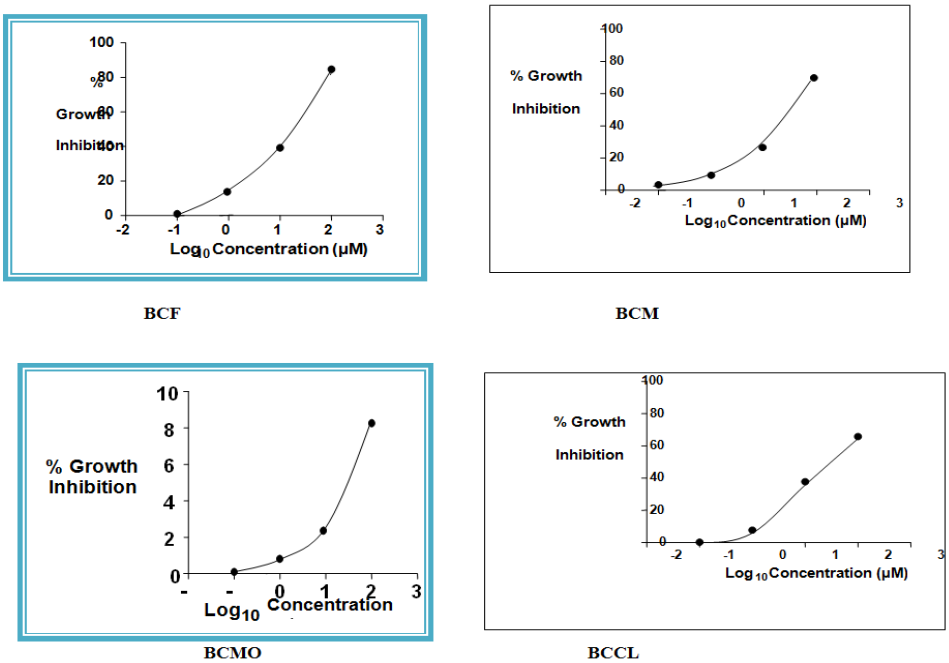


Figure 5: Percentage Growth Inhibition of Selected Chalcone Derivatives in MDA-MB-468 Breast Cancer Cells.

DISCUSSION

The present research work involved the preliminary *in silico* screening of various chalcone analogues for quantifying their drug likeness using Molinspiration software. The candidates with not more than one violation for Lipinski Rule of Five were taken for docking studies. All the proposed derivatives were docked with various protein targets obtained from PDB, using ARGUS LAB software and satisfactory docking energy scores were obtained. From the analogues showing highest energy score, eleven compounds were synthesized by conventional method. Purity was ascertained by consistency in melting point and R_f value and are characterized by IR and NMR spectral studies. A preliminary anticancer screening of the synthesized compounds was performed. The analogues namely BCF (2*E)-1-(1H-benzimidazol-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one*, BCCL (2*E)-1-(1H-benzimidazol-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one*, BCM(2*E)-1-(1H-benzimidazol -2-yl)-3-(4-methylphenyl)prop-2-en-1-one*, BCMO (2*E)-1-(1H-benzimidazol-2-yl)-3-(4-methoxyphenyl) prop-2-en-1-one* which showed better docking energy scores were screened for anti-cancer activity against Human colorectal cancer cells (HCT-116) and Human breast cancer cells (MDA-MB-468). Among them, compounds BCF and BCCL were found to be more active than BCM and BCMO. Tamoxifen is used as the standard drug. So it has been noticed that derivatives like BCCL and BCF having electro negative atoms like Chlorine and Fluorine as their substituent respectively possess more activity than derivatives like BCMO and BCM which possess electro positive groups like Methoxy and Methyl groups. So in future, by incorporating more electronegative atoms or ring systems into these derivatives, their activity can be enhanced and can be marketed as a good anticancer drug.

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REFERENCES

1. Sapavat Madhavi, Reddymasu Sreenivasulu, Jyothsna Pragathi Yazala, Rudraraju Ramesh Raju * Synthesis of chalcone incorporated quinazoline derivatives as anticancer agents. Saudi Pharmaceutical Journal, 2017; 25: 275–279.
2. Aneta Modzelewska, Catherine Pettit, Geetha Achanta, Nancy E Davidson, Peng Huang, Saeed R Khan. Anticancer activities of novel chalcone and bischalcone derivatives. Bioorg. Med. Chem, 2006; 14: 3491–3495.
3. S. B. Zangade, J. D. Jadhav, Lalpod, Y. B. Vibhute*, B. S. Dawane. Synthesis and antimicrobial activity of some new chalcones and flavones containing substituted naphthalene moiety. J. Chem.Pharm. Res., 2010; 2(1): 310–314.
4. Y. Rajendra Prasad, P. Ravi Kumar, D. Jesse Smiles and P. Ajay Babu QSAR studies on chalcone derivatives as antibacterial agents against Bacillus pumilis. ARKIVOC, 2008; (xi): 266-276.
5. K L Lahtchev, D I Batovska, St P Parushev, V M Ubiyvovk, A A Sibirny. Antifungal activity of chalcones: a mechanistic study using various yeast strains. Eur. J. Med. Chem, 2008; 43(10): 2220–2228.
6. Shen-Jeu Won , Cheng-Tsung Liu, Lo-Ti Tsao, Jing-Ru Weng, Horng-Huey Ko, Jih-Pyang Wang, Chun-Nan Lin. Synthetic chalcones as potential anti-inflammatory and cancer chemopreventive agents. Eur. J. Med. Chem, 2005; 40(1): 103–112.
7. Sharma, H., Patil, S., Sanchez, T.W., Neamati, N., Schinazi, R.F. Buolamwini, J.K., 2011. Synthesis, biological evaluation and 3D-QSAR studies of 3-keto salicylic acid chalcones and related amides as novel HIV-1 integrase inhibitors. Bioorg. Med. Chem. 2011 March 15; 19(6): 2030–2045.
8. R J Anto, K Sukumaran, G Kuttan, M N Rao, V Subbaraju, R Kuttan. Anticancer and antioxidant activity of synthetic chalcones and related compounds. Cancer Lett., 1995 Oct 20; 97(1): 33-37.
9. Abdullah M Asiri, Salman A Khan. Synthesis and Anti-Bacterial Activities of a Bis-Chalcone Derived from Thiophene and Its Bis-Cyclized Products. Molecules, 2011 Jan 12; 16(1): 523-531.
10. Abdullah M Asiri, Salman A Khan. Synthesis, characterization and optical properties of mono- and bis-chalcone. Material Lett., 2011 June 65(12): 1749–1752.
11. Abdullah M Asiri, Salman A Khan. Synthesis, Characterization, and In Vitro Antibacterial Activities of Macromolecules Derived from Bis-Chalcon. J. Hetrocycl. Chem, 2012; 49(6): 1434–1438.
12. Ducki S, Antimitotic chalcones and related compounds as inhibitors of tubulin assembly. Anti-cancer Agents in Med. Chem., 01 Mar 2009; 9(3): 336-347.
13. Hassan Mirzaei, Mohammad Shokrzadeh, Mona Modanloo, Ali Ziar, Gholam Hossein Riazi, Saeed Emami. New indole-based chalconoids as tubulin-targeting antiproliferative agents. Bioorg Chem, 2017 Dec; 75: 86-98.
14. Nicholas J Lawrence and Alan T McGown. The Chemistry and Biology of Antimitotic Chalcones and Related Enone Systems. Curr. Pharm. Des., 2005; 11: 1679-1693.
15. M L Edwards, D M Stemerick, P S Sunkara. Chalcones: a new class of antimitotic agents. J. Med. Chem., 1990; 33: 1948-1954.
16. Yali Kong, Kan Wang, Michael C Edler, Ernest Hamel, Susan L Mooberry, Mikell A. Paige et al. A boronic acid chalcone analog of combretastatin A-4 as a potent anti-proliferation agent. *Bioorg Med Chem*, January-15-2010; 18(2): 971- 977.